THE THERMODYNAMICS OF PARTITIONING OF PHENOLIC COMPOUNDS BETWEEN AQUEOUS SOLUTION AND MODEL MEMBRANE SYSTEMS

by

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ABSTRACT

The distribution behaviour of phenolic compounds between aqueous solution and the bulk organic solvents, n-octanol and cyclohexane, has been investigated over a range of temperature. The temperature dependence of partitioning of the phenolic compounds between aqueous solution and dimyristoyl phosphatidyl choline (DMPC) liposomes, below the phase transition temperature, has also been examined. From the experimental data, the thermodynamic parameters of free energy, enthalpy and entropy of transfer for the substituted phenols between aqueous solution and the three lipoidal environments have been derived. The non-covalent interactions and structural changes involved in the transfer of the phenolic solutes from aqueous solution to the various model membrane system could then be determined. Analysis of the thermodynamic data showed that the overall partitioning of phenolic solutes from aqueous solution to DMPC liposomes, below the phase transition temperature, was different than the transfer of the same solutes from aqueous solution to either n-octanol or cyclohexane. There were similarities between the three aqueous/model membrane systems however. The values of the partition coefficients in the aqueous/n-octanol system more closely resembled, in both absolute and relative terms, the values in the aqueous/liposome system, than did the aqueous/cyclohexane partition coefficients. Solute-lipid, as well as solute-water interactions were found to be important for partitioning in both the aqueous/liposome and aqueous/n-octanol systems, whilst only solute-water interactions were significant for partitioning in the aqueous cyclohexane system. The transfer of phenols from aqueous solution to DMPC liposomes

was entropy driven, whilst partitioning into n-octanol and cyclohexane was either enthalpy or entropy controlled depending on type of substituent on the molecule. Enthalpy-entropy compensation was found to be present for partitioning of phenols in the aqueous/liposome and aqueous/cyclohexane systems, but not in the aqueous/n-octanol system.

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TO OUR FATHERS

WHO LACKED COLLEGE DEGREES

BUT NOT THE VISION AND WILL

TO GUARANTEE OURS

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INTRODUCTION

CHAPTER 1

USES OF PARTITION COEFFICIENTS IN THE PHARMACEUTICAL SCIENCES.

1.1. Structure Activity Relationships (SAR).

It has always been the desire of medicinal chemists to correlate drug action with chemical structure and to provide a more reliable means of deducing potentially pharmacological agents (Cammarata and Martin, 1970). Any sound method for the prediction of a molecular structure that gives maximum biological activity will have wide applications and result in a considerable saving of time and money. As a result there has been a great upsurge in activity in this field over the last fifteen years or so. The topic of SAR has been reviewed many times in recent years and the literature abounds with excellent surveys of the field (Hansch, 1970, 1978; Ariens, 1971; McFarland, 1971; Tute, 1971; Verloop, 1972; Valkenberge, 1972; Purcell et al., 1973; Redl et al., 1974; Martin, 1978, 1974; Osman et al., 1979; Saxena and Ram, 1979; Kubinyi, 1979).

The hydrophobicity of a drug molecule, as defined by its partition coefficient has been one of the most successful parameters relating structure to biological activity. Early studies on structure activity were those made by Overton (1901) and Meyer (1899), who related narcotic activity to partition coefficients. In a normal homologous series, biological activity increases by a factor of 2.5 to 3.3 upon the addition of a methylene group depending on the nature of the drug series

and the test organism. Ferguson (1939) introduced a more rigorous concept when he suggested that an equilibrium exists between the extracellular phase and the phase at the site of action such that substances of about equal thermodynamic activity produce equal degrees of narcosis. The thermodynamic approach was further developed by Crisp and Marr (1957) who concluded that the mechanism of narcotic activity is only consistent with an equilibrium condition between the narcotic in the biophase and that in the external medium, an approach which has been recently adapted by Higuchi and Davis (1970).

The introduction of the free energy related approach to structure activity correlation by Hansch and colleagues (1962, 1963) has found wide acceptance and has prompted much work in this field.

There are numerous examples in the literature in which the biological activity of drug molecules is related to their lipophilicity; examples involving phenolic compounds will be discussed briefly. The physiological activity of substituted phenols was correlated with substituent constants by Fujita (1966). He examined the toxicity of phenols to plants and bacteria and the uncoupling activity of phenols on oxidative phosphorylation and found good correlation with hydrophobicity (and pKa). Tollenaere (1973) also investigated the structure activity relationships involved in the uncoupling of oxidative phosphorylation by phenols. Dunn and Hansch (1974) surveyed the literature and found linear correlations of the type :

$$\log \frac{1}{C} = a \log K + b$$

where C = concentration required for biological action,

K = partition coefficient,

and a, b are constants,

for the bactericidal action of phenols in S. Aureus and S. Typhosa. The haemolytic and anti-bactericidal activity and toxicity of substituted pehnols have been studied by Biagi et al. (1975). Rittich et al. (1980) have been successful in relating Hansch π values for phenols with fungicidal activity. Structure activity relationships for the inhibition of prostaglandin cyclo-oxygenase by phenolic compounds have also been successful (Dewhirst, 1980). Hydrophobicity of substituted phenols was linearly correlated with UDP-glucuronsyl-transferase activity in a study by Schefer et al. (1981).

1.2. Drug Absorption, Transport and Distribution.

Drug absorption, whether it be from the gastro-intestinal tract or from the buccal mucosa, requires the passage of the drug, in a molecular form, across the barrier membrane. Most drugs are presented to the body as solid or semisolid dosage forms and these must first release their drug content. The drug must then dissolve and if it has the appropriate physical properties, will pass from a region of high concentration to a region of low concentration across a membrane.

Biological membranes are composed of small amphiphatic molecules, phospholipids and cholesterol, which associate into lipoidal bilayers in aqueous media. Also associated with the membranes are proteins and carbohydrates embedded in the matrix of the lipid molecules. The membrane has a hydrophilic

exterior and a hydrophobic interior. The most widely accepted model for biological membranes is the Fluid Mosaic structure of Singer and Nicholson (1972) (Fig.1). Permeation of non-electrolytes across biological membranes can be explained on the basis of the membrane behaving as a continuous hydrophobic phase, although there may be a small fraction of the membrane composed of aqueous pores. It is assumed that drug absorption proceeds by passive diffusion. The permeability of solutes across biological membranes as defined by the equation :

$$P = \frac{K \cdot D}{d}$$

where P = permeability coefficient,

K = partition coefficient,

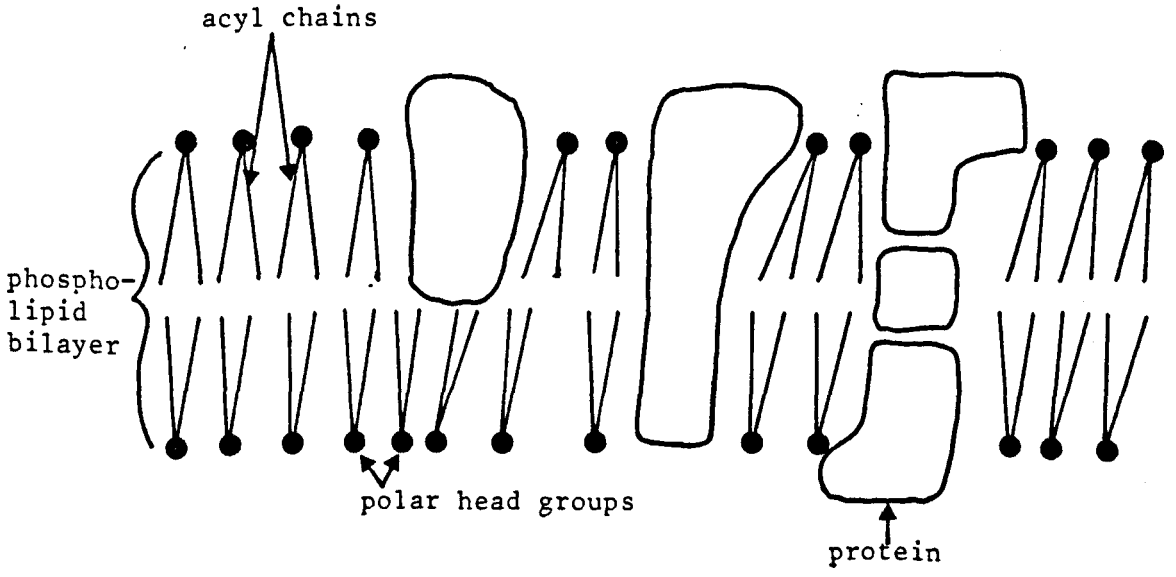
D = diffusion coefficient,

d = membrane thickness.

Thus the absorption of a drug molecule across a biological membrane is directly proportional to its partition coefficient.

The course of passage through membranes can be considered as a partitioning between alternating hydrophilic and hydrophobic phases. Hydrophilic molecules possessing very small partition coefficients tend to concentrate in hydrophilic phases, whereas lipophilic molecules, having very large partition coefficients, will be localised in hydrophobic phases. In both circumstances, ability to be transported to the site of action is impaired, resulting in reduced biological activity. Only compounds with an optimal partition coefficient will be efficiently transported to the site of action and thus show high biological activity.

Fig.1. Fluid Mosaic Model.



As most drugs are weak electrolytes it is to be expected that the unionised form of either acids or bases, the lipid soluble species, will diffuse while the ionised forms will be rejected. This is the basis of the pH-partition hypothesis in which the pH-dependency of drug absorption and transport across membranes is treated (Shore et al., 1957). Roberts et al. (1977, 1978) studied the permeability of human epidermis to phenolic compounds and found a linear relationship to water/octanol partition coefficients. Wood et al. (1979) found a parabolic relationship between partition coefficients and intestinal absorption of aliphatic carbamates. A linear relationship between water/octanol partition coefficients of 27 compounds and rat brain capillary permeability was found by Levin (1980). Cornford (1982) found a linear correlation between lipid partition coefficients and surface permeation in Schistosoma japonium.

1.3. Drug Metabolism and Elimination.

Many of the processes of drug metabolism and elimination can be considered as being nonspecific processes that are highly dependent on the lipophilic nature of the drug molecule (Lien and Hansch, 1968) and in structure activity studies both processes can be thought as a lipoidal trap (Hansch, 1971) or compartment (Higuchi and Davis, 1970). A group contribution approach to metabolism has been presented by Martin and Hansch (1971), who correlated the Michaelis constant for mixed function oxidases of rat liver microsomes with the octanol-water partition coefficient and π values. Similarly,

correlations between tubular reabsorption, lipophilicity and pKa have also been demonstrated (Seydel and Wempe, 1971).

Thus the lipophilicity of drug molecules is particularly important in pharmacokinetics, which describes the process and rates of drug movement from the site of absorption into the blood, distribution into the tissues and elimination by metabolism and/or excretion. Cheng and Grass (1979) used the ratio of tissue drug concentration to the concentration of drug in arterial or effluent plasma in development of pharmacokinetic models. A similar method was developed by Delfina and Moreno (1981) for intestinal absorption-partition relationships.

1.4. Thermodynamics of Drug Molecules in Solution.

The thermodynamic properties of functional groups of drug molecules in solution have been extensively reviewed by Davis et al. (1974). The basic assumption made is that the thermodynamic properties of a drug molecule in solution are an additive-constitutive property of the various functional groups. Based on this assumption group values for the free energy, enthalpy and entropy of transfer can be determined from experimental data and used subsequently in a priori prediction of a multiplicity of solution properties of interest to the pharmaceutical scientist. These include partitioning behaviour, solubility, compatibility, stability, absorption and duration and specificity of drug action.

1.5. Preformulation and Formulation.

Since the process of drug absorption can be regarded as the passage of a solute from an aqueous environment to a hydrophobic region, a knowledge of the partition coefficient in a suitable biphase solvent system leads to a prediction of the absorption characteristics. Many drugs are either acidic or basic, and it is usual to study the partition coefficient at 2 or more pH's in order to estimate the extent of absorption at the various pH environments of the gastro-intestinal tract.

The aqueous solubility of either a liquid or crystalline drug can be estimated from its partition coefficient. Hansch (1968) correlated the solubilities of some 150 liquid non-electrolytes with their partition coefficients and found :

$$\log S_w = -1.339 \log K_d + 0.978$$

Yalkowsky and Valvani (1980) found that the aqueous room temperature solubility of crystalline non-electrolytes, which do not have long flexible polymethylene chains, can be estimated by

$$\log S_c = - \log K_d - 0.01 MP + 0.5$$

where MP = melting point of the crystalline form in °C.
For alkyl phenols the aqueous solubility can be determined by the following (Valvani and Yalkowsky, 1980) :

$$\log S_w = - 0.997 K_d - 0.008 MP + 1.43$$

Good agreement between observed and predicted values are obtained, as shown in Table 1.

The dissolution rate of any drug given in solid form is

Table 1. Physical Properties of Alkylphenols.

Solute	Melting Point, °C	Experimental log K_d	Observed log (Molar Soly.)	log (Soly.) Predicted
Phenol	43	1.46	-0.08	-0.35
o-Cresol	30	1.95	-0.65	-0.74
m-Cresol	25	1.95	-0.71	-0.70
p-Cresol	35	1.96	-0.81	-0.79
2-Naphthol	122	2.78	-2.25	-2.26
3,4-Xylenol	64	2.42	-1.43	-1.46
3,5-Xylenol	64	2.33	-1.40	-1.37
2,6-Xylenol	42	2.36	-1.36	-1.24
2,4-Xylenol	26	2.30	-1.40	-1.06
2,5-Xylenol	75	2.33	-1.54	-1.46
o-Ethylphenol	25	2.47	-1.36	-1.22
p-Ethylphenol	47	2.42	-1.35	-1.33
Thymol	52	3.30	-2.21	-2.25
Eugenol	25	2.99	-1.40	-1.74

directly proportional to its aqueous solubility and has a marked influence on the amount absorbed (Leo and Hansch, 1971).

Since a drug molecule is required to cross a large number of membranes before it reaches the receptor site, then its lipophilicity should be of a suitable value ($\log K_d \cong 2$) in order that it should not remain in membranes. However a high lipophilicity is usually desirable for optimal binding at the receptor site. A pro-drug could be designed which would have an optimal partition coefficient for both transport and binding processes. This could be a hydrophilic derivative of a lipophilic drug, which would revert back to the original form, by enzyme hydrolysis or another mechanism, at the active site (Yalkowsky and Morozowich, 1980).

The partition coefficient is also important in the design of topical vehicles in which there exists a competing partitioning process between the vehicle and the skin. For any particular application, the topical preparation requires an optimising of the partition coefficient. Another example in which the partition coefficient is important in formulation is in liposome encapsulated drugs. In such systems the drug has to partition (or permeate) out of the bilayers of the liposome before it can interact with the receptor. The composition of the liposome can be adjusted to give the required sustained release characteristics. Rahman et al. (1978) and Juliano and McCullough (1980) have used liposomes for the controlled delivery of anti-tumour drugs and found targetting of drugs to specific tissues improved and toxicity greatly reduced. Reviews on the targetting of drugs by liposomes

(Gregoriadis, 1977) and on the pharmacological properties of liposomes (Toffano and Bruni, 1980) provide more information.

Many attempts have been made to understand the physical-chemical factors affecting emulsion formation and stability. Griffin (1949) suggested that the properties of surface-active materials could be assessed by empirically derived hydrophilic-lipophilic balance values (HLB). The HLB values of a molecule will depend on the type and number of groups. Davies (1957) has derived a quantitative relationship between HLB values and partition coefficients using data from studies on coalescence kinetics of emulsion droplets. His basic equation was of the form :

$$(HLB - 7) = - 0.36 \ln K_d$$

HLB values were calculated from group numbers which were defined as the free energy of transfer of a group from water to oil phase divided by a constant (1680).

1.6. Interaction of Drugs with Macromolecules.

Hydrophobic aspects of binding have been reviewed briefly by Rekker (1977).

1.6.1. Protein binding.

The interaction of drug molecules with proteins (including enzymes) is an important example of nonspecific hydrophobic interactions and their implication in biological systems.

Leo et al. (1971) have summarised the available data on the correlation of protein binding with log K_d and π values

using a linear free energy approach. A detailed study of the interaction of hydrocarbons and other species with albumin has been carried out by Franke (1968, 1969, 1970a and 1970b), who has attempted to correlate the free energy of binding with free energy of solution and partition coefficient. More recently Coulson and Smith (1980) found a linear relationship of hydrophobicity with protein binding with clorobiocin analogues. Protein binding has an important influence in various pharmacokinetic parameters and on the therapeutic dose necessary, since the bound fraction of drug is not available for therapeutic effect.

1.6.2. Interaction of drug with receptor site.

Modern concepts of drug action and drug specificity are all based on the assumption that the initial process in drug action is the formation of some type of reversible complex between the drug and the receptor site (Burgen, 1966). The occupation of the receptor by the drug brings about a change in some property of the cell, and this change persists so long as the drug occupies the receptor (Mackay, 1966). Rocha e Silva (1969) and Higuchi and Davis (1970) have suggested that the receptor can be split into two regions. A specific region designed to fit with the active group of the drug molecule, in which electronic and steric interactions are deemed important, and a nonspecific site where hydrophobic groups are attached mainly through hydrophobic interactions.

1.7. Solvent Extraction.

The determination of partition coefficient values, as well as the kinetics leading to equilibrium, form an essential part of the solvent extraction procedures. The efficiency of a solvent extraction process can assume major economic proportions when applied to large scale product units found throughout the pharmaceutical and chemical industry. An example in the pharmaceutical industry is the extraction and purification of antibiotics from fermentation liquors, using centrifugal counter-current extractors. A thorough study of the many parameters involved leads to optimised conditions for solvent extraction. The parameters involved include the solvent system, pH, temperature and rates of partitioning. One particular aqueous/organic solvent system may extract the component more efficiently than other systems. If the solute molecule being extracted is ionisable, pH may have a considerable effect on the extraction process. The temperature at which the extraction process is being carried out may be important if there is a large change in the enthalpy of transfer. The thermodynamics involved in the extraction of rare metals has been studied in great detail by Marcus et al. (1973, 1975, 1976). Finally the rate at which equilibrium is achieved is very important in order to know when the extraction process is complete.

Many assay procedures adopt a partitioning process to extract a drug from an aqueous phase, such as blood plasma, into a non-aqueous organic solvent in which the drug can then be assayed.

1.8. Pharmaceutical Analysis.

Many of the analytical techniques employed in analysis rely on a partitioning process. These include thin layer, paper, gas and liquid chromatography, as well as ionic exchange and ionic selective processes. The relationship between the partition coefficient and the various chromatographic parameters will be discussed in Chapter 3.1.3. In gas liquid chromatography temperature programming is often used to improve separation procedures. This procedure is now being adopted in high pressure liquid chromatography to improve the efficiency of separation. A recent review by Melander and Horváth (1980) discusses in great detail the effect of temperature on equilibria in HPLC.

Hydrophobic interaction chromatography (HIC) is a new non-biospecific liquid chromatographic method for the separation of proteins and other biological macromolecules. In this technique the mobile phases are aqueous and the absorbents are agarose beads coated or bound with ionogenic or non-ionogenic, hydrocarbonaceous ligands. The mechanisms involved in retention and elution in HIC are chiefly hydrophobic and electrostatic interactions between the protein and the adsorbent. The technique has been reviewed recently by Hjertén (1980) and Srinivasan and Ruckenstein (1980).

Aims and Objectives.

The overall aim of the thesis is to study the thermodynamics of partitioning of substituted phenolic compounds between aqueous solution and liposomes and the bulk organic solvents, n-octanol and cyclohexane. The thermodynamic parameters,

free energy, enthalpy and entropy of transfer between aqueous and lipid will be determined by studying the temperature dependence of partitioning. The thermodynamics of partitioning of phenolic compounds into DMPC multibilayer liposomes will be determined according to the method of Diamond and Katz (1974). Two techniques will be investigated to study the temperature dependence of partitioning in bulk organic solvents : these are the filter probe technique (Kinkel et al., 1981) and the AKUFVE technique (Davis et al., 1976). The relevance of each technique to a pharmaceutical application will also be evaluated. The derived thermodynamic parameters of transfer for solute molecules and their individual substituent groups will be used to explain the non-covalent interactions involved in the transfer of a solute molecule from an aqueous environment to a lipid environment. Most authors have studied the effects of the methylene and hydroxyl group contributions to the transfer process. However in this study, several other functional groups, with varying hydrophobic and hydrophilic characteristics, will be employed. This may lead to a better understanding of the intermolecular forces involved in the transport of solutes across biological membranes. By studying a simple system, it may be possible to gain a greater knowledge of the non-covalent interactions involved in biological processes, such as drug-receptor interactions, enzyme-substrate interactions or in protein structure.

The data may also help to decide whether a non-polar solvent such as cyclohexane serves as a better model system

for partitioning into biological membranes (liposomes) than a polar solvent such as n-octanol.

The determination of the enthalpy of transfer by the van't Hoff method, as in this case, has been criticised by several authors, because the heat of transfer may not be temperature independent as is assumed. This may be especially true where the composition of the two immiscible phases changes with temperature, as in the n-octanol/water system (Kinkel et al., 1981). By comparing the van't Hoff heats of transfer, as obtained in this thesis, with data obtained by calorimetric methods, the reliability of the van't Hoff plot can be assessed.

The phenomenon of enthalpy-entropy compensation (Lumry and Rajender, 1970) may be present in these partitioning processes. A linear relationship between the enthalpy and free energy of transfer is characteristic of enthalpy-entropy compensation.

Substituted phenolic compounds will be used in this study, for a number of reasons. There is a wide range of substituted phenols commercially available, and the effect of various substituent groups on the transfer process can be investigated. Phenolic compounds can be assayed at low concentrations (10^{-5} M) using ultra-violet spectroscopy. Many of our important drug molecules and metabolites are amphiphatic like phenols, with a distinct hydrophobic and hydrophilic region. Phenols are precursors of many drug molecules, and they have been used as anti-bacterial agents and in many SAR studies.

CHAPTER 2

THE THEORY OF THE PARTITIONING PROCESS.

Many physical and chemical processes involved in pharmacy can be explained in terms of thermodynamics. One such process is the partitioning of solutes between two immiscible phases. Partitioning is an equilibrium process and can be described fully in terms of equilibrium thermodynamics. A brief outline of the thermodynamics relevant to partitioning will be discussed in this section. A more rigorous treatment of the subject can be found by consulting standard texts on this subject (e.g. Klotz, 1964; Lewis et al., 1965; Prausnitz, 1969; Moore, 1972; Atkins, 1978). It is important to note that Ben Naim (1980) has presented a statistical mechanical treatment of this subject.

2.1. Enthalpy, Entropy and Free Energy.

The basic thermodynamic properties we are interested in are temperature, T, Gibbs free energy, G, enthalpy, H, and entropy, S.

These properties are related by the following equations :

$$G = H - T.S$$

and

$$\Delta G = \Delta H - T\Delta S \quad \text{(Gibbs-Helmotz equation).}$$

The enthalpy term, H, defines the heat content of a system, and the change in enthalpy, ΔH, indicates the heat absorbed or evolved in a process. A process is said to be exothermic when

heat is evolved and the enthalpy change for such a process is negative. Where heat is absorbed, the process is endothermic and the change in enthalpy is positive. In a process in which bonds need to be broken, energy is required and heat is absorbed to give an overall positive enthalpy change. Conversely, where bonds are formed in a process, energy is evolved in the form of heat and a negative change in enthalpy is observed.

The entropy term, S, characterises the degree of disorder or randomness of a system. The higher the entropy the greater the degree of disorder. A positive entropy change for a process denotes an increase in disorder, and a negative entropy change denotes a decrease in disorder. Statistically there is a greater probability of a system being arranged in a disordered, rather than an ordered manner. Changes tend to occur in the direction which produces a state of chaos, rather than a state of order.

The energy available to do useful work is termed the Gibbs free energy, G, and the change in the free energy for the process allows us to predict where the position of equilibrium will be. Reactions tend to go in the direction which results in a decrease in free energy. In a process in which there is a negative free energy change (exergonic), equilibrium lies on the side of the products, and the process is said to occur spontaneously. For a process or reaction with a positive free energy change (endergonic) equilibrium lies on the side of reactants. The free energy change must be less than or equal to zero for a process or reaction to occur spontaneously.

2.2. Partial Molar Free Energy and Chemical Potential.

Properties such as enthalpy, entropy and free energy which depend on the quantity of substance are called extensive properties. In contrast, properties such as temperature and density, which are independent of the amount of material, are referred to as intensive properties.

To apply free energy considerations to reactions in solution we need to know the dependence of free energy on composition. A term introduced by Gibbs in 1875 to describe the change in free energy with respect to composition is the chemical potential, μ_i. This can be defined as

$$\mu_i = \left(\frac{\delta G}{\delta n_i}\right)_{T,P,n_i} = \overline{G}_i$$

where μ_i = chemical potential,

$\overline{G}_i$ = partial molar free energy,

and n_i = number of components i.

The chemical potential is the partial molar free energy at constant temperature and pressure. It is an intensive potential and can be regarded as the force which drives a process to equilibrium and governs mass transfer.

2.3. Phase Equilibrium.

When two phases are brought into contact, they tend to exchange their constituents until the composition of each phase remains constant, and there is no tendency for further change. Such a system is said to be in a state of equilibrium. The composition of phases at equilibrium depend upon temperature and pressure, and the chemical nature and composition of

various substances involved.

Where a solute is distributed between two immiscible phases, then it can be described as a heterogeneous closed system, in which each phase is considered as an open system, within an overall closed system. In such a system, heat and mass transfer can take place. For systems at constant temperature and pressure, equilibrium is achieved when the free energy is no longer changing, i.e. $\Delta G = 0$, and the chemical potential of any component can no longer be changing, i.e. $\Delta\mu_i = 0$, and consequentially $\Delta n_i = 0$. The chemical potential of a given component must be equal in all parts of a system at equilibrium. For a solute species, i, distributed between two immiscible phases, α and β, at equilibrium with respect to the transfer process, then

$$\mu_i^{(\alpha)} = \mu_i^{(\beta)} ,$$

where superscript, in parenthesis, denotes phase, and subscript denotes species.

2.4. Ideal and Non-ideal Solutions.

The chemical potential of component, i, in an ideal solution can be expressed as follows :

$$\mu_i = \mu_i^o + RT \ln x_i ,$$

where μ_i^o is the chemical potential of component i in its standard state, and is a function of temperature and pressure only, and x_i is the mole fraction concentration of component i. An ideal solution can be defined as one, where at constant temperature and pressure, the activity of every component is

equal to some suitable measure of concentration, usually mole fraction.

Since most solutions are not ideal, a concept has been introduced which describes the departure of behaviour from ideality. In any real solution, interactions occur between the components, and reduce the effective concentration of the solution. The activity is a way of describing this effective concentration. The ratio of activity, a, to concentration, c, is called the activity coefficient, γ.

$$\frac{a}{c} = \gamma \qquad \text{and} \qquad a = c\gamma$$

The extent to which the activity coefficient differs from unity can be considered as a measure of the deviation from ideality. Thus the chemical potential of component, i, in a real solution can be expressed as follows :

$$\mu_i = \mu_i^o + RT \ln a_i = \mu_i^o + RT \ln \gamma_i x_i.$$

The activity of a substance in its standard state is equal to unity. There are two common standard states in use, one for the solvent and one for the solute component of the solution.

The standard state for the solvent is taken to be the pure liquid (or solid) at one atmosphere pressure and at a specified temperature. The solvent is said to behave ideally when it approaches behaviour specified by Raoult's Law. According to Raoult's Law, for an ideal solution the partial vapour pressure of a volatile component in equilibrium with the solution is equal to the product of the vapour pressure of the pure component and its concentration in the solution.

Thus $$\frac{P_i}{P_i^o} = x_i \ ,$$

where P_i is partial vapour pressure of component i,

P_i^o is vapour pressure of pure component i,

x_i is concentration of component i, in mole fraction units.

As the mole fraction concentration approaches unity, so does the activity and therefore the activity coefficient.

The standard state for the solute component is a hypothetical state in which the solute has properties of one molar, one molal or one mole fraction solution, behaving as if it were at infinite dilution. In this case, the solute behaves ideally when it obeys Henry's Law. Henry's Law states that the solubility of a gas or solute in a liquid is proportional to its partial pressure in the gas phase.

Thus $p = kx$ or $\frac{p}{k} = x$,

where p = vapour pressure of gas or solute,

x = mole fraction concentration,

k = proportionality constant or Henry's Law constant (also given symbol $H_{2,1}$).

(Raoult's Law may be regarded as a special case of Henry's Law).

As mole fraction concentration approaches zero, then activity equals concentration, and activity coefficient is unity. Thus it follows that if a solvent of a solution obeys Raoult's Law, the solute will obey Henry's Law.

The chemical potential of the solute component, i, of a real solution is usually expressed as :

$$\mu_i = \mu_i^{\theta} + RT \ln \gamma_i x_i \ ,$$

where μ_i^θ is the chemical potential of a solute in its standard state.

Infinitely dilute solutions usually behave ideally, and therefore activity equals concentration of solute.

2.5. Nernst Distribution Law.

At equilibrium the chemical potential of a solute, i, distributed between two immiscible phases, α and β, will be the same in each phase. If the chemical potential of solute, i, in each phase is represented as :

$$\mu_i^{(\alpha)} = \mu_i^{(\alpha)\theta} + RT \ln x_i^{(\alpha)}$$

and

$$\mu_i^{(\beta)} = \mu_i^{(\beta)\theta} + RT \ln x_i^{(\beta)} ,$$

then at equilibrium

$$\mu_i^{(\alpha)} = \mu_i^{(\beta)}$$

and

$$\mu_i^{(\alpha)\theta} + RT \ln x_i^{(\alpha)} = \mu_i^{(\beta)\theta} + RT \ln x_i^{(\beta)}$$

$$\Delta\mu_i^\theta = \mu_i^{(\alpha)\theta} - \mu_i^{(\beta)\theta} = RT \ln x_i^{(\beta)} - RT \ln x_i^{(\alpha)}$$

$$= RT \ln \left(\frac{x_i^{(\beta)}}{x_i^{(\alpha)}} \right)$$

Thus at a given temperature $x_i^{(\beta)}/x_i^{(\alpha)}$ reaches an equilibrium value, which is independent of initial concentrations: it is the ratio of final concentrations which determines the equilibrium position.

$$\frac{x_i^{(\beta)}}{x_i^{(\alpha)}} = \text{constant}, K_d.$$

Where K_d is called the partition coefficient or distribution coefficient of solute, i, distributed between immiscible solvents, α and β. The difference in standard chemical potentials $\mu_i^{(\alpha)\theta} - \mu_i^{(\beta)\theta}$, represents the standard free energy change for the transfer of component i from phase β to phase α. The free energy of transfer of component i from α to β is given by $\mu_i^{(\beta)\theta} - \mu_i^{(\alpha)\theta}$.

Since at equilibrium, the overall free energy change is zero. Then

$$\mu_i^{(\beta)\theta} - \mu_i^{(\alpha)\theta} = - (\mu_i^{(\alpha)\theta} - \mu_i^{(\beta)\theta})$$

and

$$\Delta_\alpha^\beta G_i^\theta = - RT \ln \frac{x_i^{(\beta)}}{x_i^{(\alpha)}}$$

A little consideration will show that the ratio $x_i^{(\beta)}/x_i^{(\alpha)}$ is the ratio of Henry's Law coefficients of solute i in the two solvents. Consider the distribution of solute, i, between two immiscible liquid phases, α and β. Each of these solutions is in equilibrium with a common vapour phase. Despite the fact that the solute can be a material of low volatility and this may have a very low vapour pressure, there will be some finite, numerically expressible, even if very small, pressure of solute vapour in the common vapour phase. In order that the two liquid phases may coexist, each must have a solute concentration that corresponds to the same solute vapour pressure; otherwise solute would transfer from one phase to the other. If Henry's Law applies to both solutions, at equilibrium there results the equation,

$$p = \frac{x_i^{(\alpha)}}{k^{(\alpha)}} = \frac{x_i^{(\beta)}}{k^{(\beta)}}$$

Rearranging yields

$$\frac{x_i^{(\beta)}}{x_i^{(\alpha)}} = \frac{k^{(\beta)}}{k^{(\alpha)}} = K_d$$

The Nernst Equation holds only for a small range of concentration (Treybal, 1963; Moelwyn-Hughes, 1964) and the effect of solute concentration and miscibility of two solvents have been considered in detail by Treybal (1963), Buchowski (1962) and Rozen (1967). One of the solvents used is always water, and there is a wide selection of bulk organic solvents to choose as other phase.

The partition coefficient is normally written as

$$K_d = \frac{C_o}{C_w}$$

where C_o and C_w are equilibrium concentrations of solute in organic and aqueous phase respectively. If the solute can ionise in aqueous phase and/or associate in organic phase then modified equations will be required so as to obtain the partition coefficient of the monomeric, unionised species. (Leo et al., 1971; Rekker, 1977).

2.6. Thermodynamic Analysis of Partition Data.

Partitioning is an equilibrium process and the free energy of transfer (ΔG_{trans}) of a solute from one phase to the other can be calculated using the van't Hoff equation :

$$\Delta G_{trans} = - RT \ln K_d = -2.303 RT \log K_d$$

A free energy term simply tells one whether some process, such as transfer, will happen, but it tells one nothing about the

driving forces or mechanisms involved. According to Franks (1975) the free energy term, for processes in aqueous systems, hides more than it reveals. This is due to thermodynamic compensation (Lumry and Rajender, 1970; Exner, 1973, 1975; Krug et al., 1976a, 1976b) in which trends in enthalpies are almost exactly matched by similar trends in entropies, leaving the free energy term almost unaffected. Thus free energy measurements are not a very potent tool for highlighting differences between processes taking place in slightly different solvent media or involving a range of related substances. Nevertheless most speculation is based solely on free energy measurements at single temperatures. The resolution of the free energy term into its constituent enthalpy and entropy contributions could be very worthwhile.

The free energy of transfer is related to the enthalpy and entropy of transfer by the Gibbs-Helmholtz equation for a system at equilibrium and constant temperature and pressure :

$$\Delta G_{trans} = \Delta H_{trans} - T\,\Delta S_{trans}$$

where ΔH_{trans} is the enthalpy of transfer,

ΔS_{trans} is the entropy of transfer,

and T is the absolute temperature in degrees Kelvin.

$$\Delta G_{trans} = - RT \ln K_d$$

$$\therefore \quad - RT \ln K_d = \Delta H_{trans} - T\,\Delta S_{trans}$$

$$\ln K_d = - \frac{\Delta H_{trans}}{RT} + \frac{\Delta S_{trans}}{R}$$

$$\ln K_d = \frac{1}{T} \cdot \frac{-\Delta H_{trans}}{R} + \frac{\Delta S_{trans}}{R} .$$

Thus a plot of ln K_d versus 1/T will give a slope of $-\Delta H_{trans}/R$, enabling the enthalpy of transfer to be obtained. The entropy of transfer can be obtained from the intercept on the y axis or from the relationship

$$\Delta S_{trans} = \frac{\Delta H_{trans} - \Delta G_{trans}}{T} .$$

2.7. Concentration Units.

The partition coefficient has no units, but its value will be dependent on the concentration scale used for its determination. Partition coefficients obtained on the mole fraction, molar and molal scales will all be different. However conversion from one scale to another is usually a simple matter, unless the lipid phase is a complex oil (Davis, 1973).

The molar partition coefficient (K_d^m) can be written as

$$K_d^m = \frac{C_o}{C_w} .$$

The 'thermodynamic' partition coefficient (K_d^x) has for a reference state, in each phase, the hypothetical ideal mole fraction of solute, such that

$$K_d^x = \frac{x_o}{x_w} .$$

It is an easy matter to convert partition coefficients on the molar scale to the mole fraction scale and vice versa :

$$K_d^x = K_d^m \cdot \frac{V_o}{V_w}$$

where V_o and V_w are molar volumes of organic and aqueous phases respectively.

The mole fraction and molar partition coefficients are the most common units for partitioning between water and bulk organic solvents, however for partitioning into membranes the molal scale is the most appropriate.

The merits of the various units have been discussed frequently in the literature. According to Franks (1977) the correct way of comparing solute environments in different solvents is through the molarity scale. This opinion is held by Ben-Naim (1978), who states that the molar unit is the only standard quantity, which has the property of sensing the local environment of the solvent. Gurney (1953), Kauzman (1959) and Tanford (1980) have given reasons why the free energy of transfer should be expressed in mole fraction units. The standard chemical potential, μ^{o}, expressed in this scale represents only the internal free energy of solute molecules and the free energy of interaction with solvent. If molar units are used, μ^{o}, would include contributions made by the entropy of mixing. For the distribution of solutes between an aqueous phase and organic phase, the difference in unitary free energy calculated from the mole fraction partition coefficient, can be considered to be approximately an additive function of the contribution for the constituent groups (Mukerjee, 1967; Aveyard and Mitchell, 1969; Hersh, 1971). Mole fraction concentration units are also the concentration scale of choice when comparing the effects of different organic solvents on partitioning as they provide a comparison of equal numbers of molecules (Mallota and Frieser, 1967; Aveyard and Mitchell, 1969). The value of the free energy term expressed on the molar

concentration scale depends upon the molar volume of the solvent (Cratin, 1968). The change in molar volume of solvent and solute with respect to temperature can be accounted for with the mole fraction unit. Jameson (1980) has recently discussed the merits and demerits of the three concentration scales.

2.8. Choice of Solvent.

The nature of the organic phase and the choice of reference solvent to be employed for partition studies have been a topic of active discussion in recent years (Davis, 1974; Rekker, 1977; Martin, 1979). The two extremes that have been employed are inert hydrocarbon solvents, such as cyclohexane or hexane, and polar solvents such as n-octanol. The partition of a polar solute between an inert solvent and water or between octanol and water will be quite different. Octanol is probably the most widely used solvent at this time (Leo et al., 1971). It was originally chosen as a solvent for partition studies because it was felt that its hydroxy group would make it similar to a biological membrane, which also has hydrogen bond donating and acceptor properties. This has been verified in studies by several workers (Burton et al., 1964; Flynn, 1971; Hansch and Dunn, 1972; Levitan and Barker, 1972) who found good correlation between biological activity and water/octanol partition coefficients. In direct contrast other workers (Beckett and Moffatt, 1969; Bickel and Weder, 1969; Shibab, 1971) have found that the partition coefficients obtained with the system heptane (or hexane) - water (buffer) correlate biological data better than those obtained using polar solvents.

It is more difficult to measure hydrocarbon/water (buffer) partition coefficients of ordinary drugs (Smith et al., 1975) since the phases are so different, the molecules tend to partition into the water or into the hydrocarbon almost exclusively. A much broader spectrum of compounds is soluble in n-octanol than in aliphatic or aromatic hydrocarbons. Octanol, because of its hydroxyl group, has rather a high concentration of water (27% on mole fraction basis). When studying the temperature dependence of partitioning the water content of octanol would vary with temperature, which may result in a temperature dependence of the enthalpy of transfer. The very low solubility of water in non-polar solvents, such as cyclohexane (0.0025 M) would most probably eliminate this problem. Although the transfer of a polar solute from water to a non-polar solvent involves a desolvation process, however some water may be transferred alongside the solute (Johnson et al., 1965, 1967). Dissolved polar molecules tend to associate with each other rather than with the solvent in hydrocarbon solvents (Anderson et al., 1979). Octanol is sufficiently polar that there is little solute-solute interaction , only solute-solvent interactions. Linear aliphatic alcohols (including n-octanol) have been shown to exist in monomeric and polymeric forms (Anderson et al., 1978). Rekker (1977) discusses in great detail the discriminating powers of the various solvents. Cyclohexane shows a greater discriminating power for hydrophobic residues, whereas functional groups with hydrophilic character are shown more discrimination in polar solvents such as octanol.

2.9. Correlation between Log K_d Values in Different Solvents.

Collander (1954) discovered a linear relationship between log K_d values of compounds measured in different aqueous/solvent systems. This relationship is described by the Collander equation :

$$\text{Log } K_{\text{octanol}} = a \text{ Log } K_{\text{solvent}} + b \ .$$

For similar compounds which are partitioned between water and octanol, there is good correlation with partition coefficients obtained between water and polar solvents. For solvents which do not contain a polar group, cyclohexane or iso-octane, correlation will be poor, and the Log K_d values of hydrogen bond donors and hydrogen bond acceptors are correlated by significantly different equations. (Leo and Hansch, 1971). Hansch and Dunn (1972) reported that for a series of phenols the difference between the octanol-water log K_d and that between cyclohexane and water is quantitatively explained by hydrogen bonding. Seiler (1974a) found that this difference is totally explained by the substituents present in the molecules. Accordingly, a new constitutive substituent constant I_H (or K_{HB}) could be defined :

$$I_H = \log K_{d\ \text{octanol}} - \log K_{d\ \text{cyclohexane}} - 0.16 \ .$$

The value of I_H for a substituent seems to be proportional to the hydrogen bonding ability of substituents. The I_H constants, listed by Seiler (1974 a) , may be used to estimate the log K_d of a compound in a different solvent from which it was first measured.

2.10. Calculation of Partition Coefficients by Additivity.

Fujita et al. (1964) were the first to define a hydrophobic substituent constant, by analogy to the Hammett electronic substituent constant σ, as

$$\pi_X = \log \frac{K_{YX}}{K_{YH}} = \log K_{YX} - \log K_{YH}$$

where K_{YX} = partition coefficient of substituted compound,
and K_{YH} = partition coefficient of parent compound.
The term π is defined as the change in log K_d brought about by substitution of a hydrogen atom of an unsubstituted compound YH with a substituent X. A comprehensive list of π values for substituents of aromatic and aliphatic compounds is given in the book by Hansch and Leo (1979). Such values remain constant for one series of compounds to another, and as a result it is possible to predict the partition coefficient of various analogues, provided the value of the parent compound has been measured. Groups that increase lipophilicity, mainly due to their size, e.g. CH_3, halogen, are easy to deal with, whereas those that have more complex properties, e.g. NO_2 or OH, may require more closely defined rules or special sets of π values.

A similar additive-constitutive approach has been presented by Nys and Rekker, and discussed at length in the book by Rekker (1977). By means of regression analysis they reported a new parameter, f, the fragmental constant, such that

$$\log K_d = \sum f \ .$$

The choice of π or f values for predictive purposes is usually a matter of individual preference and the nature of the prediction. π and f values are inter-related and can be inter-

converted (Martin, 1978). Both schools have attempted to extend their methods to account for specific interactions or situations where substituent groups can affect one another, such as the ortho effect due to intramolecular interaction. It is well worth mentioning that other approaches have also been used to predict partition data. These include molecular orbital calculations, parachor, molecular surface area and R_m values from chromatography.

2.11. Models relating Hydrophobicity to Biological Response.

The importance of hydrophobicity in determining biological activity was recognised many years ago by various workers who related drug potency to oil-water partition coefficient. Interesting correlations were reported, but the simple relationships did not always hold. A major break through came in 1963 when Fujita, Hansch and others introduced the linear free energy approach (Hansch, 1973). They reasoned that a change in a substituent may produce a major change in the electronic, steric and hydrophobic properties of the molecule. They proposed that these factors could be summed up in an equation :

$$\log \frac{1}{C} = a \log K + b E_s + c' \sigma + d \qquad (1)$$

where C is the concentration producing a given biological response;

E_s is the Taft steric parameter;

σ is the Hammett electronic parameter,

and a, b, c' and d are constants.

This equation formed the basis of the Hansch approach to structure-activity.

Although some molecules give a linear response between drug activity and hydrophobicity, others do not. To overcome this problem, Hansch and colleagues introduced a second equation,

$$\log \frac{1}{C} = a \log K + b \log K^2 + c' E_s + d \sigma + e \qquad (2)$$

Large numbers of these parabolic relationships have been reported (Hansch and Clayton, 1973) and an example is shown in Fig.2.

Equation (2) is an empirical correlation and tells us nothing about the underlying mechanism responsible for a parabolic relation between drug activity and hydrophobicity. As a result various other models have been proposed. These can be classified as equilibrium models and kinetic models. The strengths and weaknesses of the various alternatives have been discussed in great detail by Martin (1978) and Kubinyi (1979).

The equilibrium models consider the drug partitioned between various aqueous and lipid compartments in the body and the affinity of the drug for each compartment, including the receptor site. It is the amount reaching the receptor that determines response. The shapes of the resultant curves determined from the model are parabolic in nature in certain circumstances (Fig.3).

An alternative kinetic approach has also been explored by Kubinyi (1979) and more recently by van de Waterbeemd(1980). Here the rates of transfer of the solute across various water/oil interfaces is considered. The rates depend upon the hydrophobicity of the drug molecule. The relation between kinetics of transfer and hydrophobicity has three distinct regions (Fig.4). A linear relation between transport and hydrophobicity

Fig.2. Biological Response and Lipophilicity

Experimental Data.

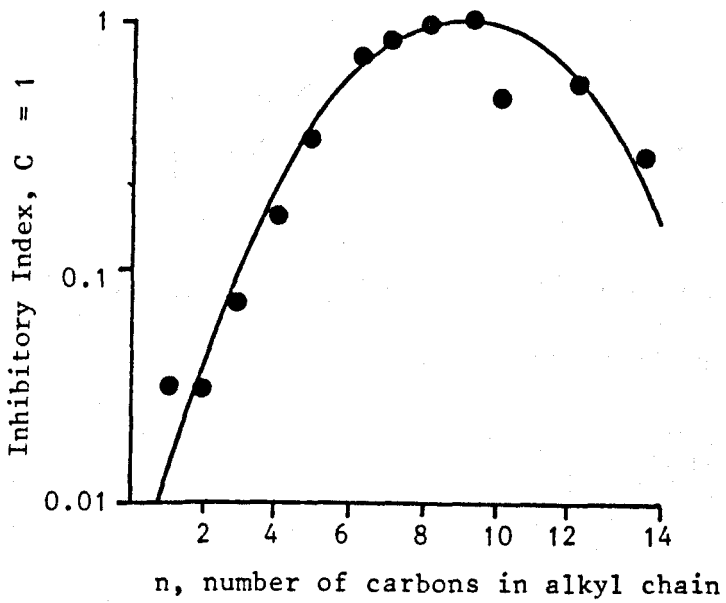
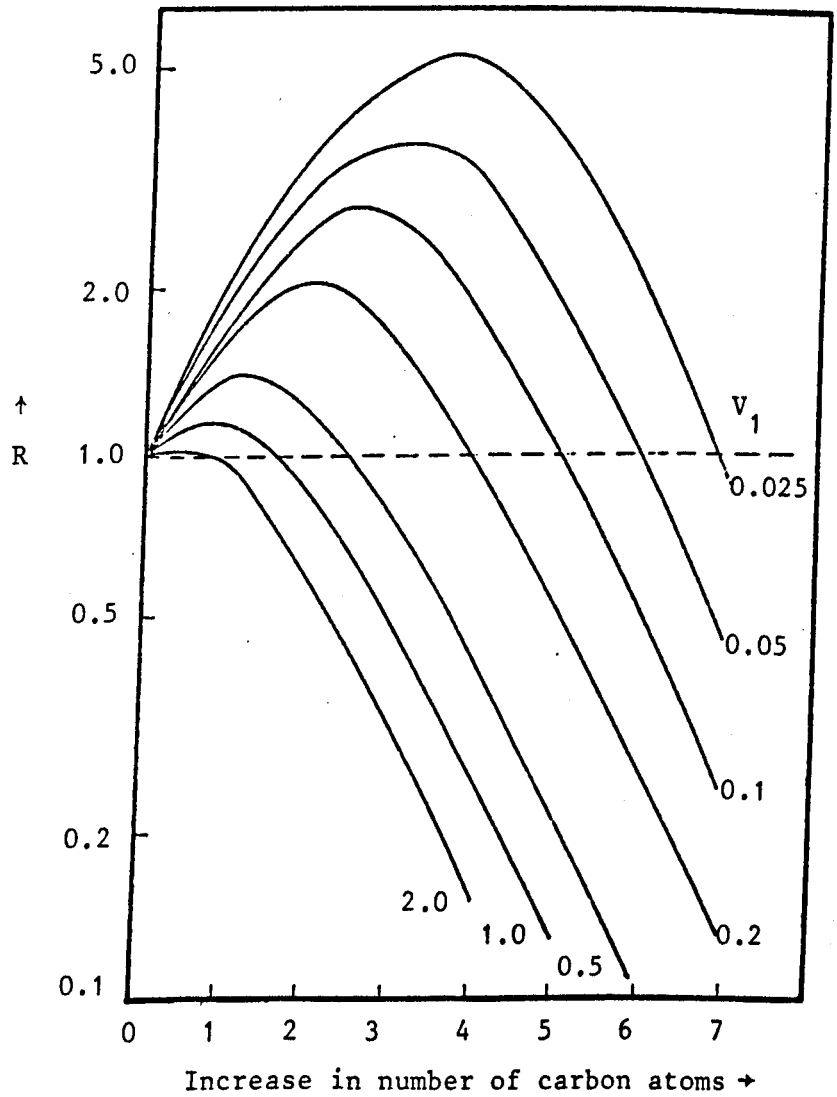


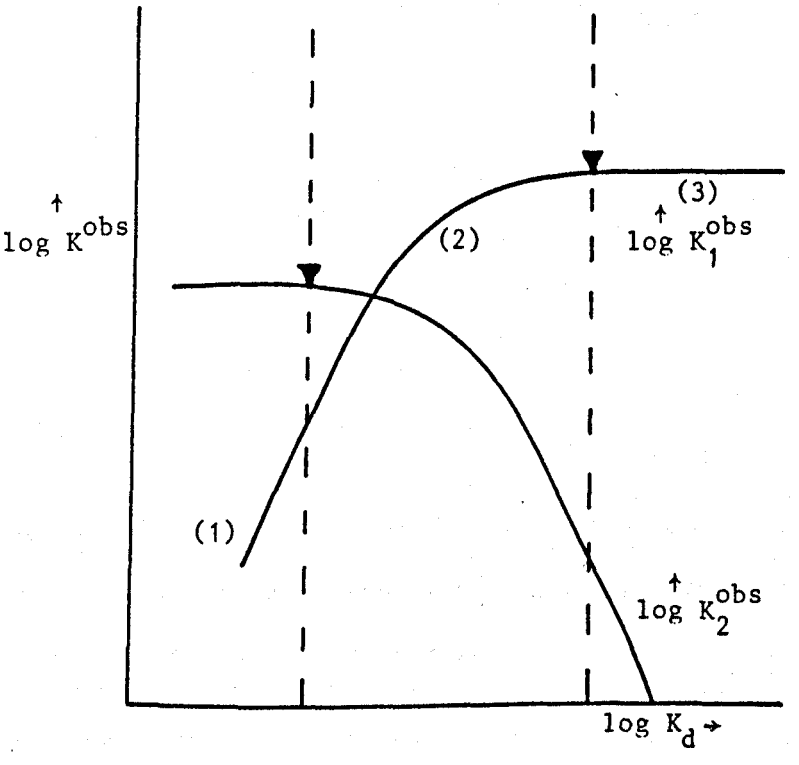
Fig.3. Biological Response and Lipophilicity Equilibrium Model Analysis.



Two-compartment model analysis of drug distribution $F_{CH_{2(r)}} = 2$, $F_{CH_{2(1)}} = 4$. (After Higuchi and Davis, 1970).

Fig.4. Biological Response and Lipophilicity

Kinetic Model (van de Waterbeemd, 1980)



is expected when K_d is small, a curvilinear region (parabolic) when K_d is intermediate and then at high K_d values the transport is independent of hydrophobicity. In this region transport is thought to be determined by the stagnant water layer adjacent to the interface or membrane.

2.12. Non-covalent Interactions Involved in the Partitioning Process.

2.12.1. Hydrogen Bonding.

This subject has been dealt with in great detail by Pimental and McClellan (1960), Vinogradov and Linnell (1970) and Joesten and Schaad (1974).

Hydrogen bonding occurs between a proton donor group, A-H, and a proton acceptor group, B, where A is an electronegative atom, O, N, S, X (F, Br, Cl, I) or C, and the acceptor group is a lone pair of an electronegative atom or an electron orbital of a multiple bond (unsaturated) system.

$$A\text{-}H + B \longrightarrow A\text{-}H \text{ ---- } B.$$

Generally a hydrogen bond can be characterised as a proton shared by two lone electron pairs. The hydrogen bond is unique to hydrogen, because hydrogen is the only atom which can carry a positive change while covalently bonded in a molecule, and which is also small enough to allow a close approach of a second electronegative atom.

Hydrogen bonding is a distinct, directional and specific interaction. Hydrogen bonds are linear, but appreciable variation in the angle A-H --- B can occur. The total H bond

length is equal to or less than the sum of the van der Waals radii of atoms A and B.

Since hydrogen bonding is an association phenomena, it causes a decrease in the total number of free molecules and an increase in the average molecular weight. In hydrogen bonding a specific covalent A-H group interacts with a specific acceptor site, B. The A-H bond is thereby weakened but not broken, and the properties of the acceptor group are also affected.

A useful classification of compounds according to hydrogen bonding type has been described by Pimental and McClellan (1960) (see Table 2). In polar solvents solute-solute hydrogen bonds (Klotz, 1965) are effectively masked by the competing solute-solvent hydrogen bonds. In non-polar solvents hydrogen bonds contribute appreciably to the energy of inter or intramolecular interactions. Changes in structure change the strength of a hydrogen bond to a common donor or acceptor in direct proportion to the effect of the structural modification on the charge of the electronegative atom which is involved in the hydrogen bond. Such substituent effects are well parameterised by Hammett sigma (σ) values. A typical correlation of substituent effects on the stability of hydrogen bonds between a series of substituted phenols and pyridines in carbon tetrachloride, is shown in Fig.5.a, 5.b (Rubin, 1964, 1965). In this example the hydrogen bond donor is a phenol and the acceptor is pyridine. The measurements are made in carbon tetrachloride so that there is little solute-solvent interactions. Fig.5.a shows the influence of substituents on the phenol of the strength of the hydrogen bond.

Table 2

Classification of compounds by hydrogen bonding type, according to Pimental and McCellan (1960).

Class of Compound	Molecule contains		Examples
	Proton Donor Groups Acid	Electron Donor Groups Base	
A	Yes	No	Mainly halogenated compounds with enough halogens to activate the hydrogens : $CHCl_3$, C_2HCl_5, etc., and possibly C_2H_2 protons
B	No	Yes	Ketones, aldehydes, ethers, tertiary amines, esters, olefins
AB	Yes	Yes	Water, alcohols, phenols, carboxylic acids, primary and secondary amines
N	No	No	Saturated hydrocarbons, CS_2, CCl_4

Fig.5.a. Log K vs. Hammett σ values for phenols (Rubin et al., 1964)

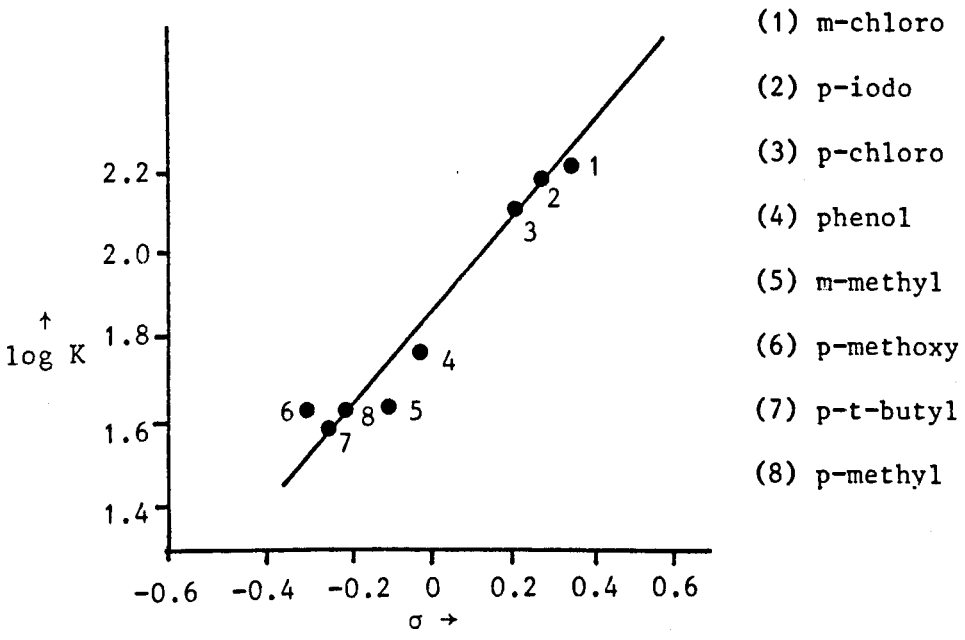
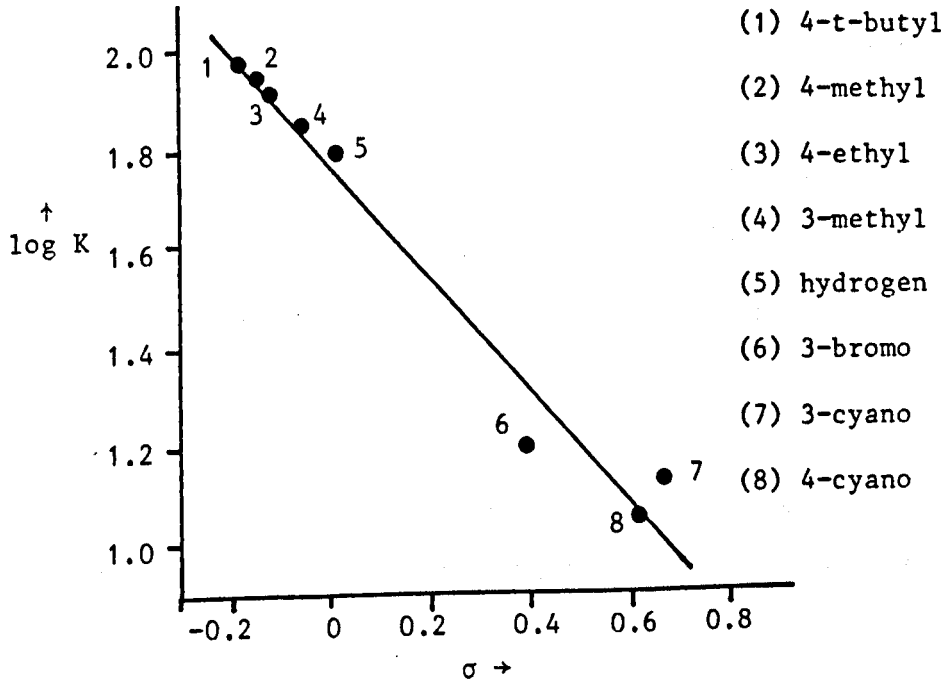


Fig.5.b. Log K vs. Hammett σ values for pyridines (Rubin et al., 1965)



(The equilibrium or association constant of hydrogen bonding, $K = \frac{[B\text{----}HA]}{[HA][B]}$ and σ is the Hammett sigma constant (Clarke and Perrin, 1964)). Electron withdrawing substituents in the phenol, make the oxygen more positive, decrease its affinity for the proton and correspondingly increase the strength of the hydrogen bond. Electron donating substituents in the phenol decrease the strength of the hydrogen bond. There is a positive correlation between sigma value of a substituent and its affects of log K of association.

Fig.5.b shows the effects of substituent effects of hydrogen bonding in substituted pyridines. Electron donating substituents which make the nitrogen of the pyridine more negative, increase the strength of the hydrogen bond. Electron withdrawing substituents have the reverse effect. There seems to be some confusion as to which Hammett sigma constant gives best correlation with hydrogen bonding. According to Hine (1975) the equilibrium constants for hydrogen bonding are best correlated with σ^n values, whereas the ionisation constants of phenols are best correlated by use of σ^- values. Sources of sigma constants are given by Martin (1978).

Hydrogen bond formation is characterised by a negative enthalpy, whereas hydrogen bond breaking is shown by a positive enthalpy. The enthalpy of hydrogen bond formation generally falls in the range of -4 to -40 kJ/mole . For the bond -O-H---O , the enthalpy of bond formation is usually -16 to -25 kJ/mole. A comprehensive list of thermodynamic data for hydrogen bond adducts, including substituted phenols with bases, is included in the appendix of the monograph by

Joesten and Schaad (1974). A correlation between σ and ΔH has been reported for adducts of para substituted phenols (Neerinck and Lamberts, 1966; Drago and Epley, 1969 and Vogel and Drago, 1970). Neerinck and Lamberts (1966) derived the equation

$$-\Delta H = 1.87\ \sigma + 6.50$$

from enthalpies for pyridine adducts of para substituted phenols.

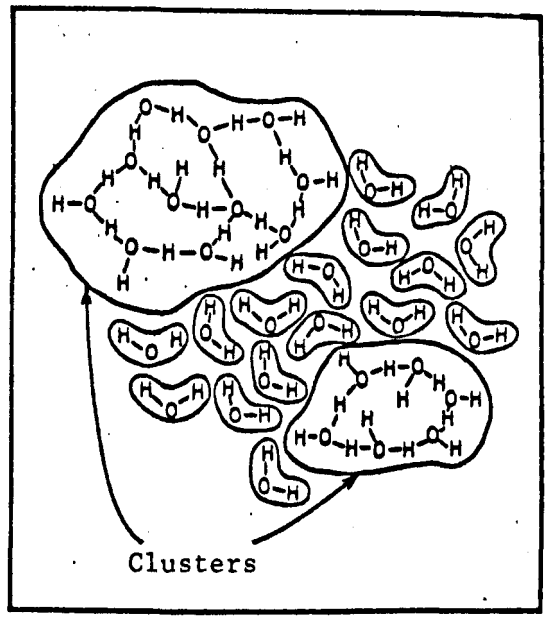
2.12.2. Water Structure.

Although a number of models for the structure of liquid water have been proposed, so far none have been found to be entirely satisfactory, in the sense of being able to account for all the properties of liquid water. Generally speaking the models can be divided into two types : the uniformistic or continuum models, which assume that only one type of molecule corresponding to the average of all the molecules need be considered, and mixture models which require the existence of two or more different structural units. The continuum type of models of liquid water structure, include the bent or distorted hydrogen bond model of Pople (1951) and the related random network model of Bernal (1964). In Pople's model water molecules are arranged tetrahedrally as in Ice I, but the majority of hydrogen bonds are bent or distorted to varying degrees, rather than intact and/or broken. According to this model, the hydrogen bonds, even in ice, bend as the temperature is raised, until the long range orientation in the crystal lattice breaks down. The random network model is an extension of the distorted hydrogen bond model. Each water

molecule is hydrogen bonded to four neighbours as previously, and the hydrogen bonds may be distorted. The hydrogen bonded, tetrahedrally coordinated water molecules form irregular networks of cyclic structures. The mixture models of water structure can be sub-divided into network/cluster models and cell/cage models. The earliest mixture model envisaged regions having a structure much like ice, called icebergs which were floating in a disordered liquid having relatively few hydrogen bonds. This concept was modified by Frank and Wen (1957) who took into account the impossibility of the continuous existence of any such ice-like regions, and pictured instead "flickering clusters" - regions in which water molecules are joined by hydrogen bonds, but which are formed suddenly, have a very short half-life, and disappear suddenly. Another version of the mixture model was developed by Nemethy and Scheraga (1962) (see Fig.6). Emphasis is on the equilibrium number of hydrogen bonds that exist in the liquid, a number related to cluster size, for water molecules on the outside of a cluster have but two or three, rather than four hydrogen bonds. Increasing temperature decreases somewhat the number of hydrogen bands and size of cluster. It is calculated that at 20°C there are about 57 molecules to the cluster on average, with 50% of total possible hydrogen bonds present, and this decreases to 25 molecules per cluster at 70°C.

In the cell/cage model the water is assumed to contain two components. One of them is a bulky ice-like framework of tetrahedrally coordinated water molecules. The other component consists of single water molecules in the cavities of this

Fig.6. Model of the Structure of Water as a Mixture of Clusters and Single Water Molecules. The Water molecules in the Clusters are linked by Tetrahedral Hydrogen Bonds.



framework, where they rotate without restraint because they do not participate in any hydrogen bonding. Pauling (1959) has suggested a cage model based on two structural units which are both water polyhedra. One unit is a pentagonal dodecahedron comprising 20 water molecules with a cavity diameter of 5.2 °A; the other is a tetrakaidecahedron containing 24 water molecules having a cavity of 5.9 °A in diameter. According to Pauling these polyhedra can be combined in various ways to give the framework structure of liquid water. Another interstitial model assumes that liquid water possesses a structure similar to Ice I, but with single water molecules inside cavities formed by the layers of hexagonal rings of water molecules (Samoilov, 1965). According to Samoilov, when ice melts some of the water molecules break their hydrogen bonds and move into neighbouring cavities. Marchi and Eyring (1964) have proposed another cage model, which assumes that when ice melts, the water molecules of ordinary ice structure rearrange themselves into a new structure, but in which the coordination is still tetrahedral.

Although most of the theories of liquid water structure mention tetrahedrally coordinated or single water molecules, Frank and Quist (1961) have shown clearly that it is necessary to consider a third state of water molecules. There are aggregates formed from 2, 3, 4 and possibly up to 6 molecules of water. They may only be present in small amounts at the melting point, but their concentration increases with rising temperature.

All the models of liquid water have one common feature : namely that water molecules do not move round as free separate

molecules but as complexes in which the molecules are kept together by hydrogen bonds. This complex structure of liquid water becomes less ordered with increasing temperature due to decrease in hydrogen bonding.

Another very important aspect of structure is the effect of solutes on the arrangements of water molecules. In general, non polar solutes tend to increase the amount of order, these are termed structure formers, but polar and ionic materials tend to reduce the amount of order, these are termed structure breakers (Franks, 1973).

Excellent monographs on water structure have been written by Kavanau (1964), Eisenberg and Kauzman (1968), Horne (1972), Franks (1972-1979) and Ben-Naim (1974) and the subject has been reviewed by Wicks (1966) and Frank (1970).

2.12.3. The Hydrophobic Interaction.

Many biological processes are influenced by the hydrophobic interaction or hydrophobic effect. These include membrane bilayer and micelle formation; protein and nucleic acid structure; drug-protein binding, drug-receptor and enzyme-substrate interactions. In fact 'hydrophobic forces' are probably the most important single factor providing the driving force for non-covalent interactions in aqueous solution (Jencks, 1969). Thus a knowledge of the hydrophobic interaction will lead to a fuller understanding of the many complex processes occurring in biological systems.

Much information on the hydrophobic interaction has been gained by studying the solution properties of non-polar solutes,

such as hydrocarbons, in water (see Table 3). The solution process is thought to take place in 2 stages. Firstly in order to insert the apolar solute into the solution, hydrogen bonds need to be disrupted to separate the strongly interacting water molecules and create a cavity for the solute (Butler, 1937; Sinanoglu and Obdulnar, 1962). This leads to a positive increase in enthalpy. The initial disruption of hydrogen bonds is immediately followed by the formation of new hydrogen bonds, and a negative decrease in enthalpy. This leads to a more ordered structure of the water molecules around the solute, and a restriction in the movement of the solute. The resulting loss in entropy (negative) makes a dominant contribution to the positive free energy change and hence renders the dissolution unfavourable. The origin of the large negative entropy of solution is usually ascribed to an increase in water structure around the apolar solute (Frank and Evans, 1945; Frank and Wen, 1957), although a contribution from the decrease of rotational freedom of solute molecules cannot be excluded (Frank and Franks, 1968; Franks and Watson, 1969). For the solution of a hydrocarbon, such as methane, there is an overall decrease in entropy and enthalpy, and an unfavourable free energy change. This process is termed hydrophobic hydration (Fig.7.a). The hydrophobic interaction can be regarded as a partial reversal of the unfavourable solution process. The hydrophobic association is thought to occur as a result of the favourable positive entropy change that accompanies the destruction of part of the ordered region as two or more hydrocarbon groups come into contact (Fig.7.b). Ben-Naim (1971) describes the

Table 3

Thermodynamic parameters for the solution of alkanes, at infinite dilution (Franks and Reid, 1972).

Alkane	$-\Delta H$ soln cal.mole^{-1}	$-\Delta S$ soln cal.mole^{-1}deg.$^{-1}$	ΔG soln cal.mole^{-1}
Methane	2250-2860	18.4-16.8	2510-3150
Ethane	2370-1270	19.5-16.8	3320-3860
Propane	2090-1450	23.5-21.3	4900
Butane	960-720	22.7-21.9	5820-6000
Pentane	300±1600	24	6840

Table 4

Thermodynamics of hydrophobic pairing at 20°C (Ben-Naim, 1971).

	2 CH_4	2 C_2H_6	4 CH_4	2 C_6H_6
ΔG (cal.mole.$^{-1}$)	-2112	-1500	-5724	-1136
ΔH (cal.mole.$^{-1}$)	1300	3000	5600	3200
ΔS (cal.mole.$^{-1}$deg.$^{-1}$)	11	15	37	14

Fig.7.a. Hydrophobic Hydration.

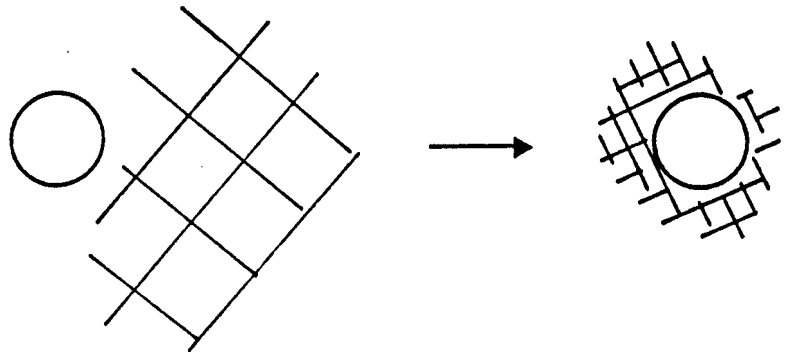


Fig.7.b. Hydrophobic Interaction.

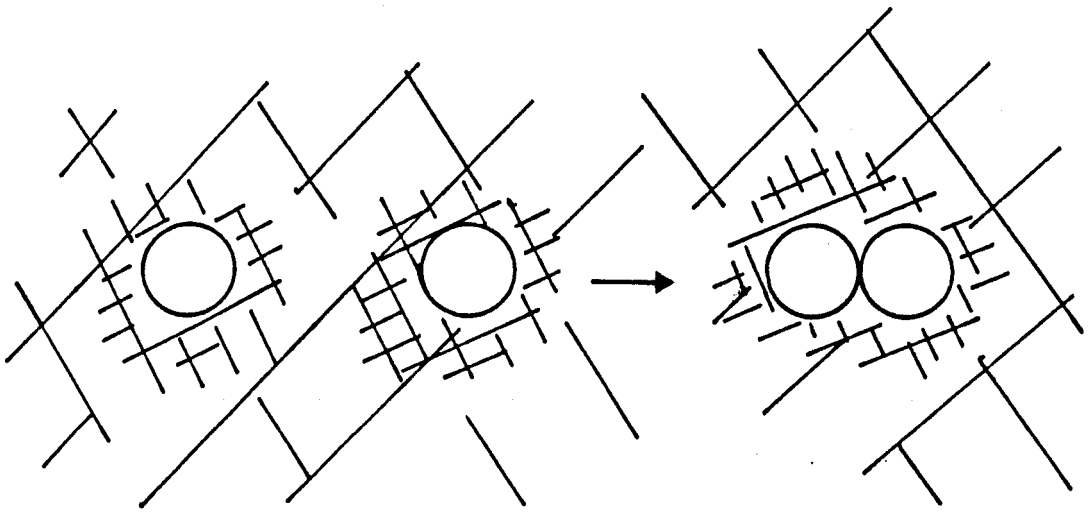
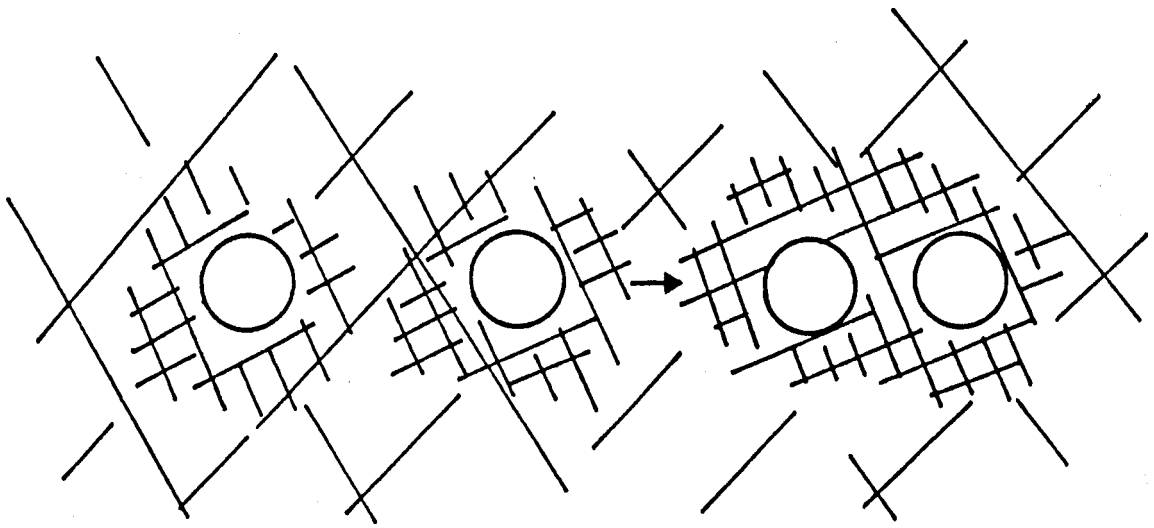


Fig.7.c. Long Range Interactions.



hydrophobic pairing of methane molecules at 20°C (Table 4). There is a favourable negative free energy change and positive entropy change for the process. Franks (1975) has suggested that in predominantly aqueous environments, hydrophobic groups may be stabilised not by contact interactions, but by longer range interactions (Fig.7.c). The view that the hydrophobic interaction is an entropically driven process is held by most authors, and was first put forward by Franks and Evans (1945), Kauzman (1959) and Nemethy and Scheraga (1962). However, recently Abraham (1980) has reasoned that the dissolution process of apolar solutes in water is essentially an enthalpic effect and is controlled by hydrogen bonding. This is a view shared by several other authors : Eley (1939), Sinanoglu (1968), and Shinoda (1977, 1978). This implies that the association of non-polar entities in water is also enthalpically controlled.

The hydrophobic interaction is formed when two or more solute molecules interact with each other in an aqueous environment, as a result of the mutual attraction between water molecules, rather than any specific interactions, e.g. van der Waals, between the solute molecules. The hydrophobic interaction can be thought of as the squeezing out of solute molecules by water molecules. A direct way to demonstrate the dominant role of water attraction is to study the properties of the hydrocarbon/water interphase. The free energy of attraction between water and hexane at 25°C is about -40 erg/cm^2 of contact area and the free energy of attraction for hydrocarbons themselves is also about -40 erg/cm^2. (Tanford, 1980). However the free energy of attraction of water molecules is -144 erg/cm^2.

It is clearly the latter alone that leads to a thermodynamic preference for the elimination of hydrocarbon-water contacts. This is supported by evidence from an earlier paper by Tanford (1978) in which he mentions the very weak intermolecular attractive forces between hydrophobic solutes, the irregular partial molar heat capacity of hydrocarbons in water as compared to other solvents, and the uniqueness of water as an extremely poor solvent for hydrocarbon solutes. From dielectric relaxation measurements (Hollenga et al., 1980) concluded that the thermodynamic quantities which characterise hydrophobic interactions were directly related to changes in the dynamic properties of water. The interpretation of the hydrophobic interaction in terms of the properties of water was originally given by Hartley (1936), Frank and Evans (1945), Kauzman (1959) and Nemethy and Scheraga (1962) and the current view held by Ben-Naim (1980), Tanford (1979) and Franks (1977).

However many authors believe that the hydrophobic interaction is due to solute-solute interactions rather than due to the unique properties of water. Hildebrand (1968, 1969, 1979) believes that the mechanism responsible for the hydrophobic interaction is due to van der Waals forces between hydrophobic molecules, rather than a "squeezing out" of the solute by water molecules. He puts forward several pieces of evidence to support his claim, but perhaps the most satisfactory describes the diffusivity of methane in water and carbon tetrachloride. The viscosities of water and carbon tetrachloride at 25°C are nearly the same, 0.880 and 0.895 c.p. respectively, and the diffusivity of methane in water is

1.72 cm^2 sec^{-1} at 25°C and in carbon tetrachloride is 2.89 cm^2 sec^{-1}. If the molecules of methane were surrounded by structured water, they could not diffuse six tenths as rapidly in water as in carbon tetrachloride. Analyses by Butler et al. (1935), Mukerjee (1967), Amidon et al. (1975), and Wolfenden and Lewis (1976) and more recently the work of Cramer (1977) indicate that for the aliphatic hydrocarbons a major portion (80%) of the free energy of solution is a result of solute-solute interactions in the solute or non polar phase only 20% being a result of solute-solvent interactions. It is noteworthy that, according to spectral studies, little if any effect on the structure of water accompanies the dissolution of non-polar molecules (Hertz, 1970). The positive N.M.R. shifts which are usually observed (Clifford and Pethica, 1964) are rather more suggestive of a decrease in hydrogen bonding, although there is also a modest decrease in the conformational liability of water molecules (Clifford and Pethica, 1964). Van Oss and colleagues (1980) have shown thermodynamically that the preferred attraction between hydrophobic molecules in water, can be entirely ascribed to van der Waals interactions.

The nature of the hydrophobic effect has been a source of considerable discussion over the past few years and no doubt will continue to be so, until there are definite criteria to distinguish between the two mechanisms outlined.

Ben-Naim (1980) has summarised the models used in the study of the hydrophobic effect. There are essentially two different concepts discussed in the literature under the term hydrophobic interaction or effect. One is represented by the

transfer of a solute between water and some non-aqueous solvent (Table 5) and it measures the relative tendency of the solute to prefer one environment over the other (Fig.8.a). The second measures the tendency of two or more solute molecules to aggregate in aqueous solution (Figs.8.b, c, d and Table 4).

Both quantities express some kind of phobia for the environment. In discussing the relevance of both models to biological situations, it can be said that the transfer studies are appropriate if the "real" process involves the transfer of a molecule or group from an aqueous medium into an effectively non polar medium, e.g. micelle formation, phospholipid bilayer formation and globular protein folding. Whereas on the other hand, for a hydrophobic interaction which takes place in water without removal into a non-polar environment, e.g. clusters of small molecules, α helix formation by polypeptides, binding of small molecules by biopolymers and sol-gel transition of biopolymers, solute aggregation in aqueous solution might be appropriate (Franks, 1975).

The effect of temperature and solutes on the nature and magnitude of the hydrophobic interaction are very important. Nemethy and Scheraga (1962) have computed the strength and the temperature dependence of pairwise hydrophobic interactions and found that it increases, with increasing temperature up to about 60°C. However according to Ben-Naim (1980) the temperature dependence of the hydrophobic interaction is represented by the entropy and not the enthalpy change as envisaged by Nemethy and Scheraga. Based on this assumption, the strength of the hydro-

Table 5

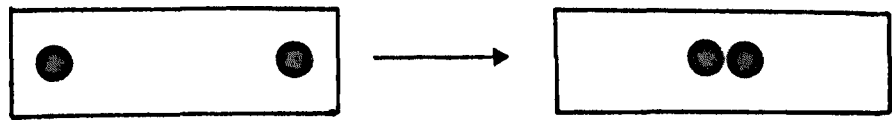
Thermodynamic parameters for the transfer of aliphatic alcohols from water to pure liquid at 25°C (Arnett et. al., 1969).

	$\Delta G_{w \to \ell}$ cal.mole^{-1}	$\Delta H_{w \to \ell}$ cal.mole^{-1}	$\Delta S_{w \to \ell}$ cal.mole.$^{-1}$deg^{-1}
Ethanol	-760	+2430	+10.7
n-propanol	-1580	+2420	+13.4
n-butanol	-2400	+2250	+15.6
n-pentanol	-3222	+1870	+17.1

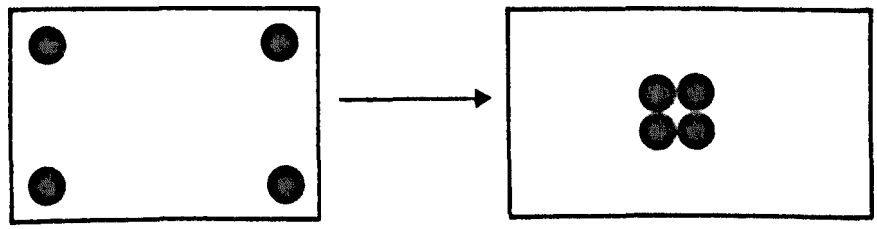
Fig.8. Model Processes used to Study Hydrophobic Interactions (Ben-Naim, 1980).

(8.a) Transfer of simple solute from water into hexane.

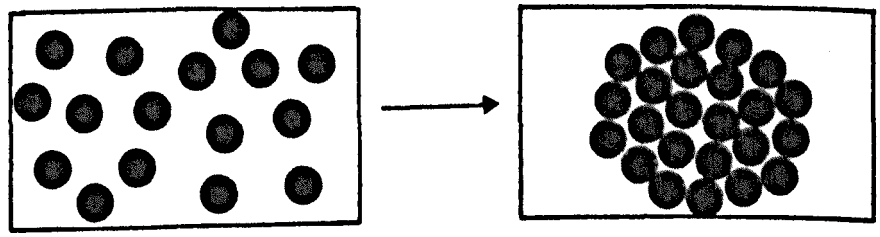
(8.b) Dimerisation of two simple solutes in an aqueous solution.



(8.c) Formation of tetramer.



(8.d) Aggregation of large number of solute molecules.



phobic interaction increases with respect to temperature up to 85°C. The strength of the hydrophobic interaction is affected by structural changes in water. Water structure decreases with rise in temperature and this leads to an increase in the strength of the hydrophobic interaction. Solutes which increase the structure of water will weaken the strength of the hydrophobic interaction and vice versa. Alcohols and electrolytes decrease the structure of water and strengthen the hydrophobic interaction.

Excellent monographs on the hydrophobic effect have been written by Ben-Naim (1980) and Tanford (1980) and the biological implications of hydrophobic forces have been considered by Jencks (1969). The subject has been reviewed by Franks (1975), Franks and Reid (1973) and Suggett (1977).

CHAPTER 3

THE EXPERIMENTAL DETERMINATION OF PARTITION COEFFICIENTS AND THE THERMODYNAMIC PARAMETERS OF TRANSFER.

3.1. Experimental Determination of Partition Coefficients.

3.1.1. Shake Flask Technique.

The most commonly used, and simplest method is the "shake flask" technique. Precise details of this technique have been given by Corwin Hansch in Purcell et al. (1972), Rekker (1977) and Martin (1978). A brief outline will be given here. For the actual determination of the partition coefficient, the mutually saturated aqueous and organic phases are pipetted into centrifuge tubes, with screw caps. The pure solute is dissolved in the phase in which it is most soluble, and to prevent ionisation of solute, the aqueous phase contains a suitable buffer. The solvent ratio is so arranged that almost equal amounts of solute are found in each phase. The solute concentration is kept to a minimum to prevent association. The centrifuge tubes, containing solvents and solute, are tightly stoppered and placed in a thermostated water bath, at a specified temperature, and shaken until equilibrium is reached. The tubes are then centrifuged, at moderate speeds, to achieve complete phase separation of solvents. This is particularly important with water/octanol phases, which are more difficult to separate than water and non polar solvents, such as cyclo-

hexane. Then samples from each phase are removed, and the solute concentration in each phase is determined by some suitable analytical method, e.g. UV spectroscopy, GLC or HPLC. The partition coefficient is determined from the ratio of solute concentration in organic phase to aqueous phase. In many cases, glass stoppered conical flasks or even separating funnels are used for equilibrating the solvents and solute.

Recent examples of the use of this technique are given by Nahum and Horvath (1980), Levin (1980), Wong and Lien (1980), Banerjee and Gupta (1980), Inagi et al. (1981), Anliker et al. (1981), Terada et al. (1981) and Shun et al. (1981).

The 'shake flask' technique has the obvious advantage of being inexpensive and easy to use. However the method is very tedious and time consuming, especially when one wishes to study partition coefficients under different experimental conditions. The values determined can often be inaccurate, especially for solutes with very high or very low log K_d values. Precise temperature control is difficult and it is often difficult to obtain partition coefficient values at temperatures other than room temperature. For a project involving a study of the temperature dependence of partitioning the shake flask technique would not be the most suitable.

3.1.2. Counter-Current Distribution.

Craig's counter-current distribution apparatus is described in great detail by Saunders (1971). In this technique a small amount of solute (5-25 mg) is subjected to several transfers between a properly chosen pair of immiscible solvents. It has

been mentioned that such a procedure yields valuable partition coefficient data. However for purposes of characterising or separating a solute, it is desirable to have a partition coefficient near to one (Craig et al., 1947). This is often accomplished through the use of mixed solvents. Also when a clean separation of solutes is desired, concentrated buffers are used to give maximum shift of partition coefficient with pH. As a consequence the results obtained by this method cannot be compared with other results. The method has the disadvantage that the apparatus is cumbersome and difficult to clean. The technique has the advantages that the solute need not be pure and is applicable to a wide range of log K_d values. Saha et al. (1963) have successfully used this technique to determine partition coefficients of monohydric phenols.

3.1.3. Chromatographic Methods.

There have been many attempts to develop chromatographic techniques, many of which depend on the partitioning process, to determine partition coefficients. Paper, thin layer, gas and high pressure liquid chromatography have all been used with success. An excellent review on this topic has been written by Kaliszan (1981).

3.1.3.(i) Thin Layer Chromatography.

Thin layer chromatography was one of the first widely used techniques in this context. Martin (1949) deduced, on theoretical grounds, a relationship between partition coefficient, K_d, and R_f values for liquid-liquid partition chromatography expressed

in the equation

$$K_d = k\,[(1/R_f) - 1]$$

where $k = \frac{V_m}{V_s}$ and V_m = volume of mobile phase, V_s = volume of stationary phase.

Bate-Smith and Westall (1950) defined R_m by

$$R_m = \log\,[(1/R_f) - 1]\ ,$$

from which it follows that

$$\log K_d = \log k + R_m$$

so that R_f and R_m values are related to $\log K_d$. The solute under investigation is applied to a glass plate coated with an inert support, such as silica gel, and impregnated with a suitable stationary phase, e.g. liquid paraffin and eluted with an aqueous solvent. (This process is termed reversed phase T.L.C.).

The polar mobile phase is usually a mixture of aqueous buffer and acetone, or another organic solvent that is miscible with water, e.g. methanol, saturated with the stationary phase. The mixing ratio of water to organic solvent depends on the nature of the compounds under investigation : the higher their hydrophobicity, the lower the percentage of water to be used in the mobile phase. The plates are developed in a chromatographic chamber using an ascending technique. After drying the plates are examined by an appropriate detection method, and the R_f values determined. Much effort has been directed to preparing suitable lipoidal phases. Silica gel, Kieselguhr G or cellulose have been impregnated by allowing solutions of reverse phase compound, in volatile solvent, to cover support and then evaporating the solvent. Hexane has commonly been used as solvent

for paraffin oils after Boyce and Millborrow (1965). Fatty acids and their derivatives have usually been dissolved in diethyl ether (Fujii et al., 1978) or toluene (Duran and Plá Delfina, 1974), n-octanol in acetone (Bird and Marshall, 1971; Yamana et al., 1977) and silicone oil in ethanol (Biagi et al., 1970). Hulshoff and Perrin (1976, 1977) have used Kieselguhr G impregnated with oleyl alcohol, and Kuchar et al. (1979) have used silica gel G impregnated with silicone oil. Dearden et al. (1974) used polyamide plates.

Biagi et al. (1975, 1979) determined the R_m values of phenols and acetophenones and obtained good correlation with biological activity. They found that R_m values obtained on silica gel layers impregnated with silicone oil were much more closely related to log K_d values in an octanol-water system, than R_m values determined on polyamide layers. Gasparic (1980) used reverse phase T.L.C. to assess hydrophobicity in studies of structure activity relationships of sulphonamides. Silica gel and cellulose sheets impregnated with paraffin oil, in n-hexane, or 1-octanol in ethanol were used as stationary phase. They found that not all compounds chromatographed were partitioned between the organic stationary phase and the aqueous mobile phase. Some compounds were adsorbed by the 'inert' support. Thus as a precaution, a blank chromatograph, with mobile phase, but on an untreated support, should be run simultaneously to show that the mechanism involved in separation is a partitioning process.

A recent innovation in T.L.C., is the use of C_8 and C_{18} alkyl chains chemically bonded to silica gels as the organic

stationary phase. Ellgehausen et al. (1981) used C_{18} chemically bonded silica plates to determine R_m values of 15 common pesticides and found good correlation with log K_d water/octanol values.

According to Tomlinson (1975) reverse phase TLC has several distinct advantages :

(a) It is simple to use, less tedious and very rapid, e.g. up to 25 different solutes can be developed simultaneously;

(b) Only small amounts of sample needs to be used;

(c) Solute to be examined need not be ultrapure;

(d) There is no need for quantitative analysis of solute.

The method has several disadvantages :

(a) Precise temperature control is difficult;

(b) Overloading of solute onto plates may produce streaking, which leads to errors in measuring R_f values;

(c) Sometimes the separating mechanism is not only a partitioning process, but adsorption onto inert support may occur.

(d) Range of log K_d values not very large.

3.1.3.(ii) Gas Liquid Chromatography.

The determination of partition coefficients of volatile substances is often very difficult and time consuming. For this reason Bŏcek (1979) developed a gas liquid chromatographic method, using water and oleyl alcohol as liquid stationary phases independently. Bŏcek assumed the partition coefficient of a compound in the system oleyl alcohol-water to be equal to the ratio of partition coefficients in the system oleyl alcohol (saturated with water) - nitrogen and water (saturated with

oleyl alcohol) - nitrogen which could be determined by GLC. A similar procedure was adopted by Sato and Nakajima (1979) to determine partition coefficients of chlorinated hydrocarbons, and by Kuhne et al. (1981) to determine K_d values of alcohols, ketones, esters and nitro-alkanes. Earlier attempts by Fujita et al. (1964) and Hansch and Anderson (1967) to determine partition coefficients by GLC also proved successful.

The method is complicated as care must be taken to eliminate the influence of absorption, and thus determine the true value of the partition coefficient.

3.1.3.(iii) High Pressure Liquid Chromatography.

The use of reverse phase HPLC for the determination of partition coefficients was first suggested by Haggerty and Murrill (1974). Various parameters of HPLC retention have been utilised, and these may be related to K_d by the general equation :

$$\log K_d = \log (\text{HPLC retention}) + \text{constant},$$

where log (HPLC retention) = log k' or log t_c or log V_r. The retention term has been expressed as log k', where k' is calculated from retention times; t_R, the elution time for a retained peak, and, too, the eluted time for an unretained reference solute. (Carlson et al., 1975; McCall, 1975).

$$\log k' = \log \left(\frac{t_R - t_o}{t_o} \right) .$$

The corrected elution time, t_o (Muirlees et al., 1976), or the retention volume, V_R (Henry et al., 1976), have also

been used :

$$\log t_c = \log (t_R - t_o) ,$$

and $$\log V_R = \log [(t_R - t_o)(\text{flow rate})] .$$

The terms k' and $\frac{1}{R_f}$ are analogous. Therefore

$$\log K_d = \log k' + \log k ,$$

where k = constant for system.

In general there is very good correlation between log K_d and log k'; often the slope is approximately 1.0 (Yamana et al., 1977).

The principle of operation is similar to TLC. An organic layer forms a stationary phase on an inert support medium, whilst the mobile aqueous phase is forced under high pressure down a column packed with the organic stationary phase. The solute is injected onto the column and eluted by the mobile phase. Various detectors, e.g. UV-visible spectroscopy, fluorescence, refractive index or polarography, can be used to assay the solute as it is eluted off the column.

Octadecyl chains chemically bonded to pellicular silica gel were first used as hydrophobic phases for HPLC by Haggerty and Murrill (1974), McCall (1975) and Carlson (1975). Tanaka and Thornton (1977) used silylated Corasil C_{18} and Bondapak C_{18} bonded columns, and mobile phases of water-methanol to determine log K_d's of numerous organic compounds, when studying structural and isotopic effects in hydrophobic interactions.

Octadecyl chemically bonded stationary phases, with mobile phases of ethanol-water mixtures, have been used by Ellgehausen et al. (1981) to determine octanol/water partition

coefficients of 20 common pesticides. Rittich et al. (1980) and Butte (1981) have determined R_m values of substituted phenols using C_{18} chemically bonded stationary phases, and a mobile phase of water/methanol mixture. Tomlinson et al. (1978, 1981) used octyl and octadecyl groups bonded to silica as stationary phases and a mobile phase of aqueous-methanol mixtures. The alkyl group of the methanol undergoes hydrophobic bonding with the alkyl groups of stationary phases, so that hydroxyl groups of methanol are free to interact with solute molecules. So in effect the solute is presented with an "aliphatic alcohol" stationary phase.

Mirrlees et al. (1976) have argued that the only true model for n-octanol is n-octanol itself. According to them, a bonded ODS support must have essentially the same partitioning characteristics of a non-polar hydrocarbon. In such a situation, Mirrlees et al. (1976) coated the column packing (Kieselguhr) with water saturated n-octanol, and used buffer, saturated with n-octanol, as mobile phase. Miyake and Terada (1978) described a method for preparing a n-octanol coated column that had high stability. Hot Corasil I was mixed with n-octanol, the mixture was slurry packed and excess of n-octanol was removed by elution with buffer saturated with octanol. Unger et al. (1978) prepared a n-octanol coated ODS column by injecting n-octanol directly onto the column under pressure until droplets appeared. The column was then flushed with octanol saturated buffer until eluate appeared clear. As octanol itself is very lipophilic it bonds strongly to ODS and gives a stable column. Moreover octanol is bonded to octadecyl

chains with its free hydroxyl end free to interact with the solute, as is expected in shake flask octanol-water partitioning.

Reverse phase chromatography of very lipophilic compounds cannot be performed with n-octanol as stationary phase, because the polar solvent concentration in the mobile phase, necessary for obtaining measurable retention times, would solubilise n-octanol (Hulshoff and Perrin, 1976a). For this reason other stationary phases have been proposed. Hulshoff and Perrin (1976b) impregnated Porasil C and Chromosorb PNAW with oleyl alcohol. A similar technique has been applied for coating Pellicular Corasil II silica with squalene (Henry et al., 1976).

A series of molecules characterised by a wide range of physico-chemical properties cannot be easily measured with a single set of experimental conditions. Several studies are underway, however, with the aim of developing a system of standard methods and values. Baker and May (1979) have recently reported the establishment of a retention index, which in principle would allow the use of a range of solvent systems in HPLC, all related to a continuous scale.

The advantages claimed for this technique are :

(a) Results are accurate and reproducible;

(b) Can use impure solutes;

(c) Technique is very rapid to use and because of this unstable compounds can be determined;

(d) Only small amount of solute is required;

(e) There is no need for specific quantitative analysis and a wide range of detectors can be used - UV-spectroscopy, fluorescence, refractive index, etc.;

(f) Technique is relatively easy to use and is common in many analytical laboratories, where it can be easily adapted to determine partition coefficients.

However there are a number of disadvantages involved with HPLC (Elson, 1978; Anderson et al., 1981);

(a) Temperature control is difficult;

(b) The effect of pH and ionic strength of buffered phase on column are not well understood, and large variations in these parameters are not advised;

(c) Range of log K_d is limited to values of 0.5 to 2.5;

(d) The solvent range is limited;

(e) The system does not operate under strict thermodynamic conditions, since the solute is partitioning at an ever decreasing concentration between its own phase and 'pure' extracting phase, a phenomenon which is unpredictable.

3.1.4. Titrimetric Methods.

Most drugs are either weak acids or weak bases. If partition coefficients of such compounds are determined, the degree of ionisation at the pH value of the aqueous phase must be considered. For a partially ionised solute the apparent partition coefficient or distribution coefficient, D, and the true partition coefficient, K_d, are related by the following equations :

Acids : $$AH + H_2O \rightleftharpoons A^{\ominus} + H_3O^{\oplus}$$

$$D = \frac{[AH]_{org}}{[AH]_{aq} + [A^-]_{aq}} = (1-\alpha)\, K_d = \frac{K_d}{1+10^{pH-pKa}} \qquad (1)$$

Bases : $B + H_3O^{\ominus} \rightleftharpoons BH^{\oplus} + H_2O$

$$D = \frac{[B]_{org}}{[B]_{aq}\,[BH^+]_{aq}} = (1-\alpha)\,K_d = \frac{K_d}{1+10^{pKa-pH}} \qquad (2)$$

where α = fraction of ionised species.

Only the unionised species is assumed to partition from the aqueous phase into the organic phase. True partition coefficients, K_d, and pKa values can be determined simultaneously from distribution coefficients, D, at different pH values, using equation (1) for acids and equation (2) for bases.

Seiler (1974b) described the simultaneous determination of partition coefficients and pKa values by potentiometric titration. Titration of the drug in aqueous phase was performed, as usual, until approximately half the amount of titrant had been added. A definite volume of a water saturated organic solvent was added and the titration was finished in the presence of this solvent. The true partition coefficient, K_d, and pKa value could then be calculated by non-linear regression analysis, from the volumes of aqueous and organic phases, the amounts of added titrant and the corresponding pH values. This method is an extension of the titrimetric method developed by Brandstrom (1963) for bases, and that used by Bird and Marshall (1967) for use with acids.

A similar procedure has been used by Kaufman and colleagues (1975) for the micro-electrometric titration of various narcotics and narcotic antagonists. After determination of the pKa value by normal titration and in the presence of a known amount of organic phase, the true partition coefficient, K_d,

could then be determined from the apparent shift of the pKa value. The scope and limitations of these methods have been discussed by Martin (1979). These titrations must be carried out in thermostated vessels. The ionic form must be soluble to the extent soluble for typical titrations (molar solubility must exceed the K_a). It is important that the water and octanol used are mutually saturated (otherwise wrong volumes would be used in calculations), that the phases are equilibrated before readings are taken, and that none of the octanol or drug evaporates during titration. It is not known if the octanol, dissolved in the water, changes the pKa of dissolved solutes. Theoretically, water in such a mixed solvent would preferentially solvate, and hence alter the pKa, of lipophilic compounds. This could limit the usefulness of these methods for the determination of log K_d and pKa values.

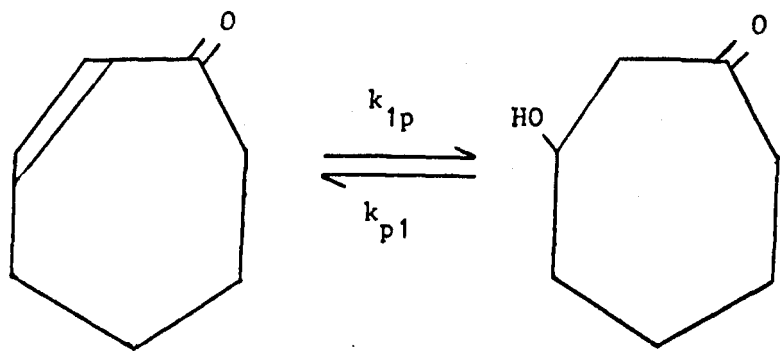
Johansson et al. (1976) used a two phase titration method to determine partition coefficients and pKa's of amines and organic acids. More recently, Li Wan Po and Irwin (1980) have developed an automated method for the potentiometric titration of weak acids and bases. The process enables the rapid, accurate and precise determination of pKa and partition coefficient values, and is based upon the high degree of control afforded by a Hamilton Microlab P microprocessor controlled pipette. These methods are only of value for drugs which are weak acids or bases. However they are relatively simple to use, and relatively small quantities of solute are required for experimentation.

Cantwell and Mohammed (1979) have recently developed a

technique for photometric acid-base titrations in the presence of an immiscible solvent, which can simultaneously measure distribution coefficients. (This will be dealt with in more detail in the filter probe technique).

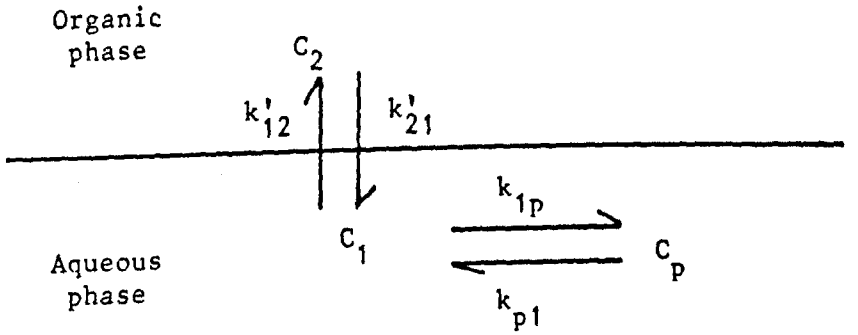
3.1.5. Kinetic Methods.

Determining the partition coefficient of an unstable species presents certain problems. However these may be overcome by employing kinetic methods. Byron *et al.* (1980) demonstrated that the partition coefficient of an unstable compound may be determined using a stirred transfer cell, containing equal volumes of liquid paraffin and an aqueous phase, by kinetic analysis. Cyclohept-2-enone (I) was chosen as solute, since it is a neutral molecule, and its partition coefficient should be pH independent. It undergoes acid catalysed hydration to 3 hydroxy-cycloheptanone (II), but is sufficiently stable at neutral pH to determine the partition coefficient.



I II

The model system chosen represents first order transfer between the aqueous (C_1) and organic (C_2) phases with simultaneous reversible first order hydration.



The transfer constants, k'_{12} and k'_{21}, were determined at 37°C, in the absence of degradation, where asymptotic values for C_1 agreed with the observed equilibrium values in non kinetic partitioning studies. The first order rate constants for hydration in 0.1 N HCl were determined at 37°C, in the absence of the organic phase. Partitioning with simultaneous hydration was then studied using 0.1 N HCl and liquid paraffin. Data were analysed by non-linear regression based on the equation for C_1 as function of time.

The values for k'_{12} and k'_{21} from these experiments were comparable to the estimates obtained under stable conditions. This agreement demonstrates that simultaneous degradation and partitioning can be analysed for k'_{12} and k'_{21}, thus permitting calculation of the partition coefficient (i.e. $K_d = k'_{12}/k'_{21}$) that would be observed if the drug were stable.

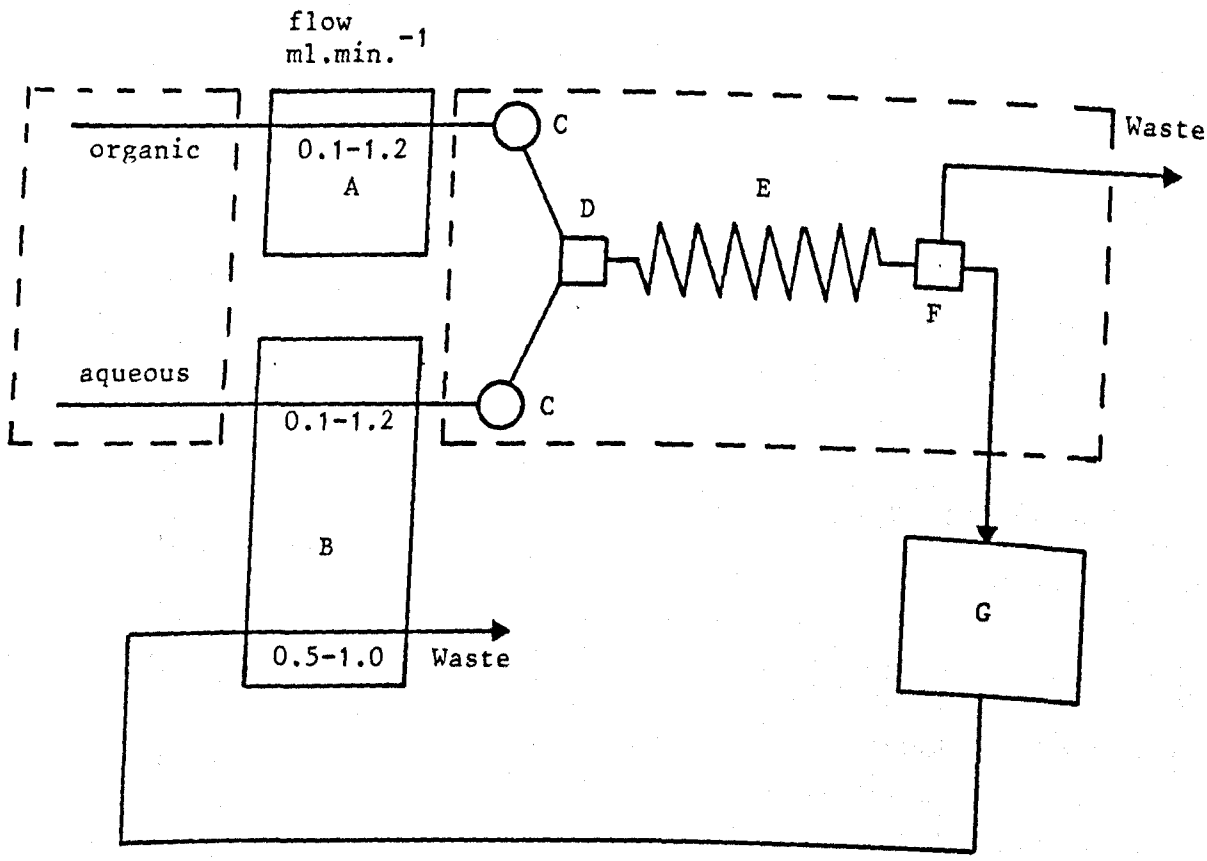
The same authors (Tomlinson et al., 1980) have also studied the simultaneous partitioning and degradation kinetics of amoxicillin and ampicillin by a similar method.

3.1.6. Segmented Flow/Phase Splitting System.

Kinkel and Tomlinson (1980) have described a method for the rapid determination of liquid-liquid partition coefficients.

The approach is based upon a fast solute phase equilibration in oil/water segmented flow through helically coiled tubes, with phase splitting based on dissimilar phase wetting of hydrophobic and hydrophilic surfaces. The method has a capacity for up to 45 determinations per hour, with a precision error of less than 2.0%. The method is most suitable for single one off determinations of the partition coefficient, but it can be used to study the effect of various environmental factors affecting distribution (e.g. pH, ionic strength, temperature). The segmented flow/phase system is shown in Fig.9, and is comprised of 5 principal parts : reservoirs and pumping devices, injector, segmentor, mixing coil and phase splitting device. The aqueous phase was pumped via a peristaltic pump and the oil via a HPLC pump. Segmented flow was achieved by pumping both phases through a T piece of PTFE. Segments were then pumped through a helical coiled PTFE tube of variable length. Wall drag, and coiled aspect induces turbulent flow in the extraction coil (Snyder and Adler, 1976) and rapid solute distribution occurs between the two phases. After equilibration, phases were separated in a phase splitter device, which acts on the basis of dissimilar wetting of hydrophilic and hydrophobic surfaces, and was a modification of that designed by Copsey (1977). The phase splitter (Fig.10) consisted of two surfaces, PTFE and silica glass, having entrance and exit ports, cut as discs, clamped together by a steel jacket to avoid leakage. Sample injection was accomplished by two methods. Firstly solute was dissolved in the aqueous reservoir, and after extraction and phase splitting was assayed

Fig.9. Construction of segmented flow/phase splitter apparatus for determination of partition coefficients.



A double reciprocating pump

B peristaltic pump

C injection valve

D PTFE T piece

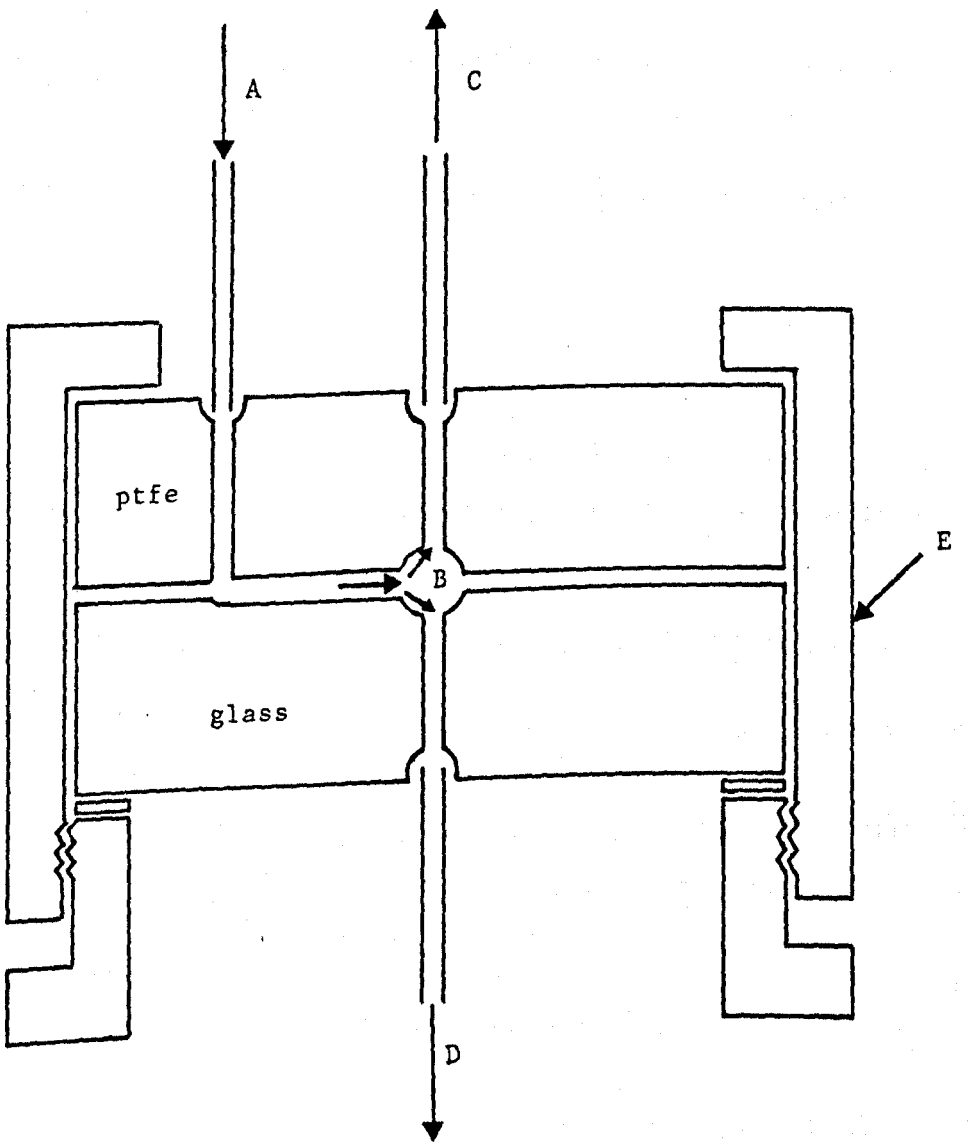
E extraction coil

F phase splitter

G flow cell

---- area enclosed by dashed lines is thermostatted

Fig.10. Construction of phase splitter.



A segmented flow inlet

B splitting chamber

C oil phase outlet

D aqueous phase outlet

E stainless steel jacket

off line. Secondly the solute was introduced, via a HPLC valve (C), either into the polar or non-polar stream, and after leaving phase splitter, a fraction of phase to be assayed was pumped through a flow cell of a spectrophotometer. (For temperature studies the components of the apparatus enclosed in dashed line of Fig.9 were thermostated by immersion in a temperature controlled water bath). The partition coefficient was then determined from the ratio of concentration of solute in organic and aqueous phases respectively. The partition coefficient values obtained were found to be in good agreement with those from conventional shake flask method. The rapidity of method makes it suitable for the determination of partition coefficients of unstable species. The system is appropriate for log K_d values in the range -2.5 to +2.5.

The technique has several disadvantages however :

(a) Operation of technique is somewhat difficult and depends on very precise operation of the pumping systems.

(c) Viscous solvents cannot be used.

(c) The two phases cannot be recycled.

3.1.7. AKUFVE - A Continuous Extraction System.

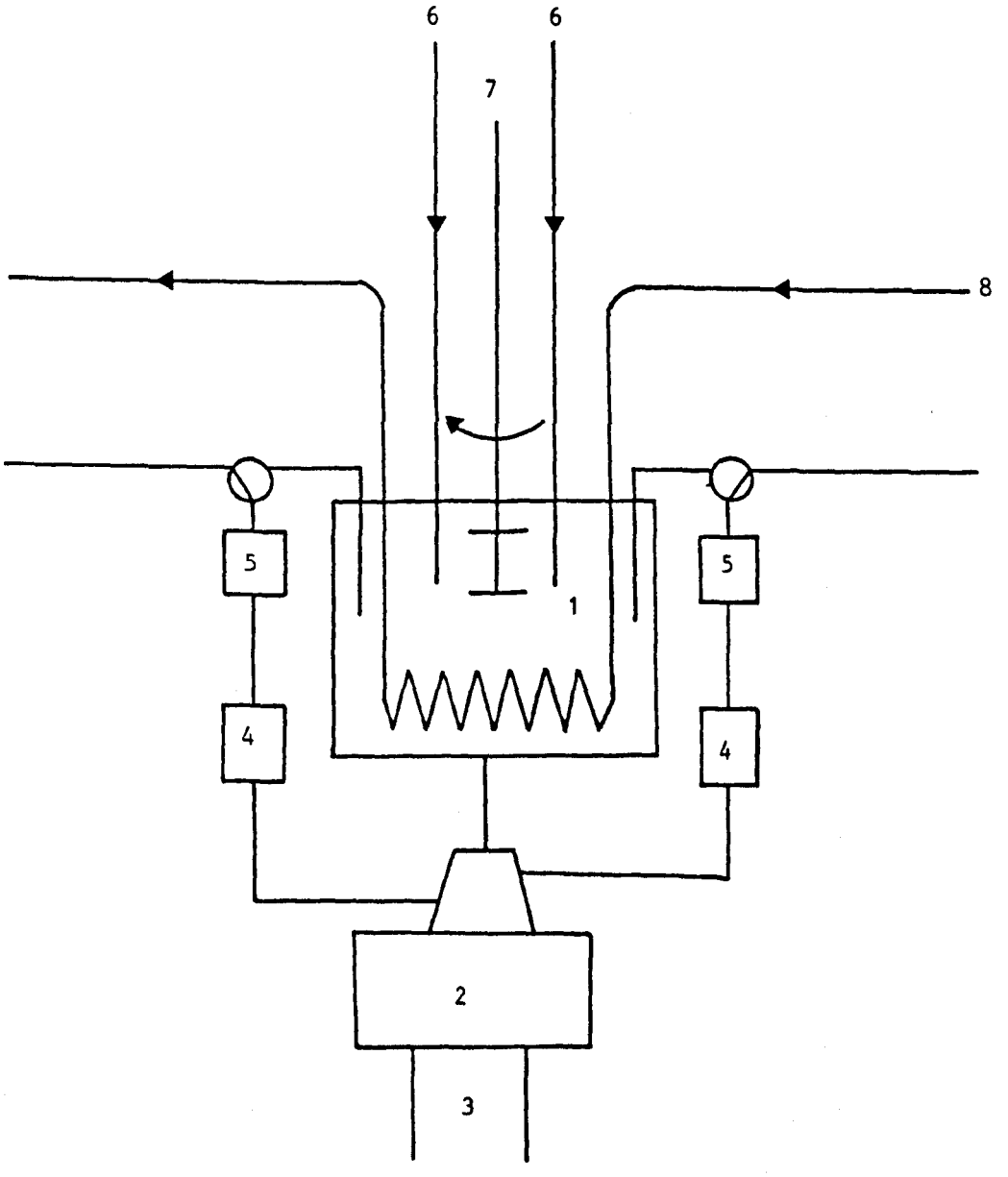
(The term AKUFVE is a Swedish abbreviation for apparatus for continuous measurement of distribution factors in solvent extraction).

This apparatus was originally developed by Reinhardt and Rydberg (1969) to obtain distribution data for the solvent extraction of rare metals. It facilitates the continuous, rapid and accurate measurement of the distribution of a solute

between two immiscible solvents, as a function of variation in simultaneously measured physical and chemical conditions. Full details of the development and evaluation of the AKUFVE technique in solvent extraction has been discussed by Reinhardt and Rydberg (1969, 1970, 1972). Davis et al. (1976) and Elson (1978) have demonstrated that the AKUFVE H-33 could be adopted for studies in the pharmaceutical field and be used for the rapid determination of partition coefficients under a variety of experimental conditions, including pH, temperature and ionic strength. The mechanism of liquid-liquid phase separation depends on density differences in the two immiscible solvents.

A schematic diagram of the AKUFVE apparatus is given in Fig.11. It consists of a mixer that gives rapid and efficient contact between the two immiscible phases, a centrifugal separator that ensures rapid separation of the two phases, and connections permitting sampling or on-line measurement of solute concentration in both solvent phases. Inlets are provided into mixing chamber for addition of solute, solvent, acids and alkalies. The operation of the apparatus is dependent upon the specially designed H centrifuge that provides "absolute phase separation". (The centrifuge provides light and heavy phases that contain few or no droplets of the other phase). It is a simple matter to use on-line detection methods, such as UV-visible spectroscopy. Alternatively the apparatus can be fitted with rubber septa, whereby samples can be removed by syringe and assayed off line. The pH of the aqueous phase can be monitored via a pH electrode, which

Fig.11. Schematic diagram of AKUFVE apparatus.



1. mixing chamber
2. H-centrifuge
3. motor
4. flow meters and regulators
5. detection unit
6. feeds for solvents and reagents
7. stirrer/mixer
8. thermostat

may be connected to a 'pH stat' if required.

The AKUFVE is operated by placing the two mutually saturated solvents, normally 500 ml. of each, into the mixing chamber. The stirrer speed is set to provide efficient mixing and the centrifuge is then started. The optimal stirring and centrifuge speeds are dependent on the nature of the two phases, and to some extent on solute and pH. Excessive speeds for each can lead to emulsification. The mixed system is run into the centrifuge and the flow rates to and from the centrifuge are adjusted to provide clear aqueous and organic phases. Equilibrium conditions are reached in 30 seconds or less. The separated phases are then passed through detectors and returned to the mixing chamber. The separation process is cyclic and continuous. The solute can then be introduced into the mixing chamber. The solute concentration in each phase can be determined, if necessary, by analysing solvents on line or by taking samples, via septa.

Table 6 shows the variables which affect the distribution of a solute between an aqueous and organic solvent. All of these factors have been studied by Davis et al. (1976) using the AKUFVE H-33 system. Advantages of the AKUFVE are :

(i) Absolute phase separation takes place very quickly, and partitioning of unstable species can be studied.

(ii) A large selection of detection systems may be used - spectrophotometric, radiometric, refractive index, pH.

(iii) A large range of experimental conditions can be investigated.

Although the AKUFVE has been used to provide large quantities

Table 6

Factors affecting the distribution of a solute between an aqueous and an organic phase.

Solute

Concentration

Molecular structure

Ionisation

Complexation

Association

Solvent

Chemical nature

Density

Mixtures

Phase ratios

Experimental

pH

Ionic strength

Temperature

of reliable data in a short period of time, it also has some disadvantages :

(i) It is expensive.

(ii) Setting up and operation of AKUFVE system is difficult.

(iii) The apparatus needs to be cleaned after each partition study. In order to do this the AKUFVE system needs to be stripped down and cleaned, which is very time consuming. Thus the AKUFVE is not suitable for the routine determination of a large number of one off partition values.

(iv) Relatively large quantities of solvents and solutes are required (500 ml. of each), which is a great disadvantage with dangerous and expensive chemicals.

(v) The AKUFVE gave unsatisfactory separation of water and organic solvents, with a density between 0.88 and 1.05 gm/ml.

(A smaller version of the AKUFVE H-33, the mini-AKUFVE ADMCS H-10 trS, is now available in the Department of Pharmacy, Nottingham University, and it will be set up to study the thermodynamics of partitioning of substituted phenols between water and bulk organic solvents).

3.1.8. The Filter Probe Technique.

Recently in the literature an apparatus has been described (Cantwell and Mohammed, 1979; Mohammed and Cantwell, 1979, 1980) which permits the photometric acid-base titration of solutes in the presence of an immiscible solvent - this is the filter probe technique. This was then modified by Kinkel et al. (1981)

to study the temperature dependence of distribution of substituted benzenes and methyl benzoates between aqueous buffers and 2,2,4-trimethylpentane. Partition coefficients were determined using a rapid mix/filter probe system (see Experimental for more details). It consisted of a thermostatted (± 0.1°C) mixing chamber (200 ml. volume) whose contents would be vigorously stirred, using a magnetic stirring bar and motor. Using filters of hydrophilic (cellulose) or hydrophobic (teflon or silicone treated phase separating paper) material, the aqueous or oil phases, could be probed by linking the filters to a HPLC pump or peristaltic pump, and then examining the phase under study by on-line detection using a UV-visible spectrophotometer, fitted with a thermostatted flow through cell. Flow exiting from this cell was returned to the mixing chamber. All connecting tubes were made of narrow bore stainless steel or teflon tubing. To check mass balance the hydrophobic probe was used occasionally; normally only the aqueous phase was probed for partition coefficient determinations. Solvents were mutually saturated with one another before experimentation. The buffered aqueous phase was added to the mixing chamber and probed to a blank reading (A_b); then a second addition of aqueous phase containing solute was made to obtain an unextracted sample reading (A_u). The organic phase was then added and the absorbance of the aqueous phase was monitored continuously (A_e). To examine the effect of temperature on solute distribution, the temperature of mixing chamber could be raised several times, over a range of 10-50°C, and the A_c values determined at equilibrium. Thus

$$K_d = (A_u - A_e) \cdot (A_e - A_b)^{-1} R^{-1}$$

where R is the oil/water phase volume ratio.

The accuracy and precision of method compares favourably to conventional shake flasks. The filter probe technique has several distinct advantages :

(i) It is cheap, quick and simple to use.

(ii) Gives accurate, precise, reproducible results.

(iii) Can be used to determine partition coefficients of unstable species.

(iv) A large range of log K_d values, -3.0 to +3.0, can be determined.

(v) Good temperature control can be achieved.

Tomlinson (1982) has recently published a paper describing the practical details in using the filter probe technique, and its application in studying molecules of a pharmaceutical interest. (The filter probe technique will be used to study the thermodynamics of partitioning of substituted phenols between water and organic solvents).

3.2. Experimental Determination of the Free Energy, Enthalpy and Entropy of Transfer.

3.2.1. Free Energy of Transfer, ΔG_{tr}.

The free energy of transfer, ΔG_{tr}, is related to the partition coefficient by the equation :

$$\Delta G_{tr} = -RT \ln K_d$$

where R = gas constant, 8.314 $J.K.^{-1}mole.^{-1}$

T = temperature, units of Kelvin.

Thus the free energy of transfer can be obtained from the partition coefficient at a particular temperature. Methods of measuring liquid/liquid partition coefficients have been discussed in Chapter 3.1.

The free energy of transfer of a solute from the gaseous or vapour phase to the liquid phase is also important. The transfer of a solute from the gaseous phase to the liquid phase, i.e. the gas/liquid partition coefficient can be determined in a number of ways. Kuhne et al. (1981) and Sato and Nokajima (1979) determined the gas/liquid partition coefficient by gas liquid chromatography. Cramer (1977), Ben-Naim and Wilf (1980) and Tanford (1980) showed that this parameter could be obtained from solubility measurements. Amidon and Anik (1980) and Tucker et al. (1981) used vapour pressure measurements to determine the gas/liquid partition coefficient. If the gas/water and gas/organic solvent partition coefficient of a solute are determined, then the water/organic partition coefficient can be obtained from this data.

3.2.2. Enthalpy of Transfer.

The enthalpy of transfer can be determined indirectly by studying the temperature dependence of distribution or solubility - the van't Hoff method, or directly by micro-calorimetry.

3.2.2.(i) The Van't Hoff Method.

(a) Temperature Dependence of Distribution.

If the liquid-liquid partition coefficient of a solute is

determined between two immiscible solvents, over a range of temperature, then the enthalpy of transfer can be obtained from the slope of the graph of ln K_d versus 1/T.

This method has been employed by several workers in studying the thermodynamics of partitioning of drug molecules and their functional groups between water and bulk organic solvents. The shake flask technique has been employed in this way by Harris et al. (1971), Crugman (1976), Brodin et al. (1976), Lundberg (1979), Rogers and Wong (1980), Korenman (1980) and Ueda et al. (1982). Using the AKUFVE technique, Elson (1978) obtained the enthalpy of transfer of alkyl phenols between water/octanol and water/cyclohexane.

Several workers have employed HPLC methods to determine the enthalpy of transfer between aqueous and organic phases. These include Melander (1978), Chmielowiec and Sawalzsky (1979), Vigh and Varga-Puchony (1980) and Tomlinson et al. (1981). Kinkel and Tomlinson (1981) have used the filter probe technique to determine the enthalpy of transfer for substituted benzenes and methyl benzoates between aqueous buffer and isooctane.

(b) Temperature Dependence of Solubilities.

Ben-Naim (1974) determined the heats of solution of methane in water and several organic solvents, by studying the temperature dependence of solubility in the appropriate solvent. The enthalpy of transfer is obtained from the difference in heat of solution in aqueous and in organic phase.

(c) Criticism of the Van't Hoff Method.

In determining the enthalpy of transfer by the van't Hoff

method, it is assumed that the enthalpy of transfer, ΔH_{tr}, is temperature independent over the range of temperature in which the distribution measurements are made. If the enthalpy of transfer is temperature independent then a plot of ln K_d versus 1/T would yield a straight line, whereas if it were temperature dependent, a curved line would be obtained.

According to Kinkel et al. (1981) if the composition of the aqueous-organic phase system changes dramatically with temperature, due to mutual solubility, as would be expected with the aqueous buffer/octanol system, then the van't Hoff relationship would fail. However they claim that by using a biphasic solvent pair, such as aqueous buffer-isooctane, with low mutual solubility the van't Hoff plot method would be suitable since the system would not change with temperature. Franks and Reid (1973) discussed the problems involved in the van't Hoff treatment of equilibrium constants in great detail :

(a) Since the evaluation of enthalpy of transfer involves a differentation step, it makes severe demands on the accuracy of experimental partition data.

(b) It is assumed that solute activity is equal to its concentration, and solvent activity is sensibly unchanged. If this is not true, the van't Hoff heat will not agree with calorimetric heat.

(c) It is assumed that no cooperative effects are involved in the expression for ln K_d.

(d) Whereas calorimetry yields integral heats, the van't Hoff method gives rise to the differential heat. King (1965) discusses the precision of the van't Hoff method in determining

ΔH_{tr}, with reference to the temperature dependence of the equilibrium constant, K_a - the acidity constant. The standard deviation in the enthalpy and entropy term is greatly reduced when the pKa value is measured to an accuracy of standard deviation, ± 0.001, with a large number of determinations (10) over a large range of temperature (5°-50°C).

There is direct evidence from Reid et al. (1969) for the value of the van't Hoff plot. These workers measured the ΔH solution for benzene, n-hexane and cyclohexane by calorimetry, and found good agreement with van't Hoff heats, thus indicating the usefulness of the van't Hoff plot. Similarly, Huyskens and Tack (1975) compared the enthalpies of transfer for phenol derivatives between water and cyclohexane, obtained by van't Hoff and calorimetric procedures and found good agreement.

3.2.2.(ii) Direct by Calorimetry.

(a) Flow Micro Calorimetry.

Breslauer et al. (1973, 1974, 1978) have described a method of determining the enthalpy of transfer by flow micro-calorimetry. The main components of the instrument were a precision fluid delivery system and a thermopile; enclosed within a massive aluminium sink. The fluid delivery system consisted of two glass syringes equipped with gas tight Teflon tipped plungers which were independently driven by two variable speed motors. The two immiscible solvents, one containing solute, were delivered to the calorimeter system by passage through Teflon tubes. At the entrance to the heat sink, the Teflon tubing

was changed to platinum tubing, which ensured proper equilibration of liquids prior to their entering the thermopile. On reaching the thermopile, the two platinum tubes were brought together, by means of a Y junction, which served to initiate the mixing process. The mixed solvents were then pressed tightly against the inside surface of the thermopile. Any heat evolved or absorbed upon mixing the two solvents, was recorded as an electrical output, amplified and recorded. The extraction was accomplished by flowing a dilute alkaline (or acidic) aqueous solution with an organic solution of the compound to be transferred. The enthalpy change for the extraction process, not only includes the enthalpy of transfer, but also heat of ionisation, heat of protonation or deprotonation, heat of mixing and heat of water formation, and these must be eliminated by further experimentation. Thus the enthalpy of transfer is not determined 'directly' but a series of deductions. This leads to errors, especially if the enthalpy of transfer is low in any case. It is very important for the two phases and solute to reach equilibration, otherwise an error will be incurred in enthalpy value.

Riebesehl and Tomlinson (1981) have presented a similar method for determining the enthalpy of transfer. However they have improved the experimental set up by including a phase splitting device (Kinkel and Tomlinson, 1980) at the flow mix cell output to determine the extent of distribution.

(b) Batch Calorimetry.

The thermodynamics of extraction of rare metals from aqueous solutions has been dealt with in great detail by

Marcus and Kolarik (1973, 1975, 1976). In their studies they have used a calorimetric method for determining the enthalpy of transfer of metal salts (e.g. lithium halides and uranium nitrate) between water and organic solvents. The experimental set up is shown in Fig.12. One of the phases, containing the solute, was placed in the Dewar vessel and the other phase was placed in the bulb of the apparatus. The two phases were allowed to equilibrate, at a particular temperature. The two phases were allowed to mix and the temperature change was then measured by the Quartz thermometer. The enthalpy of transfer is proportional to the change in temperature. The heat change measured also contains the heats of dilution and interaction as well as heat of transfer, and these parameters have to be taken into account by further experimentation.

(c) Heats of Solution.

Tanford (1980) has shown that the enthalpy of transfer can be obtained from heats of solution :

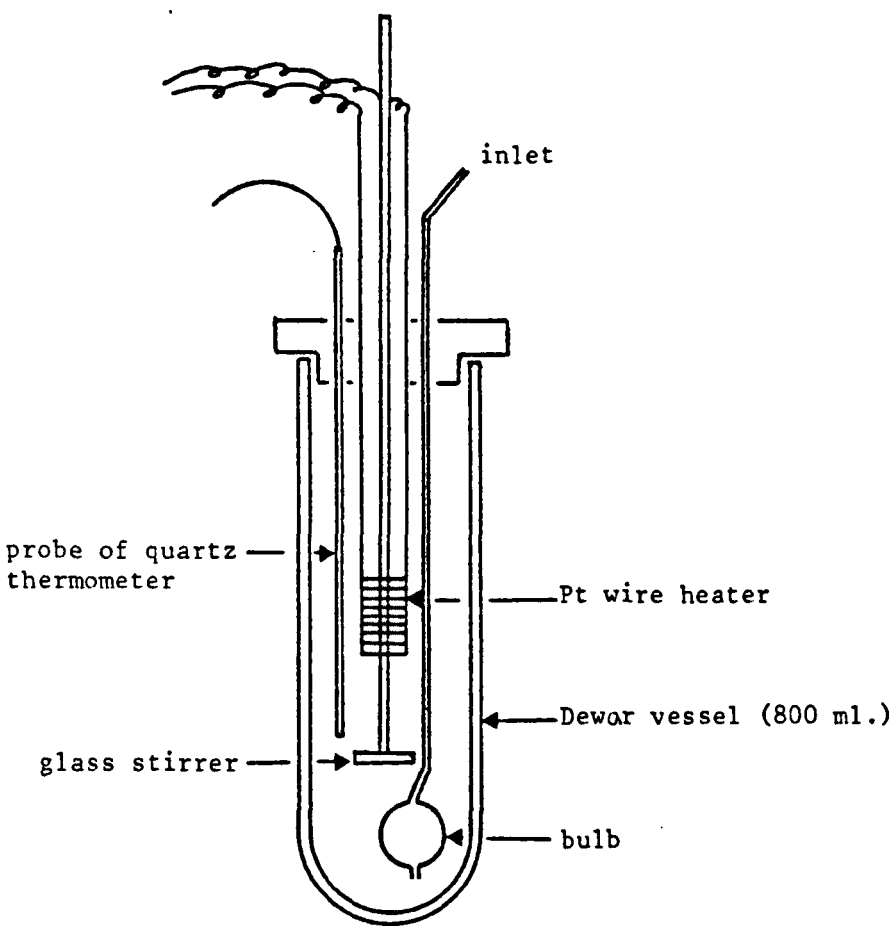
$$\Delta H_{tr} = \Delta H(o) - \Delta H(w)$$

where $\Delta H(o)$ is heat of solution of solute in organic phase,

$\Delta H(w)$ is heat of solution of solute in aqueous phase.

Using calorimetric data of Gill et al. (1976), he derived the enthalpy of transfer of hydrocarbons from organic solvents to water at 25°C. He also derived the enthalpy of transfer of aliphatic alcohols from the pure liquid to water at 25°C using the precise calorimetric data of Arnett et al. (1969). Aveyard and Mitchell (1969, 1970) performed similar calculations for aliphatic acids and alcohols partitioned between alkanes and water.

Fig.12. Calorimetry apparatus of Marcus and Kolarik (1973).



3.2.3. Entropy of Transfer.

The entropy term cannot be determined experimentally, but is obtained by substitution in the equation :

$$\Delta G_{tr} = \Delta H_{tr} - T\,\Delta S_{tr}$$

where the free energy and enthalpy of transfer are obtained experimentally.

CHAPTER 4

THE STUDY OF TRANSPORT OF SOLUTE MOLECULES ACROSS MODEL MEMBRANES.

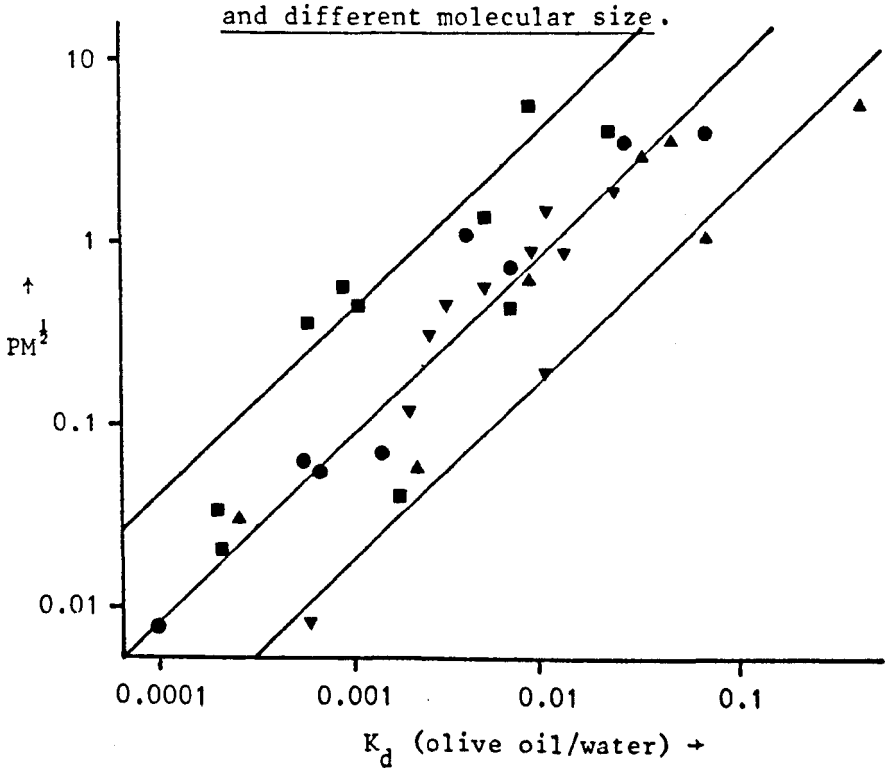
Various approaches have been employed to study the transport of solute molecules across membranes, and these will be discussed briefly in this section. Particular attention will be paid to those studies in which a thermodynamic approach has been adopted to explain the mechanisms involved in the transfer of a solute from an aqueous environment to an hydrophobic environment. The various model membrane systems which have been used to study solute transport can be subdivided into biological membranes and bulk organic solvents. It is assumed that the mechanisms involved in the transfer of a solute from an aqueous to an hydrophobic environment are essentially the same in bulk (isotropic) and membrane (anisotropic) phases (Higuchi et al., 1970; More and Schowsky, 1969).

4.1. The Transport of Solute Molecules Across Biological Membranes.

The permeability of biological membranes to non-electrolytes depends intimately upon molecular structure, so that a small change in the structure of a solute often causes a profound change in its permeating power. At the turn of the century, empirical relations between molecular structure and permeability were formulated in broad outline by Overton (1896, 1899, 1902). He investigated the permeability of a large number of solutes across the plasma membrane of plant

(Overton, 1896) and muscle cells (Overton, 1902). The rate of penetration was compared with hydrophobicity, measured as the distribution coefficient between oil and water, for each substance. He noticed that the more hydrophobic the substance, the faster was its permeability rate. This offered the first indication that lipids play an important role in the permeability characteristics of membranes, and formed the main selectivity pattern for non-electrolyte permeability. Ultimately this observation led Davson and Danielli (1943) to their well known lipid bilayer model for biological membranes. The studies of Overton were extended by other workers in the first half of this century. The work of Collander on the permeability of solutes across giant algal cells of the Characea family and on model membranes was of great significance. In particular the study by Collander and Barlund (1933) on the permeability coefficients for forty five non-electrolytes in the alga Chara ceratophylla, and by Collander (1954) on the permeability of seventy non-electrolytes in cells of the alga Nitella mucronata yielded reliable and accurate permeability data. Collander found a nearly linear relationship between the oil/water partition coefficient and the permeability of the solutes and this led to the conclusion that the permeability of a solute was proportional to its partition coefficient (see Fig.13). Other studies by Collander were of equal importance (Collander 1937, 1947, 1949a,b,c, 1950a,b, 1951, 1956, 1957, 1959a,b, 1960). Close examination of the results showed that there were exceptions to the main pattern of selectivity. Larger molecules permeated much more slowly than was expected on the basis of their lipid

Fig.13. The permeability of cells of C. ceratophylla to organic non-electrolytes of different oil solubility and different molecular size.



■ MR_D < 15

● MR_D 15-22

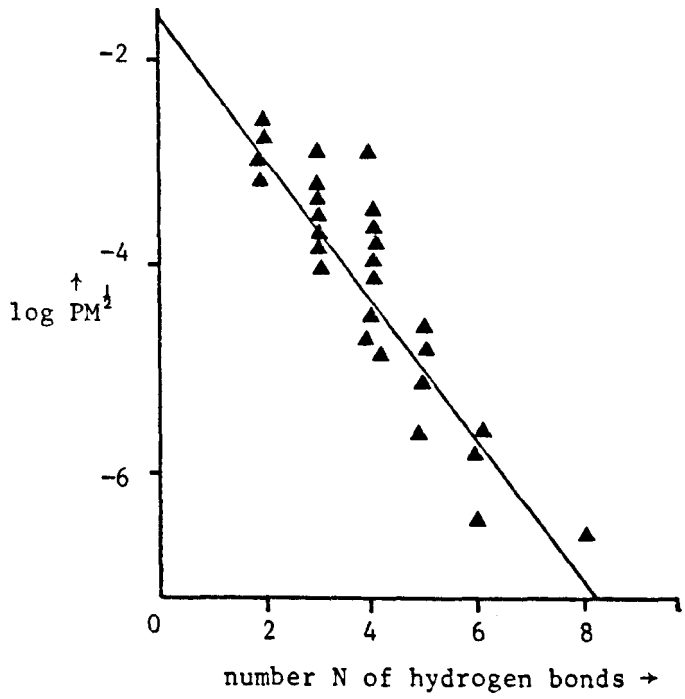
▼ MR_D 22-30

▲ MR_D > 30

solubility, indicating that another factor plays a role in permeation, namely the molecular volume (or size) of the solute molecules. It was found that very small polar molecules, penetrated cell membranes faster than would be expected from distribution coefficient values. In order to explain the rapid penetration of these molecules, e.g. urea, it was suggested that cell membranes, although lipoid in nature, were not continuous but were interrupted by small channels or pores through which polar, low molecular weight compounds could permeate quickly. Branched solutes permeated much more slowly than would be expected from their partition coefficients. This is due to the fact that a branched chain molecule would have a larger disruptive effect on the closely packed chains of the bilayer and encounter a greater steric hindrance than a straight chain molecule (Jain, 1972).

Another important step involved the investigation by Stein (1967) on the effect of hydrogen bonding on permeation. He postulated that hydrogen bonds between water and solute must be broken when a solute is transferred to the bilayer. Hydrogen bonds offer interfacial resistance and the membrane interface is the rate limiting step in permeation. Using data from Collander (1949a) he plotted the logarithm of $PM^{\frac{1}{2}}$ as a linear function of the number of hydrogen bonds to be broken (Fig.14), where P equals the permeability coefficient and $M^{\frac{1}{2}}$ is the square root of molecular weight. From the slope of the curve he calculated enthalpy changes of 17-21 kJ per mole per hydrogen bond. This value agreed with the value previously calculated in other ways for the breaking of single hydrogen

Fig.14. Relationship between permeability (log $PM^{\frac{1}{2}}$) and number of hydrogen bonding groups in solutes.



bonds, suggesting that penetration does indeed require previous breaking of hydrogen bonds.

The publication of work by Diamond and Wright (Wright and Diamond, 1969a,b; Diamond and Wright, 1969a,b) provided a further major contribution to the understanding of the molecular forces governing non-electrolyte permeation through cell membranes. These authors studied permeability by determining the reflection coefficient, σ, for 206 non-electrolytes across rabbit gall bladder epithelium, and found good agreement with the work of Collander. However they took the work one step further by looking at the effect of individual substituent groups on permeability and interpreting these in terms of solute/water and solute/lipid intermolecular interactions. A summary of their work is given in Table 7. They also adopted a thermodynamic approach by calculating the changes which substituent groups produced in the partial molar free energies and in some cases, enthalpies and entropies of partitioning and solution in water and lipids. The data were obtained from other sources and a summary of the incremental free energies of transfer from water to lipid is given in Table 8. The incremental free energy values for most substituents are positive, except for the methylene group; that is every substituent, except the methylene group, reduces the partition coefficient. These results can be interpreted by saying that all the functional groups, except methylene, increase solute-water interactions and effectively reduce solute-lipid interactions, thereby reducing partition coefficients. The methylene group contribution has the reverse effect. No further conclusions can be drawn, unless the free energy term

Table 7

Influence of Chemical Substitution on the Membrane Permeability of Several Series of Nonelectrolytes.

Substituent Group	Influence on membrane permeability
Alcoholic hydroxyl group (-OH)	(a) At any given chain length there is a decreased permeability as the number of -OH groups increases (b) Intramolecular H-bonds formed between -OH groups on adjacent carbons will result in greater permeability compared to the same compound with nonadjacent -OH groups due to decreased H-bond formation with water
Ether group (-O-)	Has less of an influence on decreasing permeability compared to an -OH group
Carbonyl group (a) Ketone (-C=O) (b) Aldehyde (-HC=O)	Has less of an influence in decreasing permeability compared to -OH group; difficulty in measuring permeability of these compounds per se as many are unstable in solution forming diols and enolic tautomers
Ester group O ‖ (-C-O-)	Has less of an influence in decreasing permeability compared to -OH group
Amide group O ‖ ($-C-NH_2$)	Causes a greater decrease in permeability than any of the above groups
Amino nitrogen ($-NH_2$, -NHR, $-NR_2$)	Causes a large decrease in permeation rate
Urea derivatives O ‖ $R-NH-C-NH_2$	These compounds have lower permeability than amides with the same number of carbons and are about as impermeable as the corresponding dihydroxyl alcohols
α-amino acids R-CHCOOH ‖ NH_2	These compounds have the lowest $K_{o/w}$ values of all organic molecules and are essentially impermeable due to large dipole-dipole interaction with water
Nitrile group	Decreases permeability, but less than hydroxyl group

contd...

Table 7 contd.

Substituent Group	Influence on membrane permeability
Sulphur Functions	
Sulphur replacement of oxygen	(a) Sulphur compounds have greater $K_{o/w}$ values and permeate membranes more readily than the corresponding oxygen compound; this is a result of poor H-bond formation between sulfur and water compared to the oxygen analog (b) Sulphoxides ($R_2S=O$) are less permeable than the corresponding ketone ($R_2C=O$) due to stronger H-bond formation with water
Halogens Nitro groups	Increase permeability
Double and triple bonds	Effect of unsaturation is variable - causes decrease, no change or increase in permeability
Aromatic compounds	Reduces permeability
Methylene or alkyl groups	Increasing hydrocarbon chain length increases permeability
Chain branching	Reduces permeability

Table 8

Incremental Free Energies ($\delta\Delta G$, in cal./mole.) for Transfer from Water (Diamond and Wright, 1969b).

Substituent	Iso-butanol	Ether	Olive Oil	Nitella
-OH	1,000	2,100	2,800	3,600
-O-	600	1,400	1,400	800
-C- " O	-	2,100	2,200	2,500
-C-OH " O	1,100	1,700	2,800	-
-C-OR " O	1,200	1,200	1,400	1,400
-C-NH_2 " O	1,700	4,900	4,800	6,200
-NH-C-NH_2 " O	1,900	5,500	5,300	7,300
-C≡N	-	1,100	-	-
-NH_2	1,100	3,500	-	-
-CH_2-	-530	-670	-660	-610

can be resolved into its constitutive, enthalpy and entropy contributions. The authors concluded that the quantitative differences between the different membrane systems originate with interactions between solute and membrane lipids, since the intermolecular forces in the aqueous phase are identical for these systems. Also they assumed that the interior of the Nitella cell membrane was much closer to the pure hydrocarbon than olive oil or oleyl alcohol, since the hydroxyl group reduced the permeability of Nitella by a larger factor than for partition coefficients of oleyl alcohol.

Ever since the classical studies of Meyer (1899) and Overton (1899) it has been known that the potency of a general anaesthetic is directly proportional to its oil/water partition coefficient. Seeman and colleagues used the erythrocyte membrane as a quantitative model for studying the effects of anaesthetics on membranes. Seeman (1969) investigated the temperature dependence of erythrocyte membrane expansion by alcohol. He found that an increase in temperature enhanced the amount of drug induced expansion of the ghost membrane, and also increased the ghost membrane/water partition coefficient for benzyl alcohol. Machleidt et al. (1972a) studied the hydrophobic expansion of erythrocyte membranes by nineteen phenol anaesthetics. They found a linear correlation between the anti-haemolytic effect (AH 50%) of the phenols and the octanol/water partition coefficient, indicating an hydrophobic interaction between the membrane and phenol anesthetic. For those phenols studied, there was also a linear correlation between the membrane/buffer partition coefficient and octanol/water partition

coefficient, and between the membrane/buffer partition coefficient and anti-haemolytic effect. Seeman et al. (1971) investigating the partitioning of a series of alcohols in the erythrocyte/water system found the incremental free energy of transfer of the methylene and hydroxyl group to be -743 ± 14 and 3000 ± 57 calories per mole. respectively. Roth and Seeman (1972) investigated the membrane/buffer partition coefficients of forty-fouranaesthetics (neutral, positive and negative) in erythrocyte, synaptosome (nerve) and sarcoplasmic reticulum (muscle) membranes. Good correlation was found between the membrane/buffer partition coefficient and the octanol/water partition coefficient. For neutral molecules the membrane/buffer partition coefficient was found to be one fifth of the octanol/water partition coefficient, indicating the membrane is much less hydrophobic then octanol. The membrane/buffer partition coefficients in nerve and muscle membranes were the same as those for the erythrocyte membrane.

A further notable contribution to this field was provided by Diamond and Katz, who investigated the use of dimyristoyl phosphatidyl choline (DMPC) liposomes as model membranes for the partitioning of solute molecules, and presented their findings in a series of papers (Katz and Diamond, 1974a,b,c; Diamond and Katz, 1974). The temperature dependence of partitioning of several solutes between water and DMPC liposomes was studied, and from these data the partial molar free energy, enthalpy and entropy of partitioning for each solute were obtained. The incremental thermodynamic functions for various substituent groups were determined and these are summarised in Table 9. The contribution of individual substituent groups to partitioning was discussed,

Table 9

Incremental thermodynamic functions for DMPC liposome/water system.

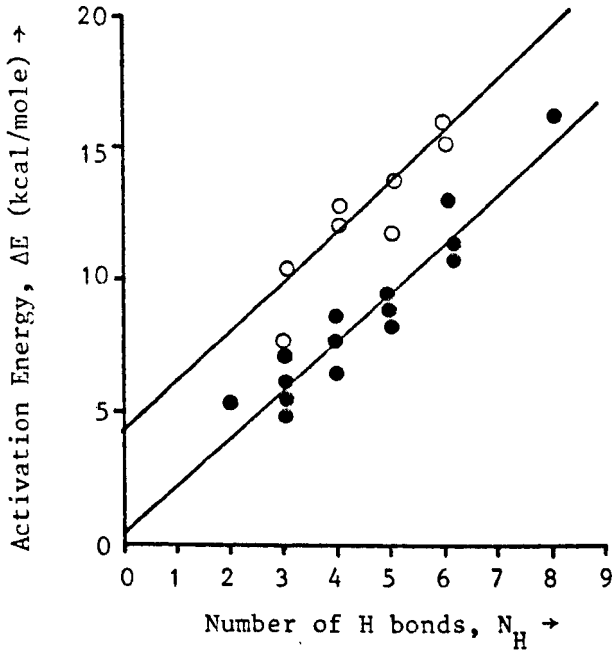
Group	$\delta\Delta G_{w\to\ell}$ cal./mole.	$\delta\Delta H_{w\to\ell}$ cal./mole.	$\delta\Delta S_{w\to\ell}$ cal./mole./K
$-CH_2-$	-450 to -650	-1,260 to -1,630	-2.06 to -3.95
-OH	790 to 970	1,290 to 2,950	1.67 to 6.66
=O vs. -OH	-240	-	-
$-O-\overset{\vert}{C}=O$ vs. -OH	130	-	-
$-\overset{\vert}{C}-$	370	1,660	4.33
$-\overset{\vert}{\underset{\vert}{C}}-$	630	-	-

and interpreted in terms of hydrogen bonding and hydrophobic interactions. The overall conclusion they made was that the effects of substituent groups on partitioning in DMPC liposomes were qualitatively similar to those summarised by Overton's rules for permeation in biological membranes and partitioning in bulk organic solvent systems. The average solvent properties of lecithin were similar to those of lower alcohols, particularly iso-butanol.

Wright and Pietras (1974) studied the permeation of sixteen non-electrolytes across three epithelial membranes; toad urinary bladder, frog choroid plexus and rabbit gall bladder. They found that the patterns of non-electrolyte permeation were similar for all three membranes, and similar to those in other biological membranes. The permeability of the solutes was found to be proportional to bulk phase oil/buffer partition coefficients.

Cohen (1975a,b) investigated the effect of temperature on the permeability of non-electrolytes across liposomal membranes, above and below their transition temperature, using an osmotic method. The activation energy of permeation, obtained from an Arrhenius plot of permeability versus the reciprocal of temperature, was found to be linearly correlated to the capacity of the solutes to form hydrogen bonds in water (Fig.15). This indicates that dehydration of the solute plays an important role in the permeation process. This correlation is only true for the permeation across a continuous bilayer, and is not valid where a bilayer contains an alternative route of permeation, such as an aqueous pore. The magnitude of the activation energies, on the other hand, have been found to be related to the

Fig.15. Relationship between activation energies for permeation and capacity of solute to form hydrogen bonds in water.



○ egg lecithin/PA (96:4) liposomes

● egg lecithin/cholesterol/PA (66:30:4) liposomes

physical state of hydrocarbon chains in the bilayer, since energy is required to form a 'hole' in the bilayer for solute diffusion. The permeability of toad urinary bladder to twenty two non-electrolytes was studied by Wright and Bindslev (Bindslev and Wright, 1976a; Wright and Bindslev, 1976b) using radioactive tracers. The permeability coefficients (P's) were found to be proportional to the olive oil/water partition coefficients for the majority of the molecules ($P \alpha K_d^{1.3}$).

The permeability coefficients (P's) and activation energies (E_a's) were analysed in terms of the thermodynamics of partitioning between membrane lipids and water. Particular attention was paid to the contributions made by $-CH_2-$ and -OH groups: on average the addition of one $-CH_2-$ group to a molecule increased the partition coefficient four fold, while the addition of one -OH group reduces the partition coefficient five hundred fold. The incremental free energies ($\delta\Delta G$), enthalpies ($\delta\Delta H$) and entropies ($\delta\Delta S$) for partition, hydration and solution in membrane lipids were determined. The results for toad bladder were compared and contrasted with those extracted from literature for red blood cells, lecithin liposomes and bulk phase lipid solvents (Table 10). The partition of methylene groups into toad bladder and red cell membranes were dominated by entropy effects. This process resembles that in "frozen" liposome membranes. In "melted" liposomes and bulk lipid solvents, the free energy of solution in the lipid is controlled by the enthalpy of solution. Partition of hydroxyl groups in all systems was governed by hydrogen bonding between the hydroxyl group and water. According to Wright and Bindslev (1974b), overall the

Table 10

Incremental thermodynamic functions for the transfer from water to lipid of :

$-CH_2$ group

	$\delta\Delta G_{w\to\ell}$ cal./mole.	$\delta\Delta H_{w\to\ell}$ cal./mole.	$\delta\Delta S_{w\to\ell}$ cal./mole./K
(1) * toad bladder	-780	2,460	11.0
(2) * red blood cell	-630	9,500	34.0
(3) ** liposomes above T_c	-550	-1,450	-3.0
(4) ** liposomes below T_c	-550	1,630	4.0
(5) ** benzene	-780	540	4.3
(1) ** olive oil	-650	2,020	9.0
OH group			
(1) * toad bladder	3,780	-8,650	-42.3
(3) ** liposome above T_c	970	2,950	6.7
(6) * liposome above T_c	940	3,600	9.0
(5) ** benzene	4,500	4,100	-4.8
(1) ** olive oil	2,520	-1,600	-14.0

* permeability studies, ** partition studies

(1) Bindslev and Wright (1976)

(2) Galey et al., (1973)

(3) Diamond and Katz (1974)

(4) Katz and Diamond (1974c)

(5) Butler and Harrower (1937)

(6) Cohen (1975a)

partitioning of solutes into the plasma membrane resembles partitioning into "frozen" liposomes, where the enthalpy of partition is high (up to 27 kcal./mole) and the entropy of solution is positive (up to 90 cal./mole/k). Rogers and Davis (1980) investigated functional group contributions to the partitioning of phenols between DMPC liposomes and water. They studied the temperature dependence of partitioning of alkyl and halo phenols, and obtained the partial molar free energies, enthalpies and entropies of partition into DMPC liposomes, above and below phase transition. The incremental free energy change and π value for each substituent group were also calculated. The results were interpreted in terms of solute-water and solute-membrane interactions.

More recently, Arrowsmith (1981) investigated the thermodynamics of partitioning of a series of 21-carbon steroids between water and dipalmitoyl phosphatidyl choline (DPPC) liposomes, and Ahmed (1981) studied the temperature dependence of partitioning of phenothiazine derivatives between DMPC liposomes and water. Both authors investigated methylene and hydroxyl group contributions to partitioning.

Several workers have used lipoproteins and detergent micelles to study solute transport. Stone (1975) found that the thermodynamics of alkane transfer to lipoproteins, resembled that found for detergent micelles or liposomes, rather than for non-polar solvents. In a similar study by Simon et al. (1977), the thermodynamic transfer parameters of hexane from aqueous to liposomes, bulk hydrocarbons and micelles were compared. The results showed that a bulk hydrocarbon was

not a good model for the interior of a bilayer, whereas micelles had approximately the same thermodynamic transfer parameters as egg lecithin liposomes.

The studies mentioned so far in this section, refer to the major contribution to the field of solute transport. However, other biological membranes have been used to study group contributions, mainly methylene and hydroxyl, to solute transport. These are summarised in Table 11.

Liposomes as Model Membranes for Drug Transport.

Having reviewed the various approaches to solute transport across membranes, it would seem reasonable to assume that multi-lamellar, dimyristoyl phosphatidyl choline liposomes would provide a suitable model system for investigation into drug transfer across membranes. Therefore in this section a brief resumé of liposome preparation, and characteristics will be discussed. More comprehensive details can be obtained from the literature (Bangham, 1968, 1972, 1974, 1980, 1981; Chapman, 1967; Popahadjopoulos, 1974, 1978; Tyrrell et al., 1976; Ryman and Tyrrell, 1979; Pogano and Weinstein, 1978; Allison and Gregoriadis, 1980; Knight, 1981; Szoka and Popahadjopoulos, 1980, 1981).

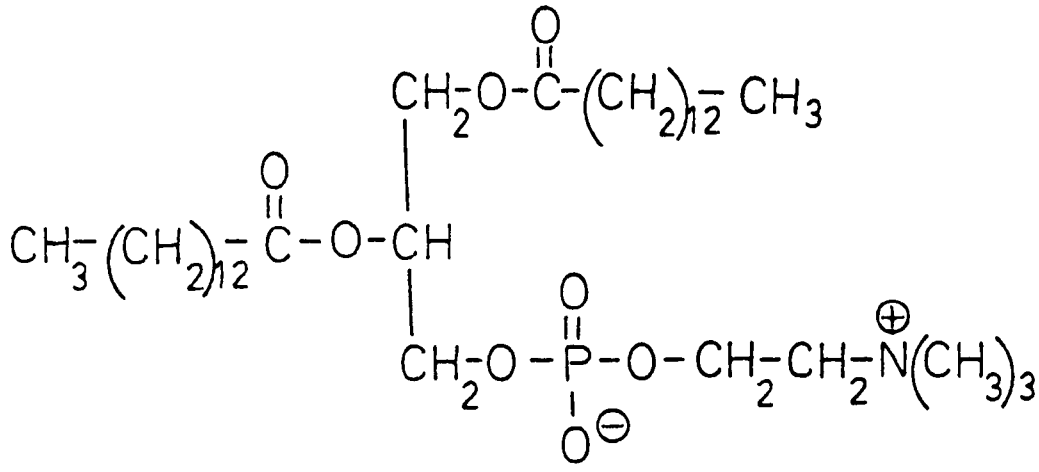
Phospholipid molecules, such as dimyristoyl phosphatidyl choline (DMPC) (Fig.16), are amphiphilic in nature, which means that they have a discrete hydrophobic region, the hydrocarbon chains, and a discrete hydrophilic region, the polar head groups. Thus each molecule behaves as though it were oil soluble at one end and water soluble at the other. These compounds when

Table 11

Incremental free energy values for the $-CH_2$ and -OH groups in various cell membranes.

Membrane	$\delta\Delta G_{w\rightarrow\ell}^{-CH_2}$ (cal./mole.)	$\delta\Delta G_{w\rightarrow\ell}^{-OH}$ (cal./mole.)	Reference
Rabbit gall bladder	-143	+422	Smulders and Wright, 1971
Rabbit jejunum	-258	+564	Westergaard and Dietschy, 1974
Rat jejunum	-356	-	Sallee and Dietschy, 1973
Rat adipocyte	-547	+1,225	Sherrill and Dietschy, 1975
Human erythrocyte ghost membranes	-686	+3,000±57	Seeman, Roth and Schneider, 1971
Rabbit sarcoplasmic reticulum vesicles	-796	-	Roth and Seeman, 1972 Koudo and Kasai, 1973
Adipocyte triglyceride	-830	+2,070	Sherrill and Dietschy, 1975
DPPC liposomes	-745±52	+2,810±196	Jain and Wray, 1978
Egg lecithin liposomes	-630±26	+1,510±62	Jain and Wray, 1978
Intestinal brush border	-820	-	Sallee, 1978.

Fig.16. Dimyristoyl phosphatidyl choline (DMPC).

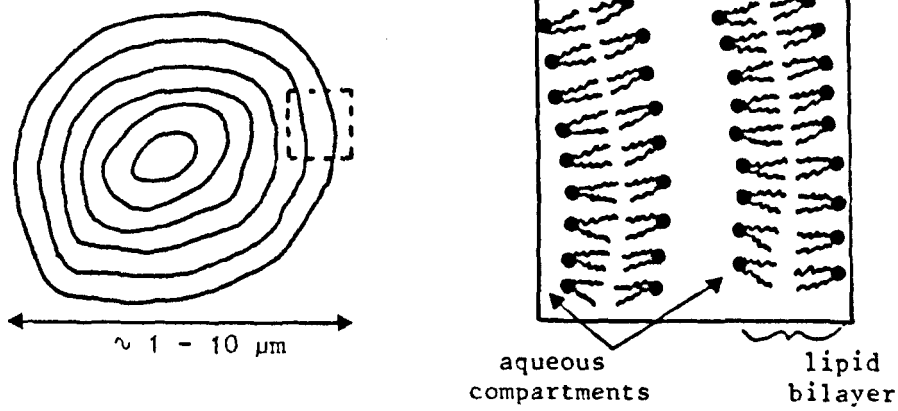


confronted with an aqueous environment, undergo a spontaneous sequence of molecular rearrangements, yielding a series of concentric, closed membranes, each membrane representing an unbroken bimolecular sheet of molecules intercalated by aqueous spaces (Fig.17.a). These systems are called liposomes or more correctly smectic mesophases (Bangham, 1980). These structures are formed due to the entropically unfavourable interaction of water molecules with the acyl chains of phospholipids, coupled with aqueous interactions of their polar head groups. According to Bangham (1981) liposomes provide a good system for measuring partition coefficients of various compounds, despite their highly specialised structure. The "melting" of a gram molecular weight of dipalmitoyl lecithin, as liposomes, has a latent heat of 8.66 kcals. at 41°C (Chapman et al., 1967). From bulk thermodynamics it would be calculated that the colligative property of an ideal solute dissolving in the membrane would cause a one degree depression in the melting temperature at a concentration of 0.0442 mole per mole of phospholipid. Liposomes give such a result (Hill, 1974).

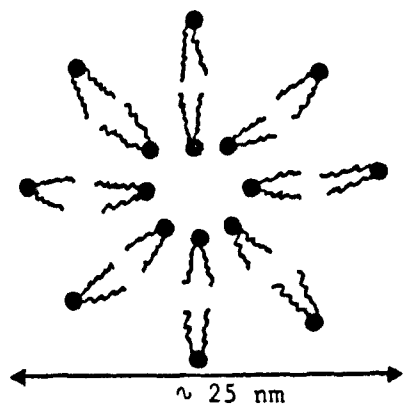
Liposomes can be classified into basic types : multilamellar vesicles (MLV) and unilamellar vesicles. MLV liposomes are comprised of a large number of bilayers, which vary in size from 0.1 μm to 5 μm (Fig.17.a) and are oblate or prolate cylinders and/or spheroids. Unilamellar vesicles consist of one bilayer only, and can be sub-divided into small unilamellar vesicles (SUV) (Fig.17.b), which are less than 100 nm in diameter, and large unilamellar vesicles (LUV) which have larger diameters. Unilamellar vesicles are usually spherical with a

Fig.17.

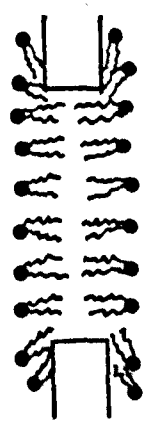
(a) Multilamellar liposome MLV.



(b) Small unilamellar liposome SUV.



(c) Bilayer lipid membrane (BLM).



minimum surface area to volume ratio. One of the attractions of using the multilamellar liposome models is that they are exceedingly easy to prepare, requiring only the minimal amount of laboratory equipment. The phospholipids are deposited from organic solvents in a thin film on the wall of a round bottom flask, by rotatory evaporation under reduced pressure. Then isotonic aqueous buffer is added and the lipids are hydrated at a temperature above the phase transition temperature, T_c, of the liposome (The phase transition temperature is the temperature at which the liposome changes from the gel to liquid state). A thin film of lipid is desirable to facilitate the efficient hydration of the bilayer. The smectic mesophase of the liposomes has formed when the lipid film whitens and disperses into excess aqueous phase. Attention to the hydration time and conditions of agitation are important for obtaining a reproducible size range of multilamellar vesicles. The hydration time, method of resuspension of lipids and thickness of lipid film can result in markedly different preparations of MLV, in spite of an identical lipid concentration and composition, and volume of suspending aqueous phase (Szoka and Popahadjopoulos, 1981). The radius of curvature of MLV liposomes is more similar to living cells than unilamellar veicles (Foster and Yguerabide, 1979). Multilamellar vesicles provide ample material on a small volume, necessary for a thermodynamic study (Wilkinson and Nagle, 1981). Multilamellar vesicles as models for study have been criticised for a number of reasons (Johnson and Bangham, 1969), sometimes, but not always with justification. Undeniably, the most valid criticism is that it is not easy to prepare a

suspension of liposomes having a uniform size population, because of magnitude of size range. Recent work of Chowhan et al. (1972) however seems to indicate that the heterogeneity of these preparations is not of great importance. One disadvantage of MLV liposomes is that experimentally they only allow access to one side, such that transmembrane phenomena cannot be studied. Also solute transfer may take place across a number of bilayers. Small unilamellar vesicles are normally prepared by sonication (both sonicator or sonic probe) of MLV liposomes, under an inert atmosphere and cooled to prevent degradation of lipid. Small unilamellar vesicles consist of a bilayer of phospholipid surrounding an aqueous space. They have seven fold encapsulation capacity, in terms of litres of aqueous space per mole lipid, as compared with multilamellar vesicles, which is important when hydrophilic solutes are to be incorporated. The unilamellar liposome is in a strained meta-stable state and is not as stable as the MLV liposome. Experimentally, small unilamellar liposomes are difficult to separate from their aqueous suspending phase by ultracentrifugation because of their low molecular weight, which makes them unsuitable for partitioning studies. Large unilamellar liposomes have not been well characterised up to date, and their preparation is somewhat difficult (Szoka and Popahadjopoulos, 1981). Despite the criticism of multilamellar liposomes, it is quite evident from the literature, that they have been used quite successfully as simple model membranes to study solute partitioning and solute permeability. Another bilayer preparation, which has the advantage of separating the surrounding water into two compartments is the black lipid membrane (BLM) (Fig.17.c). The

methods of preparation and properties of black lipid membranes have been described in great detail by Bangham (1968), Tien (1968, 1974), Jain (1972) and Tredgold (1977). Although black lipid membranes are invaluable for many transport studies, e.g. movement of ions across membranes, they are unsatisfactory for thermodynamic purposes for several reasons (Wilkinson and Nagle, 1981). Firstly, they offer a very small area of membrane surface and there is never enough material to do bulk thermodynamic measurements. Secondly, since solvents, e.g. decane, are required for the formation of these membranes, these may be present and the black lipid membrane cannot be considered as a pure phospholipid bilayer. Finally, the black lipid membrane is unstable at higher temperatures, and at lower temperatures, the components of the film may crystallise out.

4.2. The Thermodynamics of Transfer of Solute Molecules between Aqueous Solution and Bulk Organic Solvents.

Liquid-liquid distribution coefficients have been studied since the end of the last century, and are well documented in the literature (Leo et al., 1971; Hansch and Leo, 1979; Hansch Pomona College Data Bank). Since the introduction of the free energy related approach to structure activity relationships by Hansch and colleagues (1962, 1963) much data has been published on the free energy of transfer. However there are few data in the literature from which one can derive the enthalpy and entropy of transfer, as well as the free energy term, for the transfer of solute molecules and functional groups from aqueous phase to organic phase.

A major impetus to this work came from the studies of Davis (Davis et al., 1972; Davis, 1973a,b,c) who investigated the thermodynamics of drug molecules in solution, using data derived from other sources. He derived the free energy of transfer for methylene, methyl, halogen, hydroxyl and carboxyl groups between aqueous and a large number of organic solvents. This led on to a more rigorous treatment of the subject (Davis et al., 1974) in which the importance of deriving the complete thermodynamics of transfer, i.e. free energy, enthalpy and entropy terms was stressed.

A comprehensive group contribution study was presented by Harris (1971) who investigated the temperature dependence of partitioning of alkyl sulphate ion pairs between water and chloroform/carbon tetrachloride. The values she obtained for the thermodynamic values for various functional groups is given in Table 12. The effect of carbon number on free energy enthalpy and entropy of transfer for the temperature range 20-30°C is shown in Fig.18. At the same time Crugman (1971) investigated the thermodynamics of transfer from water to cyclohexane of p-n-alkyl phenols and p-hydroxy benzoic acid esters. The free energy change per methylene group in the p-n-alkyl phenols increased as the chain length increased. However neither the enthalpy team nor the entropy term was additive in terms of group contributions. Plots of free energy, enthalpy and entropy terms against carbon number for the p-hydroxy benzoic acid esters show that all three functions were linear and therefore additive (Fig.19). The mean values for the methylene contributions were $\Delta(\Delta G)CH_2 = -877$ cal.mole^{-1}, $\Delta(\Delta H)CH_2 = -345$ cal.mole^{-1} and

Table 12

Incremental Thermodynamic Functions for Partitioning of Alkyl-Sulphate Ion-Pairs.

Functional Group	$\delta\Delta G_{w\to\ell}$ (cal.mole^{-1})	$\delta\Delta H_{w\to\ell}$ (cal.mole^{-1})	$\delta\Delta S_{w\to\ell}$ (cal.mole^{-1}K^{-1})
Neohexyl	-4831	-2860	6.53
Cyclohexyl (Et)	-4405	-3661	2.45
(Pr)	-4414	-3280	3.49
Neopentyl	-4036	-1976	6.79
Cyclopentyl	-3699	-3126	1.89
Sec-butyl	-3376	-2723	2.15
t-butyl	-3136	-2702	1.43
Phenyl (Bu)	-2872	-2274	1.97
(Pr)	-2828	-3436	-2.01
(Et)	-2976	-3748	-2.55
Phenoxy	-2897	-3602	-2.33
Isopropyl	-2507	-1777	2.41
Butoxy	-2053	-92	6.47
Ethylene	-947	-1891	-3.09
Methylene	-916	-378	1.77
Bromo	-807	-1789	-3.24
Chloro	-439	-1203	-2.52
4-Fluoro (arom)	-1.79	247	1.41
Fluoro (aliph)	-38	-449	-1.36
Methoxy (-O-)	303	-87	-1.50
MeO Pr-	1214	1139	-0.25
BuO t-	1541	3208	5.50
PhO Et	79	146	0.22

Fig.18. Plot of free energy, enthalpy and entropy of transfer versus carbon number for the transfer of alkyl sulphate ion-pairs between water and chloroform/carbon tetrachloride (Harris, 1971).

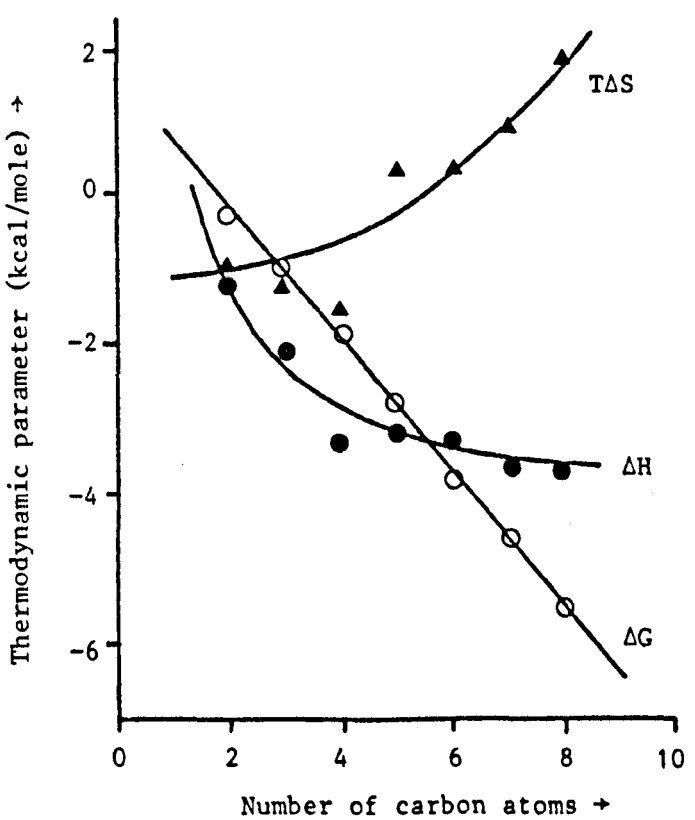
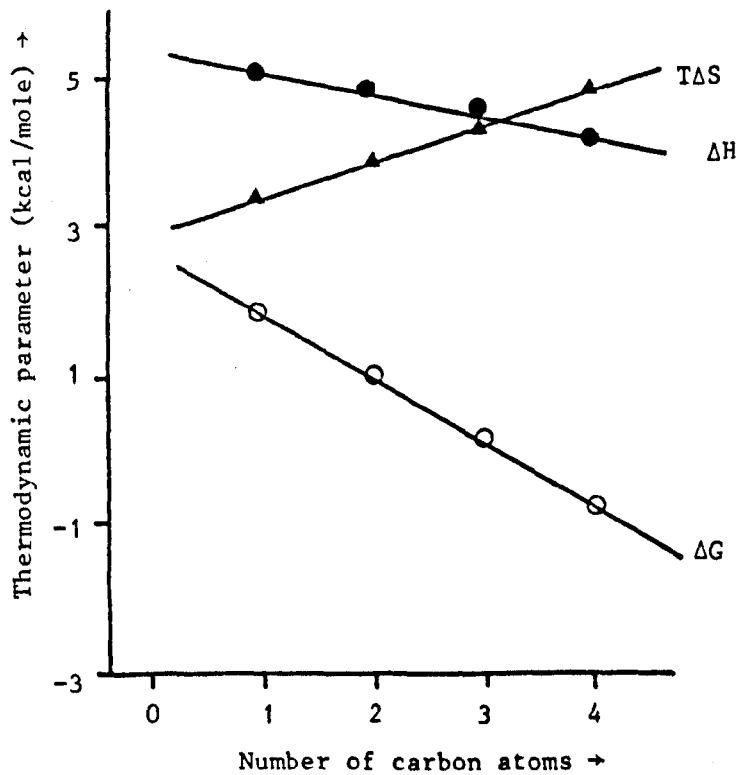


Fig.19. The change of free energy, enthalpy and entropy with carbon number for the transfer of p-hydroxy benzoic acid esters between water and cyclohexane (Crugman, 1971).



$\Delta(T\Delta S)CH_2$ = 532 cal.mole^{-1}deg^{-1}. An incremental free energy of transfer $\Delta(\Delta G)$-O- for an 'isolated' oxygen was found to be 2,729 cal.mole^{-1}.

The thermodynamics of partitioning of several solute molecules between aqueous and three bulk organic solvents, octane, toluene and octanol, was studied in depth by Breslauer (Breslauer, 1973; Breslauer et al., 1974, 1978). (The enthalpy of transfer was determined by calorimetry and the free energy term by a distribution method). Full thermodynamic parameters were derived for all the solute molecules, and for methylene and hydroxyl groups and amino acid residues. The results were interpreted in terms of solute-solvent interactions.

Korenman (1974, 1980) has investigated the influence of temperature on the distribution of phenols between water and organic solvents, and has derived full thermodynamic parameters for the transfer process. However he has not interpreted these results in terms of solute-solvent interactions.

The transfer of anilines and phenols between water and cyclohexane has been studied (Tack and Huyskens, 1974; Huyskens and Tack, 1975) by investigating the effects of methyl and chloro group contributions to the free energy and enthalpy of transfer.

A rather unique approach to this work has been adopted by Brodin et al., (1976) who investigated the temperature dependence of transfer of Methamphetamine and Ephedrine between water and octanol and water and cyclohexane. Not only did they determine the thermodynamic parameters of transfer, but also the enthalpy and entropy of activation for the process. The results were

interpreted in terms of hydrogen bonding and hydrophobic interactions. Dearden (1976, 1981, 1982) used the thermodynamics of partitioning to study intra-molecular hydrogen bonding and steric effects, which are responsible for non-additivity of hydrophobic substituent constants. Lundberg (1979) studied the temperature dependence of partitioning of several steroid molecules between water and octanol. The thermodynamic parameters of transfer were derived and these were discussed in terms of molecular structure. They also went on to discuss such parameters in the elucidation of micellar solubilisation and physiological function.

Beezer et al. (1980) obtained the thermodynamic parameters of transfer for resorcinol and several resorcinol mono alkyl-ethers from water to n-octanol. The effect of side chain length on free energy, enthalpy and entropy of transfer can be seen in Fig.20. A more systemic study on the effects of substituent groups on the thermodynamics of partitioning has been done by Rogers and Wong (1980). They studied the temperature dependence of the n-octanol-water partition coefficients of a series of substituted phenols. The transfer process for each solute molecule was discussed in terms of hydrogen bonding and hydrophobic interactions. The incremental thermodynamic functions for individual groups are given in Table 13. A critical investigation of the thermodynamics of partitioning has been undertaken by Kinkel and colleagues (Kinkel et al., 1981). The temperature dependences of the distribution of substituted benzenes and methyl benzoates between aqueous buffer and 2,2,4-trimethyl pentane (iso-octane) were determined using the filter probe technique. They found a true linear enthalpy-

Fig.20. Relationship between the free energy, enthalpy and entropy of transfer and side-chain length of resorcinol monoethers from water to n-octanol.

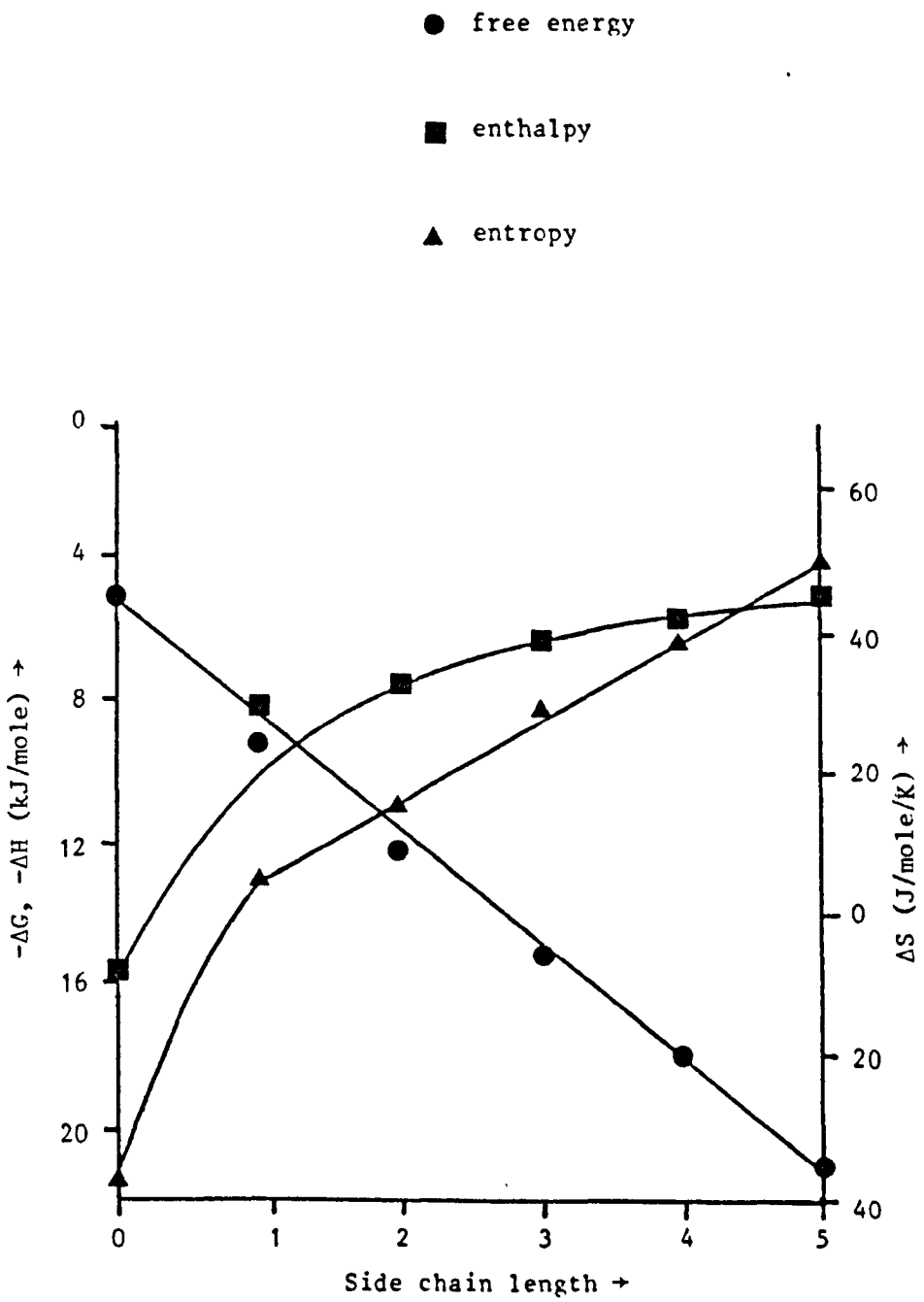


Table 13

Incremental Thermodynamic Functions for Partitioning of Phenols at 20°C.

Functional Group	$\delta\Delta G_{w\to\ell}$ ($kJ.mole^{-1}$)	$\delta\Delta H_{w\to\ell}$ ($kJ.mole^{-1}$)	$\delta\Delta S_{w\to\ell}$ ($kJ.mole^{-1}K^{-1}$)
o-methyl	-2.5	+2.8	+18.0
m-methyl	-2.8	+1.8	+15.6
p-methyl	-3.1	-0.4	+9.2
p-ethyl	-5.8	-2.8	+10.2
p-n-propyl	-9.3	-6.4	+10.1
p-n-butyl	-11.8	-2.9	+30.4
2,6-dimethyl	-4.8	+5.1	+33.8
2,4-dimethyl	-5.6	+5.2	+47.4
3,5-dimethyl	-5.7	-2.5	+10.7
p-fluoro	-2.0	-7.0	-17.1
p-chloro	-5.6	-8.6	-10.2
p-bromo	-5.8	-8.5	-9.0
p-iodo	-7.1	-7.1	+0.1
p-nitro	-3.0	-7.3	-14.5
p-methyl ester	-2.6	-13.5	-37.4
p-methoxy	0	+24.5	+84.6
p-ethoxy	-2.2	+18.9	+71.9

entropy compensation effect for distribution in aqueous/non-polar hydrocarbon systems, but not in aqueous/octanol systems. (Their own data were compared with that of the literature). Anderson et al. (1981) have investigated the thermodynamics of partitioning of substituted phenols between aqueous solution and liposomes, and compared this with the thermodynamics of partitioning in water/n-octanol and water/cyclohexane systems. They found that with respect to the partition coefficient, there was some correlation between n-octanol and liposomes, although the thermodynamics of the partitioning process was most similar in the cyclohexane and liposome systems.

Recently, Ueda et al. (1982) determined the thermodynamic parameters of transfer for procaine and lidocaine between aqueous solution and three organic solvents (heptane, benzene and octanol). The results showed that the transfer of these anaesthetics from aqueous to oil phase was an entropy-driven process, and they preferred a polar solvent over an apolar solvent.

Chapter Five

EXPERIMENTAL METHODS

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5.1. Materials and Reagents.

n-Octanol - (i) Spectrograde, special for determination of partition coefficients, Fison's Scientific Apparatus, Loughborough, Leicester, England.
(ii) Puriss grade, Koch Light Laboratories Ltd., Slough, Berkshire, U.K.

Cyclohexane - Analytical reagent, BDH Chemicals Ltd., Poole, Dorset, U.K.

Di-n-butyl ether - Analytical reagent, BDH Chemicals Ltd.

Chloroform - Puriss analytical reagent, BDH Chemicals Ltd.

Sodium chloride - Analar reagent, BDH Chemicals Ltd.

Methanol - Rathburns HPLC grade, James W. Turner Ltd., Liverpool, U.K.

Dimyristoyl-L-α-phosphatidyl choline - Synthetic, (approx. 98%) prod. no. P0888, Sigma Chemical Company, St. Louis, U.S.A.

Substituted phenols, anisoles and benzene compounds.

phenol - BDH Chemicals Ltd.

o-cresol (redistilled 2x) - Koch Light Laboratories Ltd.

m-cresol (redistilled 2x) - Fisons Scientific Apparatus.

p-cresol (recrystallised) - Koch Light Laboratories Ltd.

p-ethyl phenol (recrystallised) - Aldrich Chemical Company Inc., Gillingham, Dorset, England.

p-isopropyl phenol (recrystallised) - Aldrich Chemical Co. Inc.

p-n-butyl phenol (redistilled 2x) - Eastman Kodak Co., Rochester, New York, U.S.A.

p-t-butyl phenol - Aldrich Chemical Company Inc.

p-bromo phenol - BDH Chemicals Ltd.

p-chloro phenol - BDH Chemicals Ltd.

p-iodo phenol - Aldrich Chemical Company Inc.

p-fluoro phenol - Aldrich Chemical Company Inc.

p-methoxy phenol - BDH Chemicals Ltd.

p-nitro phenol (recrystallised) - Aldrich Chemical Co. Inc.

resorcinol - Eastman Kodak Co.

m-methoxy phenol (redistilled) - Eastman Kodak Co.

m-ethoxy phenol (redistilled) - Eastman Kodak Co.

p-amino phenol - Aldrich Chemical Co. Inc.

p-dimethyl amino phenol, hydrochloride - ABC Products, Alfred Bader Chemicals, now Aldrich Chemical Company Inc.

p-hydroxy phenol (hydroquinone) - Koch Light Laboratories Ltd.

p-cyano phenol - Research Chemicals, Ralph N. Emanual Ltd., now Aldrich Chemical Co. Inc.

p-hydroxy benzo-trifluoride - Fluorochem Ltd., Glossop, Derbyshire, England.

p-hydroxy methyl benzoate - BDH Chemicals Ltd.

p-hydroxy acetophenone - BDH Chemicals Ltd.

p-hydroxy acetanilide (paracetamol) - K and K Greeff Chemicals Ltd., Croydon, U.K.

p-hydroxy benzamide - Pfaltz and Bauer Inc., Connecticut, U.S.A.

p-hydroxy benzaldehyde, anisole - Aldrich Chemical Co. Inc.

p-methyl anisole - Aldrich Chemical Co. Inc.

p-propyl anisole - ABC Products

p-bromo anisole - Aldrich Chemical Co. Inc.

p-chloro anisole - Aldrich Chemical Co. Inc.

p-fluoro anisole - Aldrich Chemical Co. Inc.

p-iodo anisole - Aldrich Chemical Co. Inc.

p-nitro anisole (recrystallised) - BDH Chemicals Ltd.

benzoic acid - Fisons Scientific Apparatus

acetophenone - Koch Light Laboratories Ltd.

formanilide - BDH Chemicals Ltd.

benzaldehyde - Aldrich Chemical Co. Inc.

5.2. Apparatus and Equipment.

1. Mini-AKUFVE ADMCS 10 trs - MEAB Metellextraction AB. Vestra Frolunda, Sweden.
2. Filter probes - obtained from Prof. E. Tomlinson, Laboratorium voor Farmacie der Universiteit van Amsterdam, Amsterdam, Nederland.
3. Digital thermometer, Model 2180A (RTD 100Ω) - Fluke International Corp., Watford, England.
4. Platinum resistance temperature probe (100Ω, 10.5 cm x 2.5 mm) - Jenway Ltd., Essex, England.
5. Spectrophotometers :
   (i) Cecil CE 292 Digital Ultraviolet Spectrophotometer - Cecil Instruments Ltd., Cambridge, England.
   (ii) Varian-Cary 210 Spectrophotometer - Varian Associates Ltd., Surrey, U.K.
   (iii) Beckman Model 25 Spectrophotometer - Beckman - RIIC Ltd., High Wycombe, Bucks, U.K.
6. Flow-through cells :
   (i) Cecil quartz 1 cm. path length,
   (ii) Varian quartz 1 cm. path length.
7. Gallenkamp Total Immersion Thermometers (0-40°C; 40-70°C) - Gallenkamp and Co. Ltd., Christopher St., London, U.K.
8. Peristaltic pumps :
   (i) Watson-Marlow H.R. Flow Inducer - Watson-Marlow Ltd., Falmouth, Cornwall, U.K.
   (ii) L.K.B. 2120 Varioperspex® II Pump - L.K.B. Instruments, South Croydon, U.K.

9. Silicone rubber and viton peristaltic pump tubing - Watson-Marlow Ltd.

10. Thermostated Centrifuge, M.S.E. High Speed 25 - M.S.E. Scientific Instruments, Crawley, England.

11. Centrifuge tubes (10 ml., polycarbonate) - M.S.E. Scientific Instruments.

12. Magnetic stirrers :

    (i) Baird and Tatlock Magnetic Stirrer - Baird and Tatlock (London) Ltd., Romford, Essex.

    (ii) Monotherm Heater-Stirrer - Rodwell Scientific Instruments Ltd., Brentwood, U.K.

13. Shaking water bath, Grant type US3, SS30 - Grant Instruments (Cambridge) Ltd., Cambridge, England.

14. Water baths; Grant type U3.

15. Water circulator and cooler :

    (i) Grant type LC10.

    (ii) Haake FK Water Circulator - Haake, Karlsruke 41, West Germany.

    (iii)Churchill Water Circulator - Jenning's Laboratory Apparatus and Instruments, Nottingham, England.

16. Differential scanning calorimeter, Perkin-Elmer DSC2 - Perkin-Elmer Limited, Beaconsfield, Berks, England.

17. Coulter Counter Model TA - Coulter Electronics Ltd., Luton, Bedfordshire.

18. Nano-Sizer TM - Coulter Electronics Ltd.

19. Particle Size Analyser comprised :

Malvern	Spectrometer RR102,
	Digital Correlator Model K7025
	Temperature Controller RR56
Liconix	Helium-Cadmium Laser, Model 4000 ps
Gould	15 mHz Oscilloscope OS 255
Commodore	Professional Computer, Floppy Disc and Tractor Printer.

20. Mitsubishi Moisture Meter, Model CA-02 - Mitsubishi, Tokyo, Japan.

21. Corning Model 130 pH Meter - Corning Medical and Scientific, Corning Limited, Halstead, Essex, England.

22. Corning Combination Glass/Reference pH Electrode - Corning Medical and Scientific.

23. Bath sonicator, Ultrasonic - L and R Manufacturing Co., New Jersey, U.S.A.

24. Probe sonicator, Dawe Soniprobe Type 7532B - Ultrasonics Ltd., London, U.K.

25. Rotary evaporator, Buchi Rotavapor R - Glasapparate Fabrik, Flowil, Switzerland.

26. Thermostatted vacuum oven - Townson and Mercer Ltd., Croydon, England.

27. Heating coil - Morton Radiators, Nottingham.

28. Electric fan - R.S. Components Ltd., London.

29. Quickfit glassware (conical flasks, round bottom flasks, Micro-distillation apparatus) - Corning Ltd., Stone, Staffordshire, England.

30. Whatman Quantitative Cellulose Filter Papers, Type 54 - Whatman Chemical Separation Ltd., Maidstone, Kent.

31. Teflon tubing - Jencons Scientific Ltd., Hemel Hempstead, Hertfordshire, England.

32. Three way tap, with PTFE bore - Youngs Scientific Glassware Ltd., Acton, London.

33. Heat exchange coil, Quickfit Graham coil condenser, cat. no. C3/13 - Corning Ltd., Laboratory Division, Staffordshire, England.

34. HPLC pump, Waters Associates Chromatography Pump, Model 6000A Solvent Delivery System - Water Associates (Instruments) Ltd., Northwich, Cheshire.

35. Liquid chromatograph UV detector - Pye Unicam Ltd., Cambridge, England.

36. HPLC injection valve - Altex Scientific Inc., California, U.S.A.

37. Chart recorder - Perkin-Elmer Limited, Beaconsfield, Bucks, U.K.

5.3. The Thermodynamics of Partitioning of Substituted Phenols between Aqueous Solution and Liposomes.

The approach adopted in this thesis to study the thermodynamics of partitioning of solutes between aqueous solution and liposome membranes, followed very closely to that adopted by Diamond and Katz (1974) in their studies. However there were a number of differences.

Substituted phenolic solutes, with a wide range of hydrophobic and hydrophilic groups and partition coefficient values, were to be studied. The analytical technique adopted to determine the aqueous solute concentration, was ultraviolet spectroscopy. According to Bindslev and Wright (1974) the partitioning of solutes into liposomes below the phase transition temperature, resembles more closely partitioning into biological membranes. Therefore it was reasoned that partitioning studies should be carried out below the phase transition temperature.

1. Preliminary Experiments.

It was necessary to carry out certain preliminary experiments to determine the most suitable experimental conditions for the study. Initially dimyristoyl phosphatidyl choline (DMPC) liposome suspensions were prepared according to the method of Rogers and Davis (1981).

(1) Microscopic Examination.

A liposome suspension was prepared (50 mg. lecithin dissolved in 5 ml 0.15 M NaCl) and a spot was smeared onto a clean microscope slide. A cover slip was then placed over the smear. The slide was then viewed under the

microscope (60x magnification). A heterogeneous suspension of rods, cylinders and spheres, of varying size, was observed.

The liposome suspension was then centrifuged at 25,000 rpm for 1 hour in a high speed centrifuge. The supernatant was removed and a sample taken from the residual 'pellet'. This was then smeared onto a microscope slide, covered with a cover slip and viewed under the microscope. The same structures as before were observed. Thus the centrifugation process did not apparently break down the liposome bilayer. This is supported by Reeves and Dowben (1970) who have concluded that the liposomal bilayer is mechanically stable and can withstand pipetting and centrifugation.

(2) The Equilibrium Time for the Partitioning Process.

A liposome suspension was prepared by adding a known volume of p-cresol solution (10 ml of 10 mg/100 ml .15 M NaCl) to a DMPC lecithin film (100 mg). The suspension was divided into two 5 ml samples. The samples were then centrifuged and the absorbance of the supernatant determined at 276 nm. The liposome pellets were resuspended and placed in shaking water bath. This process was repeated at 12 hourly intervals until the absorbance of the supernatant remained constant, i.e. when equilibrium of the solute in the liposome suspension had been achieved. The equilibrium time for the partitioning process was found to be 48 hrs. (The equilibrium of the solute in the unilamellar liposomes of the

suspension was achieved much quicker than in the multi-bilayer liposomes).

(3) Solute and Lecithin Concentrations.

Various solute and lecithin concentrations were examined to provide the most suitable combination to study a series of substituted phenolic compounds with a wide range of partition coefficient values. The most suitable solute concentration was found to be 2 mg solute per 100 ml of ·15 M sodium chloride solution. The most appropriate lecithin weight was found to be 20 mg. Thus the liposome suspension was formed from 20 mg lecithin dissolved in 5 ml of 2 mg solute/100 ml 0.15 M sodium chloride solution. This gave an overall reduction in the number of moles of solute per total number of moles of lecithin as compared with the liposome suspension of Rogers and Davis(1981). (Later experiments showed that the lowest solute concentration possible was recommended because it caused less perturbance of bilayer). This combination of solute and lecithin enabled a wide range of partition coefficient values to be studied.

(4) Determination of Solute Concentration in Supernatant.

Ultra-violet spectroscopy was chosen as analytical technique, not only because it was convenient but also because it allowed the detection of phenols down to concentrations of 10^{-5} mol dm^{-3}. The majority of the substituted phenols under study have λ_{max}'s at approx. 220 nm and 275 nm (see Table 14 for exact values). The

Table 14

Phenol (in .15M NaCl)	λ_{max} nm	M.W.
Phenol	215 271	94.11
o-cresol	215 268	108.13
m-cresol	215 271	103.13
p-cresol	220 278	108.13
p-ethyl phenol	220 276	122.16
p-propyl phenol	220 275	136.19
p-isopropyl phenol	220 276	136.19
p-butyl phenol	220 276	150.22
p-t-butyl phenol	221 275	150.22
p-bromo phenol	226 280	173.01
p-chloro phenol	226 280	128.56
p-fluoro phenol	209 278	112.11
p-iodo phenol	232 280	219.93
p-nitro phenol	220 318	139.11
p-methoxy phenol	222 290	124.14
p-cyano phenol	246	119.12
p-hydroxy benzo-tri-fluoride	225 278	162.11
resorcinol	215 275	110.11
m-methoxy phenol	215 275	124.14
m-ethoxy phenol	215 275	138.17
p-hydroxy acetophenone	220 272	136.15
p-hydroxy acetanilide	202 240	151.16
p-hydroxy benzoic acid, methyl ester	210 253	152.14
Anisole	215 270	108.14
p-methyl anisole	222 277	122.17
p-propyl anisole	222 276	150.22
p-nitro anisole	220 318	153.14
p-bromo anisole	226 280	187.04
p-chloro anisole	226 280	142.59
p-iodo anisole	226 280	234.04
p-fluoro anisole	210 280	126.13

extinction coefficient at 220 nm was found to be the largest in most cases. So it was decided to analyse most of the phenols at this λ_{max}. However, this required certain precautions when using the spectrophotometer (Cecil CE 292). The wavelength range for the instrument was 200-900 nm. However, using the instrument at lower wavelengths required a very efficient output from the deuterium lamp. In fact an HPLC deuterium lamp was used. The output from the lamp was checked regularly using electronic means. Also the instrument was calibrated regularly with a solution of known absorbance at a certain wavelength. (A dilute solution of phenol was used with known extinction coefficient at 215 nm).

Initially ultra-violet scans of the solutes under study were performed in the Beckmann recording spectrophotometer. Then the precise λ_{max}'s of the solutes were determined on the Cecil spectrophotometer. (Experience showed that wavelength controls on the instruments were only accurate to ± 1 nm, so that it was necessary to determine the λ_{max} of the solute concerned with the particular instrument being used). The calibration graphs for the solutes were then determined on the Cecil spectrophotometer.

(5) Purification of Solutes.

It was found necessary to purify several of the solutes obtained from commercial sources. This was done by distillation, using a semi-micro distillation

apparatus, for liquids and by recrystallisation for solids using appropriate solvents. The m.p.t. or b.p.t. of the purified solute was determined as a measure of purity. Those solutes for which it was necessary to purify are mentioned in the reagents section of this thesis.

(6) Effect of Repeated Shaking, Centrifugation, Resuspension and Pipetting on the Partition Coefficient.

A DMPC lecithin film (40 mg) was dissolved in a known volume of p-cresol solution (10 ml of 2 mg p-cresol/100 ml 0.15 M NaCl). Then volumes (5 ml) of this suspension were pipetted into 25 ml conical flasks, with glass stoppers, and shaken at 22°C for 48 hours. After this time the two liposome suspensions were centrifuged at 25,000 rpm for 1 hour and the solute concentration in the supernatant analysed. The partition coefficient of the solute at 22°C was then determined by mass balance. The liposome pellets were resuspended and the process was repeated twice more. The partition coefficient was found to remain constant (within experimental error) after repeated pipetting, shaking, centrifugation and resuspension.

(7) Degradation of Solute Solution and Lecithin with Time.

Since the overall time period for the experiment was 10 days, degradation of the solute in solution, with time could be a problem, and a major source of error. The degradation of the solute in solution could easily be determined by preparing solutions of known

absorbance (calibration solutions used), storing in the dark at room temperature and determining the absorbance at regular time intervals after preparation. For those solutes, which are quoted in Results sections, degradation was not a problem. However the following solutes, when in solution, were found to degrade, as determined by measuring the absorbance of the solutions at regular time intervals after preparation, p-hydroxy-benzaldehyde, p-hydroxy benzamide, hydroquinone, p-amino phenol, p-hydroxy dimethyl amino phenol and p-hydroxy benzoic acid. Anisole and methyl anisole, which were to have been studied, were found to be too volatile and excessive evaporation took place. (p-n-butyl and p-t-Butyl phenol, and p-iodo anisole had partition coefficients which were too high for the optimised conditions of the experiment.)

The dimyristoyl phosphatidyl choline was stored below 4°C in an inert atmosphere of nitrogen. DMPC lecithin is fully saturated and is unable to undergo oxidation.

2. Experimental Conditions.

Stock solutions of the phenols were prepared at a concentration of 2 mg of phenol per 100 ml of isotonic (0.15 M) sodium chloride solution. These solutions rendered a pH of between 5.5-6.0. Known weights of DMPC (40 mg) were dissolved in small volumes (10 ml.) of chloroform in glass stoppered, round bottomed flasks (50 ml.). The solvent was removed by rotary evaporation under pressure at 35°C for 1 hour. A thin uniform lipid film, was deposited on the inside walls of the

flask. Care was taken to ensure that all lipid films produced were of similar thickness and surface area. Any residual solvent was removed by overnight drying in a vacuum oven at 35°C. Then a known volume of solute solution (10 ml.) was added to the round bottom flask, and the lecithin hydrated by gentle hand-shaking in a water bath at 35°C. The temperature of hydration needed to be above the phase transition temperature for the lecithin in order for liposome formation to occur. A thin, uniform film was desirable to facilitate the efficient hydration of the bilayer. The smectic mesophase structure of the liposome was formed, when the lipid film became white and dispersed into the excess aqueous phase. Attention to the hydration time and method of agitation were important for obtaining a reproducible size range of multilamellar vesicles. Slight variation in hydration time, method of resuspension of lipids and thickness of lipid film could result in markedly different preparations of multi-lamellar liposomes, in spite of an identical lipid concentration and composition, and volume of suspending aqueous phase. Besides alterations in liposome size or the average number of lamellae per liposome, the intra-lamellar volume and hence the entrapped volume of the multi-lamellar liposome could also be increased.

The liposome suspension was divided into two aliquots (5 ml) and pipetted into glass stoppered, conical flasks (25 ml). These were duplicates for each phenolic compound to be studied. Three or four solutes were studied simultaneously, depending on the rotor head available. (The rotor heads

accommodated either six or eight centrifuge tubes). p-Cresol was used as a control throughout the study. The flasks were placed in a shaking water bath (± 0.2°C) at the desired temperature, and left to equilibrate, shaking, for 48 hours. After equilibration, the liposome suspensions were transferred to polycarbonate centrifuge tubes (10 ml) using Pasteur pipettes, and then placed inside the rotor head (6 x 10 ml or 8 x 10 ml), precooled or prewarmed to the desired temperature. These were centrifuged at the desired temperature at 25,000 r.p.m. for 1 hour. The centrifuge was thermostatically controlled to maintain temperature within ± 0.5°C. After completion of the centrifugation step, samples of the supernatants were carefully removed with a Pasteur pipette, and transferred directly to silica cells. Absorbances were measured using the Cecil spectrophotometer at the λ_{max} for each phenol. (See Table 14). (The true aqueous absorbance reading was obtained by subtracting a blank of 0.065 absorbance units). Following spectrophotometric analysis of the supernatants the solutions were returned to the same centrifuge tubes and the liposomal pellets were redispersed. This was accomplished by repeatedly withdrawing and expelling the dispersions, whilst warming to 35°C in a water bath. The contents of the centrifuge tubes were then returned to their original conical flasks (25 ml). The conical flasks were returned to the shaking water bath, and the contents equilibrated to the next desired temperature.

This procedure was carried out at the following temperatures : 5° 10° 14° 18° and 22°C.

The pH of each suspension was determined at the end of the experiment, and the overall pH range was between 4.5 - 5.5. This procedure was necessary to account for any ionisation of solute.

The aqueous solute concentration was obtained from previously prepared calibration graphs. The lipid solute concentration was obtained by mass balance. From these values, the partition coefficient of the phenol, at the particular temperature under study, could be determined.

Precautions.

A number of precautions were taken to reduce errors :

(a) A rapid transfer of suspension from flask to centrifuge tube to rotor head to prevent temperature drop or increase. Also a rapid transfer of supernatant from centrifuge tubes to quartz cells.

(b) Careful transfer of centrifuged suspensions in the rotor to prevent disturbance of the pellet.

(c) Careful pipetting of supernatant from centrifuge tubes to prevent disturbance of the pellet.

(d) Careful balancing of the rotor head.

(e) Careful monitoring of the temperature of shaking water bath (± 0.2°C).

(f) A precise and reproducible method for preparation of liposomes.

(g) Polycarbonate centrifuge tubes were used because the solution did not adsorb (as determined by experiment) to their surface. However, after continual use they developed small cracks in their base and had to be

replaced regularly.

(h) Adequate temperature equilibrium of the rotor head.

(i) Adequate shaking of the liposome suspensions to prevent floculation.

(j) When reconstituting the liposomal pellet, it was important to ensure that all the lipid and supernatant was removed from centrifuge tubes.

Errors.

(a) Weight losses due to a small portion of the liposomal suspension being left down the side of the flasks, in the Pasteur pipettes, in the centrifuge tubes and in the curvettes.

(b) Possible evaporation and/or partitioning of the solute into the air space above the suspension in the conical flask.

Since relatively low temperatures were used (5° - 22°C), this error was minimal.

Calculation of Partition Coefficients.

Molal concentrations of the solute in the lipid phase (C_o^m) and the aqueous phase (C_w^m) are given in the following equations;

$$C_o^m = \frac{(C_T - C_w)\ W_1}{d\ M_r\ W_2}$$

$$C_w^m = \frac{C_w}{d\ M_r}$$

where C_T = initial aqueous solute concentration after equilibration;

C_w = final aqueous solute concentration;

d = density of initial aqueous phase;

M_r = molecular weight of phenol derivative;

W_1 = weight of aqueous phase in the sample;

W_2 = weight of DMPC in the sample.

Thus, the molal partition coefficient, Kd is given by :

$$K_d = \frac{(C_T - C_w)\ W_1}{C_w\ W_2}$$

The enthalpy of transfer can be derived from the temperature dependence of the partition coefficient. The mole fraction scale cannot be used for biological membranes because it requires knowledge of the change in molar volume of the organic phase (liposome) with temperature.

3. The Effect of Solute Concentration on the Enthalpy of Transition and Phase Transition Temperature (Tm) as Determined by Differential Scanning Calorimetry (DSC).

Excellent reviews on fluidity and phase transitions in biological membranes have been written by Chapman (1975) and Melchior and Stein (1976). The principles of calorimetry and its application to biological systems have been described in great detail by Wadso (1970), Sturtevant(1971), Mabrey and Sturtevant (1978), Spink (1980) and Mabrey-Gaud (1981).

In this study attempts have been made to characterise the changes in bilayer organisation, caused by increasing the solute (p-cresol) concentration, by examining the phase transition profiles produced by differential scanning calorimetry. The differential scanning calorimeter consists basically of two cells of equal volume, one containing the sample and the other an inert reference material, which can be heated at a programmed rate. In order to maintain a

constant rate of temperature increase in both cells, the temperature difference between the sample and reference is monitored and additional power is applied via a feedback circuit to the cell, which is at a lower temperature. Data output is the heat capacity of the sample relative to an equal volume of solvent plotted as a function of temperature. If an endothermic process occurs in the system under study, then more heat is supplied to the system in the sample cell, to maintain a zero temperature difference between sample and reference cells. The enthalpy of phase transition can be determined from the area of the resulting DSC spectra.

Experimental Conditions.

The method adopted followed closely the study of Fildes and Oliver (1978).

(1) Preparation of Sample.

Known amounts of DMPC (20 mg) were weighed out into round bottomed Quick fit flasks (25 ml) and dissolved in chloroform (5 ml). Each solution was evaporated to dryness, on a rotary evaporator at 40°C, to form an even film on the flask wall. Final traces of solvent were removed at room temperature, under vacuum, overnight. Known volumes (3 ml) of p-cresol solution (0 mg/100 ml; 0.1 mg/100 ml; 0.5 mg/100 ml; 1 mg/100 ml; 2 mg/100 ml and 10 mg/100 ml, in 0.15 M NaCl) were added to each flask, which were then sealed and mechanically shaken, for periods of 30 seconds only. Suspensions were then placed in a shaking water bath at 35°C for 1 hr. The liposome suspensions were transferred to dry, tared polycarbonate centrifuge tubes, with the aid of two

washings (1 ml.) of solute solution. Then they were centrifuged for 1 hour at 25,000 r.p.m. The bulk of the supernatant was removed and the final weight of suspension was adjusted to 200 mg with saline. (This gave a 10% w/w phospholipid concentration). Solute loss was assessed by ultra-violet assay of the supernatant at 220 nm. The centrifuge tubes were sealed and spun briefly at 2,000 r.p.m. to collect all the material in the tip of the tube. The suspensions were left overnight to soften the pellet, then shaken on a mechanical shaker (2 x 30 sec). All samples were left at room temperature (22°C) for 3 days prior to DSC analysis.

(2) Differential Scanning Calorimetry Procedure.

(The differential scanning calorimeter used was the Perkin Elmer DSC-2. This is an instrument of moderate sensitivity which is ideal for the characterisation of the phase transition profiles of phospholipid membrane systems).

Initially, the DSC-2 was calibrated for both temperature and quantitative heat changes using a pure Indium standard (Perkin-Elmer). A freezing mixture of water, sodium chloride and solid carbon dioxide was used to cool the calorimeter head. The samples were kept in an inert atmosphere of nitrogen. Samples (approx. 6 mg) of liposome suspensions were weighed accurately, to 10^{-5} g tolerance and hermetically sealed in aluminium pans. The reference pan contained a similar weight of 0.15 M sodium chloride solution. DSC spectra of the samples were recorded on the DSC-2 over the temperature range 278 - 318 K. The scanning (heating) rate used was 5 deg./min., with a range setting of 1 mcal./sec.

Calculation.

The enthalpy of transition for the DMPC liposomes could then be calculated, using the following equation,

$$\Delta H_{tr} = \frac{4 \cdot 18 . K . A . R . MW}{W . S .}$$

where,

ΔH_{tr} = enthalpy of transition, J/mole

K = calibration constant for the instrument, = 27.53

A = area under transition peak, cm^2

R = range control setting, m.cal/sec.

M.W = mol. wt. DMPC = 695.9

W = weight of lecithin, mg.

S = chart speed, mm./min.

The constant 27.53 was predetermined using the Indium standard.

The area under the peak was calculated by cutting out and weighing the traces of the transition profile. The temperature of transition, Tm, could be determined from the DSC spectra. Small changes in the transition temperature, Tm could only be detected with sensitive instruments. It was desirable to obtain calorimetric data over a wide range above and below the transition region in order that the baseline extrapolation could be as accurate as possible. The accuracy and high sensitivity of differential scanning calorimetry depends primarily on the stability of the baseline as observed with solvent in both cells.

4. The Reproducibility of the Hand Shaken Preparation, and the Effect of Repeated Pipetting, Shaking, Centrifugation and Resuspension on the Particle Size Distribution.

Particle Size Analysis of DMPC Liposome Suspensions Using the Coulter Counter, Model TA.

The Coulter principle is based upon electrical conductivity difference between particles and an electrolyte dilu ent. Particles act as insulators, electrolytes as good conductors. The particles, suspended in an electrolyte, are forced through a small aperture through which an electrical current path has been established. As each particle displaces electrolyte in the aperture, a pulse proportional to the particle volume is produced. Thus a 3-dimensional particle volume response is the basis for all sizing, regardless of position or orientation of the particle in the aperture.

Experimental Conditions.

The particle size analysis was carried out as directed in the Coulter Counter Model TA handbook. A tube with a 50 μm orifice was used. Isoton (0.9% w/w sodium chloride solution) was used as the suspending electrolyte. The instrument was calibrated with 4.72 μm latex particles (Coulter). Several lecithin films (20 mg.) were prepared and dissolved in small volumes (10 ml.) of 0.15 M sodium chloride solution. A few drops of the freshly prepared suspension was pipetted into the analysis vessel, sufficient to give enough particles for a significant count. The volume distribution of the particles, in the differential and cumulative modes, was then

carried out. A recording was made. This procedure was carried out on several liposome suspensions.

The liposome suspensions were then subjected to repeated shaking (in water bath), centrifugation and resuspension (5x). Particle size analysis was then carried out on these suspensions, and recordings made.

Particle size analysis of the hand shaken DMPC liposome preparations was determined using the Malvern Photon Correlation Spectrometer. The principle of operation of this instrument is a measurement of the slight (a few kHz) Doppler shift in the frequency of the scattered light that is caused by Brownian motion of the scattering particle (see Coulter Nano-Sizer experimental for more details). The working range of the instrument is 30 to 3,000 nm. and it displays the diffusion coefficient and the average particle size.

Experimental conditions were the same as those used in Coulter Counter TA particle size analysis. The average particle size of the freshly prepared handshaken preparation was determined, and also the effect of repeated shaking, centrifugation, resuspension and pipetting on particle size was determined.

5. Characterisation of Liposome Suspensions Produced by Sonication and Filtration Methods Using the Coulter Nano-Sizer.

The Coulter Counter Nano-Sizer measures the average particle size of dilute suspensions and emulsions between 0.03 and 3.00 μm. The particle size, together with an indication of the size distribution width, the polydispersity factor, are presented typically in 2 - 4 minutes. The measuring

principles used are those of Brownian motion and autocorrelation spectroscopy of the resulting frequency change of scattered light. Only the refractive index and viscosity (or temperature) of the suspending liquid need be known. Only the continual time fluctuations of the scattered light are analysed. Operation of the Coulter Nano-Sizer is extremely simple. A dilute suspension of the particles, contained in a small glass test tube, is placed in the path of the low power laser beam. The light scattered at 90° is detected by a photo-multiplier. The scattering particles undergo Brownian motion caused by the random, non-uniform, striking of molecules of fluid upon each particle. The particles vibrate in an erratic fashion and the frequency of this vibration is inversely related to particle size : the smaller the particle, the faster it will move back and forth. Due to the random nature of the Brownian motion, the scattered light intensity is continuously fluctuating around its average value. The Coulter Nano-Sizer detects these rapid intensity variations and by auto-correlation analysis determines their characteristic variation correlation time which, in turn, is directly proportional to the average particle size (weight or volume). Since the intensity scattered by the whole particle population illuminated by the laser beam is measured, and not the scattering from individual particles, only the average particle size is measured. The width of the size distribution is given by the Polydispersity Index. A P.I. of 0 or 1 would indicate a mono-dispersed distribution, whereas a P.I. of 8 or 9 would indicate a wide distribution range.

(1) Calibration of the Coulter Nano-Sizer.

The Nano-Sizer was calibrated with latex particles of known particle size. The latex suspensions were diluted to an appropriate concentration, and their particle size were measured. These are given in the Results section. The actual particle size given by the instrument agreed to within 10% of the true particle size. (The average particle size of the hand-shaken preparation was greater than 3 μm, outside of the range of the instrument).

(2) Characterisation of Liposome Suspension Produced by the Sonic Probe.

A hand-shaken liposome suspension of 2 mg. DMPC lecithin/1 ml. of 0.15 M sodium chloride solution was prepared. Portions (5 ml.) of this suspension were placed in 25 ml. plastic vials. The solutions were then sonicated using the sonic probe for periods of between 10 seconds and 10 minutes. The temperature was controlled by an ice-water mixture, so as not to heat up the suspension. The energy input was 50 watts. The sonic probe was set at optimum conditions for the particular suspension. The particle size of each sample was determined on the Coulter Nano-Sizer.

(3) Characterisation of the Liposome Suspension Produced by Bath Sonication.

A hand shaken liposome suspension of 2 mg. DMPC lecithin/ 1 ml. of 0.15 M sodium chloride solution was prepared. Portions (5 ml) were then placed in 10 ml. graduated flasks. The contents of the flasks were then placed in the bath sonicator, and sonicated for various time intervals. Care

was taken for temperature of water in bath to rise above room temperature. The sonicated suspensions were then analysed for particle size, using the Coulter Nano-Sizer. Repeated centrifugation at high speeds of liposome suspensions, produced by the sonic probe, failed to produce a clear supernatant. A fine white suspension, most probably of small sized liposomes, was found floating on the surface of the supernatant. This made it impossible to assay the supernatent by ultra-violet spectroscopy.

(4) Preparation of Liposomes of Defined Size Distribution by Extrusion through Membranes.

The preparation of liposomes of defined size distribution was attempted using the method of Olson et al (1979).

A liposome suspension of 0.2 mg DMPC lecithin per 1 ml of 0.15 M sodium chloride solution was prepared. A sample (5 ml) was taken and filtered, sequentially, using the following Millipore cellulose nitrate filters of mean pore size : 1.2, 0.8, 0.6, 0.45, 0.3, 0.2, 0.1, 0.05, 0.025 and 0.01 μm. The liposome suspension (5 ml) was taken up into a glass syringe (10 ml) and forced through the filter, which was contained in a Millipore stainless-steel Swinnex filter holder. The filtrate was analysed in the Nano-sizer for particle size. Filtration was attempted above and below the phase transition temperature, 23°C. The procedure was repeated with several suspensions. The sequential filtration failed to reduce the average particle size below 3 μm.

However the same procedure was repeated using Nucleopore polycarbonate membranes of 0.6 μm and 0.45 μm pore size. This

produced liposomes of average particle size 635 nm and 453 nm respectively.

5.4. The Determination of the Thermodynamic Parameters of Transfer Using the Mini-AKUFVE Technique.

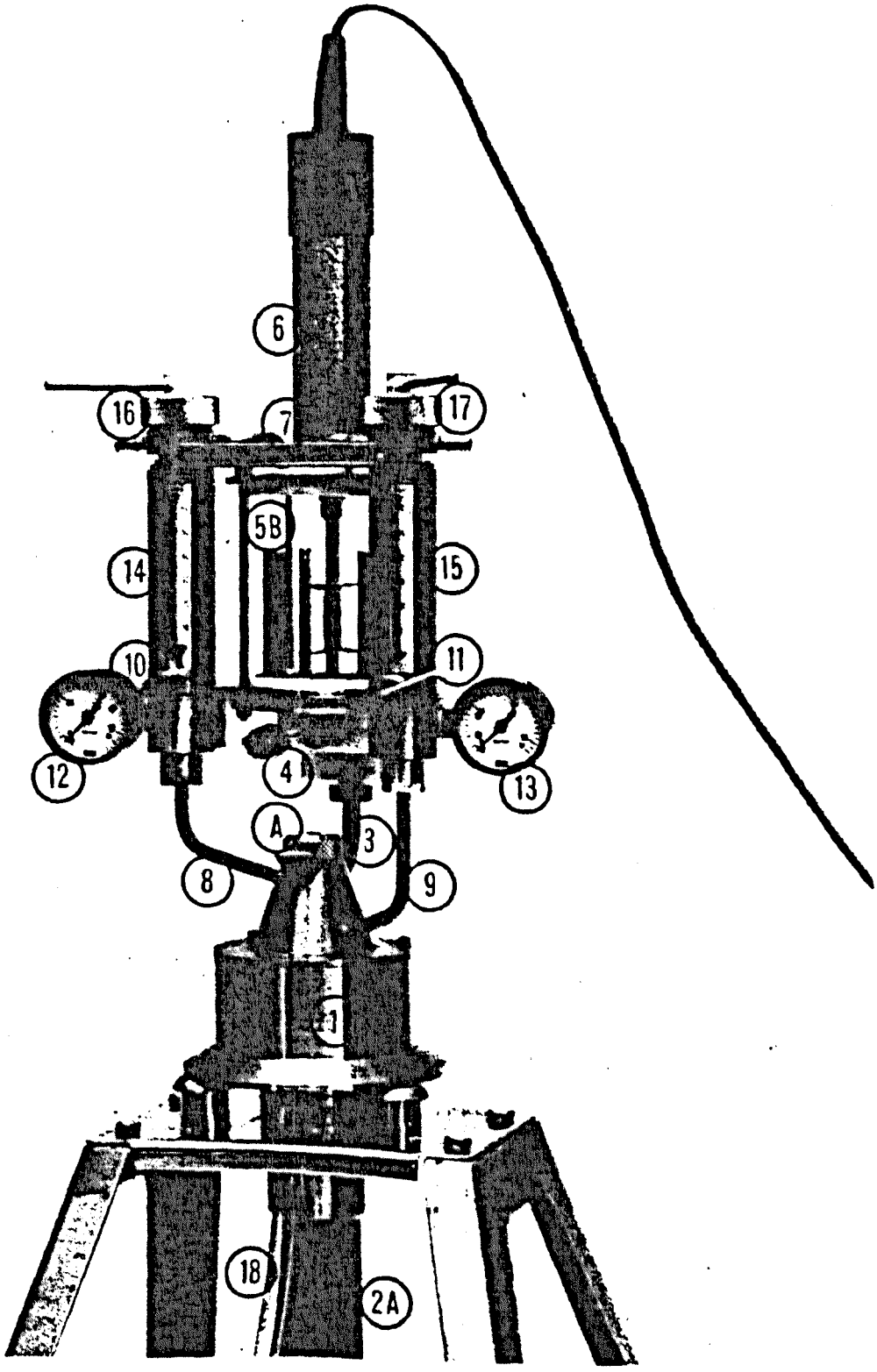
1. Technical Description.

The basic AKUFVE ADMCS 10 trs unit is shown in Figure 21. The construction and function of each major component will be described.

(1) The Inflow System.

The mixing chamber of the mini-AKUFVE consists of a glass cylinder with the top and bottom closed by two teflon-titanium lids. (5 b in Figure 21). The volume in the mixing chamber is about 200 ml. A variable speed mixer/stirrer (pneumatic) provides the mixing. This type of dynamic mixer gives a turbulent flow. In the mixing chamber, mass transfer of the species of interest between the two phases will take place according to the chemical and physical conditions of the liquid system. Heavy mixing produces small particles, large interfacial area and rapid transfer. Small particles, however, may increase the separation problems. The rotational speed of the centrifuge almost alone decides the mixing efficiency, so that mixer/stirrer speed can be kept at a minimum. Inlets are located in the top lids: one main feed inlet and inlets for heavy and light phases. The intimately mixed solvents and solute flow out peripherally to the centrifuge through a regulating valve fixed to the bottom lid. This is the only means of controlling the flow capacity of

Figure 21. The ADMCS 10 trs Mini-AKUFVE unit.



Key to Figure 21.

1. H-centrifuge (H-10 trs)
2A. Pneumatic motor drive
3. Mixture inflow to H-centrifuge
4. Regulating valve
5B. Dynamic mixer/stirrer
6. Pneumatic stirrer motor
7. Inflow to mixer, heavy and light phases
8. Outflow from H-centrifuge, heavy phase
9. Outflow from H-centrifuge, light phase
10. Throttling valve, heavy phase
11. Throttling valve, light phase
12. Pressure gauge, heavy phase
13. Pressure gauge, light phase
14. Flow meter, heavy phase
15. Flow meter, light phase
16. Directing valve, heavy phase
17. Directing valve, light phase
18. H-centrifuge drain

the centrifuge at a controlled running speed. All joints are tightened with O rings in teflon or viton. Other parts in contact with the solutions are of titanium or glass.

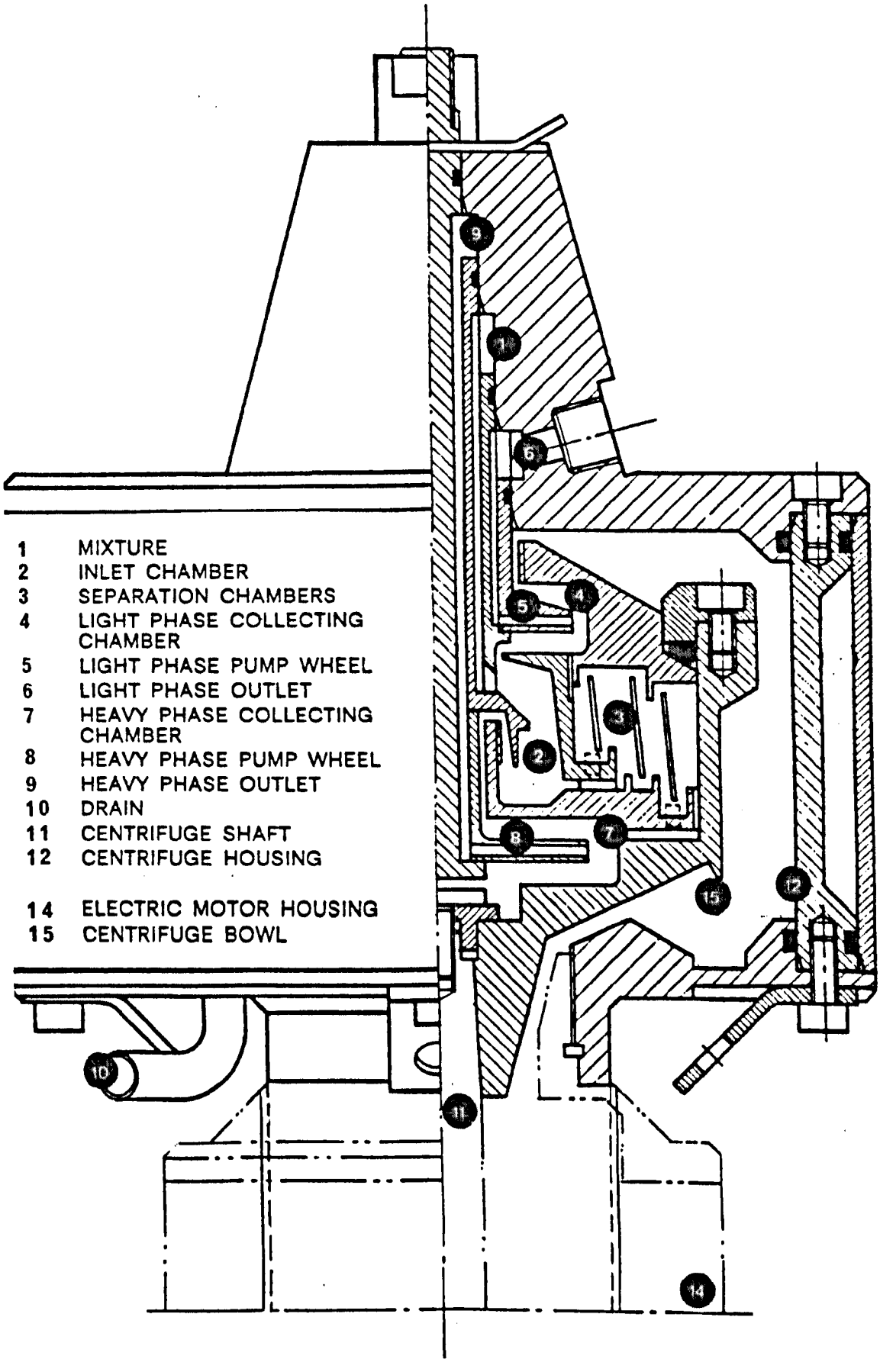
(2) H-Centrifuge.

Based on hydrodynamic and mechanical considerations the specially designed H-centrifuge was developed by Reinhardt and Rydberg (1969) to enable the rapid and 'absolute phase separation' of immiscible solvents by virtue of the difference in their respective densities. As defined by Reinhardt and Rydberg (1970) absolute phase separation means that no measurable amounts of one phase occur in the other, apart from amounts due to mutual solubility.

A section through the centrifuge is shown in Figure 22. The two-phase mixture enters the centrifuge through the central connection (1). In the inlet chamber (2) the mixture is almost instantaneously accelerated to the rotational speed of the centrifuge bowl (15). The strong turbulence produces a thorough final mixing of the two phases. Under certain circumstances, however, emulsive mixtures may result, affecting the subsequent separation process unfavourably. In such cases excessive rotational speed should be avoided.

After acceleration the mixture is forced into the separation volume (3), which contains eight chambers, symmetrically arranged around the axis and totally isolated from each other. In this arrangement the mixture has a zig-zig motion imposed by peripheral partition walls and interspersed baffle ridges. The movement of the liquids in this volume forces it to separate into its two phases, i.e.

Figure 22. H-centrifuge separator.



the light and heavy phase. Collecting chambers for the light phase (4) and the heavy phase (7) are located respectively above and below the separation volume. The pure phases are removed by pumps (5) and (8).

The heat generated by acceleration and retardation of the liquids causes a small temperature rise of the liquid. This is eliminated by the introduction of heat exchange coils (see next section) in the system.

The mini-AKUFVE H-10 trs centrifuge is driven by a pneumatic motor. The centrifuge bowl is fixed directly on the motor shaft. The upper ball bearing is placed close to the bowl in an elastic mounting of O rings, in order that critical conditions are reached and passed at low speed during starting up.

The flow capacity (for kerosene/water) of the mini-AKUFVE H-10 trs centrifuge is 100 l.p.h. The bowl volume is 15 ml. The hold up time in the centrifuge is 0.5 sec. The centrifuge has a maximum rotational speed of 19,500 r.p.m. with no liquid flow, and a rotational speed of 15,000 r.p.m. with maximum liquid flow. It has a maximum air consumption (6 bar) of 0.25 m^3/min and a maximum motor power of 200 watts.

The separation efficiency of the centrifuge can be assessed from the purity of the separated phases. This can be done in two ways. Firstly, the absorbance of the 'pure' phase in the flow cell can be compared with the absorbance of the pure solvent in a reference cell. If there is no difference then the phase can be said to be pure. Secondly, samples of the phase can be taken out and solubilities

determined, and compared with data from conventional shaking-separation technique. The separation efficiency of the H-33 AKUFVE centrifuge (large version of the mini AKUFVE) has been extensively studied using a large number of organic/aqueous systems. However the aqueous/organic solvent systems studied with the H-10 centrifuge is somewhat limited. Those studied include the nysolvin/aqueous, n-hexane/aqueous, benzene/aqueous, and methyl iso-butyl ketone/aqueous systems (Mini-AKUFVE ADMCS 10 trs manual). The H-10 centrifuge is more sensitive to changes in the flow system than the H-33, and consequently it is more difficult to find the optimal conditions, which provide the absolutely pure phases. The greater sensitivity of the H-10 centrifuge requires adjustment of the diameter of the lower pump wheel to obtain optimal separation conditions for each combination of solutions. Normally the H-10 centrifuged is equipped with a lower pump wheel of 29 mm diameter, suitable for kerosene/water mixtures. (Lower pump wheels of 31 mm and 33 mm diameter were purchased after consultation with Dr. H. Reinhardt of MEAB Metallextraktion AB, who suggested that these pump wheels might be more suitable for the separation of water/octanol mixtures).

(3) The Outflow Systems.

The outflow of separated liquids from the H-centrifuge are connected to throttle valves, directing valves, pressure gauges and flow meters for visual reading (see Figure 21).

The throttle valves are needle valves (Figure 21, 10-11) with a valve body of titanium and a needle of teflon. They are provided for the throttling of the liquid stream from

the centrifuge, in order to vary outlet pressure and facilitate control of the interfacial boundaries inside the centrifuge. The purity of one of the phases can be improved by increasing its pressure using the needle valve. Normally when the AKUFVE is started, the two needle valves are FULLY opened; if one phase is not pure, it is throttled just to improve its purity. Excessive throttling is avoided, as increase of pressure means increase of heat generated by friction. Too high a pressure may also lead to leakage, particularly of the light phase. Liquid flow rates are very little influenced by throttling.

The directing valves (Figure 21, 16-17) are double two-way tap valves with two positions, and direct the pure liquids - heavy phase and light phase - from the centrifuge to detection, heat exchanger and sampling or recirculate them to the mixer. This type of valve is important to make recirculation possible when starting up an experiment. The centrifuge does not immediately give pure phases, and entrainments in the detectors must be avoided as they may spoil subsequent measurements.

Pressure gauges (0-1.6 kg/cm^2) (Figure 21, 12-13) are connected upstream of the throttling valves. They contain a closed oil-filled cavity with a teflon membrane in contact with the process fluid to transmit pressure to the gauge and to protect it against corrosive liquids. These pressure gauges indicate the pressure of the outgoing pure, heavy and light phases from the centrifuge. The flow rates of the separated liquids are measured by ball type flow meters

(Figure 21, 14-15), with a capacity range for water of 30-300 l/h. They are made of glass with the floating ball in tantalum. A centrifuge drain (Figure 21, 18) is provided in the case of accidental overflow or leakage.

(4) Auxillary Equipment.

A compressed air control unit controls the speed of the H-centrifuge, and is a throttling valve regulating the pressure of the compressed air to the centrifuge motor. The valve can be locked by a red locking ring. The mixing action of the mixer/stirrer in the mixing chamber is regulated by the mixer control unit.

A pressure gauge showing the pressure (0-6 bar, 0-85 psi) of the compressed air to the H-centrifuge pneumatic motor is provided. The pneumatic motor of the centrifuge and mixer/stirrer is continuously lubricated by oil-fog lubricators. A specially designed silencer is equipped with the H-centrifuge. This eliminates both noise and the oil-fog.

2. Setting Up the Mini-AKUFVE System to Study the Temperature Dependence of Partitioning.

Compressed air for the pneumatic motors of the centrifuge and mixer/stirrer was provided by an hydrovane air compressor. This gave a total air pressure of 7.5 bar, and a maximum of 4.5 bar was supplied to the centrifuge and mixer/stirrer collectively.

In order to study the temperature dependence of partitioning, a number of components needed to be added to the basic ADMCS trs unit. These were as follows :

(i) An on-line detection device for measuring the solute concentration in the aqueous phase.

(ii) A method for controlling the temperature of the circulating solvents, and also the immediate environment of the mini-AKUFVE unit.

(iii) A device for accurate and precise temperature measurement.

(iv) A method for introducing solvents and solute into the mixing chamber.

(v) A method for draining and cleaning the apparatus, without dismantling.

(vi) A way of connecting all the various components.

The detector chosen was a Cecil ultra-violet-visible spectrophotometer since the phenolic compounds, which were to be studied, have relatively large extinction coefficients in the ultra-violet region. The aqueous phase was monitored by passing the aqueous phase through a flow-through cell situated in the cell compartment of the spectrophotometer. Precise temperature control of the circulating phases was achieved by passing them through heat exchange coils (Quick-fit Graham coil-condensers), which were connected to a Grant thermostatted water circulator. The cell compartment of the spectrophotometer was also thermostatted by connecting to the water circulator. An insulating polystyrene box was constructed to house the basic AKUFVE unit. An electric fan was used to blow air over a thermostatted radiator and into the insulation box. This controlled the temperature of the immediate environment of the AKUFVE unit (± 2°C).

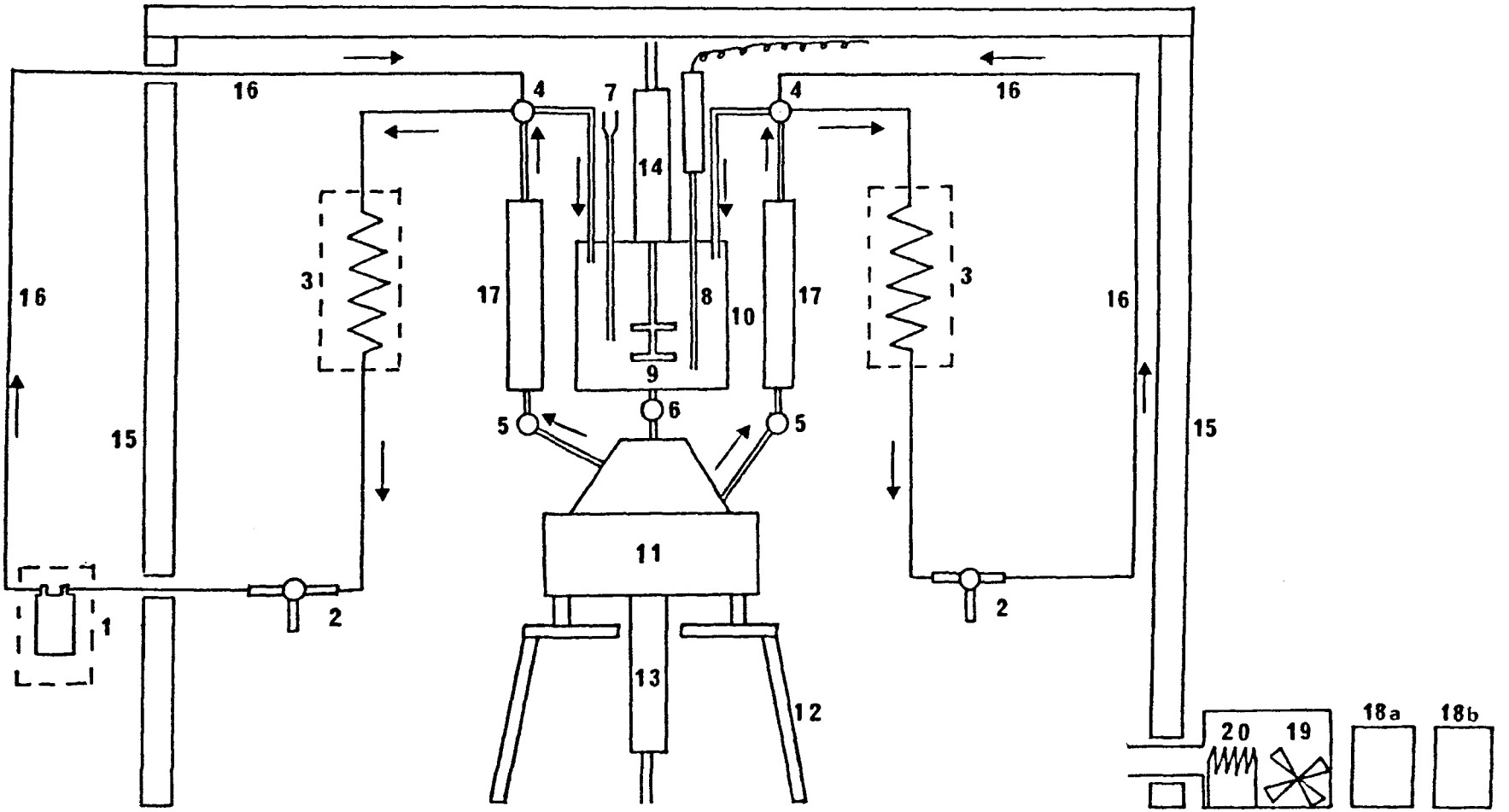
A Fluke digital thermometer was utilised for accurate and precise temperature measurement. A hole (5 mm 1D) was drilled in the lid of the mixing chamber and a special teflon nut and bolt, with drilled out core, was attached. The probe of the thermometer was then inserted into the teflon nut and bolt, and the tip was positioned near the bottom of the mixing chamber.

The solvents and solutes were introduced into the mixing chamber, via a large bore stainless steel needle. This was fitted to the already existing hole in the lid, by a teflon nut and bolt, with drilled out centre.

Two three-way taps, with teflon bores, were used to drain the apparatus. Residual solvents left in the system were removed by connecting three-way taps to vacuum line. The various components were connected up using teflon tubing (4 mm. 1D) and glass tubing (4 mm. 1D). Teflon to glass and teflon-titanium tubing connections were tightened by small jubilee clips. It was found necessary to secure titanium to titanium joints with teflon tape to prevent leaks. Only glass, titanium and teflon surfaces were in contact with the circulating solvents and solutes. PVC tubing was used to connect the water circulator to the heat exchange coils, the cell compartment and the small radiator. All tubing (teflon and PVC) outside the temperature controlled insulation box was lagged with foam rubber. The whole set up was placed in a fume cupboard, to safely remove any organic solvent vapour.

A schematic of the mini-AKUFVE apparatus is shown in Figure 23. The actual apparatus used is shown in the photo-

Figure 23. Schematic diagram of mini-AKUFVE apparatus.



Key to Figure 23.

1. Thermostatted flow through cell
2. Three way tap, with teflon bore
3. Heat exchange coils
4. Three way directing valves
5. Needle valves
6. Inlet valve to centrifuge
7. Injection port
8. Temperature probe
9. Stirrer/mixer
10. Mixing chamber
11. H-centrifuge
12. Tripod support
13. Pneumatic motor of centrifuge
14. Pneumatic motor of mixer/stirrer
15. Polystyrene insulation box
16. Narrow bore teflon tubing
17. Flow meter

18a,18b. centrifuge and stirrer controls

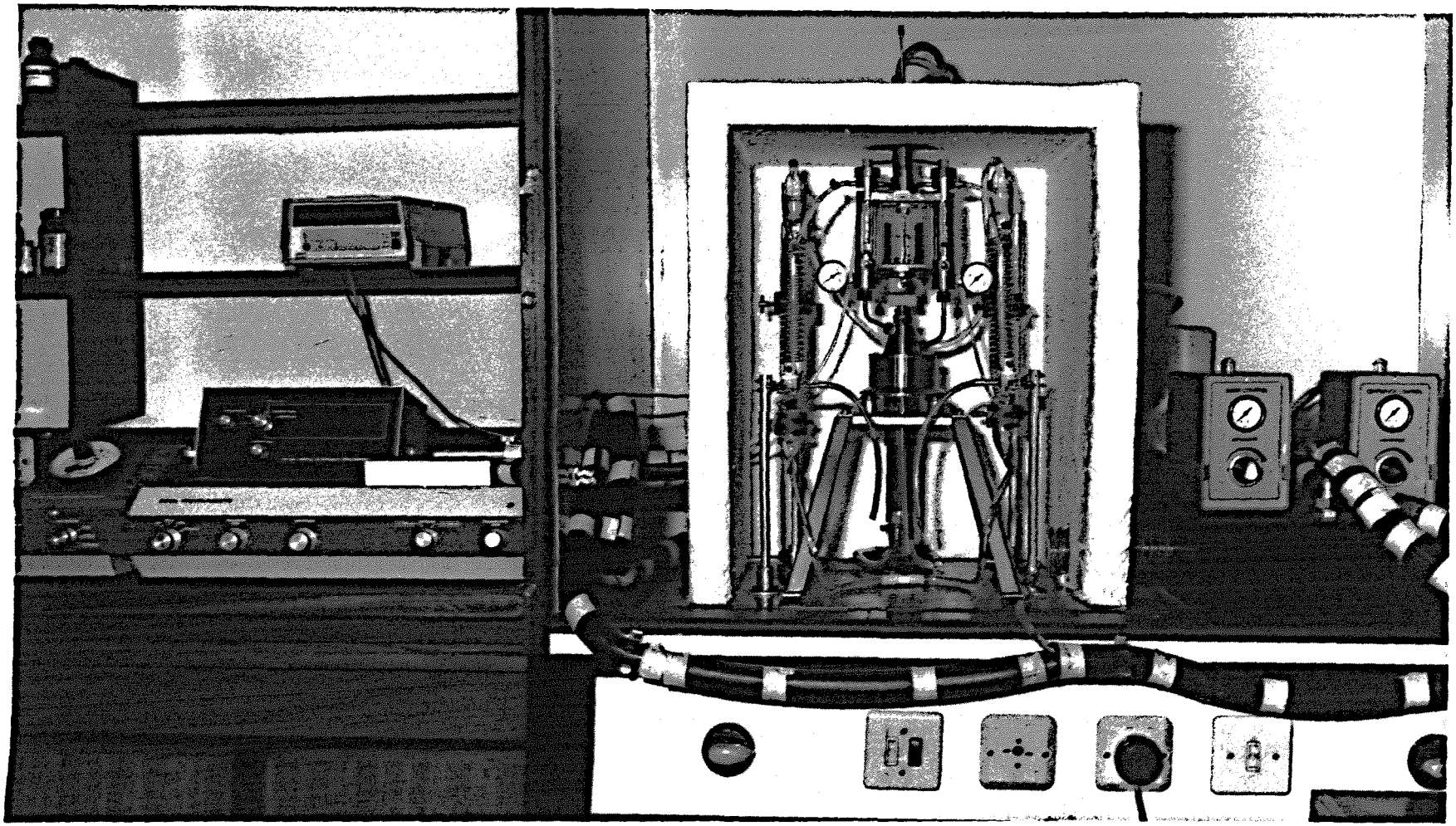
19. Electric fan
20. Small radiator.

— — — Area enclosed by dotted lines means that apparatus is connected to water circulator.

——→ Direction of solvent flow

Plate 1

Photograph of mini-AKUFVE set-up



graph following Figure 23.

3. Operation of the Mini-AKUFVE to Determine the Temperature Dependence of Partitioning.

The rapid and absolute phase separation of a particular aqueous/organic solvent system depends upon the operating conditions of the AKUFVE and the physical properties of the solvents.

The AKUFVE operating conditions can be varied as follows :

(1) Speed of rotation of the centrifuge.

(2) Stirrer/Mixer speed.

(3) Inlet volume to the centrifuge (regulates flow rate).

(4) Counter pressures on the phases leaving the centrifuge.

(5) Lower pump wheel diameters.

The H centrifuge separates solvent mixtures on the basis of a difference in densities. The differences in densities is affected by the mutual solubilities of the phases, which in turn is dependent on the temperature. The phase ratios and volumes of the solvents used also affect the separating conditions. The flow rate of a solvent in the AKUFVE system, will be influenced by its viscosity, which also varies with temperature.

The physical properties of water, octanol and cyclohexane are given in Table 15. Consequently the operation of the AKUFVE to obtain optimal separation conditions will depend on these properties mentioned.

The optimal operating conditions for the separation of water/octanol and water/cyclohexane mixtures of varying phase

Table 15 Physical-chemical properties of the solvents.

Solvent	Density g/ml	Solubility of H_2O in solvent g H_2O/100 g solvent	Solubility of solvent in H_2O g solvent/ 100 g H_2O	Viscosity cp	m.pt. °C	b.pt. °C	M.W
Water	.999 (15°C) .997 (25°C) .994 (35°C) (1)	-	-	1.139 (15°C) 1.002 (20°C) 0.798 (30°C) (7)	0 (8)	100 (8)	18.015 (9)
n-octanol	.825 (20°C) .822 (25°C) .818 (30°C) (2)	3.84 (10°C) 4.30 (20°C) 4.75 (30°C) (3)	0.052 (4) 0.042 (5) 0.038 (6)	10.640 (15°C) 8.892 (20°C) 6.125 (30°C) (7)	-15.0 (8)	195.3 (8)	130.225 (9)
cyclohexane	.779 (20°C) .774 (25°C) .769 (30°C) (1)	0.01 (20°C) (8)	0.0055 (25°C) (8)	0.980 (20°C) 0.898 (25°C) (8)	6.6 (8)	80.73 (8)	84.161 (9)

References.

(1) Landolt Bornstein 6 Auflage II Bond 2 a Bondteil;
(2) Hales and Ellender (1976);
(3) Erichsen (1952);
(4) Vochten and Petra (1973);
(5) Addison (1945);
(6) Kinoshita et al., (1958),
(7) Riddick and Bunger (1978);
(8) Sedivec and Flek (1970);
(9) Handbook of chemistry and physics (1977).

ratios and volumes were investigated.

(1) Water/Cyclohexane System.

(The aqueous phase is 0.15 M sodium chloride solution and the cyclohexane is BDH analytical reagent).

Absolute phase separation for the water/cyclohexane system was obtained relatively easily, using the lower pump wheel of diameter 29 mm. It was possible to obtain pure phases of aqueous and cyclohexane simultaneously. The purity of both phases could be assessed visually, by viewing them through the flow meters. Also a sample of the aqueous phase could be removed by draining off the system, and its optical density compared with that of pure water. The purity of the aqueous and cyclohexane phases (as assessed by viewing) was not affected by running the system over a temperature range of 15°-35° (approx.). The optical density of the aqueous phase remained constant (at zero) over the temperature range. This suggested that the physical properties of the aqueous/cyclohexane did not change dramatically with temperature. This is supported by the literature (Kinkel et. al., 1981). The majority of the substituted phenolic compounds which were to be studied have a λ_{max} at approx. 220 nm and 275 nm in the UV region (see Table 14). The extinction coefficient at 220 nm (approx.) is the strongest. Thus by assaying the solute at a λ_{max} of 220 nm (approx.) a lower concentration could be used, which is desirable when determining partition coefficients because it reduces the risk of association.

Pure cyclohexane (analytical reagent) has a very low absorbance at 220 nm (0.100 Abs units) and the small amount of

cyclohexane dissolved in the aqueous did not affect the absorbance. So it was possible to monitor the solute in the aqueous phase at 220 nm (approx.).

Experimental Conditions.

The aqueous (0.15 M NaCl) and cyclohexane phases were mutually saturated with each other. The λ_{max} of the solute under study was determined on the Beckman spectrophotometer, and on the Cecil spectrophotometer. A calibration graph of the phenol was determined on the Cecil spectrophotometer for a suitable range of solute concentration. The spectrophotometer and digital thermometer were allowed to warm up. The water circulator was allowed to equilibrate to give a circulating temperature of 10°C.

The centrifuge motor was started up and the rotation speed of the centrifuge was maintained with an air-pressure of approx. 3 bar. (The exact value was dependent on phase ratio and volumes of phases involved). The inlet valve to the centrifuge was closed, and the needle valves were fully opened. The director valves were adjusted to direct the separated heavy and light phases back into the mixing chamber.

Known volumes of aqueous phase and organic phase were introduced into the mixing chamber, through the large bore stainless steel needle, via a glass syringe. The mixer/stirrer was started up and maintained at a rotation speed sufficient to give a thorough mixing of two phases, without causing excessive agitation. (Excessive agitation caused solvents to stick to sides and lid of mixing chamber, and also caused emulsification in most severe cases).

The solvents were gradually introduced into the H-centrifuge by opening the inlet valve (Volume entering centrifuge was dependent on phase ratios and volumes used). After entering the centrifuge the separated light (cyclohexane) and heavy (aqueous) phases were passed through the flow meters and directed back into the mixing chamber. The cyclic process was repeated several times. Physical mixing is reached within 30 sec. in this closed cyclic arrangement. If necessary the counter pressures on the heavy and light phases were altered, by adjusting the needle valves, to improve the purity of the aqueous and organic phases.

Once physical mixing had reached equilibrium, the absorbance (and transmittance) of the aqueous phase was zeroed. The solute was introduced into the mixing chamber, using a syringe. The solute was dissolved in either the organic phase or aqueous phase depending in which it was most soluble. (Special care was taken to ensure that solubility in the solvent was not exceeded).

The solvents and solutes were allowed to equilibrate for between 10-15 minutes at the lower temperature of study (e.g. 15°C). When conditions were stable the absorbance of the aqueous phase was recorded. The solute concentration in the aqueous phase could be determined from the calibration graph. The solute concentration in the organic phase could then be determined by mass balance, and the partition coefficient at the particular temperature could be calculated.

The next step was to adjust the dial control of the water circulator, to raise the temperature of the circulating

solvents 3 or 4°C. Once temperature equilibrium was reached (within 10-15 minutes) the absorbance of the aqueous phase was again recorded. Again the partition coefficient at that particular temperature could be determined. This procedure was repeated several times, until several partition coefficients over the range 15°-35°C were determined.

Once the run was completed, the system was drained. This was accomplished by opening the three-way taps and collecting the spent solvents.

The next step was to clean the apparatus before the next experiment. The three-way taps and the inlet valve to the centrifuge were closed. 200 ml. of distilled water was injected into the mixing chamber. The stirrer/mixer rotation speed was increased, to thoroughly agitate the water in the mixing chamber. This helped to remove solvent and solute from sides and lid of mixing chamber. The rotation speed of the stirrer/mixer was reduced. The water was then introduced slowly into the centrifuge, by opening the inlet valve, and allowed to circulate throughout the system. By adjusting the needle valves it was possible to direct the water through the light and heavy phase compartments. The water was left to circulate through the whole system for 3-4 minutes. After which time the system was drained. This procedure was repeated several times until the aqueous phase gave a constant absorbance of zero. The system was finally drained and any residual solvents removed by vacuum.

Typical AKUFVE operating conditions were as follows :

(i) Centrifuge speed of rotation maintained by an air pressure of 3 bar.

(ii) Stirrer/mixer speed control $2\frac{1}{8}$ turns in a clockwise direction.

(iii) Needle valves adjusted to give heavy and light phase counter pressures which gave recorded flow rates of 15 and 40 l.p.h. respectively.

(iv) Inlet valve adjusted $5\frac{1}{2}$ turns in a clockwise direction :

The following phase ratios and volumes of solvents were used :

Aqueous (ml) : Cyclohexane (ml)			
1:1	2:1	3:1	4:1
100:100	120:60	150:50	200:50 (using reservoir)
75:75	100:50		
60:60			
50:50			

Several solutes were studied and the aqueous concentrations used were in the range 5×10^{-5} - 2.5×10^{-4} mol. dm^{-3}. Final organic concentrations used were in the range 2×10^{-5} - 3×10^{-4} mol. dm^{-3}. Several problems were encountered in using this technique.

Leaks were a problem at first. However these were successfully dealt with. (see previous section). Overflow from the centrifuge bowl was more of a serious and persistent problem. This was caused by running the centrifuge and mixer/stirrer at too high a rotation speed or by injecting the solute in too large a volume of solvent. This caused grease to be removed from the upper ball bearing race in the pneumatic motor of centrifuge, and initially one set had to be replaced. However by covering the ball bearing race in a solvent resistant molybdenum disulphide grease (Rocol MX44),

and with frequent oiling, this problem was solved. By carefully controlled experimental conditions and by closing inlet valve to centrifuge when injecting solute overflow from the bowl of centrifuge was eliminated. However slight overflow from centrifuge took place when draining the instrument, but this could not be avoided. The glass of the centrifuge bowl had to be replaced. Sticking of solvent onto side and lid of mixing chamber was a problem during the experiment. This could be reduced by using a rather slower mixer/stirrer speed. One of the major problems was the volume of aqueous solvent left in the system after cleaning. This could not be removed completely by vacuum. It was not possible to pass acetone through as this could attack the elastic viton O rings in the centrifuge. The only way to remove the aqueous solvent completely was to strip down the system totally and to dry thoroughly every component. This would have been a very time-consuming procedure.

The enthalpy of transfer for several solutes between 0.15 M NaCl and cyclohexane were determined by this method. However, the method did not prove to be reproducible, and the results compared unfavourably with those of the literature. The partition coefficient values at 22°C were reproducible and compared favourably with literature values. (see Results section).

(2) Water/Octanol System.

(The aqueous phase was 0.15 M sodium chloride solution and Koch-Light puriss grade n-octanol was used as organic phase). The separation of aqueous/octanol mixtures proved exceedingly

difficult. Various AKUFVE conditions and lower pump wheels (29, 31 and 33 mm diameters) were experimented with until 'optimal' conditions were found. (A phase splitter (Kinkel and Tomlinson, 1980) was introduced into the aqueous phase system to reduce the amount of dissolved octanol, but this proved unsuccessful). A lower pump wheel, with a diameter of 29 mm, proved the most efficient. A visibly clear aqueous phase would be obtained, but the octanol phase was cloudy. The absorbance of n-octanol (Koch-Light puriss grade) at 220 nm was high (1.500 absorbance units). If the aqueous phase was monitored at this wavelength, interference from dissolved n-octanol occurred. However, the absorbance of n-octanol (Koch-Light puriss grade) at 275 nm was low (0.300 units) and if the aqueous phase was monitored at 275 nm (approx.) interference from dissolved n-octanol was reduced. However, the extinction coefficient of the phenol is lower at this wavelength, so that a slightly higher solute concentration need be employed. The absorbance of the aqueous phase was found to decrease with increasing temperature, when the system was run over a range of temperature (15°-35°C). This suggested that the solubility of octanol in water decreases with temperature. This agreed with literature findings (see Table 15) and also with the findings of Y. C. Martin (personal communication), who also had been investigating the use of the mini-AKUFVE to study partition coefficients. Later studies, showed that the solubility of water in octanol increases with temperature.

The physical properties of the water/octanol system were

such that the mini-AKUFVE could not produce 'absolute phase separation' of water/octanol mixtures, when the system was run over a range of temperature. Thus the mini-AKUFVE apparatus could not be employed to study the temperature dependence of phenols between aqueous and n-octanol. However, it was possible to determine the partition coefficients of several phenolic solutes between water and octanol, at one temperature, i.e. 22°C, using the mini AKUFVE system. The procedure adopted was the same as with the water/cyclohexane system. The solute concentration in the aqueous phase was determined by monitoring the aqueous phase at the higher wavelength λ_{max}, i.e. 275 nm. (approx.). (N.B. exact value depended on each particular solute; see Table 14). Final solute concentrations in the aqueous phase were in the range $1 \times 10^{-4} - 5 \times 10^{-4}$ mol. $dm.^{-3}$. The final solute concentration in the organic phase was in the range $2 \times 10^{-2} - 4 \times 10^{-2}$ mol. dm^{-3}. The partition coefficient values obtained at 22°C are given in the Results section.

The use of the mini-AKUFVE system to study the temperature dependence of the partitioning of phenols between aqueous/n-octanol and aqueous/cyclohexane systems proved unsuccessful. Therefore an alternative method was sought. The filter probe technique seemed most suitable.

5.5. The Determination of the Temperature Dependence of Partitioning between Aqueous Solution and Organic Solvents using the Filter Probe Technique.

The procedure for determining partition coefficients using

the filter probe technique follows closely to that adopted by Cantwell and Mohammed (1979), Mohammed and Cantwell (1979, 1980), Kinkel et al. (1981) and Tomlinson (1982). However certain differences in the experimental method were employed.

The apparatus consists of a filter probe with filter paper, a thermostatted mixing chamber with lid, a magnetic stirrer, a digital thermometer with temperature probe (or glass thermometer), a peristaltic pump with silicone rubber tubing, an ultra-violet spectrophotometer and flow through cell, and fine bore, teflon tubing to connect all components. The arrangement of the components is shown in Figure 24.

Two versions of the filter probe were used; model 1 and model 2. These are shown in Figures 25 and 26 and Plate 2. Both filter probes were machined from stainless steel and worked on the same principle.

A cellulose filter paper is attached to the filter probe, via a teflon sleeve as in model 1, or via a screw-on stainless steel cap as in model 2. When the filter paper is wetted, with distilled water it becomes hydrophilic and forms a barrier to a hydrophobic organic solvent, whilst allowing an aqueous solution through. Once wetted with water it is not wettable with organic solvent. The filter probe, with attached wetted filter paper, is immersed in an intimately mixed solution of aqueous and organic phase contained in a mixing chamber. The tip of the filter probe is attached to a short piece of silicone rubber tubing of a peristaltic pump, using fine bore teflon tubing. When the pump is switched on, the aqueous phase is extracted, and this can be pumped through

Figure 24. Assembly of filter probe apparatus.

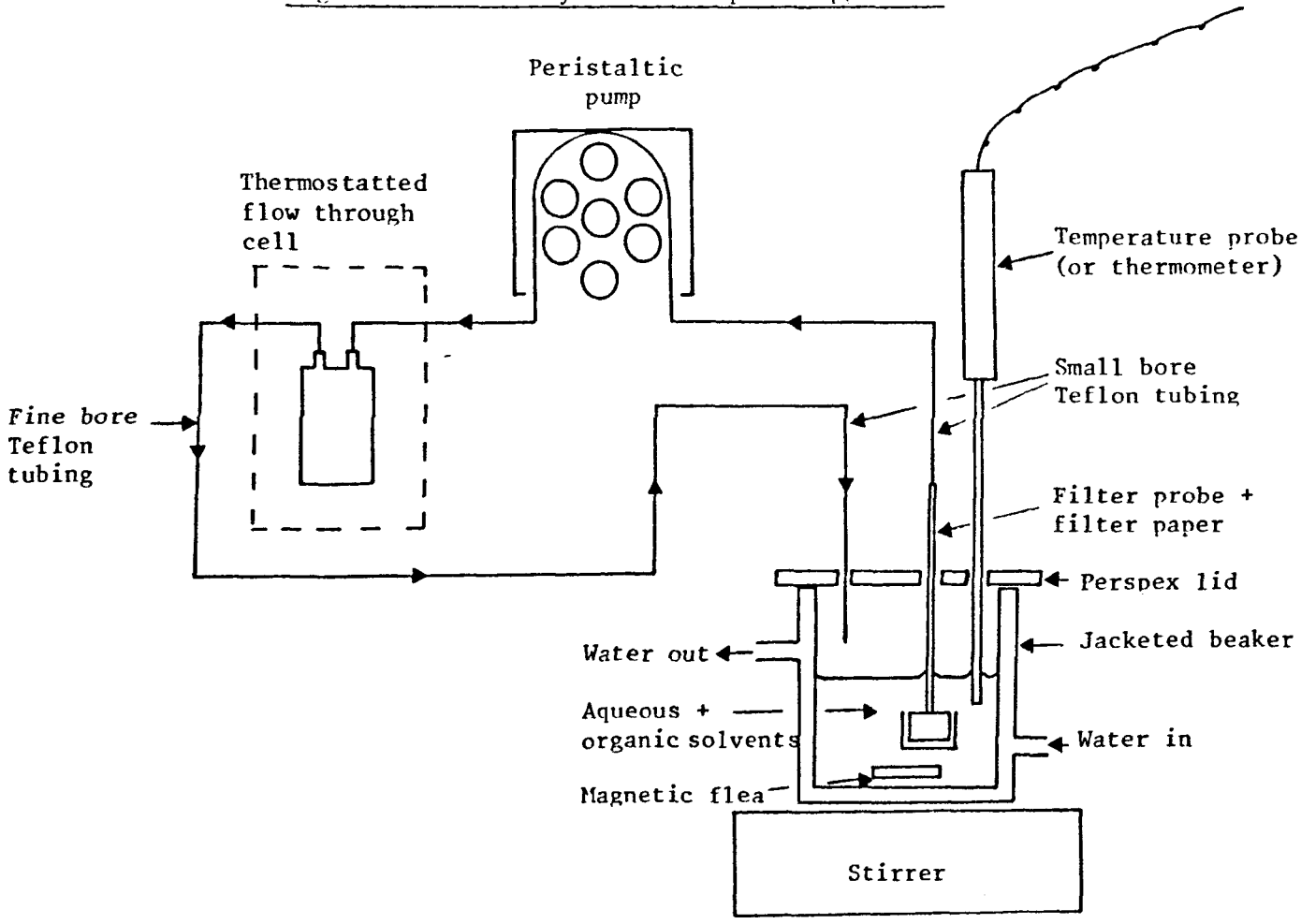
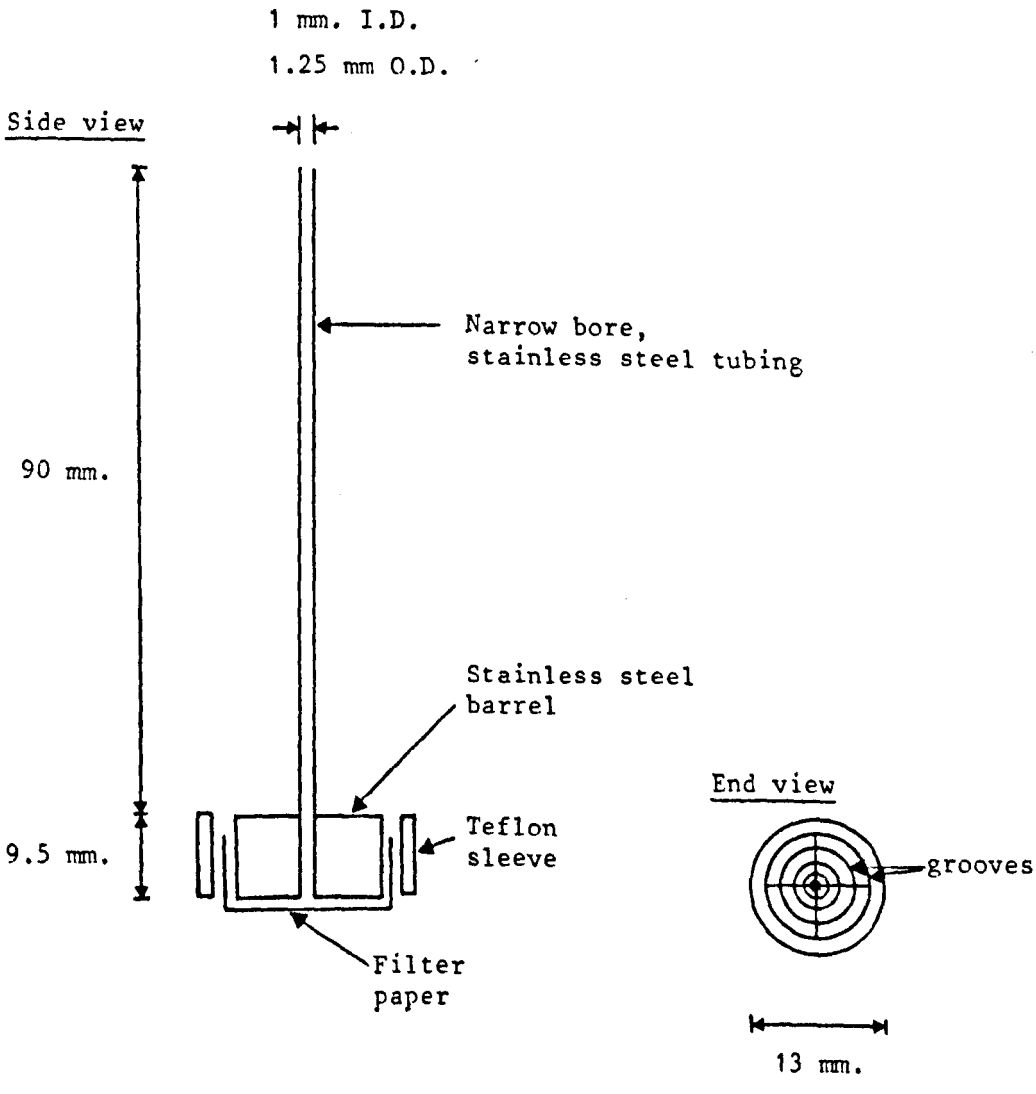
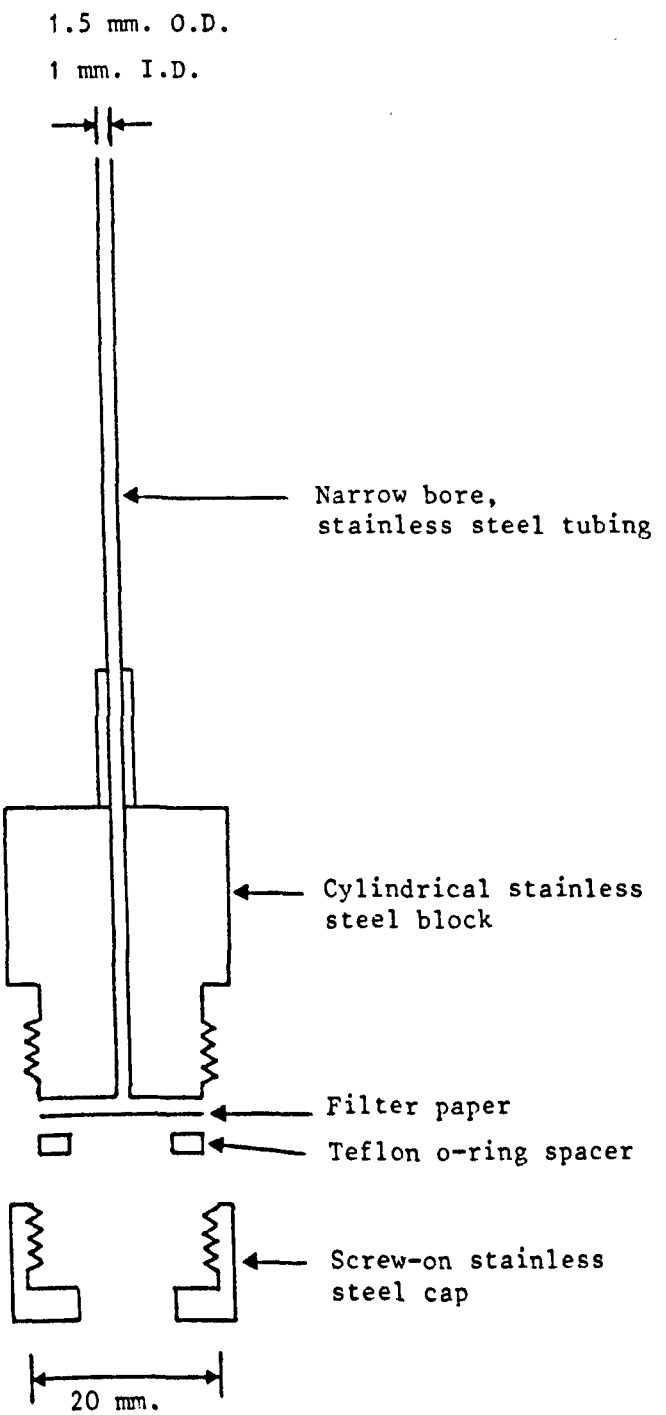


Figure 25. Filter probe model 1.



(NOT TO SCALE)

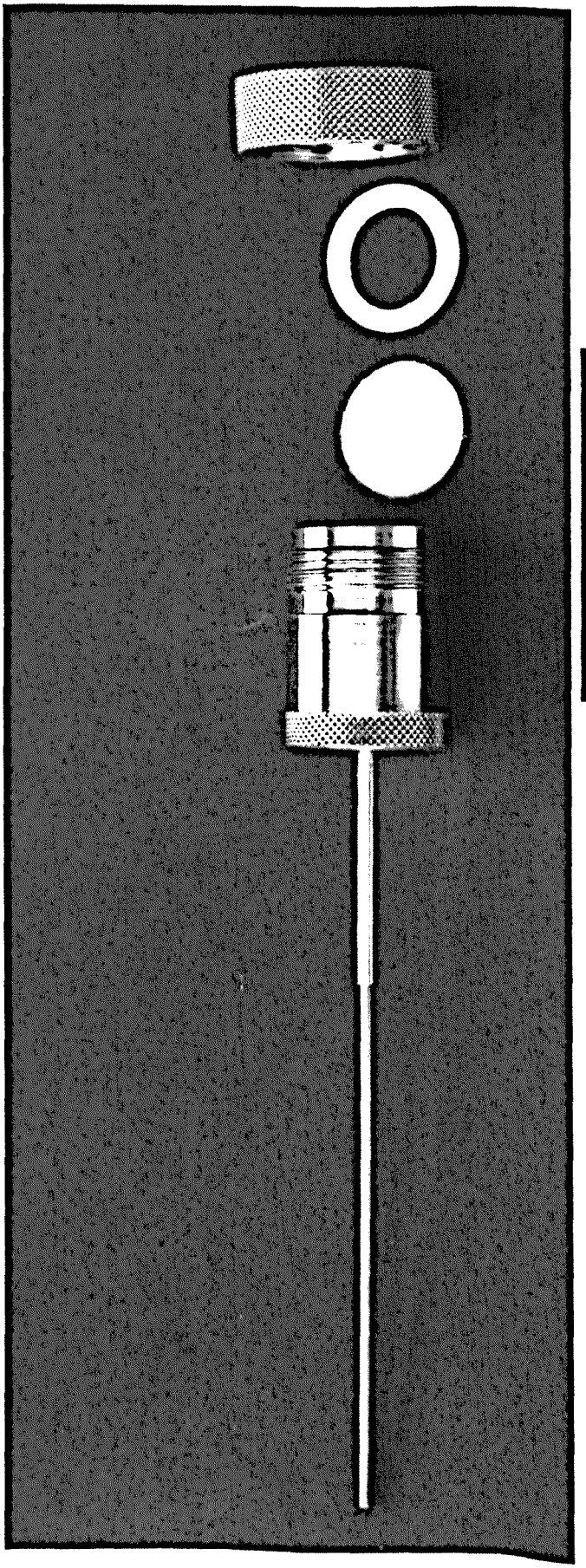
Figure 26. Filter probe model 2.

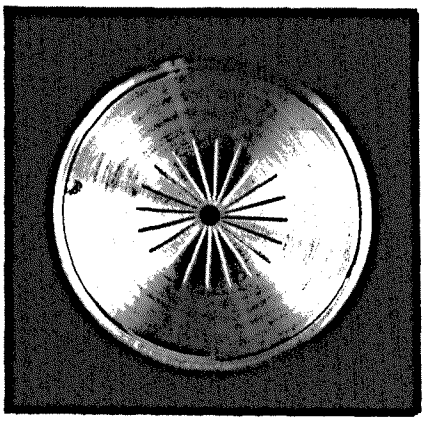


(NOT TO SCALE)

Plate 2

Filter Probe Model 2





fine bore teflon tubing to a flow-through cell of a spectrophotometer. From the flow-through cell the aqueous phase can then be pumped through teflon tubing back into the mixing chamber. If a solute is introduced into the mixing chamber, then it will distribute itself between the aqueous and organic phases until equilibrium is reached. If the solute can be assayed spectrophotometrically, then the aqueous concentration can be determined by monitoring at a suitable wavelength, and referring to a calibration graph. The organic phase solute concentration can be obtained by mass balance. From these calculations the partition coefficient can be determined. If a temperature measuring device is introduced into the mixing chamber, then the temperature of the mixed solvents could be measured, and the partition coefficient at a specified temperature determined. If the mixing chamber and cell compartment of spectrophotometer are thermostatted, then the temperature dependence of partitioning can be studied.

(N.B. The organic phase can be extracted using a hydrophobic filter (Kinkel and Tomlinson, 1981)).

1. Preliminary Experiments.

In order to determine most suitable experimental conditions for the study, certain preliminary investigations had to be made. One of the main criticisms of using a peristaltic pump is that silicone rubber tubing adsorbs solutes. However according to the manufacturer, Watson and Marlow, their silicone rubber tubing was not attacked by phenolic compounds and had a long

tube life. Subsequent permeability studies using a phenol with a relatively large partition coefficient, p-propyl phenol, showed that adsorption was not a problem. Later observations when cleaning tubing, showed that there was no leaching out of solute. (Organic solvents could not be circulated through this tubing however, because they attacked the silicone rubber). Viton tubing was tried but this had a very short tube life and split after a short time in use. Adsorption onto the teflon connecting tubing was not found to be a problem.

(The use of an HPLC pump for circulating solvent would overcome any problems of adsorption and would permit circulation of organic phases. However the pump has the following disadvantages :

(a) priming is necessary,

(b) a relatively large internal volume,

(c) requires larger volume of circulating phase).

Various filter papers and combinations were tried, until it was found that Whatman cellulose filter paper, quantitative grade 54 (hardened), was most suitable. This type of filter paper had high 'wet' strength and flexibility and did not tear easily when loading onto filter probe (with flat forceps). It had high chemical resistance, and low levels of impurities which could be extracted. Pore size was small, and this made it less likely for small droplets of organic phase to be drawn through. Pore size distribution was narrow and this gave a reproducible flow, through the pore structure and clogging effects were reduced. Filtration speed was fast so that aqueous phase could be quickly extracted from aqueous/organic

mixture. Flow rates of between 1-1.5 ml/min were found to be most suitable. Flow rates higher than this caused hydrophilic barrier to fail and small droplets of organic phase were drawn through. The Whatman grade 54 filter paper was 2.5 cm in diameter. This fitted the filter probe model 1 perfectly. The filter paper was placed onto the barrel of the probe, using forceps. The teflon sleeve was then placed onto barrel securing the filter paper and forming a water tight seal. The filter paper was wetted and ready for use. For use with filter probe model 2, the filter paper was cut to the right size using a cork borer. The filter paper was then secured to the barrel of the filter probe by means of a teflon washer and stainless steel screw-on cap. The filter paper was then wetted and ready for use.

Time for solute, partitioning between the two phases to reach equilibrium depended upon :

(i) efficiency of stirring rate,

(ii) phase ratios and volumes of aqueous and organic phases,

(iii) partition coefficient of solute.

Generally this was found to be between 2-10 minutes.

It was found that a teflon spoiler was not necessary to reduce vortex formation since this was prevented by the filter probe and temperature probe (or thermometer).

The solute concentration in the aqueous phase was to be determined by monitoring the aqueous phase, flowing through the flow cell in the spectro-photometer, at the lower wavelength of 220 nm (approx.). The effect of dissolved solvents in the aqueous phase on the absorbance was investigated. The

filter probe system was set up using only aqueous phase and organic solvent. The aqueous phase was monitored over the range 160 nm - 360 nm and there was no absorbance due to dissolved solvent. (The n-octanol used for the study was Fisons special for partition coefficient measurement, and this is of very high purity. BDH analytical grade cyclohexane was used as the other organic solvent). The effect of temperature on the aqueous absorbance, at 220 nm, was investigated by running the system over the temperature range 10° - 40°C. Again no change in absorbance was observed for both aqueous/octanol and aqueous/cyclohexane. The filter probe systems efficiently extracted the aqueous phase, and left behind the organic phase; complete phase separation was effected.

Since it was possible to monitor the solute concentration at the lower wavelength λ_{max} of 220 nm (approx.) very low solute concentrations could be used. This reduced the risk of association in the organic phase. For the aqueous/octanol system final aqueous concentrations were in the range 5×10^{-5} - 2.5×10^{-4} mol. dm.$^{-3}$, on average 10^{-4} mol. dm.$^{-3}$, and final organic concentrations were in the range 2×10^{-4} - 4×10^{-2} mol. dm^{-3}, on average 4×10^{-3} mol. dm.$^{-3}$. For the aqueous/cyclohexane system final aqueous concentrations were in the range 5×10^{-5} - 3×10^{-4} mol. dm.$^{-3}$, on average 10^{-4} mol. dm.$^{-3}$ and final organic phase concentrations were in the range 10^{-6} - 10^{-3} mol. dm.$^{-3}$, on average 10^{-4} mol. dm^{-3}.

It was necessary to use different phase ratios and volumes of solvents in order to achieve appropriate solute concentrations

in both phases. These were as follows :

Aqueous/Octanol		Aqueous/Cyclohexane	
Volumes solvents (ml)	Phase ratio	Volumes solvents (ml)	Phase ratio
200 : 2	100 : 1	200 : 5	40 : 1
100 : 2	50 : 1	200 : 10	20 : 1
120 : 4	30 : 1	80 : 20	4 : 1
100 : 5	20 : 1	50 : 50	1 : 1
100 : 10	10 : 1	25 : 50	1 : 2
100 : 20	5 : 1	20 : 80	1 : 4
		50 200	1 : 4

The use of different phase ratios and volumes of solvent necessitated changing the volume of mixing chamber between 100 ml and 500 ml.

The filter probe study was carried out in the Pharmacy Dept., Nottingham University and ICI Plant Protection, Bracknell, Berkshire. Consequently, two sets of apparatus had to be assembled. The various components are given in Table 16 and were arranged as in Figure 24. Watson Marlow peristaltic pump tubing of 3 mm. O.D, 1 mm. I.D and 15 cm length was used in both set ups. Teflon tubing of 1.25 mm. O.D. and 1.0 mm. I.D. was used to connect the various components. Short pieces of tubing were used to reduce circulating volume. A circulating volume of 1.2 ml was achieved. Teflon tape was used to secure holes in perspex lid, which were used for access of filter probe, temperature probe and returning teflon tubing, to eliminate risk of evaporation of solvents. The perspex lid formed a close fit onto mixing chamber. The filter probe was secured using a crocodile clip. The completed experimental set-ups are shown in Plates 3 and 4.

Table 16

Instrument	ICI Plant Protection Bracknell, Berks	Pharmacy Dept. Nottingham Univ.
Spectrophotometer	Varian-Cary 210 double beam	Cecil CE292 single beam
Flow-through cell	Varian 1 cm path length .75 ml. volume	Cecil 1 cm path length .5 ml. volume
Peristaltic pump	Watson-Marlow H.R. Flow Inducer	L.K.B.
Mixing chamber	Beaker (100 ml-500 ml) immersed in vessel containing water and thermostatted coil + perspex lid	Jacketed beaker (100 ml-500 ml) + perspex lid
Stirrer	Baird and Tatlock magnetic stirrer	Rodwell heater/ stirrer
Thermocirculator	Haake FK water circulator	Grant thermo-circulator
Temperature measuring device	Gallenkemp total immersion thermometers 0-40°C, 40-70°C 0.1°C accuracy	Fluke digital thermometer plus platinum resistance probe 0.01°C accuracy
Filter probe	Model 1	Model 2

Plate 3

Filter probe set-up, used at

Pharmacy Dept., Nottingham University

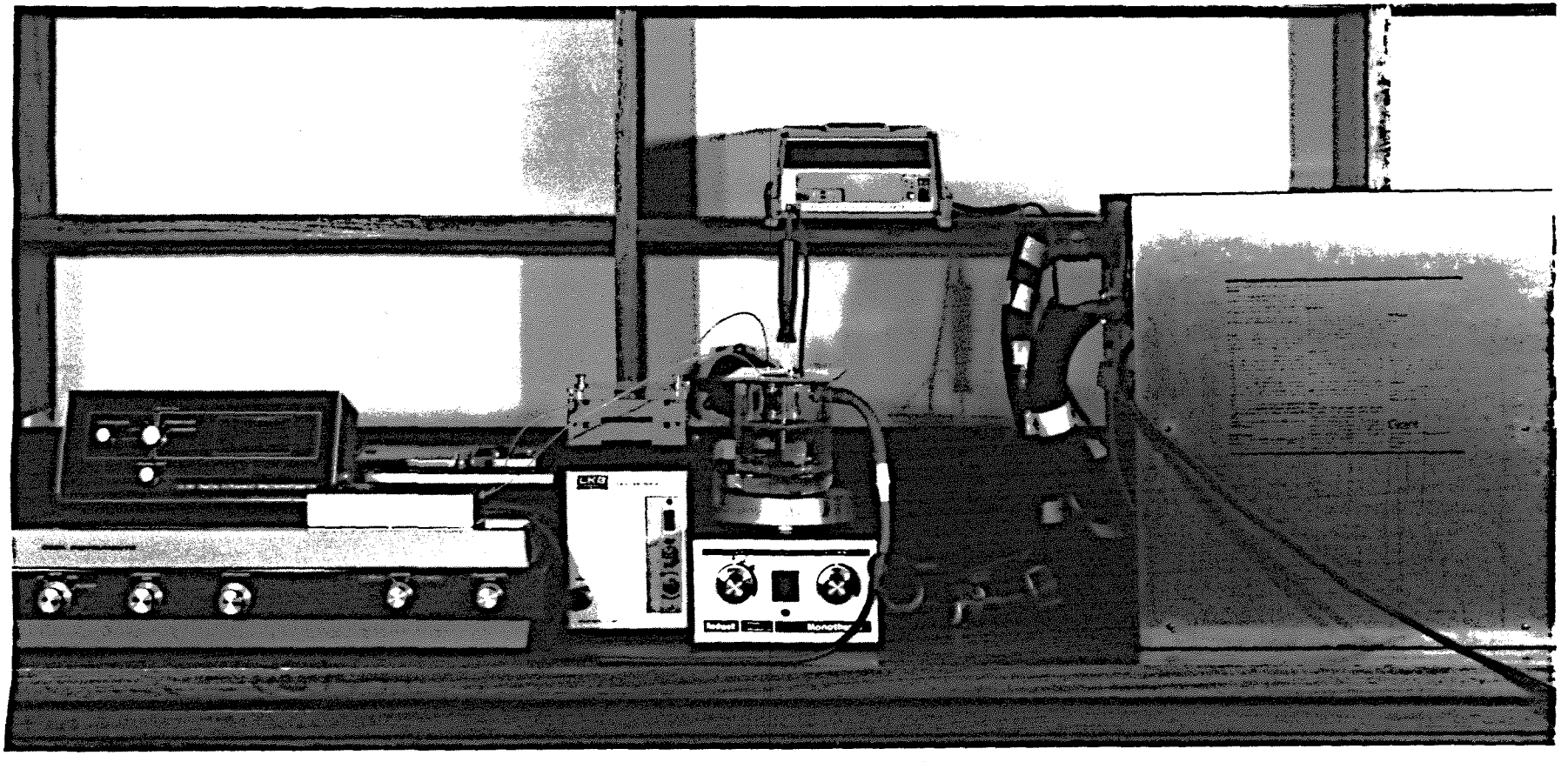
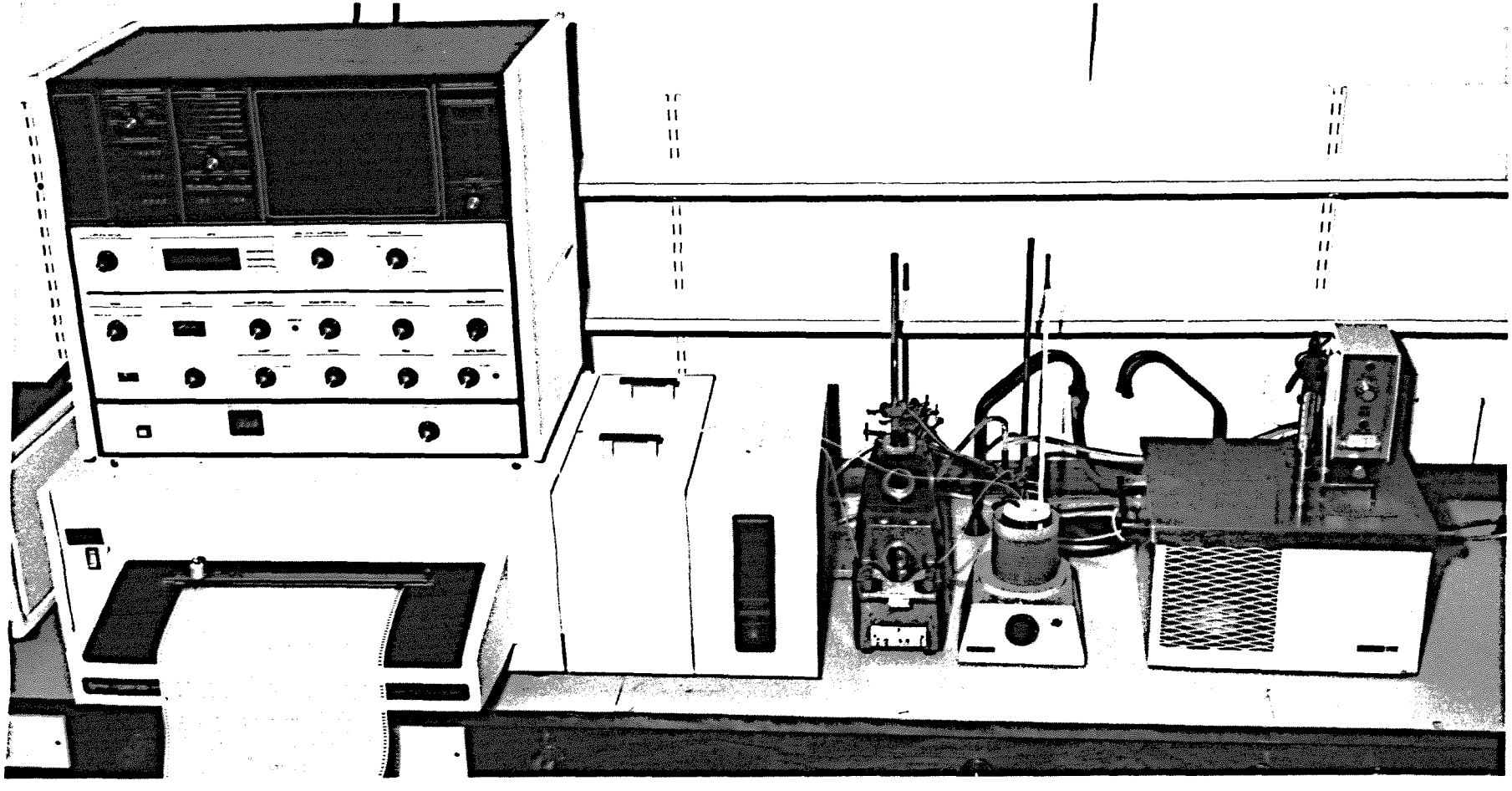


Plate 4

Filter probe set-up, used at

ICI Plant Protection, Jeolotts Hill



2. Experimental Conditions.

The procedure adopted to determine the temperature dependence of partitioning of the phenol in the aqueous/octanol and aqueous/cyclohexane system was the same.

The apparatus was assembled as in Figure 24. The aqueous and organic solvents were pre-saturated with each other. (This is particularly important in aqueous/octanol system to prevent errors in phase volume ratios). This was done by shaking the aqueous phase with the appropriate organic solvent in a separating funnel and leaving to equilibrate for 24 hours.

Solute solutions were prepared. The λ_{max} of the solute at 220 nm (see Table 14) in 0.15 M sodium chloride solution was determined on a scanning U-V spectrophotometer, and then on the spectrophotometer used in the filter probe circuit. A calibration curve was prepared for the solute at this wavelength. The spectrophotometer was maintained at this detection wavelength for the experiment.

The thermo-circulator control was adjusted to give a circulating temperature of approx. 10°C, i.e. the lowest temperature for first measurement. The magnetic "flea" was introduced into the mixing chamber. The appropriate volumes of organic and aqueous solvents were pipetted into the mixing chamber. The filter probe, with attached wetted filter paper, and temperature probe (or thermometer) were introduced into the mixing chamber. The lid was fitted and the circuit was completed. The stirrer was started and an intimately mixed solution of aqueous and organic phase was produced. The peristaltic pump was switched on and the aqueous phase

was extracted from the mixture. This was pumped through the flow cell of the spectrophotometer and returned to the mixing chamber. A flow rate of between 1 - 1.5 ml/min was established. The absorbance and transmittance controls of spectrophotometer were adjusted to give zero absorbance, and 100% transmission respectively. The temperature of circulating solvents was noted. Solute was injected when temperature remained constant. A known volume of solute solution (between 1 ml - 10 ml) dissolved in either aqueous or organic solvent (depending upon which solute was most soluble) was taken up into a glass syringe (Sigma). This was then weighed. The contents of the syringe were injected into the mixing chamber, through a small hole in the perspex lid. The empty syringe was weighed. From the two weighings the exact weight of solute solution injected could be calculated. The solute distributed itself between the aqueous and solvent phases, and equilibrium was reached (within 2 - 10 minutes) when the absorbance reading remained constant. The solute concentration in the aqueous phase could be determined from the absorbance reading, and referring to the calibration graph. The solute concentration in the organic phase could be determined by mass balance. The partition coefficient, at a particular temperature of measurement, could then be determined. The dial control of thermo-circulator was adjusted to increase temperature of circulating solvents 3 or 4°C. When equilibrium had been reached at the new temperature, and absorbance reading remained constant, the new aqueous concentration was determined. The partition coefficient was determined at this higher temperature.

The procedure was repeated several times to obtain

partition coefficient values at nine to ten temperatures in the range 10° - 40°C. From the temperature dependence of the partitioning the enthalpy of transfer could be determined.

The filter probe, thermometer, lid and magnetic flea were removed and cleaned. The mixing chamber was emptied of its contents and cleaned. The teflon tubing and flow cell were cleaned by circulating distilled water, and drained thoroughly by connecting to vacuum line. The apparatus was now ready to use for the next temperature dependence study.

The aqueous/n-octanol and aqueous/cyclohexane systems were studied, and those solutes as mentioned in Results section.

The effect of determining the partition coefficients at the highest temperature first and then working down to the lowest temperature was investigated. It was found that the same partition coefficient values were obtained, but equilibrium times were slightly different.

The filter probe technique had the following advantages :

(i) It was relatively simple and quick to use.

(ii) Relatively inexpensive instrumentation was necessary.

(iii) Very accurate and precise partition coefficient values were produced as is necessary in a thermodynamic study.

(iv) It was possible to obtain partition coefficients in the range -3.0 - 3.0 log units, by varying phase ratios and volumes, and solute concentrations.

(v) It was possible to use low solute concentrations, which eliminated the risk of solute association in the organic phase.

(vi) The partition coefficient and enthalpy values were found

to be reproducible each time.

(vii) Precise temperature control was possible and the aqueous phase was monitored at the temperature of the partitioning process.

(viii) The circulating volume in the tubing was low, so that equilibrium was reached quickly, and the temperature had no time to change.

(viv) Cleaning of the apparatus was simple and thorough, and no residual solvents were left in the system.

(x) Complete phase separation was effected for both the aqueous/n-octanol and aqueous/cyclohexane systems.

The filter probe method has a number of disadvantages :

(i) Although adsorption onto silicone rubber tubing was not found to be a problem with the phenolic compounds studied, several authors have stated that this is a problem with certain compounds (Tomlinson, 1982).

(ii) Condensation of water onto the cu vette at lower temperatures (10°C) was a problem. This was avoided by performing the experiment as soon as possible after temperature equilibrium had been reached .

(iii) Although the system was not studied at high temperatures (i.e. above 40°C), slight condensation of solvents was found to occur onto the lid of the mixing chamber.

(iv) Extraction of organic phase was not possible with the peristaltic pump.

5.6. The Determination of the Temperature Dependence of the Solubility of Water in n-Octanol by a Coulometric Karl Fischer Method - The Mitsubishi Moisture Meter Model CA-02.

The Karl Fischer titration method is widely used as one of the most reliable procedures for the determination of low concentrations of water. However, this method involves some troublesome procedures such as regular calibration of reagents and closing off the burettes and titration cell from atmospheric moisture.

The Mitsubishi Moisture Meter Model CA-02 (see Figure 27) combines coulometry with Karl Fischer Titration in order to eliminate these troublesome and time-consuming procedures.

The fully automatic instrument enables fast and accurate analysis to be carried out. The adoption of a high electrolysis current, and automatic control of that current, ensures a fast analysis time, high accuracy (within 5 μg for 10 μg - 1 mg and within 0.5% for 1 - 30 mg) and trace level sensitivity (1 μg H_2O) for the detection of water content.

1. Principle of Coulometric Karl Fischer Measurement.

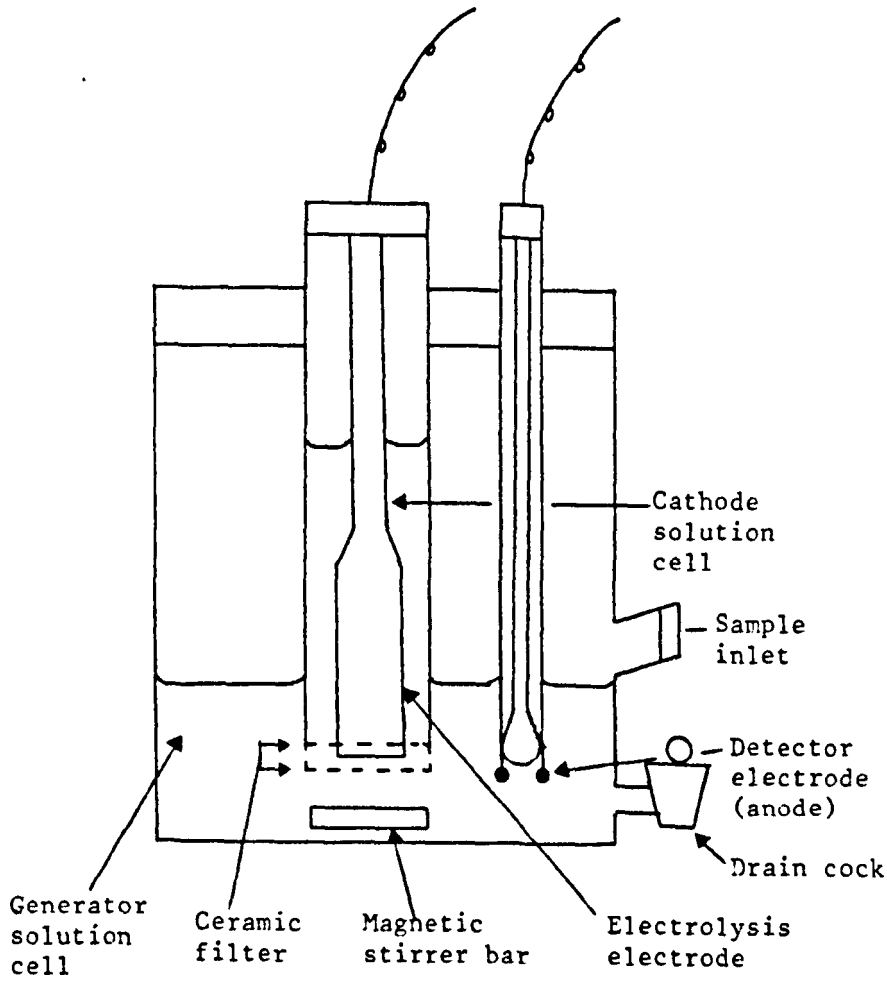
Karl Fischer reagent reacts with water as follows :

$$I_2 + SO_2 + 3\ C_5H_5N + H_2O \rightarrow 2\ C_5H_5N.HI + C_5H_5N.SO_3 \qquad (1)$$

$$C_5H_5N.SO_3 + CH_3OH \rightarrow C_5H_5N.HSO_4CH_3 \qquad (2)$$

In Coulometric Karl Fischer titration, the sample is added to a pyridine-methanol solution (with iodide ion, I^-, and sulphur dioxide as principal components). The iodine, generated electrolytically at the anode, reacts with the water in the

Figure 27. Mitsubishi Moisture Meter Model CA-02.



sample, as shown in Equations (1) and (2).

Iodine is generated in direct proportion to the quantity of electricity according to Faraday's Law.

$$2I^- - 2e \rightarrow I_2 \qquad (3)$$

One mole of iodine reacts quantitatively to one mole of water. Therefore, 1 mg of water is equivalent to 10.71 coulombs. Based on this principle, water can be directly determined from the quantity of electricity (coulombs) required for electrolysis.

2. Operation.

Volumes of generator solution (Aquamicron A) and cathode solution (Aquamicron C) were pipetted into the generator (anode) and cathode cell respectively. The voltmeter displays the potential difference (p.d.) across the dual-platinum sensor electrode (anode). The sample is injected into generator cell and excess water in the cell will produce a high p.d., up to 5.5 mV. This p.d. will decrease as the concentration of water in the cell decreases, eventually reaching zero. A high p.d. across the sensor electrolysis, will cause an electrolysis current, of up to 300 mA, to be automatically selected, according to the water content of the sample, and will decrease in direct proportion to the residual water content until the end point is reached. The end point, i.e. the point where the electrolysis current falls to the first blank current level, is indicated by a buzzer and the result, in micrograms of water is digitally displayed.

3. Sample Preparation.

Small volumes of n-octanol (2 ml) and 0.15 M sodium chloride solution (2 ml) were pipetted into scintillation vials, with screw tops and aluminium inserts. These were then incubated for 48 hrs. at the following temperatures.

10°, 20°, 30° and 40°C.

Immediately after incubation, a 20 µl sample was taken from the octanol layer, using a 100 µl. Hamilton syringe, taking care not to disturb the aqueous layer. The syringe was weighed on a microbalance, and its contents were injected into the generator solution cell of Moisturemeter. (Care was taken not to leave drops on end of the needle). The weight, in µg, of water in sample was displayed within a few minutes. The empty syringe was weighed again. From the difference in the two weighings, the precise weight of octanol injected was calculated.

This procedure was repeated at least five times for each temperature.

5.7. The Determination of Partition Coefficients in the Water/Octanol System using an HPLC Method.

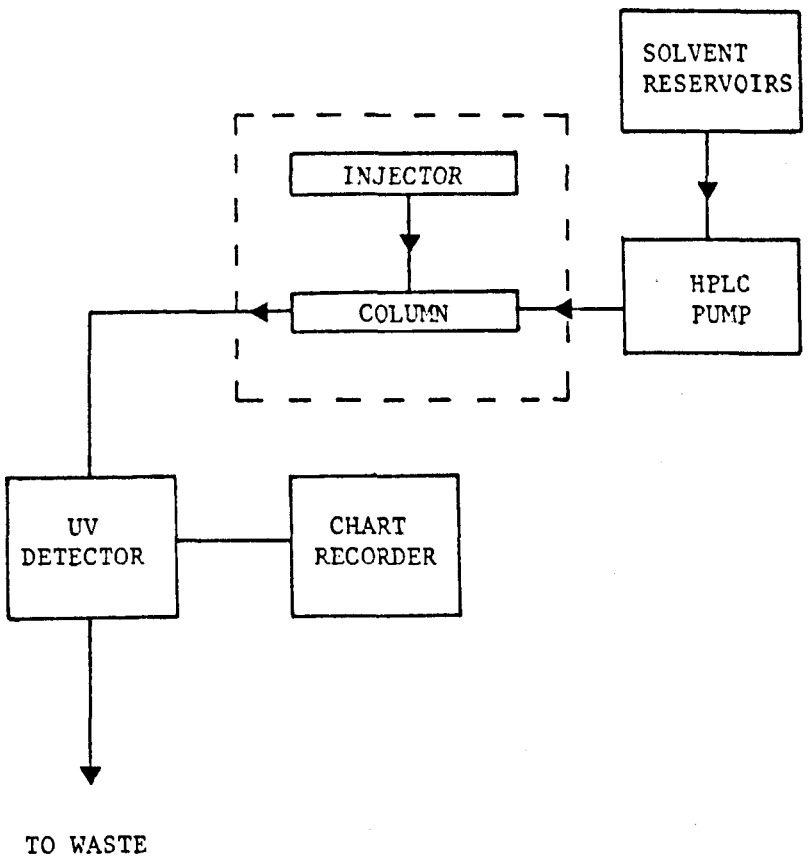
In this technique the retention time of a solute eluted on an HPLC column is related directly to its partition coefficient (Mirrlees et al., 1976; Unger et al., 1978; Miyake and Terada, 1978). The organic phase is a silica gel support, coated with n-octanol, and the aqueous phase is the eluting solvent.

The apparatus consisted basically of an HPLC pump (Waters),

a ultra-violet detector (Pye-Unicam), a chart recorder (Perkin-Elmer), an HPLC column (10 cm x 0.45 cm), an injection valve (Altex) with 10 µl. and 50 µl. injection loops and solvent reservoirs. The various components were connected together using fine bore, stainless steel capillary tubing, and assembled as shown in Figure 28. Solvents were delivered from the reservoir to HPLC pump via large bore teflon tubing. The column and injection valve were submerged in a water bath (Grant), in which the water temperature was controlled by water circulating from a thermo-circulator (Churchill). Care was taken to ensure that injection port of injection valve was above the level of water. The HPLC column (10 cm. x 0.45 cm.) was packed with Lichrosphere S.I. 1000 (Merck), a silica gel base, silanised with trimethyl silyl chloride (TMSC). The column was introduced into the system, and washed with deionised double-distilled water. (N.B. All solvents were filtered on a sinter funnel and degassed in an sonic bath before use). The aqueous mobile phase, 0.15 M sodium chloride solution saturated with n-octanol (Fisons) was eluted through the system at a flow rate of 2 ml./min. Small volumes of n-octanol (50 µl) were injected onto the column at intervals of 5 minutes until a total volume of 0.75 ml. was loaded. Initially excess octanol was found to come off the column, as observed by the erratic response of the ultra-violet detector. After 4 hours the base line remained constant, the column had been successfully coated and was ready for use.

The instrumental conditions used for the experiment were

Figure 28. Schematic diagram of HPLC system used to determine partition coefficients.



as follows :

Ultra-violet detector - λ_{max} 220 nm
Absorbance range 1.28

HPLC pump - flow rate 2 ml./min.

Recorder - 5 mm./min.

Water bath temperature - 20°C

Injection loop - 10 µl

The solute concentrations were chosen to give reasonable detector response and peak size. For the solutes under study concentrations of between 1 - 10 mg per 100 ml. sodium chloride solution were found to be suitable. The solutes benzamide, phenol, p-cresol and p-chloro phenol were dissolved in 0.15 M sodium chloride solution, together with sodium nitrate (zero marker). A small volume (10 µl) of this solution was injected onto the column using a Hamilton syringe. At the point of injection the chart recorder was started. The retention times for various solutes were recorded. (The retention time for each solute had been found previously, by injecting the solutes individually onto the column in separate experiments).

The peaks were found to be rather broad and slightly tailing. This made it difficult to detect small changes in retention time, that would be expected if the experiment was carried out at different temperatures as would be required in a thermodynamic study. Several attempts were made to record the column and improve the peak shapes, but this proved unsuccessful. Although the method was unsuitable for precise thermodynamic measurements, a good correlation between log R.T. and log K_d was observed.

Chapter Six

RESULTS

THE TEMPERATURE DEPENDENCE OF PARTITIONING OF PHENOLIC SOLUTES IN THE WATER/LIPOSOME SYSTEM

Table 17

Liposome experiment 1.

Temp °C	1/T x10^3	p-cresol		p-ethyl phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	14.55	2.678	58.44	4.068
10	3.532	51.20	3.936	184.78	5.219
14	3.483	47.62	3.863	200.45	5.300
18	3.435	212.96	5.361	331.39	3.803
22	3.388	276.32	5.622	507.58	6.229

Temp °C	1/T x10^3	p-propyl phenol		p-iso-propyl phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	318.18	5.763	519.23	6.252
10	3.532	331.39	5.803	569.67	6.345
14	3.483	359.76	5.885	659.09	6.491
18	3.435	543.65	6.298	770.41	6.647
22	3.388	659.09	6.491	1362.9	7.217

K_d is in molal units

Table 18

Liposome experiment 2.

Temp °C	1/T x10^3	p-cresol		o-cresol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	27.78	3.324	44.11	3.787
10	3.532	68.47	4.226	72.58	4.285
14	3.483	72.58	4.285	78.95	4.369
18	3.435	115.01	4.745	83.33	4.423
22	3.388	188.10	5.237	107.14	4.674

Temp °C	1/T x10^3	m-cresol	
		K_d	Ln K_d
5	3.595	13.16	2.577
10	3.532	31.07	3.436
14	3.483	51.20	3.936
18	3.435	54.88	4.005
22	3.388	76.79	4.341

Table 19

Liposome experiment 3.

Temp °C	1/T x10^3	p-cresol		p-bromo phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	14.55	2.678	507.58	6.229
10	3.532	83.33	4.423	627.19	6.441
14	3.483	102.11	4.626	659.09	6.491
18	3.435	120.37	4.79	675.93	6.516
22	3.388	170.17	5.136	770.40	6.647

Temp °C	1/T x10^3	p-chloro phenol		p-fluoro phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	305.55	5.722	83.33	4.423
10	3.532	407.89	6.011	102.11	4.626
14	3.483	416.66	6.032	134.6	4.902
18	3.435	464.29	6.141	153.22	5.032
22	3.388	496.27	6.207	173.72	5.157

Table 20

Liposome experiment 4.

Temp °C	1/T x10^3	p-cresol		p-cyano phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	15.96	2.770	17.38	2.855
10	3.532	51.20	3.936	39.01	3.664
14	3.483	60.55	4.103	66.46	4.197
18	3.435	212.96	5.361	250.00	5.521
22	3.388	235.0	5.459	270.83	5.601

Temp °C	1/T x10^3	p-hydroxy benzo-trifluoride	
		K_d	Ln K_d
5	3.595	287.5	7.964
10	3.532	1362.9	7.217
14	3.483	1923.9	7.562
18	3.435	2022.7	7.612
22	3.388	2250.0	7.719

Table 21

Liposome experiment 5.

Temp °C	1/T x10^3	p-cresol		p-nitro phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	30.89	3.430	230.77	3.441
10	3.532	76.79	4.341	250.0	5.520
14	3.483	83.33	4.422	245.05	5.501
18	3.435	120.37	4.791	276.88	5.624
22	3.388	197.35	5.285	293.48	5.682

Temp °C	1/T x10^3	p-methoxy phenol	
		K_d	Ln K_d
5	3.595	3.353	1.210
10	3.532	6.883	1.929
14	3.483	20.27	3.009
18	3.435	45.86	3.826
22	3.388	117.65	4.768

Table 22

Liposome experiment 6.

Temp °C	1/T x10^3	p-cresol		resorcinol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	18.82	2.935	78.95	4.369
10	3.532	47.62	3.863	92.47	4.527
14	3.483	58.64	4.070	112.31	4.721
18	3.435	196.43	5.280	299.45	5.702
22	3.388	281.90	5.642	359.76	5.885

Temp °C	1/T x10^3	m-methoxy phenol		m-ethoxy phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	7.732	2.045	18.81	2.934
10	3.532	44.11	3.787	83.33	4.423
14	3.483	70.12	4.250	87.83	4.475
18	3.435	204.55	5.321	230.77	5.441
22	3.388	240.19	5.483	293.48	5.682

Table 23

Liposome experiment 7.

Temp °C	1/T x10^3	p-cresol		p-hydroxy benzoic acid, methyl ester	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	17.38	2.855	382.9	5.948
10	3.532	58.64	4.071	382.91	5.948
14	3.483	66.46	4.197	375.00	5.927
18	3.435	78.95	4.369	367.28	5.906
22	3.388	117.65	4.768	359.76	5.885

Temp °C	1/T x10^3	p-hydroxy acetanilide		p-hydroxy acetophenone	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	13.16	2.577	29.33	3.379
10	3.532	14.55	2.678	27.78	3.324
14	3.483	15.96	2.770	27.78	3.324
18	3.435	14.55	2.678	21.74	3.079
22	3.388	14.55	2.678	17.38	2.855

Table 24

Liposome experiment 8.

Temp °C	1/T x10^3	p-cresol		p-bromo anisole	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	13.16	2.577	170.16	5.137
10	3.532	58.64	4.071	444.44	6.097
14	3.483	92.46	4.527	711.54	6.567
18	3.435	117.65	4.768	1000.	6.908
22	3.388	173.73	5.157	1750.	7.467

Temp °C	1/T x10^3	p-chloro anisole		p-fluoro anisole	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	163.22	5.096	221.69	5.401
10	3.532	416.66	6.032	474.64	6.163
14	3.483	791.66	6.674	1000.	6.908
18	3.435	1265.15	7.143	2527.77	7.835
22	3.388	2130.95	7.664	9750.	9.185

Table 25

Liposome experiment 9.

Temp °C	1/T x10^3	p-ethyl phenol		p-methoxy phenol	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	159.12	5.070	3.265	1.183
10	3.532	176.92	5.176	10.46	2.348
14	3.483	182.98	5.209	25.78	3.250
18	3.435	315.27	5.753	45.48	3.817
22	3.388	342.72	5.837	90.93	4.510

Temp °C	1/T x10^3	p-nitro phenol	
		K_d	Ln K_d
5	3.595	266.18	5.584
10	3.532	286.04	5.656
14	3.483	294.42	5.685
18	3.435	298.71	5.699
22	3.388	307.48	5.728

Table 26

Liposome experiment 10.

Temp °C	1/T x10^3	p-ethyl phenol		p-propyl anisole	
		K_d	Ln K_d	K_d	Ln K_d
5	3.595	126.85	4.840	1250.	7.131
10	3.532	187.46	5.234	2250.	7.719
14	3.483	212.50	5.359	2428.6	7.795
18	3.435	290.26	5.671	4437.5	8.398
22	3.388	366.66	5.904	6000.0	8.699

Temp °C	1/T x10^3	p-nitro anisole	
		K_d	Ln K_d
5	3.595	21.43	3.065
10	3.532	35.59	3.572
14	3.483	58.47	4.069
18	3.435	68.75	4.230
22	3.388	82.61	4.414

Figure 29

Van't Hoff plots for liposome experiment 1.

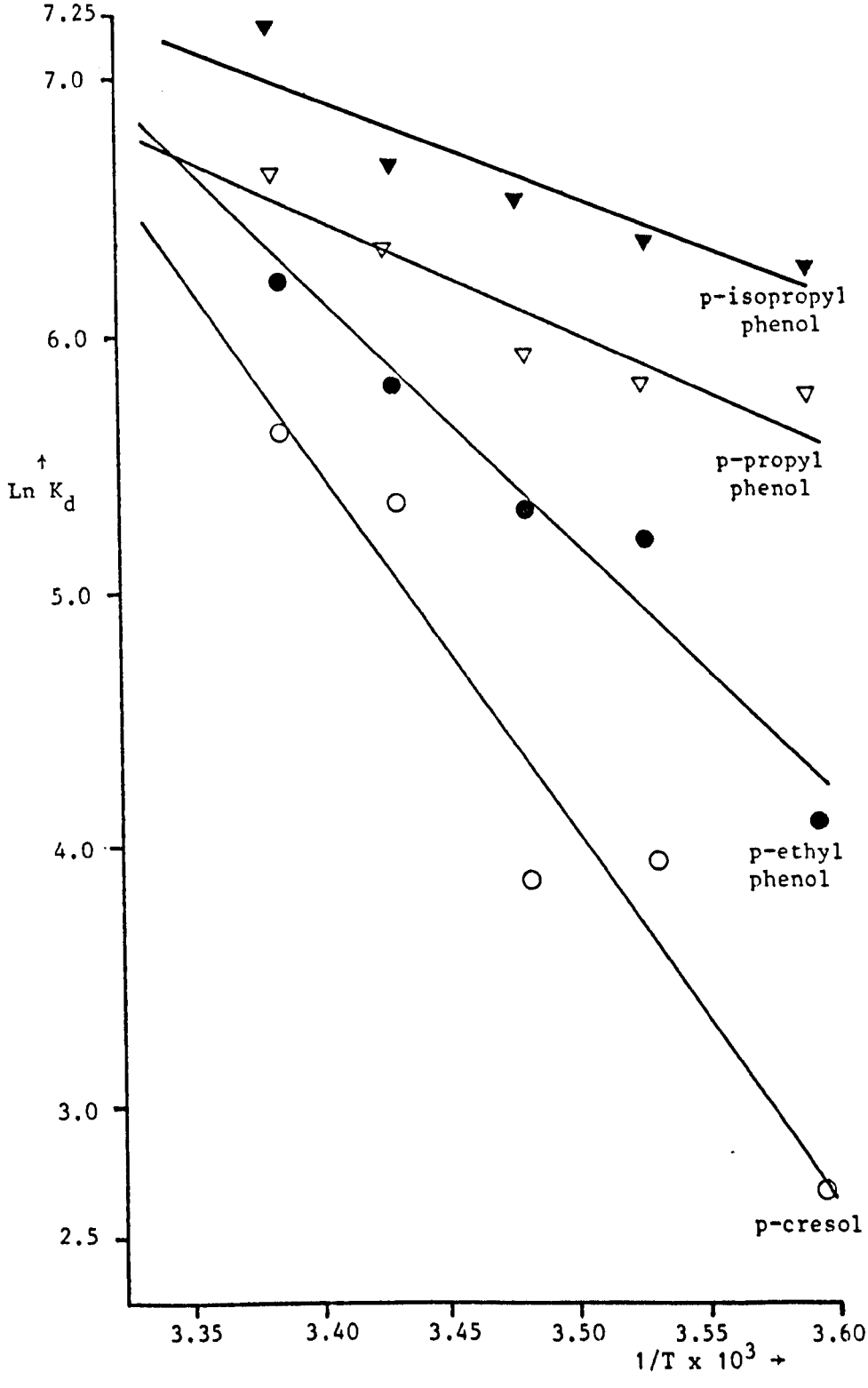


Figure 30

Van't Hoff plots for liposome experiment 2.

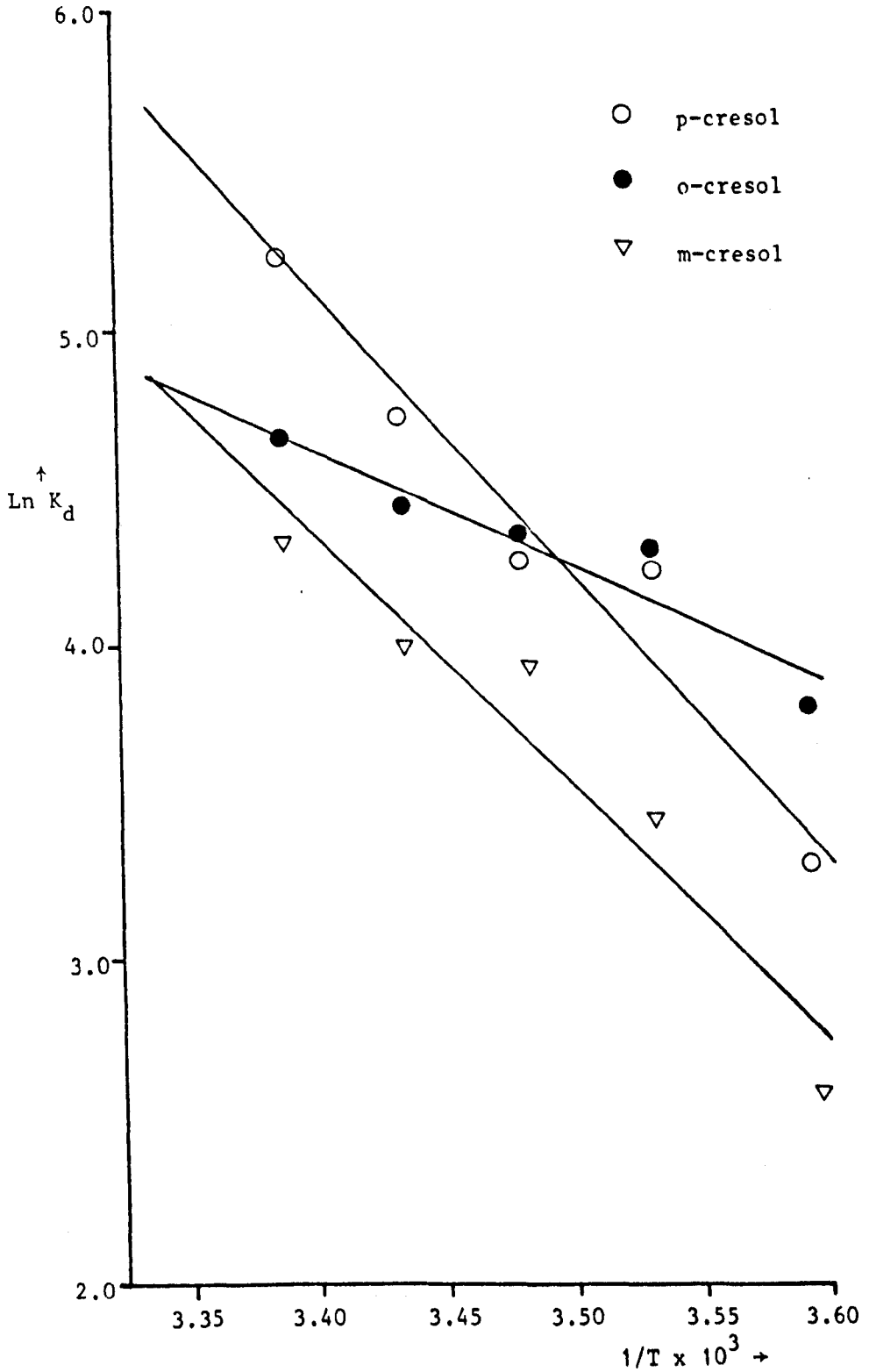


Figure 31

Van't Hoff plots for liposome experiment 3.

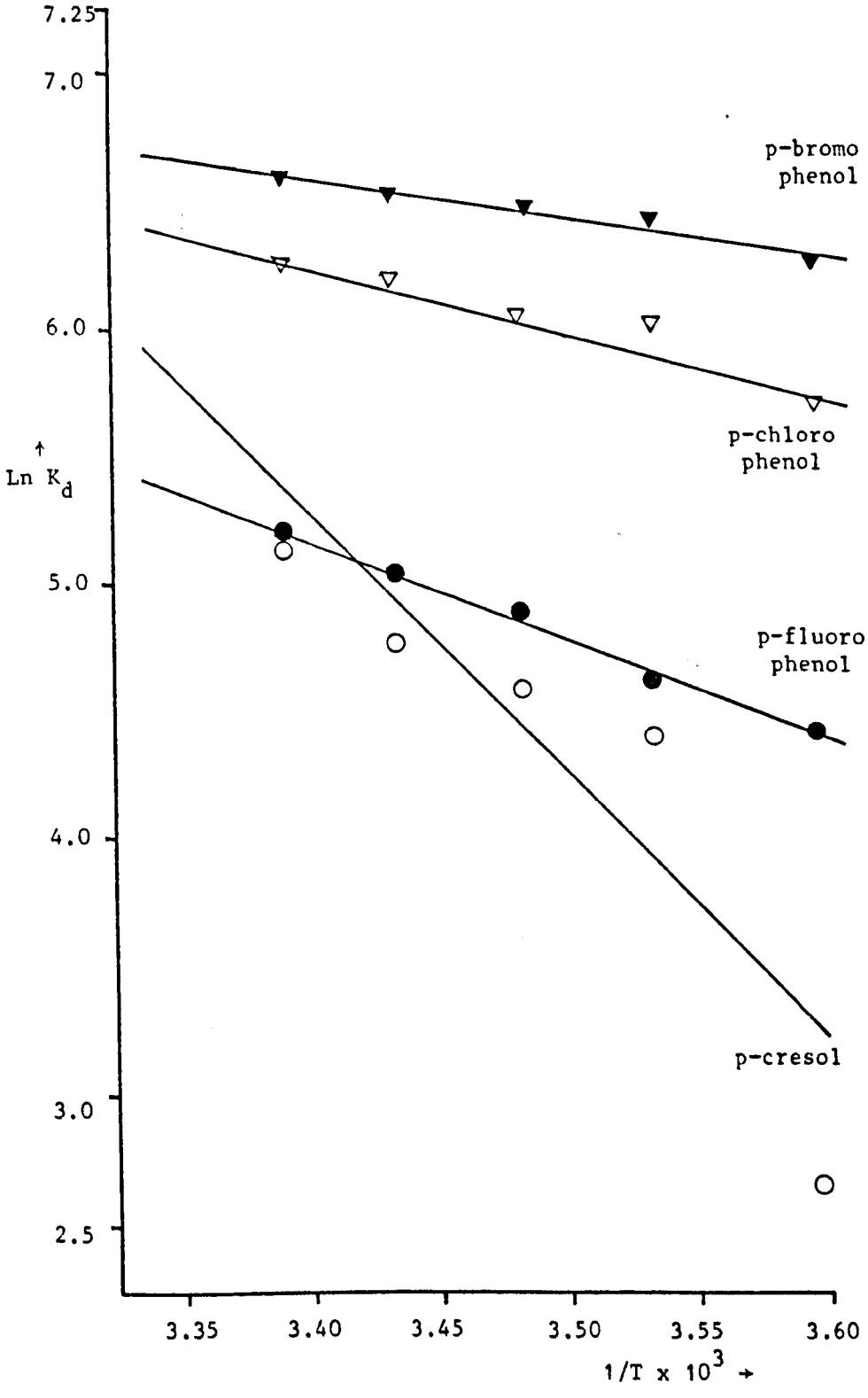


Figure 32

Van't Hoff plots for liposome experiment 4.

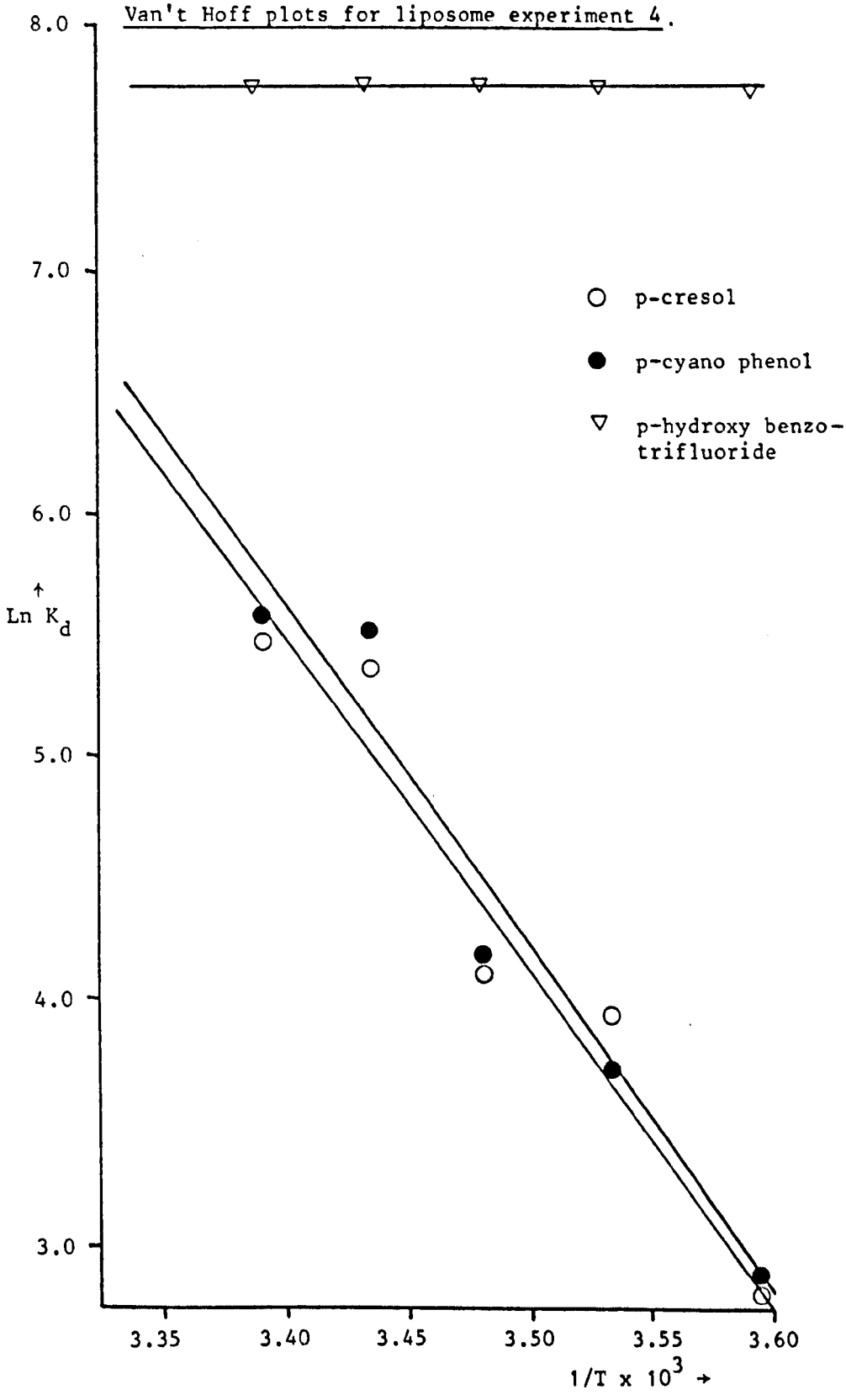


Figure 33

Van't Hoff plots for liposome experiment 5.

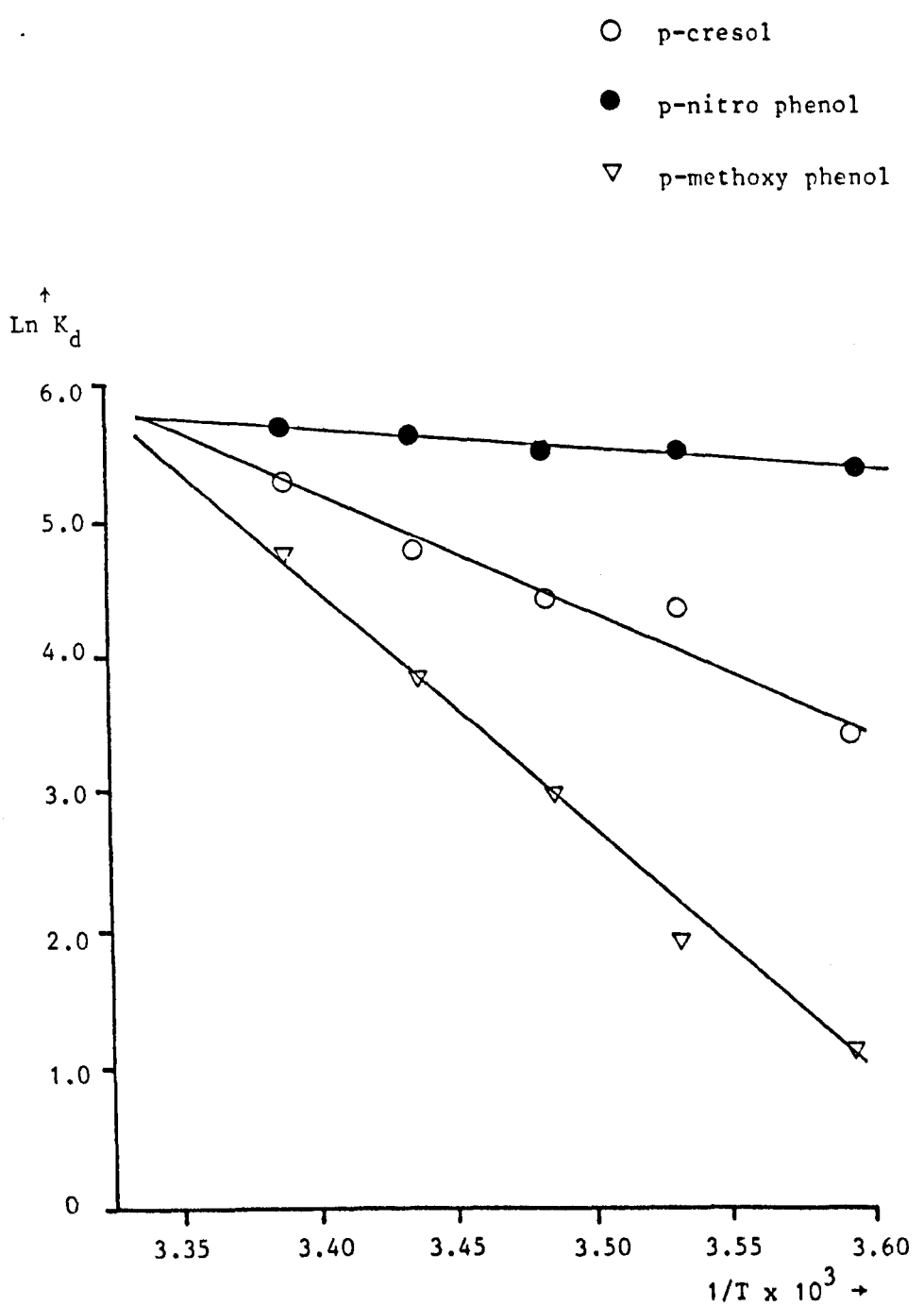


Figure 34

Van't Hoff plots for liposome experiment 6.

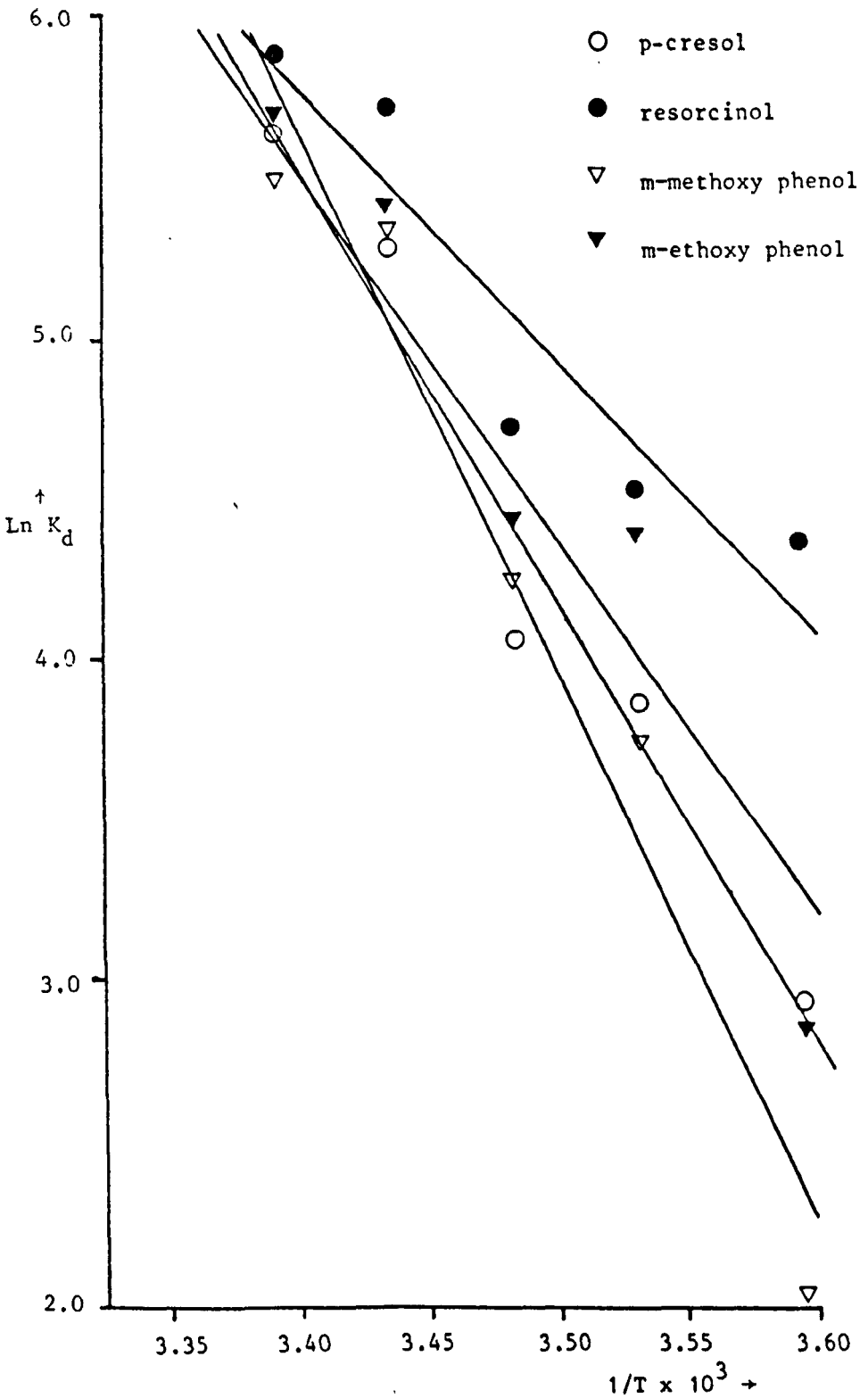


Figure 35

Van't Hoff plots for liposome experiment 7.

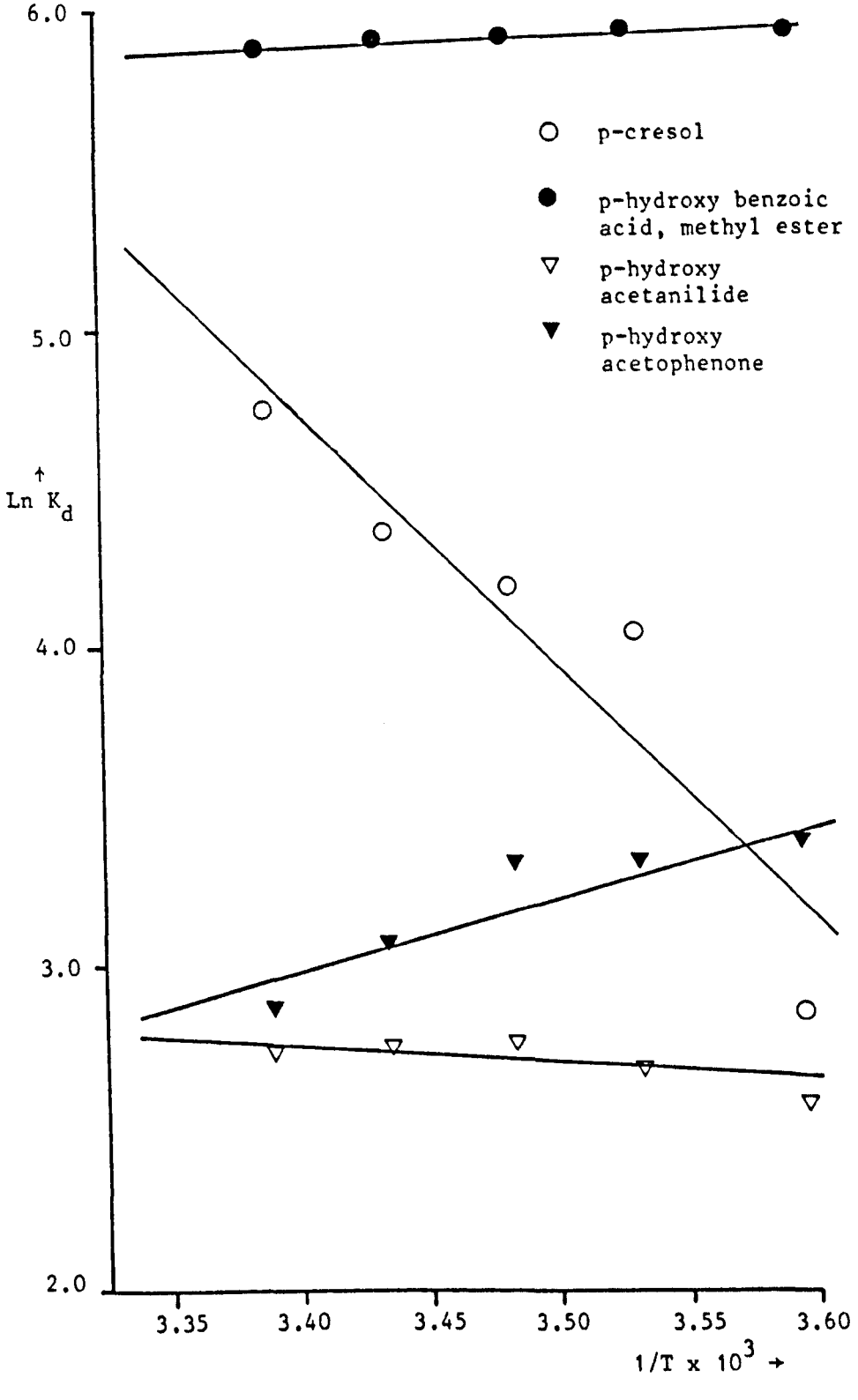


Figure 36

Van't Hoff plots for liposome experiment 8.

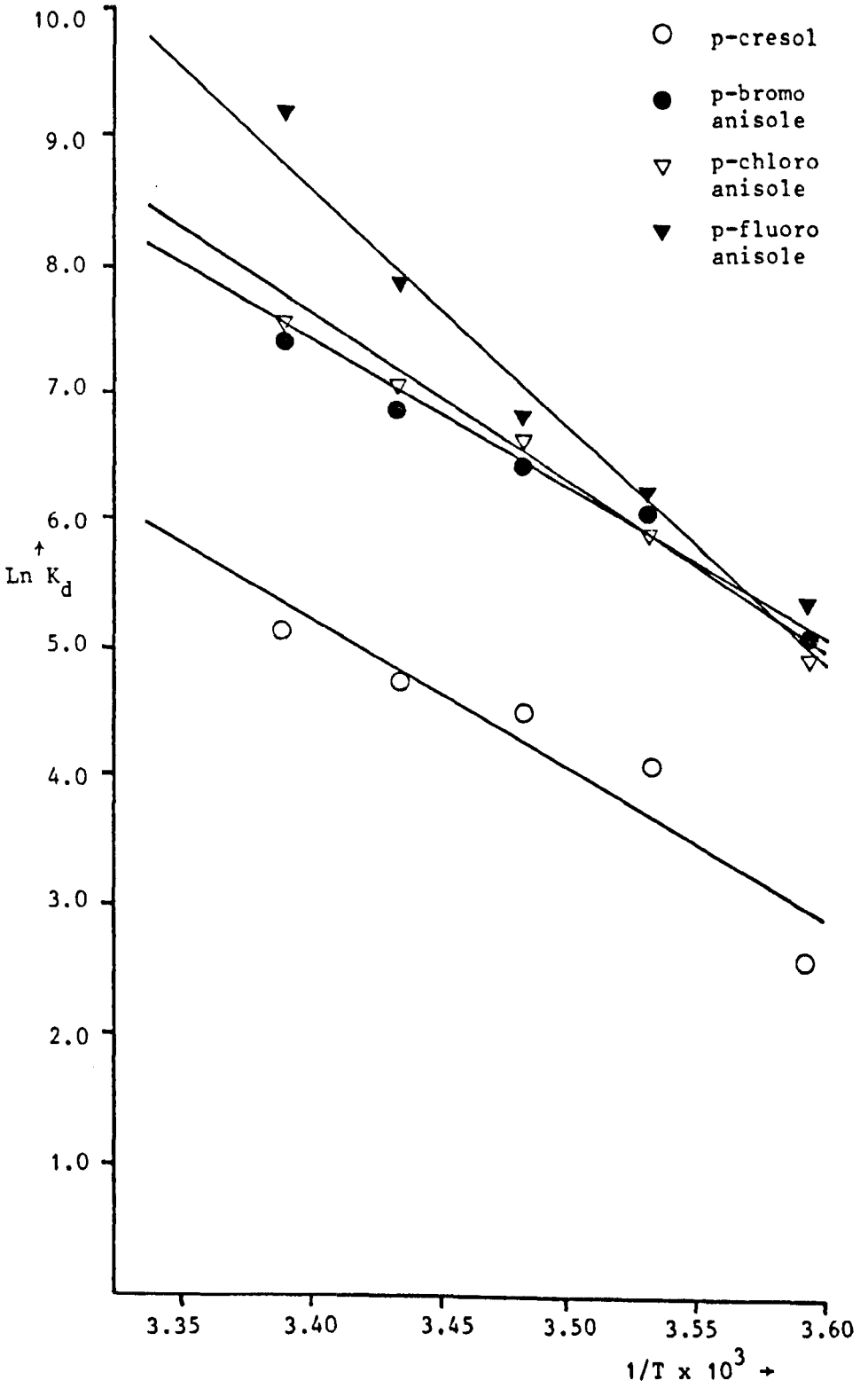


Figure 37

Van't Hoff plots for liposome experiment 9.

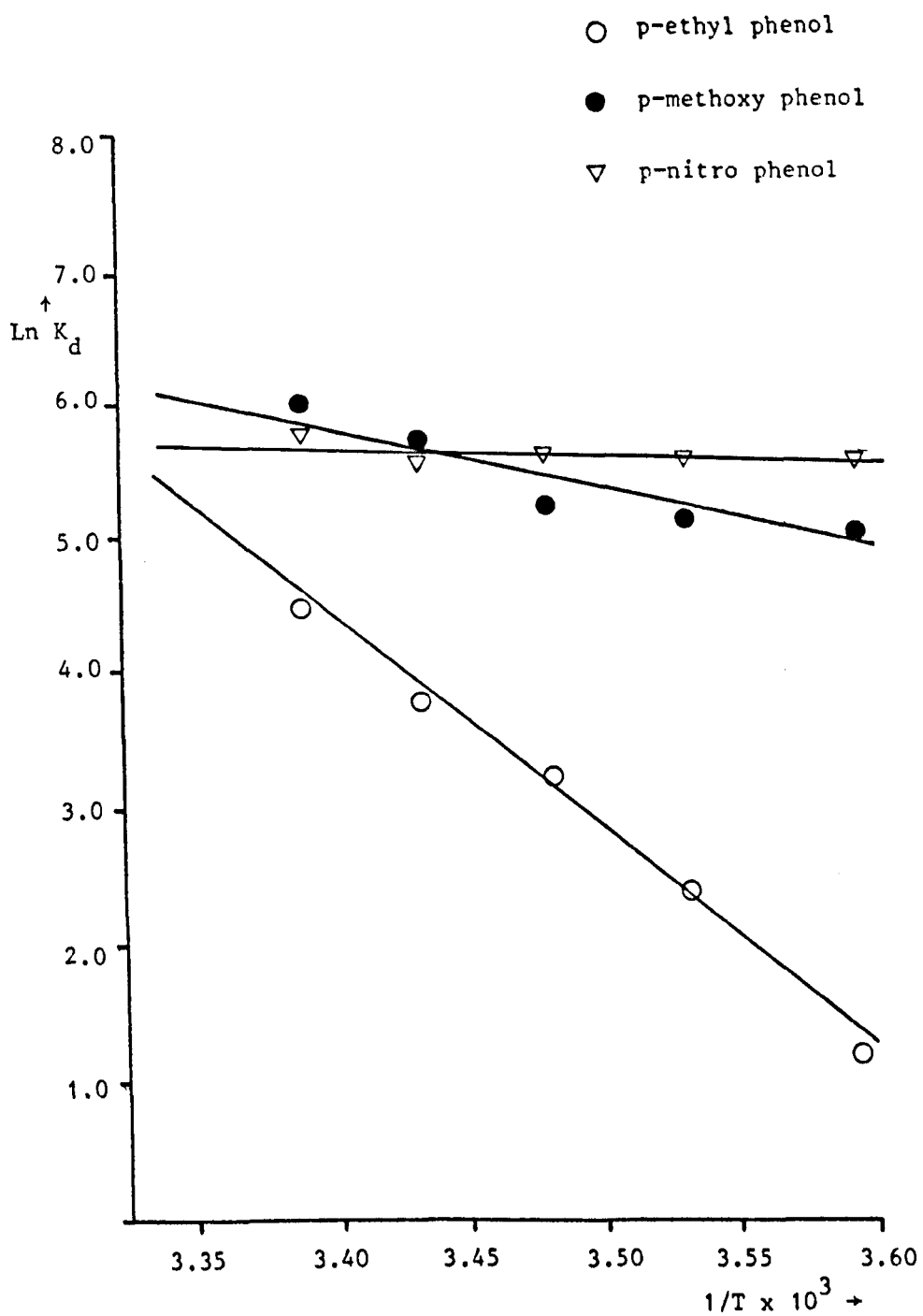
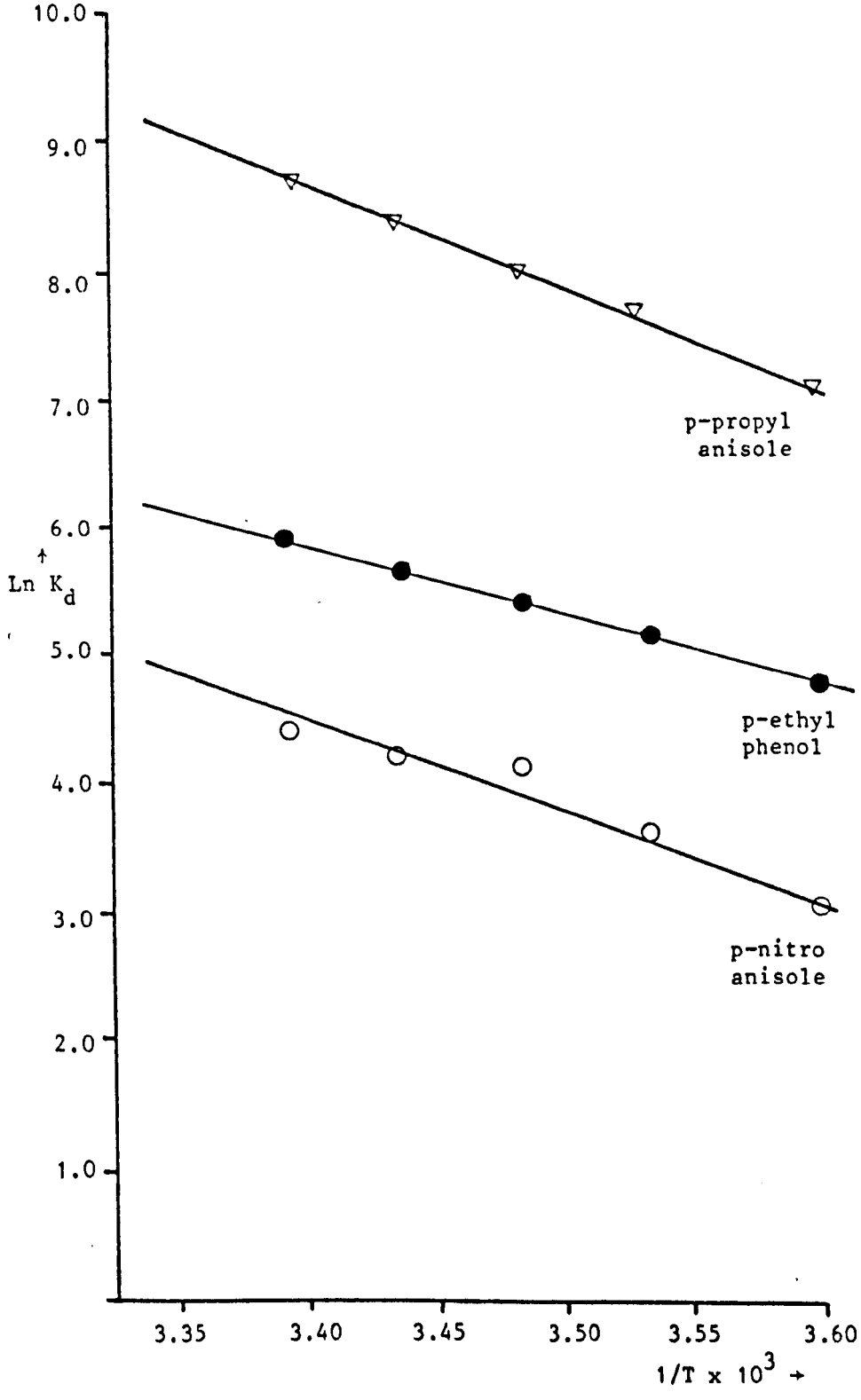


Figure 38

Van't Hoff plots for liposome experiment 10.



THE TEMPERATURE DEPENDENCE OF PARTITIONING OF PHENOLIC SOLUTES IN THE WATER/OCTANOL SYSTEM

(FILTER PROBE TECHNIQUE)

Table 27

Phenol, aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
9.25	3.541	40.92	355.35	5.873
11.65	3.512	38.89	338.45	5.824
13.75	3.486	38.08	331.71	5.804
17.0	3.446	37.29	325.45	5.785
18.5	3.429	36.68	320.44	5.769
22.2	3.386	35.79	313.57	5.748
23.0	3.377	35.21	308.42	5.731
29.2	3.307	35.27	292.36	5.678
32.95	3.267	32.22	283.86	5.648
35.40	3.241	31.71	279.81	5.634
37.20	3.222	30.72	271.23	5.603

Table 28

p-cresol, aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.2	3.517	109.83	954.65	6.861
14.2	3.480	105.17	916.62	6.820
17.6	3.439	102.24	892.25	6.794
18.3	3.431	100.83	888.27	6.780
21.6	3.393	96.79	847.09	6.742
23.3	3.373	96.14	842.37	6.736
28.0	3.321	92.40	812.24	6.699
32.0	3.277	88.34	777.55	6.656
34.6	3.249	85.61	754.74	6.626
37.2	3.222	83.02	733.00	6.597
40.6	3.187	80.07	707.82	6.562
41.7	3.176	79.59	704.11	6.557

K_d^m molar units; K_d^x mole fraction units

Table 29

o-cresol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
12.3	3.503	119.86	1042.98	6.950
14.9	3.472	116.05	1011.94	6.920
16.5	3.452	115.14	1004.94	6.913
17.5	3.441	113.37	989.88	6.898
22.5	3.382	108.21	947.97	6.854
27.0	3.332	104.22	915.35	6.819
31.0	3.283	99.05	872.06	6.771
32.9	3.267	96.28	848.21	6.743
36.0	3.235	89.29	787.77	6.669
40.5	3.188	86.38	763.65	6.638

Table 30

m-cresol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.8	3.522	119.65	1040.07	6.947
13.8	3.485	114.25	995.21	6.903
15.0	3.470	112.54	981.13	6.889
17.6	3.439	111.70	974.81	6.882
21.9	3.389	107.67	942.21	6.848
23.5	3.371	104.84	918.24	6.822
25.9	3.344	102.42	898.60	6.800
28.9	3.311	98.93	869.44	6.768
32.2	3.275	95.00	836.12	6.729
36.1	3.234	90.15	795.32	6.679
40.1	3.192	85.69	757.63	6.630

Table 31

p-ethyl phenol,aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
11.9	3.508	297.71	2587.7	7.859
16.0	3.458	294.64	2571.9	7.852
17.8	3.437	285.01	2490.1	7.820
19.4	3.418	267.30	2337.5	7.757
22.1	3.386	263.16	2302.9	7.742
24.1	3.364	255.13	2236.88	7.713
26.7	3.335	247.64	2175.0	7.685
28.0	3.321	240.73	2116.0	7.657
33.0	3.266	231.53	2039.5	7.620

Table 32

p-propyl phenol,aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
13.6	3.487	724.15	6307.90	8.749
17.4	3.442	685.40	5981.49	8.694
21.0	3.399	668.48	5851.27	8.674
27.5	3.326	612.50	5378.59	8.590
31.2	3.286	596.34	5250.00	8.566
34.3	3.253	588.55	5188.94	8.554
36.6	3.228	559.20	4932.54	8.504
40.9	3.184	532.42	4706.11	8.457
43.8	3.155	468.72	4147.68	8.330

Table 33

p-bromo phenol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.6	3.524	661.16	5747.51	8.657
12.9	3.496	663.34	5510.21	8.614
14.5	3.476	627.66	5466.79	8.606
20.5	3.405	524.71	4587.65	8.431
25.6	3.347	476.32	4179.56	8.338
27.6	3.325	455.05	3995.97	8.293
29.8	3.301	435.44	3830.57	8.251
32.0	3.277	415.12	3649.56	8.202
35.65	3.238	383.65	3385.37	8.127
37.4	3.220	359.84	3176.94	8.064

Table 34

p-chloro phenol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
14.4	3.477	434.44	3783.89	8.239
17.4	3.442	394.04	3438.79	8.143
21.7	3.392	347.14	3038.12	8.019
25.5	3.348	334.83	2938.19	7.986
31.3	3.284	302.5	2666.98	7.887
36.2	3.233	279.86	2468.84	7.812
38.0	3.214	258.46	2281.25	7.732
40.7	3.186	244.78	2163.75	7.679
43.45	3.166	219.89	1946.33	7.574
43.1	3.161	228.00	2018.45	7.610

Table 35

p-fluoro phenol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
16.5	3.462	68.37	596.73	6.391
19.0	3.423	65.11	568.70	6.343
24.0	3.365	61.16	536.25	6.285
27.0	3.332	58.73	515.82	6.246
29.8	3.301	57.00	501.43	6.217
31,5	3.282	54.81	482.48	6.179
32.9	3.267	53.77	473.70	6.161
33.3	3.263	53.25	469.05	6.151
36.8	3.226	50.79	448.49	6.106
43.5	3.158	45.13	399.46	5.990
44.3	3.150	44.77	396.66	5.983

Table 36

p-iodo phenol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.5	3.525	1;253.4	10,895.6	9.296
14.0	3.483	1,163.5	10,135.2	9.224
17.5	3.441	1,147.0	10,009.7	9.211
19.0	3.423	1,084.9	9,477.2	9.157
22.0	3.388	1,056.2	9,242.3	9.132
25.0	3.354	909.3	7,970.4	8.983
25.5	3.348	888.4	7,795.6	8.961
26.8	3.334	868.3	7,626.6	8.939
27.5	3.326	849.1	7,455.9	8.917
30.9	3.289	795.6	7,005.0	8.854
34.4	3.252	733.2	6,464.3	8.774
35.5	3.239	698.43	6,162.7	8.726

Table 37

resorcinol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.6	3.512	8.369	72.74	4.287
13.8	3.485	7.755	67.55	4.213
16.9	3.448	7.376	64.34	4.164
17.8	3.437	7.315	63.91	4.157
22.4	3.384	6.547	57.36	4.049
24.6	3.359	6.260	54.88	4.005
27.1	3.331	5.893	51.75	3.947
31.6	3.281	5.373	47.30	3.856
32.9	3.267	5.204	45.85	3.825
35.9	3.236	4.881	43.06	3.763
37.2	3.222	4.804	42.42	3.748
42.5	3.168	4.294	38.02	3.638

Table 38

m-methoxy phenol,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.5	3.513	42.63	370.54	5.915
14.9	3.472	41.55	362.31	5.893
20.9	3.401	39.26	343.66	5.840
23.6	3.369	38.08	333.91	5.811
26.3	3.339	36.73	322.22	5.775
29.1	3.309	35.45	311.53	5.742
31.3	3.285	34.25	301.51	5.709
37.1	3.223	31.67	279.62	5.633
41.3	3.180	29.68	262.63	5.571

Table 39

m-ethoxy phenol, aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
13.8	3.485	100.30	873.69	6.773
15.5	3.464	100.30	874.41	6.774
19.8	3.414	98.93	865.06	6.763
20.8	3.402	98.26	860.10	6.757
22.3	3.385	97.57	855.07	6.751
23.8	3.368	96.28	844.19	6.738
26.8	3.334	93.75	823.44	6.713
30.6	3.292	89.57	787.78	6.669
32.3	3.274	89.00	784.25	6.665
34.0	3.256	86.23	759.95	6.633
39.8	3.195	79.65	704.34	6.557
45.2	3.141	75.13	665.35	6.500
46.0	3.133	74.27	658.41	6.489

Table 40

p-methoxy phenol, aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
10.4	3.532	26.15	227.02	5.425
11.5	3.513	25.29	219.82	5.393
12.5	3.500	25.29	220.07	5.394
14.8	3.473	24.68	214.95	5.370
19.6	3.416	24.48	214.06	5.366
25.1	3.353	24.48	214.84	5.370
29.65	3.303	23.89	210.17	5.348
31.1	3.287	23.71	208.75	5.341
35.0	3.245	23.15	204.05	5.318

Table 41

p-nitro phenol, aqueous/n-octanol

Temp °C	1/T x10^3	* K_d^m	K_d^x	Ln K_d^x
12.7	3.498	141.47	1230.96	7.116
15.5	3.464	130.03	1133.72	7.033
18.8	3.425	120.42	1051.88	6.958
21.5	3.394	112.45	984.15	6.892
22.7	3.380	108.92	954.19	6.861
26.0	3.343	102.00	896.38	6.798
29.0	3.309	91.93	807.88	6.694
31.6	3.281	88.23	776.67	6.655
34.9	3.246	81.86	721.56	6.581
37.3	3.221	77.94	688.11	6.534
40.1	3.192	72.46	640.66	6.462

* corrected for ionisation

Table 42

p-hydroxy benzo-trifluoride, aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.5	3.513	1422.67	12,366.0	9.423
14.2	3.480	1378.11	12,003.7	9.393
15.3	3.467	1336.09	11,544.9	9.354
17.0	3.446	1315.96	11,485.0	9.349
21.6	3.393	1240.84	10,859.7	9.293
25.4	3.350	1142.07	10,021.9	9.213
29.1	3.309	1070.28	9,405.6	9.149
36.5	3.229	927.92	8,184.9	9.010
41.0	3.183	842.06	7,428.7	8.913

Table 43

p-hydroxy benzoic acid, methyl ester, aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
11.1	3.518	117.09	1017.82	6.925
15.0	3.470	104.69	907.08	6.810
20.3	3.405	89.58	783.22	6.663
22.8	3.379	84.10	736.71	6.602
28.2	3.318	71.52	628.69	6.444
32.7	3.269	65.24	574.83	6.354
36.8	3.226	58.62	517.66	6.249
38.4	3.210	56.54	499.58	6.214
42.8	3.165	49.65	439.59	6.085

Table 44

p-hydroxy acetophenone, aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
12.1	3.506	30.42	264.73	5.579
14.8	3.473	29.26	254.83	5.541
16.5	3.452	28.54	249.10	5.518
19.3	3.419	26.55	232.20	5.448
22.4	3.388	25.93	227.35	5.426
24.0	3.365	24.75	217.01	5.379
27.6	3.325	23.65	207.70	5.336
31.2	3.286	22.24	195.79	5.277
35.8	3.237	21.06	185.82	5.245
39.2	3.202	19.11	169.02	5.130

Table 45

p-cyano phenol, aqueous/n-octanol

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
12.2	3.504	50.12	436.13	6.078
15.35	3.466	48.00	418.53	6.037
20.0	3.411	46.50	406.60	6.008
23.8	3.367	44.73	392.20	5.972
26.55	3.337	42.82	376.12	5.930
30.0	3.299	41.14	361.89	5.891
33.2	3.264	39.90	351.50	5.862
36.3	3.232	38.42	338.93	5.826
38.3	3.211	36.98	326.79	5.789

Table 46

anisole,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.8	3.522	128.15	1113.53	7.015
13.9	3.484	125.99	1097.33	7.000
15.6	3.463	125.45	1093.92	6.998
18.9	3.424	125.45	1096.24	6.999
22.5	3.382	127.60	1117.48	7.019
26.5	3.337	128.15	1124.94	7.025
29.0	3.310	129.25	1135.57	7.035
33.2	3.264	128.69	1133.82	7.033
34.8	3.247	128.69	1134.69	7.034
38.8	3.206	128.69	1136.96	7.036
42.1	3.172	124.93	1105.37	7.008

Table 47

p-nitro anisole,aqueous/n-octanol

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.4	3.527	105.09	912.51	6.816
13.5	3.489	103.57	902.17	6.805
17.5	3.441	105.09	917.07	6.821
21.7	3.392	106.64	933.30	6.839
25.3	3.351	103.57	908.44	6.812
30.4	3.294	100.53	884.23	6.785
31.9	3.278	102.12	898.89	6.801
33.8	3.258	101.37	893.88	6.796
38.0	3.214	99.95	882.19	6.782
40.5	3.188	99.25	877.43	6.777

Figure 39

Van't Hoff plots for phenol, p-cresol, p-ethyl and p-propyl phenol distribution in the aqueous/octanol system.

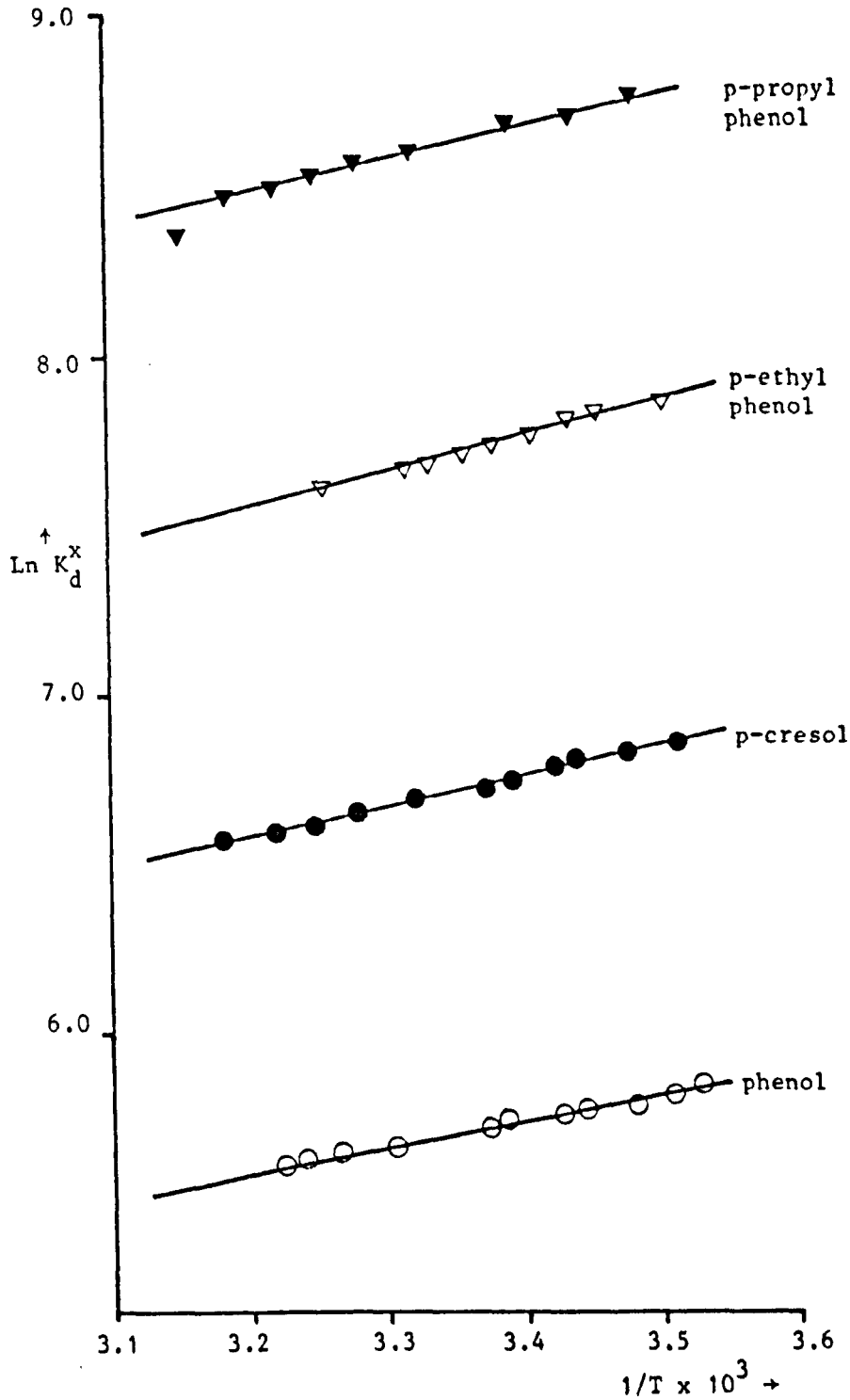


Figure 40

Van't Hoff plots for o-cresol and m-cresol distribution in the aqueous/octanol system.

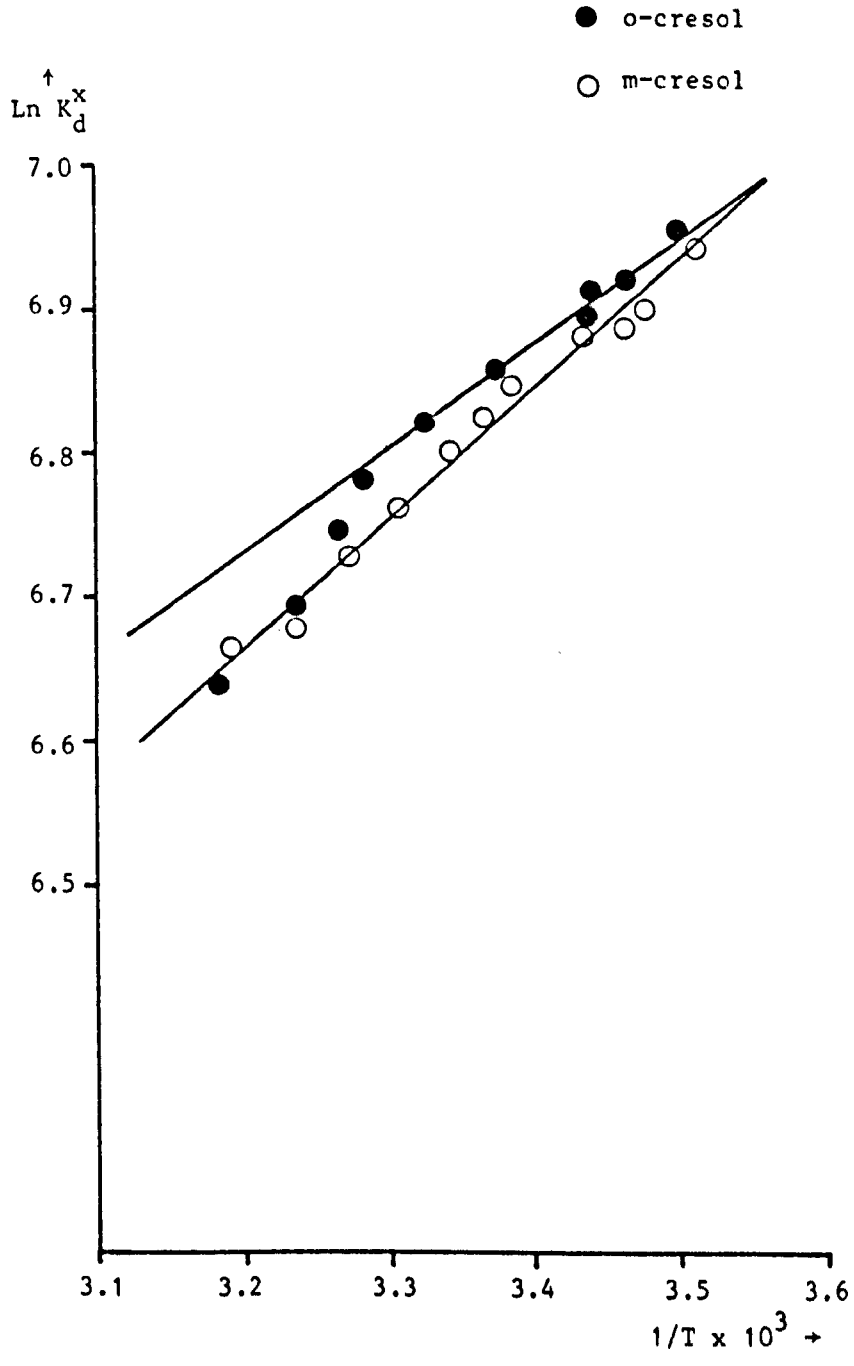


Figure 41

Van't Hoff plots for p-bromo, p-chloro, p-fluoro and p-iodo phenol distribution in the aqueous/octanol system.

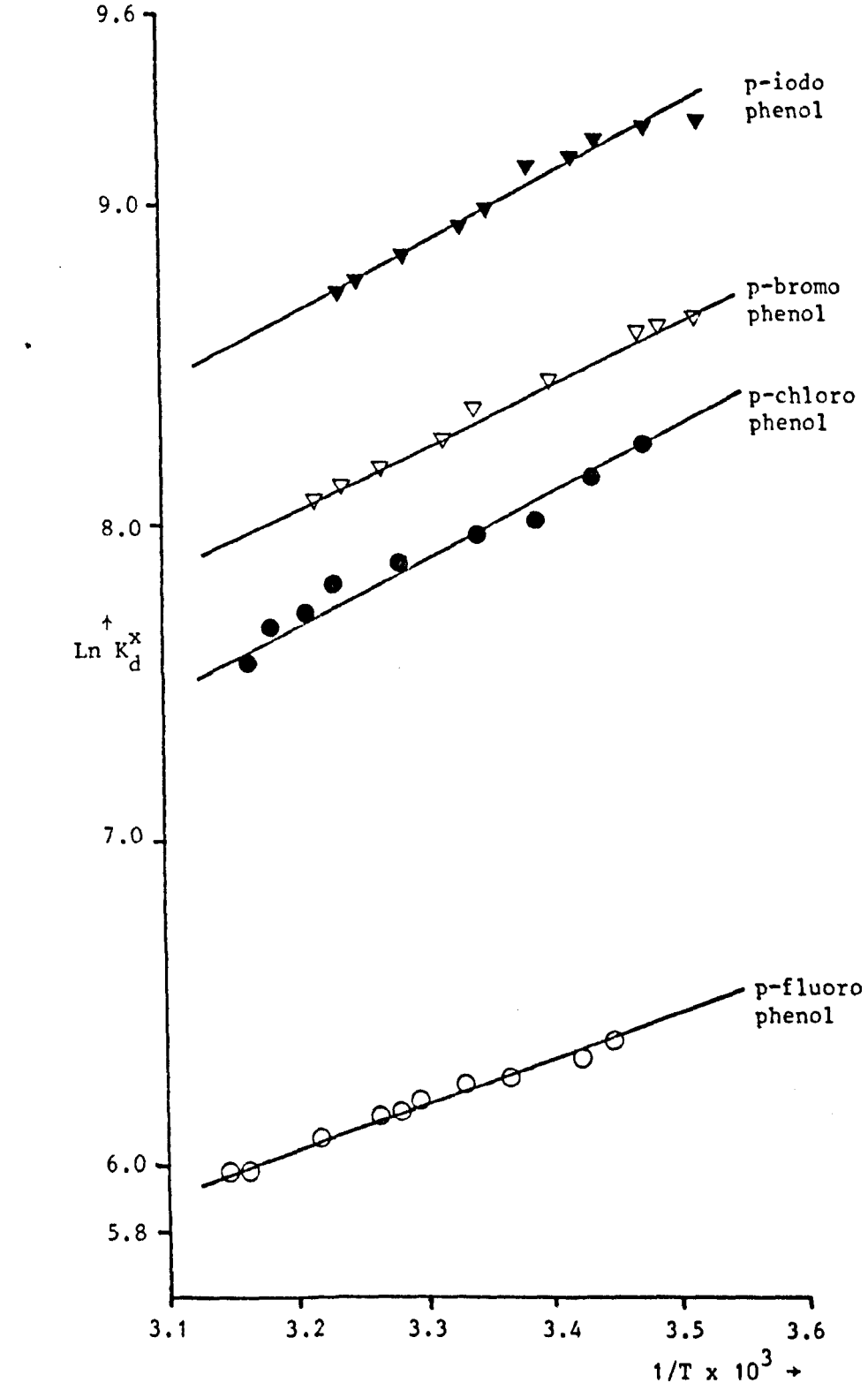


Figure 42

Van't Hoff plots for resorcinol, m-methoxy, m-ethoxy and p-methoxy phenol distribution in the aqueous/octanol system.

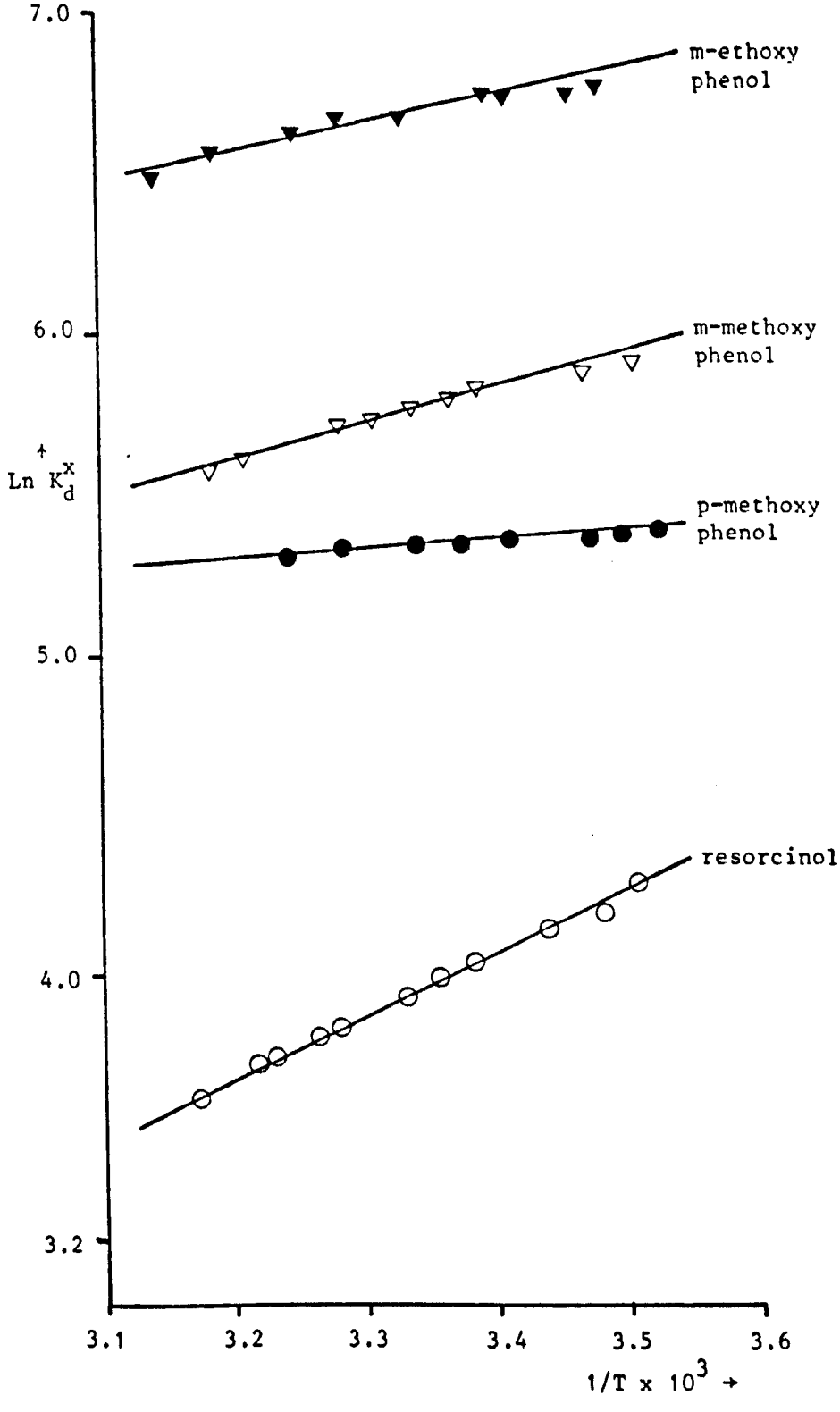


Figure 43

Van't Hoff plots for p-nitro phenol, p-cyano phenol, p-hydroxy benzo-trifluoride distribution in the aqueous/octanol system.

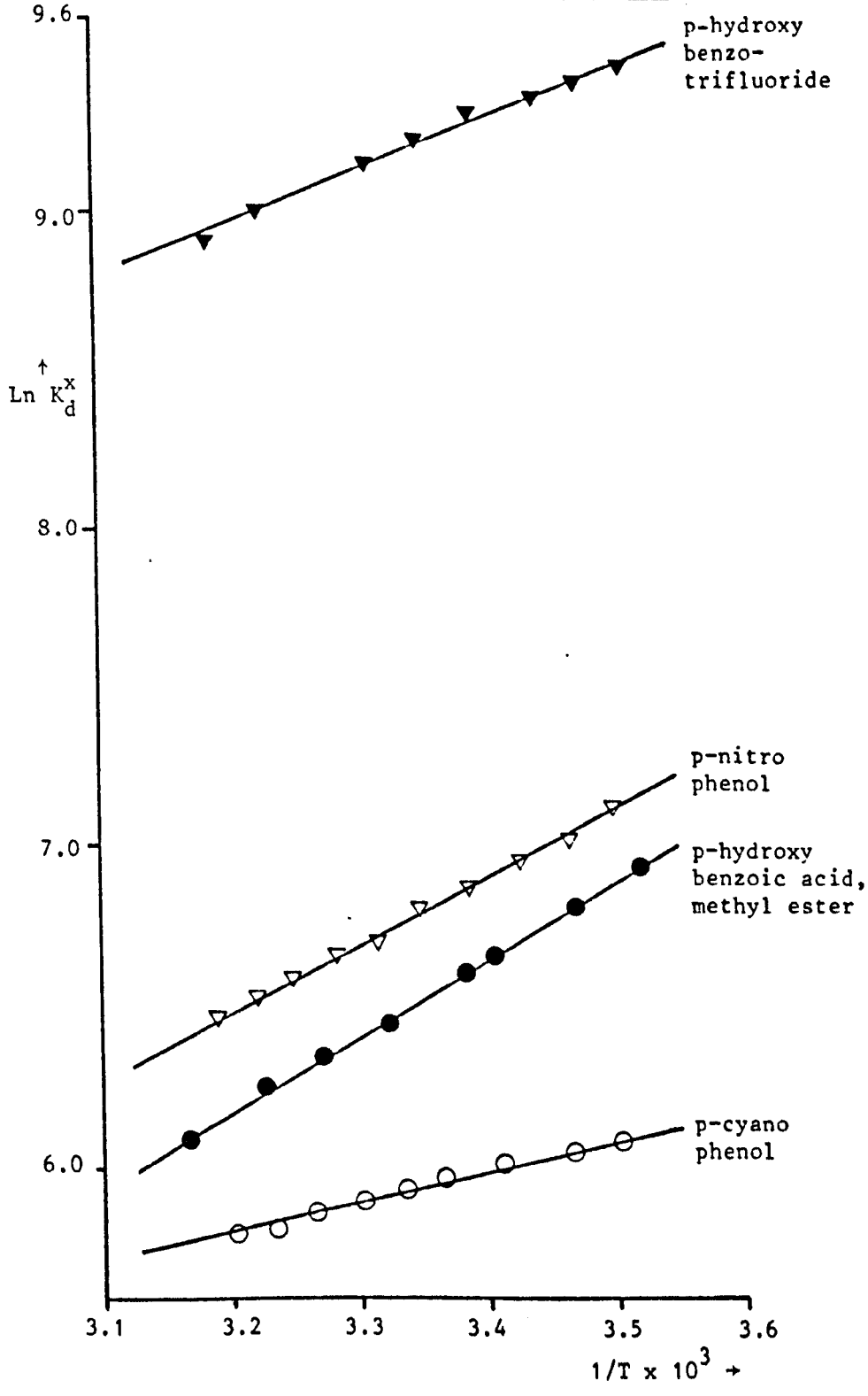
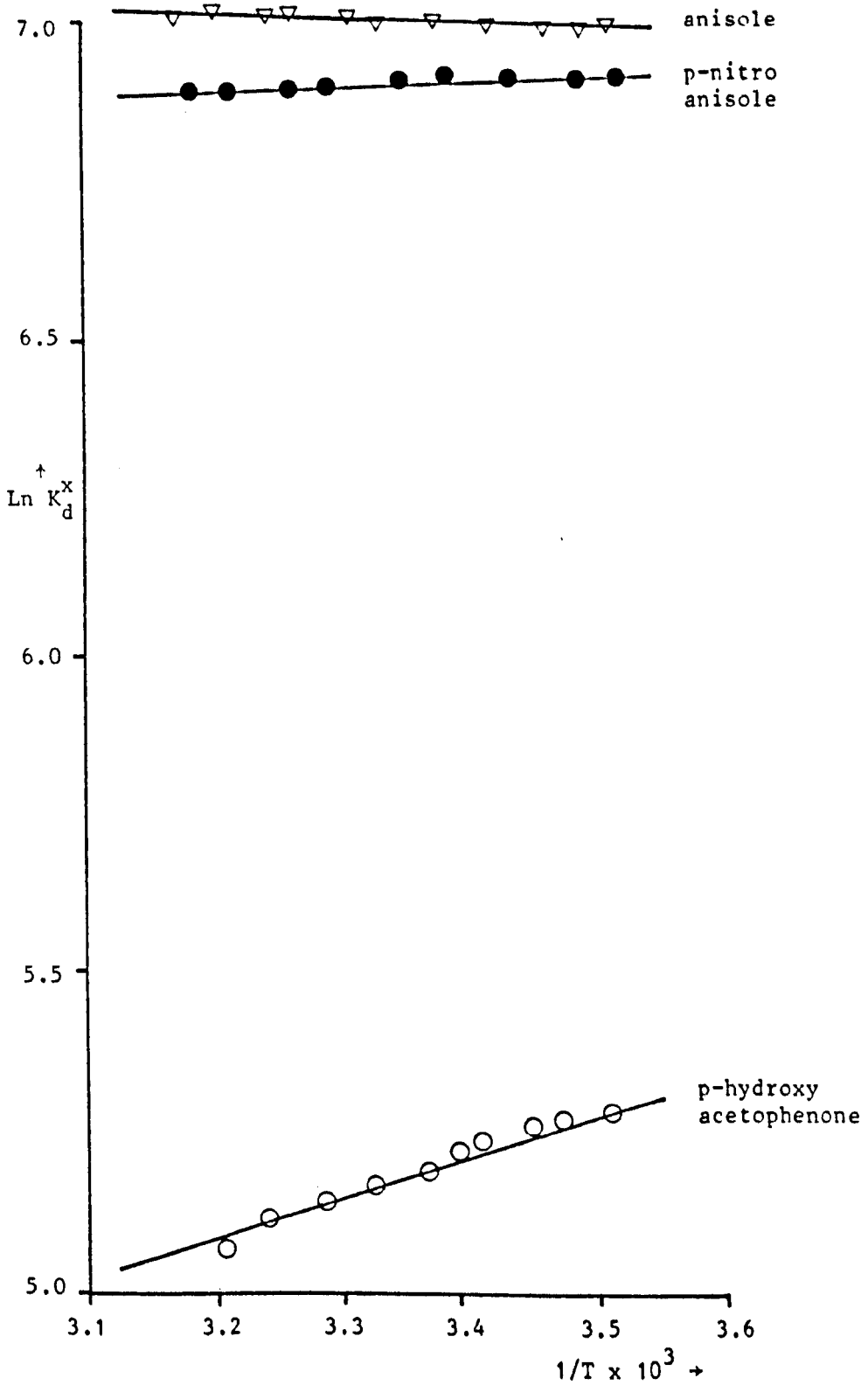


Figure 44

Van't Hoff plots for anisole, p-nitro anisole and p-hydroxy acetophenone distribution in the aqueous/octanol system.



THE TEMPERATURE DEPENDENCE OF PARTITIONING OF PHENOLIC SOLUTES IN THE WATER/CYCLOHEXANE SYSTEM (FILTER PROBE TECHNIQUE)

Table 48

phenol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
12.75	3.498	.101	.600	-0.510
17.21	3.440	.122	.729	-0.317
20.00	3.411	.129	.773	-0.258
23.66	3.369	.137	.823	-0.194
25.88	3.344	.144	.867	-0.142
28.44	3.316	.159	.959	-0.040
31.46	3.283	.167	1.011	0.011
32.42	3.273	.174	1.057	0.055
35.72	3.238	.190	1.154	0.144
38.44	3.209	.198	1.206	0.187
40.40	3.189	.206	1.257	0.228

Table 49

p-cresol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
9.87	3.533	.552	3.271	1.185
12.27	3.504	.564	3.351	1.209
15.87	3.460	.641	3.823	1.341
18.01	3.435	.673	4.022	1.392
21.16	3.398	.734	4.400	1.482
23.37	3.372	.770	4.626	1.532
26.15	3.341	.822	4.952	1.600
28.28	3.318	.862	5.203	1.649
31.35	3.284	.955	5.780	1.750
33.27	3.263	.982	5.954	1.784
35.68	3.238	1.040	6.319	1.844
38.37	3.210	1.087	6.619	1.889
41.29	3.180	1.139	6.953	1.939

Table 50

o-cresol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
12.30	3.503	0.961	5.771	1.742
16.64	3.451	1.06	6.327	1.845
20.23	3.409	1.174	7.033	1.951
22.24	3.385	1.220	7.322	1.991
24.74	3.357	1.290	7.760	2.049
27.17	3.329	1.350	8.122	2.095
31.47	3.285	1.469	8.892	2.185
34.51	3.250	1.532	8.299	2.229
39.30	3.201	1.632	9.945	2.297
42.39	3.169	1.721	10.515	2.353

Table 51

m-cresol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.35	3.515	.459	2.739	1.008
14.25	3.479	.493	2.935	1.077
18.94	3.424	.563	3.368	1.214
22.72	3.380	.613	3.681	1.303
25.58	3.348	.667	4.016	1.390
27.62	3.325	.695	4.192	1.433
31.80	3.279	.754	4.566	1.519
35.03	3.245	.802	4.870	1.583
37.29	3.221	.869	5.286	1.665
41.62	3.177	.923	5.635	1.729

Table 52

p-ethyl phenol,aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
12.36	3.504	2.207	12.281	2.508
16.16	3.456	2.467	14.793	2.694
18.00	3.435	2.603	15.56	2.745
20.03	3.411	2.773	16.682	2.814
24.68	3.358	3.008	18.096	2.896
27.92	3.321	3.219	19.424	2.966
32.77	3.269	3.525	21.364	3.062
35.64	3.238	3.755	22.816	3.127
40.03	3.193	4.067	24.801	3.211

Table 53

p-propyl phenol,aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.77	3.522	10.151	60.22	4.098
14.54	3.476	11.00	65.51	4.182
18.67	3.427	12.12	72.49	4.283
22.54	3.382	13.05	78.34	4.361
26.17	3.341	13.86	83.49	4.425
29.79	3.301	14.75	89.15	4.490
34.11	3.255	15.48	93.92	4.500
37.47	3.219	16.27	98.99	4.595

Table 54

p-bromo phenol,aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
11.26	3.516	.852	5.057	1.621
15.94	3.459	.942	5.617	1.726
19.85	3.413	1.041	6.489	1.870
22.5	3.382	1.105	6.634	1.892
26.01	3.343	1.174	7.071	1.956
28.10	3.319	1.222	7.359	1.996
33.51	3.261	1.339	8.121	2.095
39.20	3.202	1.448	8.827	2.177

Table 55

p-chloro phenol,aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
11.40	3.514	.563	3.342	1.207
16.12	3.457	.613	3.657	1.297
20.32	3.408	.695	4.164	1.426
25.32	3.350	.762	4.587	1.523
30.24	3.296	.843	5.098	1.629
34.73	3.248	.914	5.549	1.714
37.20	3.222	.961	5.846	1.766
38.75	3.206	.980	5.969	1.787

Table 56

p-fluoro phenol, aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
12.60	3.499	.135	.802	-0.221
18.06	3.434	.164	.980	-0.019
20.38	3.407	.169	1.013	0.012
24.18	3.363	.185	1.112	0.106
28.14	3.319	.202	1.219	0.198
30.29	3.296	.211	1.276	0.244
33.31	3.263	.226	1.370	0.315
37.11	3.223	.249	1.515	0.415
39.72	3.196	.256	1.561	0.445

Table 57

p-iodo phenol, aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
12.58	3.499	1.679	9.979	2.300
15.80	3.461	1.723	10.276	2.329
19.11	3.422	1.844	11.033	2.401
22.44	3.383	1.954	11.729	2.462
24.74	3.357	2.029	12.206	2.502
26.58	3.336	2.096	12.630	2.536
28.50	3.315	2.167	13.082	2.571
31.16	3.286	2.228	13.483	2.601
33.74	3.258	2.291	13.896	2.632
37.06	3.224	2.371	14.421	2.669
42.70	3.160	2.542	15.534	2.743

Table 58

m-methoxy phenol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
10.60	3.524	.106	.629	-0.464
14.75	3.473	.118	.703	-0.353
17.83	3.437	.127	.759	-0.276
22.26	3.385	.141	.846	-0.167
28.28	3.318	.162	.978	-0.023
31.22	3.285	.171	1.035	0.034
35.24	3.243	.184	1.118	0.111
48.36	3.210	.195	1.187	0.172
42.61	3.167	.209	1.277	0.245

Table 59

m-ethoxy phenol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
12.05	3.506	.365	2.168	.774
16.88	3.448	.389	2.322	.843
19.76	3.414	.418	2.503	.917
22.68	3.380	.455	2.733	1.005
25.82	3.345	.487	2.933	1.076
28.41	3.316	.515	3.101	1.132
32.03	3.277	.550	3.319	1.99
35.27	3.242	.594	3.608	1.283
39.21	3.201	.633	3.857	1.349

Table 60

p-methoxy phenol, aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
13.02	3.494	.140	.832	-0.183
17.05	3.446	.152	.908	-0.097
21.48	3.394	.166	.995	-0.005
25.88	3.344	.186	1.121	0.114
28.83	3.311	.207	1.250	0.223
31.82	3.279	.226	1.368	0.314
32.59	3.271	.231	1.399	0.336
36.13	3.233	.258	1.568	0.450
40.34	3.189	.278	1.696	0.528

Table 61

p-hydroxy benzo-tri-fluoride, aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
12.82	3.497	.980	5.826	1.762
16.99	3.447	1.041	6.216	1.827
20.42	3.406	1.150	6.890	1.93
24.02	3.365	1.299	7.810	2.055
26.86	3.333	1.381	8.324	2.119
30.10	3.298	1.500	9.069	2.205
32.93	3.267	1.597	9.677	2.270
36.02	3.235	1.703	10.349	2.337
39.31	3.200	1.768	10.775	2.377

Table 62

p-nitro phenol, aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
11.14	3.518	.029	.172	-1.763
15.83	3.460	.037	.221	-1.508
20.22	3.409	.044	.264	-1.331
22.26	3.385	.050	.297	-1.214
26.86	3.333	.057	.334	-1.067
29.05	3.309	.063	.381	-0.966
32.52	3.272	.070	.425	-0.855
36.89	3.225	.080	.486	-0.722
40.32	3.190	.087	.528	-0.638

Table 63

p-hydroxy benzoic acid, methyl ester, aqueous/cyclohexane

Temp °C	$1/T \times 10^3$	K_d^m	K_d^x	Ln K_d^x
10.88	3.521	.033	.196	-1.631
13.92	3.483	.040	.238	-1.435
17.05	3.446	.046	.275	-1.292
20.98	3.399	.053	.318	-1.147
23.81	3.367	.057	.343	-1.071
26.84	3.333	.063	.380	-0.968
27.54	3.326	.066	.398	-0.921
30.17	3.297	.071	.429	-0.846
33.14	3.265	.076	.461	-0.775
35.44	3.241	.081	.492	-0.709
39.06	3.203	.091	.555	-0.590

Table 64

p-hydroxy acetophenone, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.80	3.509	.006	.035	-3.351
16.13	3.457	.007	.044	-3.120
19.81	3.413	.009	.053	-2.933
23.66	3.369	.011	.065	-2.735
25.96	3.343	.012	.069	-2.670
30.97	3.288	.014	.085	-2.461
33.80	3.256	.016	.097	-2.333
36.02	3.234	.017	.105	-2.252
37.63	3.218	.019	.118	-2.141
39.50	3.198	.020	.122	-2.105

Table 65

p-cyano phenol, aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
11.77	3.509	.003	.017	-4.061
15.22	3.468	.004	.022	-3.842
17.93	3.435	.005	.029	-3.551
21.38	3.395	.006	.036	-3.325
24.07	3.365	.007	.044	-3.126
26.74	3.335	.009	.051	-2.968
30.16	3.297	.010	.059	-2.826
33.50	3.261	.012	.070	-2.646
35.67	3.238	.014	.083	-2.486
37.02	3.224	.015	.091	-2.394
39.58	3.198	.016	.099	-2.309

Table 66

anisole,aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
12.00	3.507	271.97	1615.56	7.3874
19.05	3.422	271.97	1627.14	7.3946
24.18	3.363	274.11	1648.09	7.4074
31.06	3.287	274.11	1658.39	7.4136
36.09	3.234	271.97	1652.84	7.4103

Table 67

p-methyl anisole,aqueous/cyclohexane

Temp °C	1/T x10^3	K_d^m	K_d^x	Ln K_d^x
13.34	3.491	425.12	2528.78	7.835
17.87	3.436	425.12	2540.60	7.840
22.34	3.386	414.55	2488.29	7.819
27.50	3.326	430.59	2597.10	7.862
31.0	3.288	425.12	2572.10	7.852
36.0	3.235	425.12	2583.70	7.857

Figure 45

Van't Hoff plots for phenol, p-cresol, p-ethyl and p-propyl phenol distribution in the aqueous/cyclohexane system.

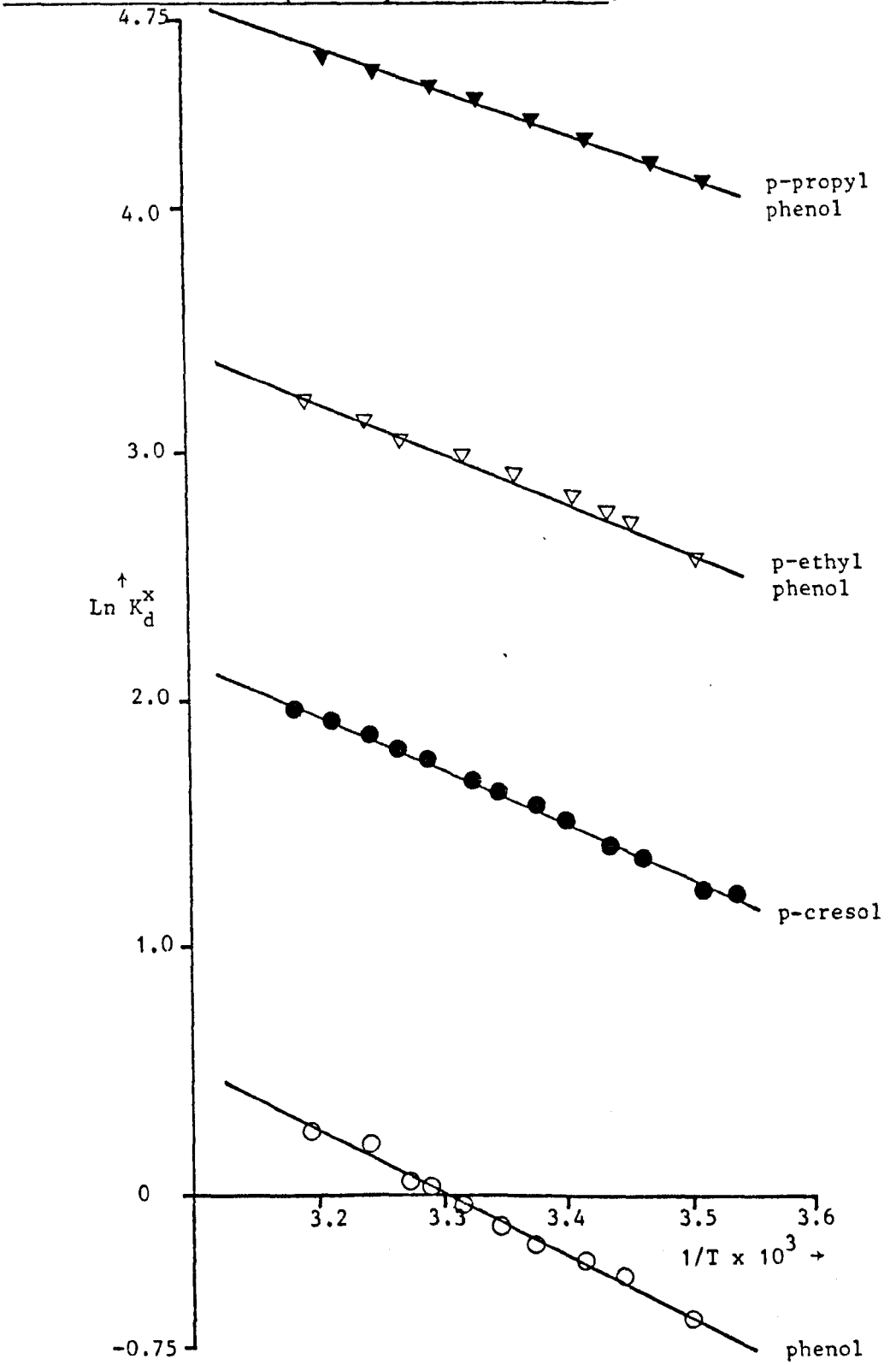


Figure 46

Van't Hoff plots for o-cresol and m-cresol distribution in the aqueous/cyclohexane system.

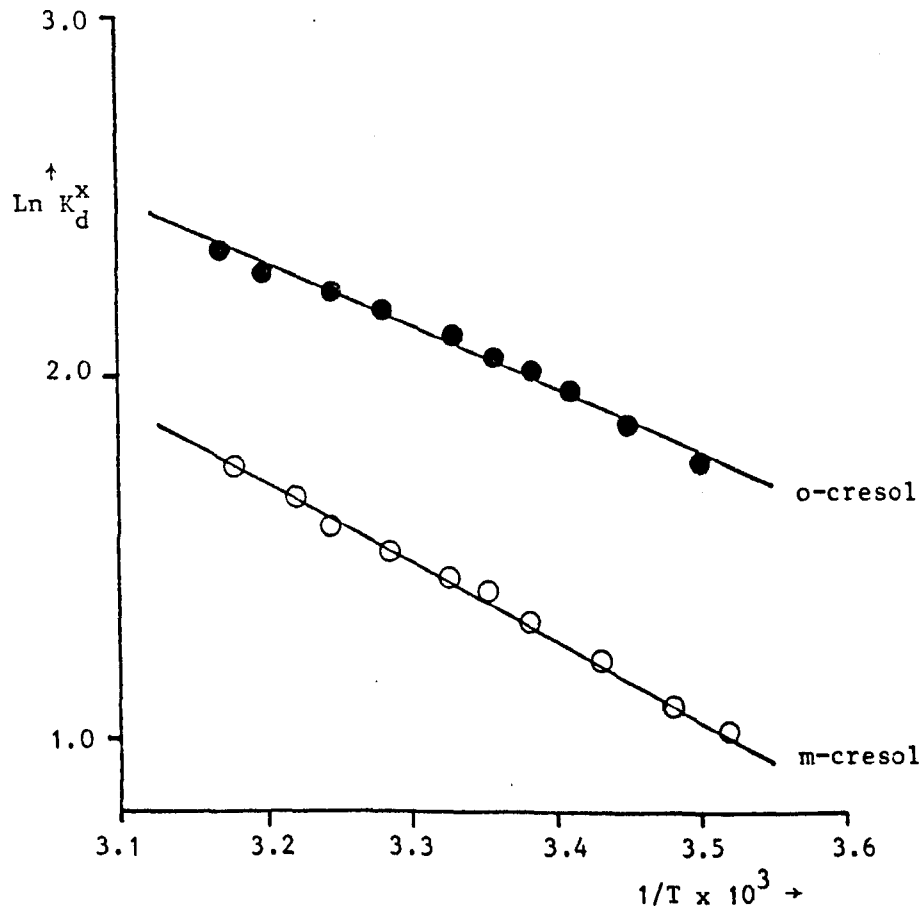


Figure 47

Van't Hoff plots for p-bromo, p-chloro, p-fluoro and p-iodo phenol distribution in the aqueous/cyclohexane system.

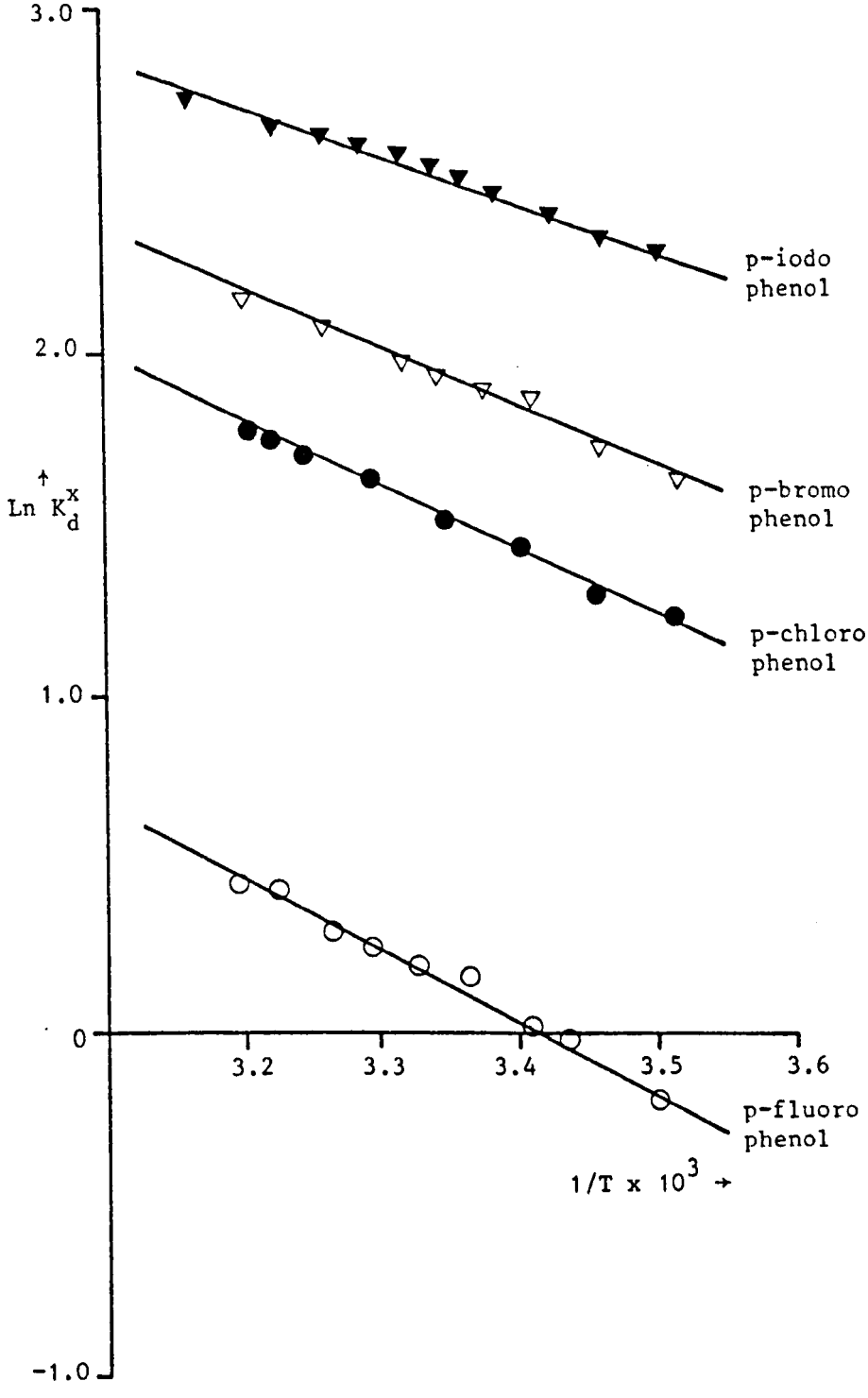


Figure 48

Van't Hoff plots for m-ethoxy, m-methoxy and p-methoxy phenol distribution in the aqueous/cyclohexane system.

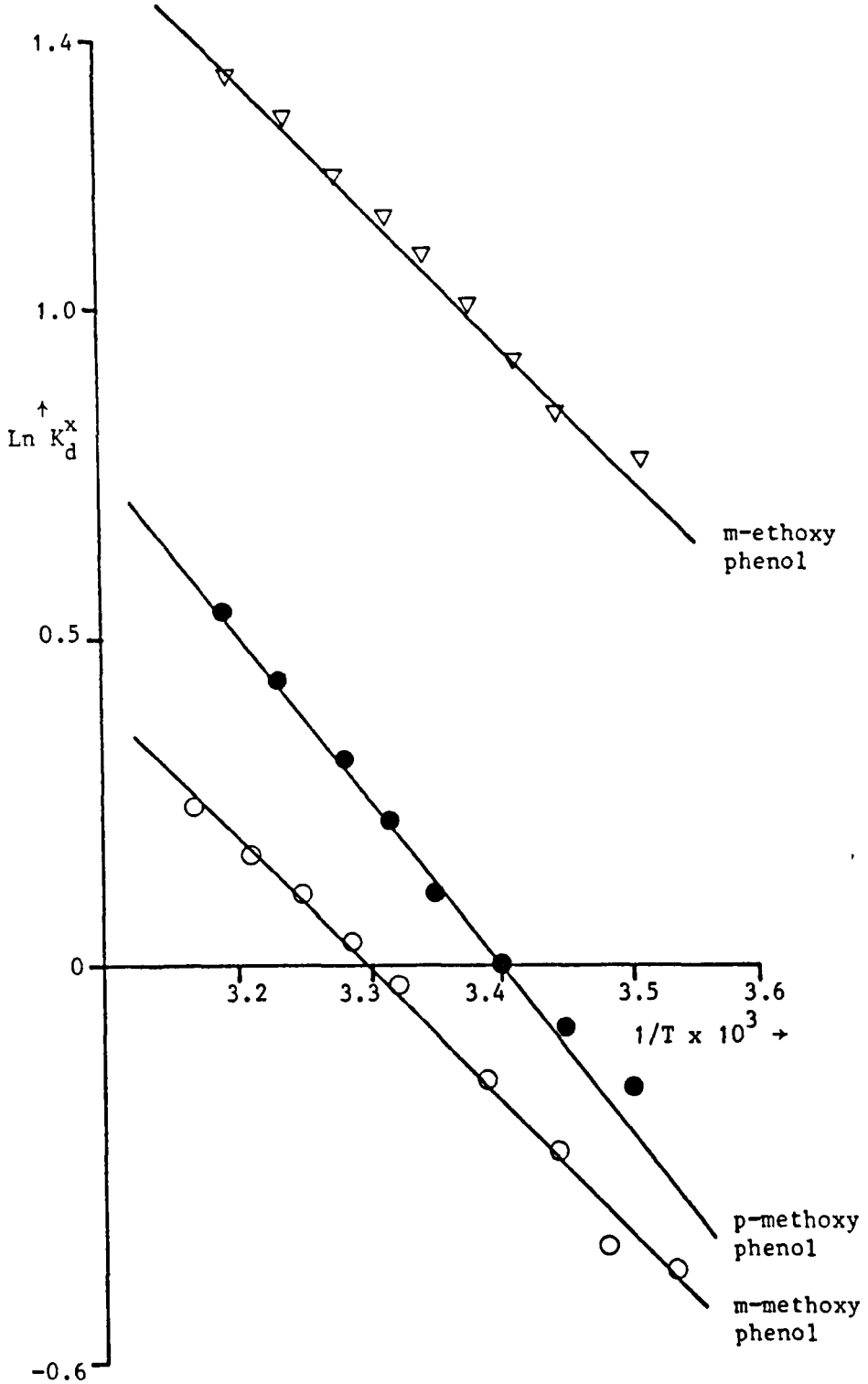


Figure 49

Van't Hoff plots for p-cyano phenol, p-hydroxy acetophenone, p-nitro phenol and p-hydroxy benzoic acid, methyl ester distribution in the aqueous/cyclohexane system.

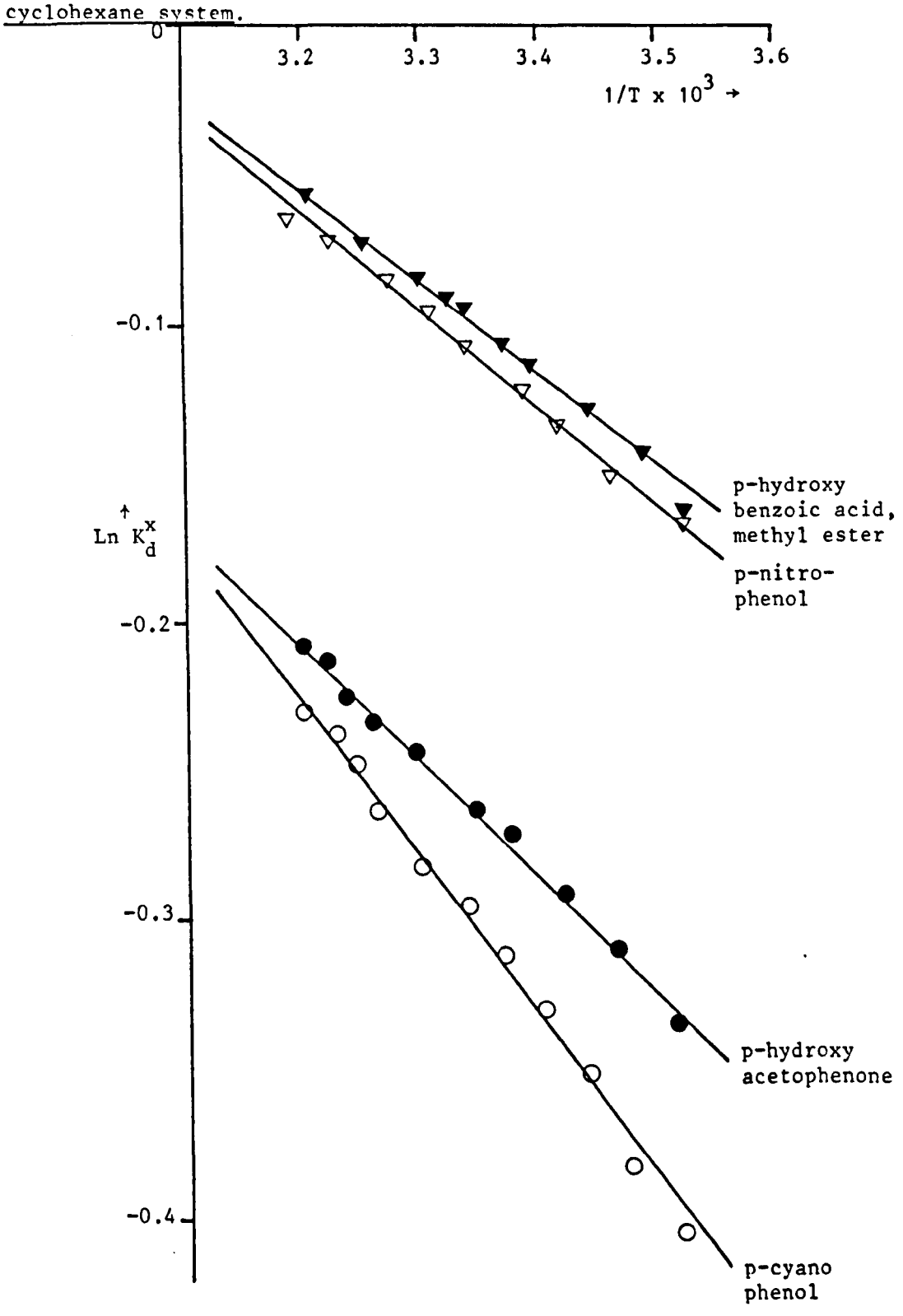
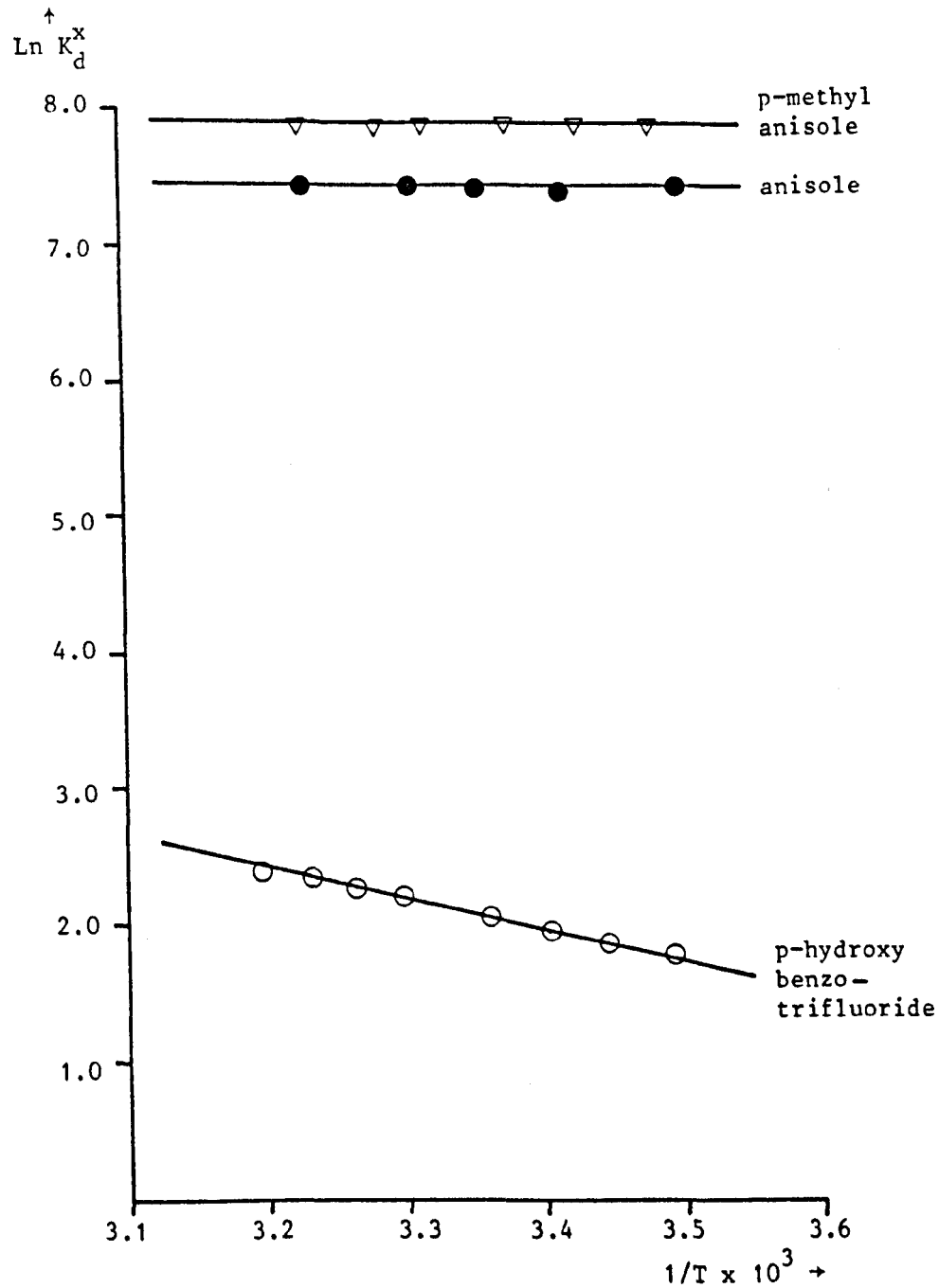


Figure 50

Van't Hoff plots for p-hydroxy benzo-trifluoride, anisole and p-methyl anisole distribution in the aqueous/cyclohexane system



DETERMINATION OF PARTITION COEFFICIENTS OF PHENOLIC COMPOUNDS USING THE AKUFVE TECHNIQUE

Table 68

	K_d^m (22°C)	Log K_d^m
Water/Octanol		
phenol	33.39	1.52
	32.14	1.51
p-cresol	89.00	1.95
	101.95	2.01
Water/Cyclohexane		
phenol	.219	-.660
	.308	-.511
	.245	-.611
	.238	-.623
	.262	-.582
p-cresol	.743	-.129
	.712	-.148
	.734	-.111
o-cresol	1.385	.141
	1.315	.119
m-cresol	.725	-.140
	.67	-.174
p-ethyl phenol	2.207	.344
	2.818	.450
	2.75	.439
p-propyl phenol	11.83	1.073
p-isopropyl phenol	8.166	.912
p-bromo phenol	1.354	.132
p-chloro phenol	.714	-.146
p-fluoro phenol	.381	-.419
p-iodo phenol	2.142	.331
p-hydroxy benzo-trifluoride	1.215	.085
p-methoxy phenol	.128	-.893
anisole	162.37	2.21

TEMPERATURE DEPENDENCE OF PARTITIONING OF PHENOLIC COMPOUNDS USING THE AKUFVE TECHNIQUE

Table 69

Phenol, aqueous/cyclohexane

Temp °C	1/T Kx10^3	K_d^m	K_d^x	Ln K_d^x
16.0	3.458	.280	1.670	0.513
18.0	3.435	.295	1.763	0.567
20.0	3.411	.295	1.767	0.569
22.0	3.388	.308	1.848	0.614
24.0	3.365	.321	1.930	0.658
26.0	3.343	.334	2.012	0.699
28.0	3.321	.348	2.100	0.742
30.0	3.299	.362	2.189	0.783
32.0	3.277	.376	2.277	0.823
34.0	3.256	.384	2.330	0.846
36.0	3.235	.398	2.418	0.883
38.0	3.214	.421	2.563	0.941
40.0	3.193	.453	2.763	1.016

Table 70

p-cresol, aqueous/cyclohexane

Temp °C	1/T Kx10^3	K_d^m	K_d^x	Ln K_d^x
14.0	3.483	.650	3.871	1.353
16.0	3.458	.679	4.051	1.399
18.0	3.435	.720	4.303	1.459
20.0	3.411	.752	4.504	1.505
22.0	3.388	.774	4.645	1.536
24.0	3.365	.819	4.925	1.594
26.0	3.343	.843	5.078	1.625
28.0	3.321	.867	5.233	1.655
30.0	3.299	.892	5.394	1.685
32.0	3.277	.918	5.559	1.715
34.0	3.256	.944	5.728	1.745
36.0	3.235	.944	5.734	1.747

Table 71

p-ethyl phenol, aqueous/cyclohexane

Temp °C	1/T Kx10^3	K_d^m	K_d^x	Ln K_d^x
16.0	3.458	2.570	15.332	2.729
18.0	3.435	2.621	15.666	2.751
20.0	3.411	2.646	15.847	2.763
22.0	3.388	2.750	16.503	2.804
24.0	3.365	2.832	17.029	2.835
26.0	3.343	2.918	17.578	2.867
28.0	3.321	3.038	18.337	2.909
30.0	3.299	3.167	19.151	2.952
32.0	3.277	3.234	19.585	2.975
34.0	3.256	3.303	20.043	2.998
36.0	3.235	3.375	20.507	3.021

Table 72

p-propyl phenol, aqueous/cyclohexane

Temp °C	1/T Kx10^3	K_d^m	K_d^x	Ln K_d^x
14.0	3.483	10.014	59.63	4.088
16.0	3.458	10.455	62.37	4.133
18.0	3.435	10.928	65.32	4.179
20.0	3.411	11.327	67.84	4.217
22.0	3.388	11.830	70.98	4.262
24.0	3.365	12.287	73.88	4.302
26.0	3.343	12.684	76.41	4.336
28.0	3.321	13.103	79.09	4.371
30.0	3.299	13.548	81.92	4.406
33.0	3.266	14.183	85.95	4.454
34.0	3.257	14.293	86.73	4.463
36.0	3.235	14.577	88.57	4.484

Table 73

p-bromo phenol, aqueous/cyclohexane

Temp °C	$1/T_3$ Kx10^3	K_d^m	K_d^x	Ln K_d^x
14.6	3.475	1.204	7.176	1.971
16.0	3.458	1.232	7.350	1.995
18.0	3.435	1.291	7.716	2.043
20.0	3.411	1.322	7.917	2.069
22.0	3.388	1.354	8.124	2.095
24.0	3.365	1.403	8.436	2.133
26.0	3.343	1.437	8.656	2.158
28.0	3.321	1.507	9.096	2.208
30.0	3.299	1.526	9.228	2.222
32.0	3.277	1.582	9.581	2.259
34.0	3.256	1.602	9.721	2.274
36.0	3.235	1.621	8.849	2.287

Table 74

p-chloro phenol, aqueous/cyclhexane

Temp °C	$1/T$ Kx10^3	K_d^m	K_d^x	Ln K_d^x
13	3.495	.597	3.392	1.221
15	3.470	.617	3.677	1.302
16	3.458	.627	3.741	1.319
18	3.435	.664	3.969	1.378
20	3.411	.692	4.144	1.422
22	3.388	.714	4.284	1.455
24	3.365	.737	4.432	1.489
26	3.343	.761	4.584	1.523
28	3.321	.786	4.744	1.557
30	3.299	.811	4.904	1.590
32	3.277	.837	5.069	1.623
36	3.235	.877	5.329	1.673

Table 75

p-iodo phenol, aqueous/cyclohexane

Temp °C	$1/T_K \times 10^3$	K_d^m	K_d^x	Ln K_d^x
14.7	3.475	1.923	11.46	2.439
16.0	3.458	1.952	11.65	2.455
18.0	3.435	2.013	12.03	2.488
20.0	3.411	2.087	12.49	2.526
22.0	3.388	2.142	12.85	2.553
24.0	3.365	2.234	13.43	2.598
26.0	3.343	2.332	14.05	2.642
28.0	3.321	2.384	14.39	2.666
30.0	3.299	2.436	14.73	2.689
32.0	3.277	2.477	15.00	2.708
34.0	3.256	2.519	15.29	2.727
36.0	3.235	2.576	15.65	2.750

Table 76

The thermodynamic parameters of transfer of various phenols distributed between aqueous (.15 M sodium chloride) solution and cyclohexane obtained using the mini-AKUFVE.

Phenol	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mole/K	Corr. Coef. r
Phenol	-1.53	15.0	56	-.994
p-cresol	-3.76	13.6	59	-.991
p-ethyl phenol	-6.89	11.7	63	-.995
p-propyl phenol	-10.44	13.9	82	-.999
p-bromo phenol	-5.15	11.3	56	-.996
p-chloro phenol	-3.53	14.0	59	-.994
p-iodo phenol	-6.28	11.3	60	-.994

Table 77

Determination of Partition Coefficients in the Aqueous/ n-octanol System Using H.P.L.C.

Solute	Retention Time (20°C)	Log R.T. (20°C)	Log K_d^m (20°C)
Benzamide	2.6 min	0.415	0.65
Phenol	21.1 min	1.342	1.477
p-Cresol	62.0 min	1.79	2.00
p-Chloro phenol	218.8 min	2.34	2.556

Figure 51. Relationship between log R.T. and Log K_d^m in the aqueous/n-octanol system.

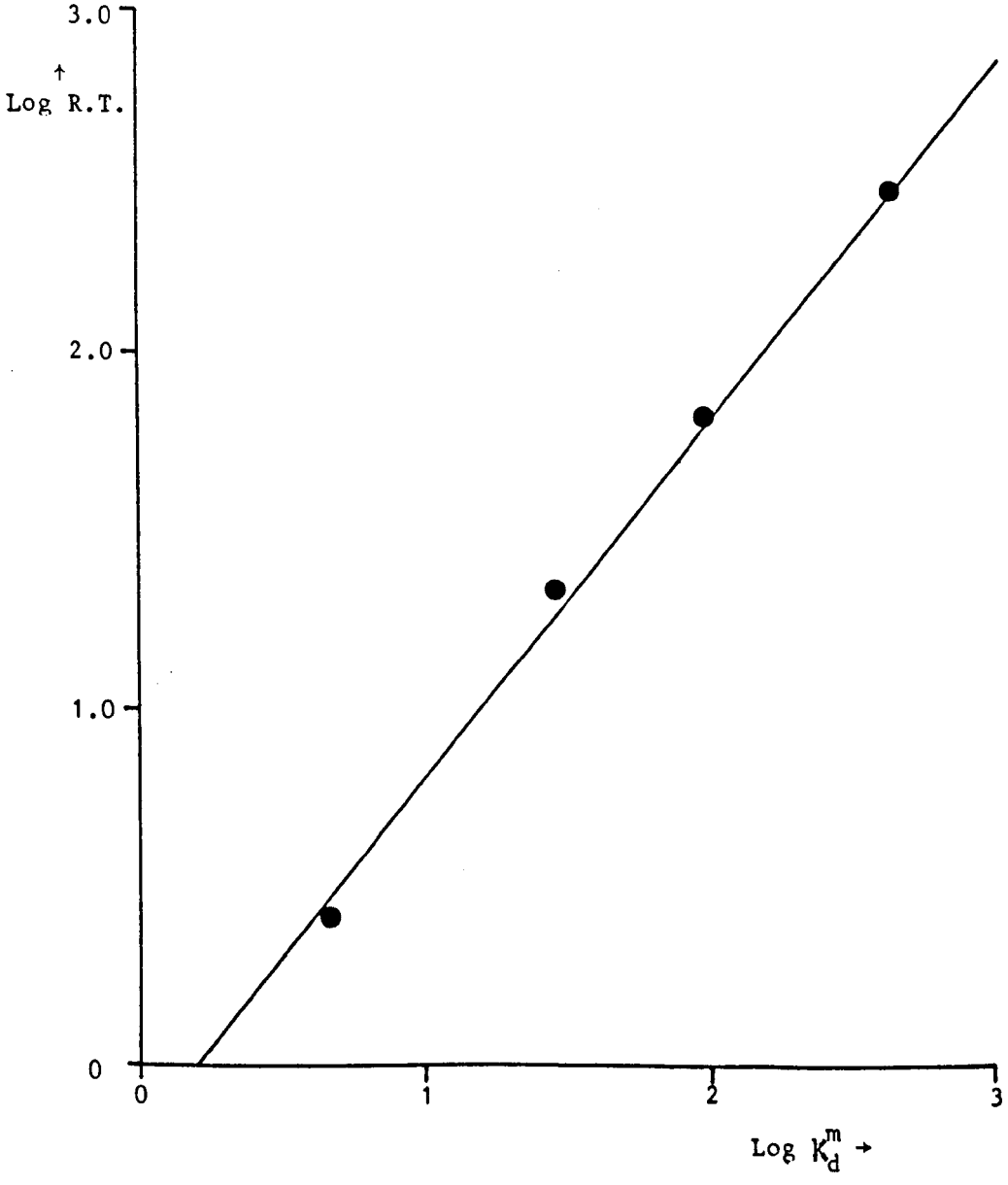


Table 78

Temperature dependence of the solubility of water in n-octanol as determined by an automated coulometric titrimetric method.

(The Mitsubishi Moisture Meter Model CA-02)

Temperature °C	Solubility of water in n-octanol g water/100 g octanol
10	4.56, 4.61, 4.59, 4.57, 4.48 mean 4.56
20	4.76, 4.76, 4.72, 4.89, 4.84 mean 4.78
30	5.44, 5.38, 5.17, 5.24, 5.30 mean 5.31
40	5.9, 5.64, 5.84, 5.85, 5.70 mean 5.79

Figure 52. Temperature dependence of the solubility of water in n-octanol.

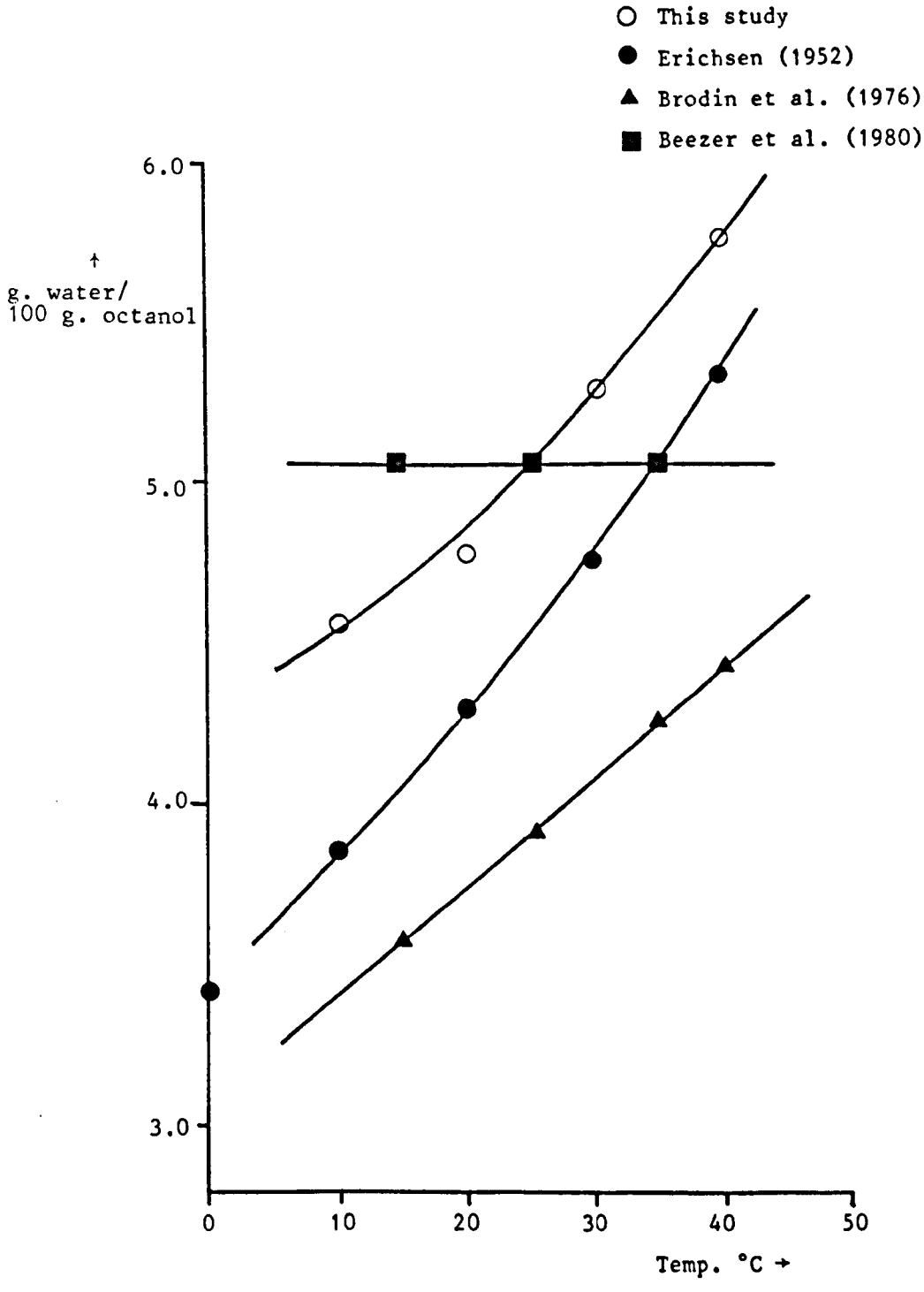


Figure 53. Effect of solute concentration on DSC thermograms of DMPC liposomes.

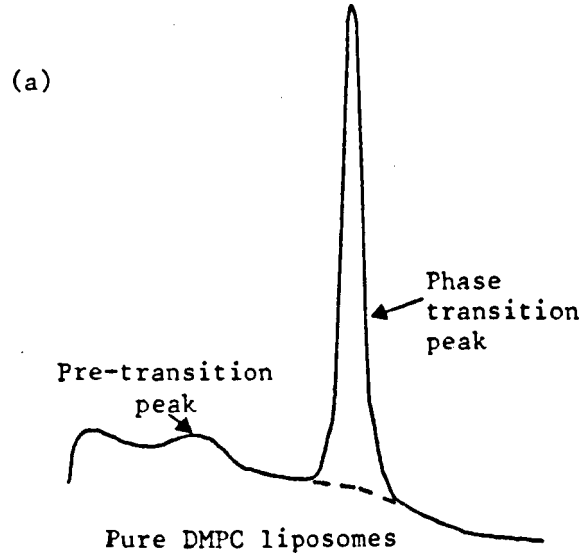


Table 79

The Enthalpy and Entropy of Transition and the Phase Transition Temperature (T_m) of DMPC Multilamellar Liposomes as Determined by DSC.

Solute Concentration mg/100 ml	ΔH_{tr} kJ/mole	ΔS_{tr} J/mole/K
0	23.9	81
0.1	22.9	77
0.5	22.8	77
1	20.1	68
2	19.5	66
10	14.7	50

The pre-transition temperature was found to be 13.5°C. The main transition temperature (T_m) for pure DMPC liposomes was found to be 23.5°C. There was no detectable change in the transition temperature (T_m) with increase in solute concentration. The main transition peak broadened and its intensity decreased as the solute concentration was increased, giving a reduced enthalpy of transition. Broadening of the transition peak meant that the onset temperature of transition (T_c) was slightly lowered with increasing solute concentration. The pre-transition peak was reduced with increasing solute concentration, until it was almost absent.

Table 80

The Enthalpy of Transfer and Transition Temperatures in DMPC Liposome Bilayers.

Pre-transition Temp. °C	Transition Temp. °C	Enthalpy of Transition ΔH kJ/mole.	
14.0	23.0	27.82	(1)
13.5	23.7	26.2	(2)
13.0	24.0	26.38	(3)
14.2	23.9	22.7	(4)
14.0	23.9	22.8	(5)

References

(1) Chapman et al. (1967)
(2) Jones (1979)
(3) Hauser and Philips (1979)
(4) Wilkinson and Nagle (1981)
(5) Mabrey and Sturtevant (1976)

Particle Size Analysis of Handshaken DMPC Liposomes using :

The Coulter Counter Technique.

The average particle size of the handshaken preparations was found to be 10 μm. Typical volume distributions are shown in Figure 54.

Repeated shaking (in water bath), centrifugation and resuspension had the effect of reducing average particle size to 8 μm. Typical volume distributions for such liposome suspensions are shown in Figure 55.

Photon Correlation Spectroscopy

Average particle size of the freshly prepared handshaken DMPC liposomes were in the range 4 - 7 μm. The average particle size of handshaken DMPC liposomes after repeated shaking, centrifugation and resuspension were in the range 3 - 6 μm.

Figure 54. Cumulative volume distribution of freshly prepared DMPC liposome suspensions (Coulter Counter).

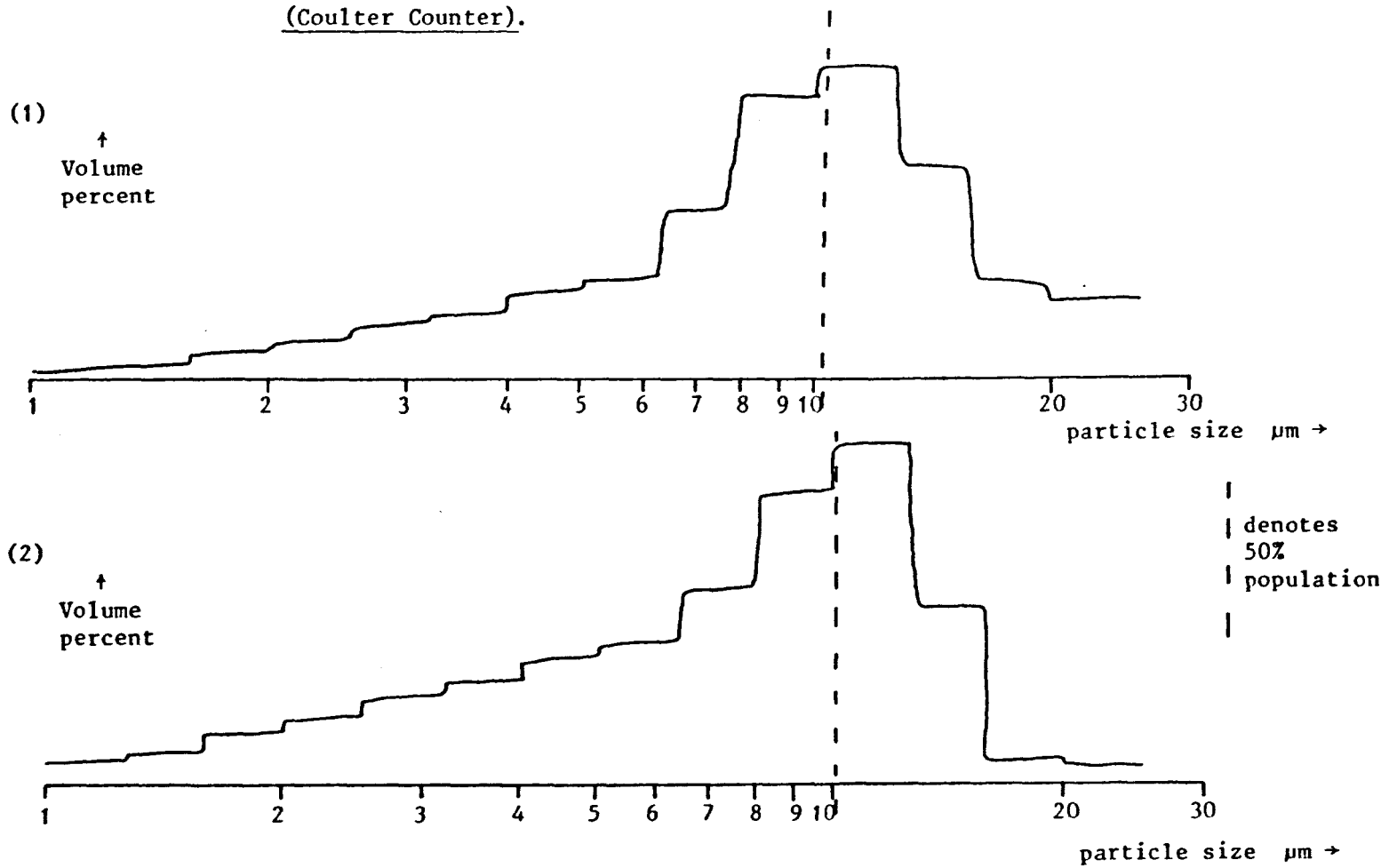


Figure 55. Cumulative volume distribution of DMPC liposome suspensions after repeated centrifugation etc. (Coulter Counter).

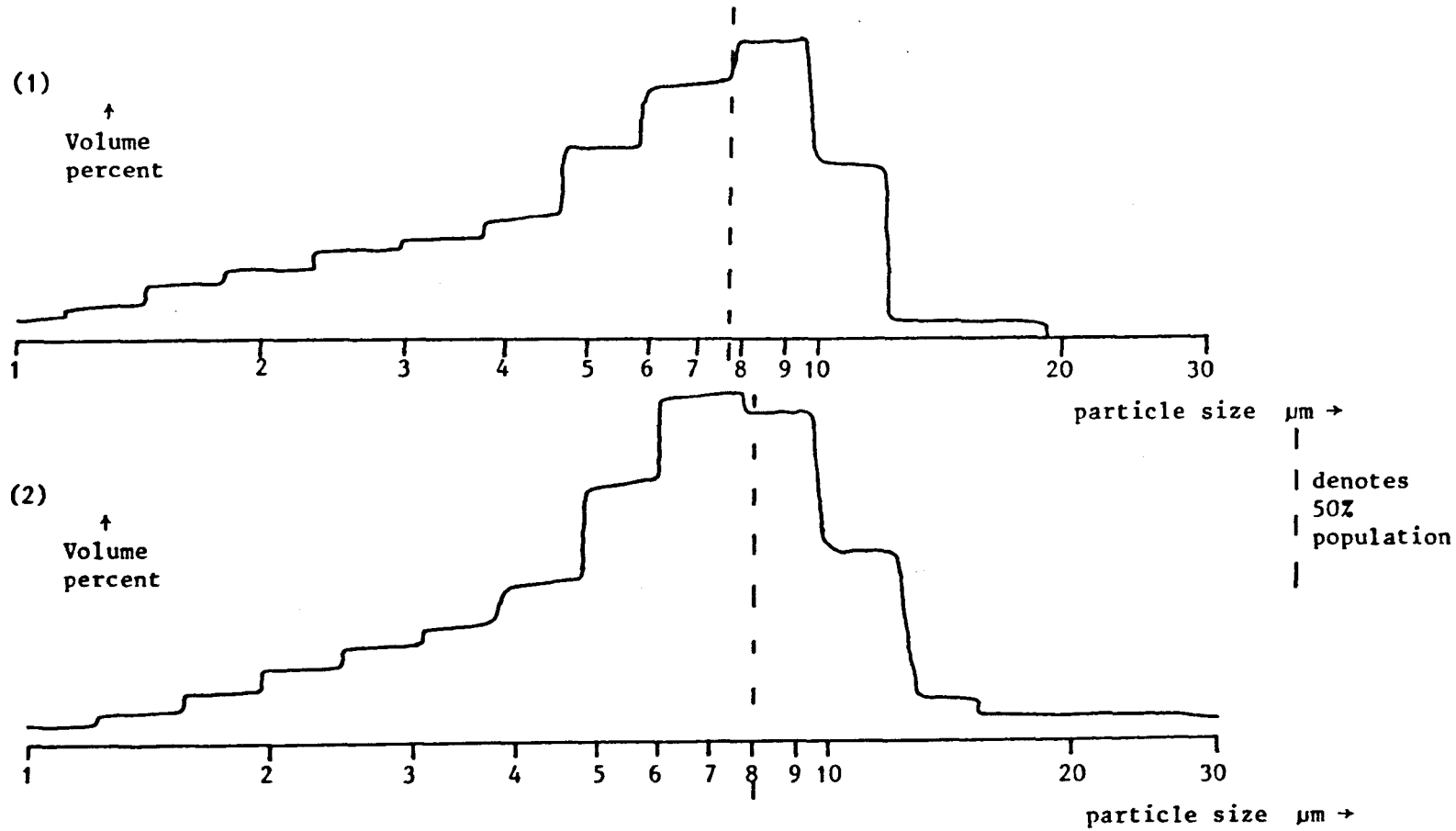


Table 81

Calibration of Nano-Sizer.

Latex Particle Size (nm)	Particle Size (nm)	Polydispersity Index
175	165	0
	167	0
280	269	0
	272	0
425	534	2
	552	2
	547	2
490	501	2
	484	2
556	605	0
	632	0
604	573	1
	671	1
	646	0
716	730	3
	780	0
1060	1150	5
	1220	3
1160	950	2
	1000	2

Table 82

Particle Size Analysis of Liposomes produced by Bath Sonication using the Nano-Sizer.

Time of Sonication (min.)	Particle Size (nm)	Polydispersity Index
0	>3	7
5	2,890	7
10	2,780	7
15	1,530	7
25	1,760	8
30	1,660	8
35	1,570	8
40	1,550	8
45	1,530	8
55	1,530	8
70	1,510	8
90	1,510	8

Both sonication of handshaken liposomes produced an average particle size of 1,500 nm.

Table 83

Particle Size Analysis of Liposome Suspension Produced by the Sonic Probe using the Nano-Sizer.

Time of Sonication	Particle Size (nm)	Polydispersity Index
10 sec	2,250	6
20 sec	1,900	6
30 sec	468	6
40 sec	462	6
50 sec	397	7
60 sec	245	7
2 min	245	8
3 min	247	8
4 min	259	9
5 min	242	9
6 min	250	9
7.5 min	240	9
10 min	239	9

It can be seen that 1 min of sonication produced an average particle size of 245 nm. For more concentrated liposome suspensions a longer period of sonication was required. This procedure gives a method of producing a liposome suspension of 250 nm. average particle size.

Chapter Seven

DISCUSSION

1. The thermodynamics of partitioning of substituted phenols in the aqueous/liposome system.

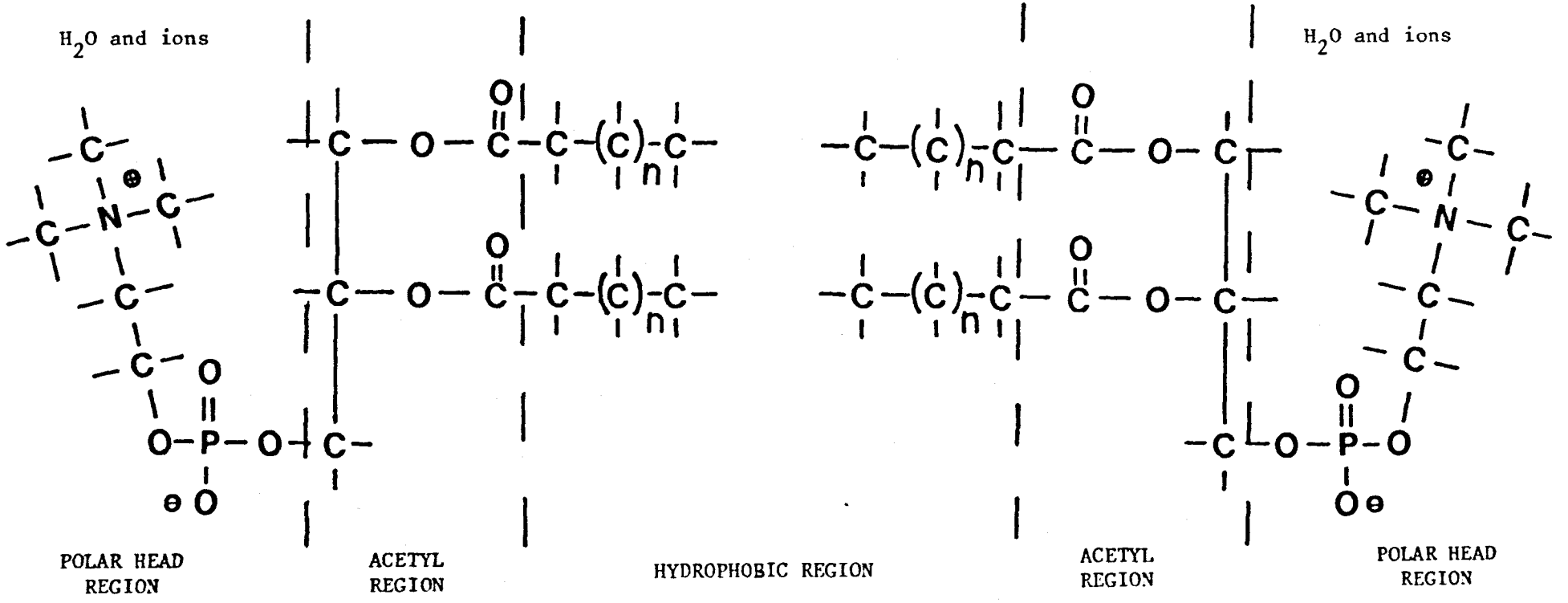
The lipid bilayer differs from bulk organic solvents, not only in composition, but also in organization. The lipid bilayer is a highly structured and ordered system. The polar head groups of the phospholipid molecules form the bilayer surface, and the hydrophobic interior of the bilayer is formed from anisotropically orientated hydrocarbon chains (Figure 56). The ordering of the hydrocarbon chains decreases from the polar head groups as one progresses towards the centre of the bilayer. Even below the phase transition temperature, where the acyl chains are packed closer together, the segments of the hydrocarbon chains near the centre of the bilayer still retain considerable flexibility (Hubbill and McConnell, 1971; Seelig and Seelig, 1974; Stockton et al., 1976). There is an increase in hydrophobicity across the bilayer from the outside towards the centre (Jain et al., 1978; Wilkinson and Nagle, 1981). The anisotropic arrangement of the lipid bilayer is in direct contrast to isotropic apolar solvents, such as alkanes, where structuring is absent. In polar solvents, such as n-octanol, hydrogen bonding between solvent molecules will lead to a more structured environment.

The structural organization and water content of the bilayer change dramatically with temperature. In the ordered gel state, below the phase transition temperature, the lipid hydrocarbon chains are in an all trans configuration, in which they are closely packed and well ordered. On raising the temperature, the mobility of the acyl chains increases, until the endothermic phase transition is reached, where a 'melting' or disordering of the hydrocarbon

Figure 56

Diagram of the liposome bilayer to show three regions of distinctly different chemical character.

(Ashcroft and Smith, 1981)



chains takes place, due to the transformation from the all trans configuration to the gauche arrangement. This results in a lateral expansion and decrease in thickness of the bilayer (Wilkinson and Nagle, 1979, 1981), and a reorientation of the hydrocarbon chains from a tilted state to a position perpendicular to the plane of the bilayer (Chapman et al., 1974; Rand et al., 1975). Phosphatidyl choline liposomes exhibit a pre-transition peak, some 5 - 10°C lower than the main transition peak, which has been attributed to rotations of the polar head groups (Ladbrooke and Chapman, 1969) or is associated with a transformation in the lamellar structure and changes in the hydrocarbon chain packing (Brady and Fein, 1977; Larsson, 1977; Janiak et al., 1976, 1979a).

The position of a solute partitioned into the bilayer may vary with temperature due to structural changes within the membrane. The water content of the bilayer, both solvent water between lamellae and non-solvent water bound to polar head groups, increases with increasing temperature (Elworthy, 1961; Katz and Diamond, 1974).

When a solute molecule partitions into the liposome bilayer from the aqueous environment, its position or localization within the bilayer will depend upon its structure. The more polar solutes will be located near the polar head groups, and a more lipophilic solute molecule would be expected to be partitioned into the hydrophobic acyl regions of the interior of the bilayer. The measured aqueous/liposome partition coefficient represents an average value for the whole bilayer (Diamond and Katz, 1974). The transfer of a phenolic solute from aqueous solution to the liposome bilayer requires first the breaking of solute-water bonds (positive enthalpy change) and subsequent reformation of

water-water bonds (negative enthalpy change). The van der Waals interactions between adjacent hydrocarbon chains, and possibly hydrogen bonding and electrostatic interactions between polar head groups need to be disrupted to accommodate the phenolic solute in the bilayer (positive enthalpy change). Insertion of the solute in the bilayer may be followed by the formation of hydrogen bonds and/or van der Waals interactions between the solute and polar head groups and hydrocarbon chains respectively (negative enthalpy change).

The derived thermodynamic parameters of transfer of substituted phenols between aqueous solution and DMPC liposomes (below T_c) are shown in Table 84. For all the phenolic compounds studied, the free energy of transfer, at 22°C, from aqueous solution to the liposome bilayer is negative, indicating that the solutes prefer the lipsome bilayer to the aqueous environment. The majority of solutes exhibit a positive enthalpy of transfer (except for p-hydroxy methyl benzoate, p-hydroxy acetophenone and p-hydroxy benzo-trifluoride), indicating an endothermic transfer process. Thus partitioning into the liposome bilayer (below T_c) is more favoured at the higher temperature. The positive enthalpy of transfer for the majority of solutes indicates the transfer process involves an overall decrease in molecular interactions due to the disruption of solute-solvent and solvent-solvent non-covalent bonds. The enthalpies of transfer of the alkyl phenols, and to a lesser extent the halo phenols, are much larger than those values obtained for the same solutes by Rogers and Davis (1980) (Table 85). Experimental conditions were not the same however as it seemed preferable to use a lower solute to lipid

Table 84 Thermodynamic Parameters for the Transfer of Phenolic Solutes Between Aqueous solution (.15M NaCl) and DMPC Liposomes, Below Phase Transition.

Phenolic compound	ΔG kJ/mole 22°C	ΔH kJ/mole	ΔS J/mole/K	corr. coef. r	Dominating. parameter
p-cresol	-14.00	+119.2	+451	-.962	entropy
p-ethyl phenol	-15.42	+80.8	+326	-.969	entropy
p-propyl phenol	-15.75	+31.1	+159	-.927	entropy
p-isopropyl phenol	-17.21	+35.6	+179	-.909	entropy
m-cresol	-10.95	+67.7	+266	-.961	entropy
o-cresol	-11.49	+31.6	+146	-.948	entropy
p-cresol	-12.78	+71.2	+285	-.977	entropy
p-fluoro phenol	-12.74	+30.5	+147	-.991	entropy
p-cresol	-13.20	+88.6	+345	-.898	entropy
p-chloro phenol	-15.31	+18.2	+114	-.953	entropy
p-bromo phenol	-16.30	+15.0	+106	-.961	entropy
p-methoxy phenol	-11.48	+145.8	+533	-.994	entropy
p-cresol	-12.92	+68.3	+275	-.974	entropy
p-nitro phenol	-13.90	+9.5	+79	-.945	entropy
p-cresol	-13.92	+111.0	+423	-.981	entropy
m-methoxy phenol	-14.28	+138.6	+518	-.971	entropy
resorcinol	-14.33	+67.4	+277	-.935	entropy
m-ethoxy phenol	-14.39	+107.3	+412	-.938	entropy
p-cresol	-13.85	+111.2	+424	-.971	entropy
p-cyano phenol	-14.19	+119.4	+453	-.977	entropy
p-hydroxy benzo-trifluoride	-18.60	-3.1	+53	.112	entropy
p-hydroxy acetanilide	-6.67	+3.6	+35	-.510	entropy
p-hydroxy acetophenone	-7.23	-20.6	-45	.904	enthalpy
p-cresol	-11.94	+68.6	+273	-.930	entropy
p-hydroxy benzoic acid, methyl ester	-14.46	-2.7	+40	.953	entropy
p-cresol	-13.19	+97.3	+374	-.948	entropy
p-bromo anisole	-18.42	+89.6	+366	-.989	entropy
p-chloro anisole	-18.98	+102.1	+410	-.996	entropy
p-fluoro anisole	-21.75	+148.9	+578	-.984	entropy
p-methoxy phenol	-11.28	+132.8	+488	-.966	entropy
p-nitro phenol	-14.07	+5.5	+66	-.971	entropy
p-ethyl phenol	-14.26	+33.8	+163	-.921	entropy

Table 84 Continued

Phenolic compound	ΔG kJ/mole 22°C	ΔH kJ/mole	ΔS J/mole/K	corr. coef. r	Dominating parameter
p-nitro anisole	-11.10	+55.1	+224	-.979	entropy
p-ethyl phenol	-14.47	-41.8	+190	-.994	entropy
p-propyl anisole	-21.31	+62.1	+283	-.984	entropy

The partition coefficient, Kd, is in molal units.
The value of Kd at 22°C is taken as the theoretical value from linear regression analysis and not as the experimental value at 22°C.
ΔG and ΔS are determined at 22°C (295.15K).

Table 85 Thermodynamic Parameters for the Transfer of Phenolic Solutes Between Aqueous Solution and DMPC Liposomes.

Phenol	ΔG	ΔH	ΔS
	kJ/mole	kJ/mole	kJ/mole/K
	18°C		
p-methyl	-10.88	+9	+68
p-ethyl	-13.39	+9	+76
p-propyl	-16.44	+6	+76
p-n-butyl	-19.0	+1	+66
	15°C		
p-fluro	-10.44	+19.6	+104
p-chloro	-13.48	+10.3	+82
p-bromo	-14.43	+2.7	+60
p-iodo	-15.32	-8.0	+26

(Rogers and Davis, 1980, molal units).

ratio to reduce the disruptive effect of the solute in the bilayer. (Later studies using D.S.C. demonstrated this effect clearly). However, enthalpies of transfer of the same order as those obtained for phenols in this study, were obtained for the solutes studied by Diamond and Katz (1974), Ahmed (1981) and Arrowsmith (1981) (Table 86). Most solutes studied possess a positive entropy of transfer (except p-hydroxy acetophenone), indicating that the transfer process involves an increase in disorder or loss of structure. The solutes p-cresol and p-ethyl phenol were used as a control throughout the experiments and large variations in the thermodynamic parameters, particularly the enthalpies of transfer, were obtained when results from each separate experiment were compared. This was most likely due to batch variations in the structural properties of each liposome suspension, although great care was taken to use precisely the same conditions of preparation each time. Particle size analysis of several freshly prepared liposome suspensions by Coulter Counter gave an average particle size of 8 μm for each preparation. However this technique was not able to detect more subtle differences in structure, such as number of lamellae, distance between lamellae and water content, all of which could affect the partitioning. The results emphasize the necessity of a control so that the thermodynamic values of the other solutes can be determined on a more reliable basis.

The free energy of transfer, ΔG, comprises contributions from the enthalpy, ΔH, and entropy, ΔS, terms according to the equation :

$$\Delta G = \Delta H - T\Delta S$$

The transfer process can be said to be either enthalpy or entropy

Table 86 The Enthalpy of Transfer of Various Solutes Between Aqueous Solution and DMPC Liposome Bilayers Below Tc

	Author	Solute	ΔH kJ/mole
(1)	Diamond and Katz (1974)	Ethyl Acetate	114.2
		Butyramide	98.7
		Acetone	99.1
(2)	Ahmed (1981)	2-Valeryl-phenothiazine	44.9
		promethazine hydrochloride	39.91
		Timeprazine tartrate	67.3
(3)	Arrowsmith (1981)	Deoxycorticosterone	95.9
		Corticosterone	74.7
		17α-Hydroxy-11-deoxycorticosterone	70.1
		Hydrocortisone	76.7
		Cortisone	35.8
		Cortsone-21-acetate	49.7

dominated depending on which term contributes most to the free energy terms. For all solutes, except p-hydroxy acetophenone, the transfer process is entropy dominated, since it is the large positive entropy values which gives rise to the large negative free energy values. The contribution of either the enthalpy or entropy term to the overall free energy of transfer varies from solute to solute.

The thermodynamics of partitioning for each solute molecule will now be discussed in more detail. For the alkyl phenols studied (p-cresol, p-ethyl, p-propyl and p-iso-propyl phenol) the negative free energy of transfer increases as the number of methylene groups is increased, i.e. as the hydrophobicity of the phenol molecule is increased. It is interesting to note that the negative free energy of transfer for p-isopropyl phenol is greater than p-propyl phenol, since the reverse is true for partitioning into bulk organic solvents. (Calculated using π values taken from Hansch and Leo, 1979).

The large positive enthalpies of transfer for the alkyl phenols from aqueous solution to the lipid bilayer reflects the energy required to insert the solutes into the bilayer. It is most probable that p-cresol would be inserted between the hydrocarbon chains nearer the polar head groups, whereas p-ethyl and p-propyl phenol would be located progressively nearer the more hydrophobic interior. The hydroxyl group of the phenol molecule would probably be directed towards the polar head groups, whilst the benzene ring and alkyl group would be directed towards the hydrophobic interior. More energy is likely to be required to prize apart the hydrocarbon chains, which are in a

more ordered state and packed closer together, nearer the polar head groups than the more flexible hydrocarbon chains towards the centre of the bilayer. Thus, the insertion of p-cresol into the bilayer would lead to a larger positive enthalpy change, than the insertion of p-ethyl and p-propyl phenol. This is reflected in the decrease in enthalpy going from p-cresol to p-ethyl and p-propyl phenol. There may also be a negative enthalpy contribution derived from van der Waals interactions between the hydrocarbon chains of the phospholipids and the hydrophobic moieties of the phenols. It is probable that the insertion of a phenol in the bilayer results in the disruption of hydrogen bonding and electrostatic interactions between polar head groups since such large enthalpy changes are involved. The enthalpy of partitioning of p-isopropyl phenol is slightly higher than p-propyl phenol because chain branching offers more resistance to insertion in the bilayer. The large positive entropies of transfer reflect the disruption of the bilayer rather than an increase in solute motion on removal from water. The larger entropy of transfer of p-cresol compared to p-ethyl and p-propyl phenol is attributed to the greater disturbance of the bilayer structure by the former, which is probably located in the polar head group region. The chain branching effect of p-isopropyl phenol is shown in its larger entropy of transfer than p-propyl phenol, indicating a greater disruption of the bilayer. The free energies of transfer for ortho, meta and para cresols show no significant trends. The slightly higher negative free energy of transfer for p-cresol indicates that it partitions into the bilayer to a slightly greater extent than m-cresol and

o-cresol. The enthalpy of transfer for m-cresol and p-cresol is significantly higher than that for o-cresol, indicating that more energy is required to insert the former solutes than the latter. The lower entropy of transfer for o-cresol indicates that this molecule causes less disordering or disruption of the bilayer than m-cresol and p-cresol. The evidence seems to suggest that o-cresol partitions into a slightly more hydrophobic, less ordered region than m-cresol and p-cresol.

The free energy of partitioning of the halo-phenols follows very closely the trend shown by the para substituted alkyl phenols. The negative free energy of transfer increases with increase in size of the halogen. The positive enthalpies of transfer for the halo-phenols decrease with increase in size of the para substituent, as in the case of the para alkyl phenols. However the values are considerably smaller, there are two possible explanations for this. Firstly, the halo-phenols are inserted in regions nearer the more mobile hydrophobic interior, necessitating less energy to prize the hydrocarbon chains apart. The entropy values seem to support this reasoning, since it would be expected that p-bromo phenol would be inserted in slightly less ordered, more hydrophobic region than p-fluoro phenol, resulting in less disruption of the bilayer and a smaller entropy value. Secondly, the large positive enthalpy change required to insert the halo-phenols in the bilayer is reduced by a contribution from a negative enthalpy term derived from hydrogen bond formation between the hydroxyl group of the phenol and the phosphate groups of the polar head region. There may also be hydrogen bonding between the hydroxyl group of the phenol and the acetyl groups of the

polar head region. However since the phosphate group acts as a much stronger hydrogen bond acceptor, hydrogen bonding to the acetyl groups may be negligible. The strength of hydrogen bond formation would increase in the order p-fluoro, p-chloro and p-bromo phenol respectively. This agrees with calorimetrically determined enthalpies of hydrogen bond formation between the halo-phenols and n-heptyl fluoride in an inert solvent (Jones and Watkinson, 1964). There may also be a small negative enthalpy contribution derived from van der Waals interactions between the hydrophobic regions of the halo-phenol, namely the benzene ring and halogen group, and the hydrocarbon chains of the phospholipid. Thus p-bromo phenol would receive the largest negative enthalpy contribution from hydrogen bond formation and van der Waals interactions, and this would lead to the largest decrease in the overall positive enthalpy of transfer.

The partitioning of p-methoxy phenol and p-nitro phenol shows interesting behaviour. In the aqueous/liposome system the negative free energies of these compounds increase in the following sequence, p-methoxy phenol, p-cresol, p-nitro phenol and p-ethyl phenol. However, in the aqueous/n octanol system the negative free energies increase in the order p-methoxy phenol, p-nitro phenol, p-cresol and p-ethyl phenol. This would seem to imply increased molecular interactions of p-nitro phenol with the liposome bilayer. The enthalpy of transfer for p-methoxy phenol is somewhat larger than that for p-cresol, indicating a larger energy required to disrupt intermolecular bonds to insert the molecule. This is consistent with the large entropy value. It is possible that the p-methoxy phenol molecule is inserted in a

more ordered, less hydrophobic region of the bilayer than p-cresol. The low positive enthalpy of transfer for p-nitro phenol reflects the negative enthalpy contribution due to hydrogen bonding of the hydroxyl group with the polar head groups. There may also be hydrogen bonding between the nitro group and water molecules bound to the polar head groups. The positive enthalpy contribution to the enthalpy of transfer is probably derived mainly from the destruction of solute-water hydrogen bonds rather than the disruption of van der Waals interactions between adjacent hydrocarbon chains and/or hydrogen bonding and electrostatic interactions between polar head groups. The strong phospholipid-nitro-phenolinteraction would tend to reduce the solute's movement and introduce a negative entropy contribution, resulting in a lower entropy of transfer.

The negative free energies of transfer for resorcinol and its mono-ethers remain constant in the aqueous/liposome system and are greater than that for p-cresol. However in the aqueous/n-octanol system (Table 87) the negative free energy terms increase in the order resorcinol, m-methoxy phenol and m-ethoxy phenol and are less than that for p-cresol. This would seem to indicate that the molecular interactions of resorcinol and its mono-ethers with the liposome bilayer are greater than those with n-octanol. The lower positive enthalpy of transfer for resorcinol suggests that hydrogen bonding occurs between both the resorcinol hydroxyl groups and the polar head groups of the bilayer. The entropy of transfer for resorcinol may receive a negative entropy contribution, derived from a loss in mobility due to strong interactions with the polar head groups, so that

there is a decrease in the overall positive entropy of transfer. Alternatively it may be possible that resorcinol causes less disruption of the bilayer than its mono-ethers or p-cresol. The higher enthalpy of transfer for m-methoxy phenol with respect to p-cresol suggests that the former is located in a more structured region, whilst the latter is located in a less ordered environment. This is supported by the larger entropy of transfer for m-methoxy phenol compared to p-cresol. The enthalpy and entropy of transfer for m-ethoxy phenol are very similar suggesting that this molecule is located in a similar region to p-cresol.

The free energy of transfer for p-cyano phenol is slightly greater than that for p-cresol in the aqueous/liposome system. The reverse is true in bulk organic solvents (Tables 87 and 90). The free energy of transfer of p-hydroxy benzo-trifluoride is greater than that for p-cresol. A similar situation is found for partitioning in n-octanol and cyclohexane. The enthalpy of transfer for p-cyano phenol is larger than expected, since substantial hydrogen bonding between this phenol and the polar head groups would be anticipated because of the strong electron withdrawing effect of the cyano group. The results seem to indicate that the p-cyano phenol may be located in a similar region of the bilayer to p-cresol and that hydrogen bonding with the polar head groups is small. Thus the motion of this compound within the bilayer will not be restricted by strong interactions with the polar head groups, and there will not be a negative entropy contribution to the overall large positive entropy of transfer, thus lowering its value. The entropy value for p-cyano phenol implies that this molecule causes significant disruption of the liposome bilayer. The location of p-cyano phenol

within the bilayer may be quite different than that of p-nitro phenol. The small negative enthalpy of transfer for p-hydroxy benzotrifluoride reflects the strong hydrogen bonding between the hydroxyl group of this compound and the polar head groups of the bilayer. The enthalpy of transfer receives a positive enthalpy contribution derived from the destruction of solute-water interactions, but it seems unlikely to receive a significant positive enthalpy contribution derived from the disruption of molecular interactions within the bilayer. This is supported by the relatively small entropy of transfer compared to that of p-cresol. It is reasonable to assume that the small entropy value derives from an increase in disorder on the removal of p-hydroxy benzotrifluoride from water, rather than a disruption of the bilayer. Although it is possible that strong molecular interactions of the phenol with the polar head groups will tend to restrict its motion, leading to a negative entropy contribution which might serve to reduce an overall larger entropy of transfer.

p-Hydroxy benzoic acid, methyl ester has a slightly higher negative free energy term than expected, but p-hydroxy acetanilide and p-hydroxy acetophenone follow the trend that would be predicted from their π values. The small negative enthalpy of transfer for p-hydroxy benzoic acid, methyl ester implies that strong hydrogen bonding occurs between the hydroxyl group of the molecule and the polar head groups of the bilayer. There may also be a small negative enthalpy contribution derived from hydrogen bonding between the carbonyl group and water molecules bound to the polar head groups. It is unlikely that the enthalpy term would receive a significant positive enthalpy contribution derived from the disruption

of the bilayer. This is confirmed by the relatively small entropy of transfer for this compound, which more than likely results from an increase in disorder on the removal of the phenol from water, rather than a disruption of the bilayer. There may be a loss in entropy due to the strong interactions of the phenol with the polar head groups which may reduce the overall entropy of transfer. The small positive enthalpy of transfer for p-hydroxy acetanilide also receives a negative enthalpy contribution derived from hydrogen bonding of the phenol with the polar head groups. Again the positive enthalpy contribution is derived mainly from the disruption of solute-water bonds. The entropy of transfer for this compound suggests that there is minimal disruption of the bilayer, and that the increase in entropy is mainly due to the removal of the solute from water. There may be a negative entropy term derived from a loss of mobility of the solute due to increased hydrogen bonding with the polar head groups, which could reduce the overall entropy of transfer. The relatively large negative enthalpy of transfer for p-hydroxy acetophenone suggests strong hydrogen bonding between this compound and the polar head groups of the bilayer. There appears to be a stronger interaction of this compound with the polar head groups, than that of p-nitro phenol or p-cyano phenol, which are potentially much stronger hydrogen bonders. The reason for this is unclear. However it is most probable that the spatial configuration or shape of the solute affects its positioning in the bilayer, and this determines the extent to which it can interact with the polar head groups and/or acyl chains. The negative entropy of partitioning for p-hydroxy acetophenone indicates an increase in order on the transfer from water to the DMPC liposome. This is most likely due

to the very strong interaction with the polar head groups, leading to a considerable restriction in motion (translational and rotational) and a consequent large negative entropy contribution.

The halogenated anisoles show a greater tendency to partition into the liposome bilayer than the corresponding p-halo phenols. This is due to the presence of the methoxy group which reduces solute-water interactions and increases the hydrophobicity of the molecule. The free energy of transfer for p-fluoro anisole is higher than expected and the reason for this is unclear. The higher enthalpies of transfer for the p-halo anisoles indicate that insertion of these molecules in the bilayer requires a considerable disruption of van der Waals interactions between adjacent hydrocarbon chains, and possibly hydrogen bonding and electrostatic interactions between the polar head groups. With the p-halo phenols hydrogen bonding with the polar head groups introduces a negative enthalpy contribution to the overall positive enthalpy of transfer. However this is not possible with the p-halo anisoles. The ease of accommodation in the bilayer is in the order p-bromo, p-chloro and p-fluoro anisole. p-Fluoro anisole is much more likely to be located in the ordered region nearer the polar head groups, whilst p-chloro and p-bromo anisole will be located nearer the less ordered hydrophobic interior. The high entropy values for these compounds with respect to the p-halo phenols may have several origins. The removal of the p-halo anisoles from water will probably lead to a greater increase in entropy due to a more substantial loss in water structure and a higher degree of mobility within the bilayer, compared to the p-halo phenols. The p-halo anisoles may cause a greater disruption of the bilayer than the corresponding p-halo phenols.

Replacing the hydroxyl group of p-propyl phenol by a methoxy group has the effect of increasing the negative free energy term significantly. This is due to reduced solute-water interactions and a greater affinity for the hydrophobic region of the bilayer. The positive enthalpy of transfer for p-propyl anisole is greater than that of p-propyl phenol. The hydroxyl group of p-propyl phenol is able to hydrogen bond to the polar head groups of the bilayer and this introduces a negative enthalpy contribution to the overall positive enthalpy of transfer

The larger entropy of transfer for p-propyl anisole is probably due to a larger increase in disorder on the removal of this molecule from water, and also to a larger increase in rotational and translational motion within the bilayer compared to p-propyl phenol. p-Propyl anisole may also cause a greater disruption of the bilayer. p-Nitro anisole has a lower negative free energy of transfer than p-nitro phenol due to reduced interactions with the polar head groups. The enthalpy of transfer reflects the energy required to insert the molecule within the bilayer. Strong hydrogen bonding between p-nitro phenol and the polar head groups introduces a negative enthalpy of transfer which reduces the overall positive enthalpy of transfer. The higher entropy of transfer for p-nitro anisole indicates a greater disruption of the bilayer than p-nitro phenol. There is also a greater increase in disorder on the removal of p-nitro anisole from water and a greater motional freedom within the bilayer. It is most likely that p-nitro anisole is located in a less polar, more hydrophobic region of the bilayer than p-nitro phenol.

The derived thermodynamic parameters for the partitioning

of phenolic compounds between aqueous solution and the liposome bilayer can be considered to be more qualitative than quantitative in nature, particularly the enthalpy and entropy values. Thus it is not possible to derive accurate incremental thermodynamic parameters and π values for the various substituent groups.

2. A Study of the Interaction of p-cresol with the DMPC Liposome Bilayer using D.S.C.

The disruptive influence of p-cresol on the liposome bilayer is shown clearly by the effect of various solute concentrations on the phase transition profiles, as studied by differential scanning calorimetry. The main transition profile for pure DMPC liposomes is very sharp, occurring over a narrow temperature range (Figure 53a). The positive enthalpy of transition is 23.9 kJ/mole (c.f. with literature values, Table 80) and indicates an endothermic process for the gel crystalline to liquid crystalline phase transition. The main transition peak broadens and its intensity diminishes (Figure 53b) as the solute concentration of the preparation is increased, giving a reduced enthalpy of transition (Table 79). With increasing solute concentration the pre-transition peak also diminishes until it is completely absent. When the main transition peak broadens, the temperature range in which the gel and liquid domains coexist in the bilayer increases. The liposome pellet equilibrated with the solute concentration used in the partitioning studies gave a broadened main transition peak, with an enthalpy of transition of 19.5 kJ/mole. The pre-transition peak was absent. The addition of p-cresol to the bilayer in the gel phase, causes a reduction in the cohesive forces between adjacent hydrocarbon chains

of the phospholipid molecules and leads to a fluidization of these chains. (Similar results were obtained by Jain and Wu (1977) and Fildes and Oliver (1978) using non-phenolic solutes). The absence of the pre-transition peak indicates a change in the electrostatic and hydrogen bonding interactions between polar head groups. Singer (1979) and Chaykowski et al., (1979) have recently shown that benzene derivatives increase lipid chain motion, below the phase transition, and that the more polar molecules are the most potent. The location of a solute in the bilayer is the main determinant of its perturbing ability, rather than its size or bilayer concentration (Singer, 1979; Chaykowski et al., 1979). It must be emphasized that partitioning studies must be used in conjunction with other techniques in order to obtain more precise information on the location and disruptive effect of solutes in the liposome bilayer. (Such techniques include differential scanning calorimetry (Mabrey-Gaud, 1981); spin label studies (Smith, 1979, Meier et al., 1982); x-ray and neutron diffraction studies (Akiyama, 1981; Buldt and Seelig, 1980) and fluorescent studies (Radda and Vanderkooi, 1972; Azzi, 1975)).

3. Particle Size Analysis of DMPC Liposomes.

Particle size analysis of freshly prepared multilamellar DMPC liposomes is possible using Coulter counter analysis. This method provides not only the average particle size of the liposomes, i.e. 10 μm in diameter, but also a particle size distribution. Repeated shaking, pipetting, centrifugation and re-suspension of the multilamellar DMPC liposomes reduces the average particle size to 8 μm.

Photon correlation spectroscopy produces an average particle size of 7 μm for freshly prepared multilamellar DMPC liposomes, and 5 μm for those liposomes which have been subjected to repeated shaking, pipetting, centrifugation and re-suspension. However, since photon correlation spectroscopy is most reliable for particle size analysis of homogeneous suspensions below 3 μm in size, the results obtained by Coulter counter analysis are probably the more accurate.

Sonication of the large multilamellar DMPC liposomes by sonic probe and bath sonication produced heterogeneous suspensions of liposomes, with an average particle size 250 nm and 1,500 nm respectively. The Coulter Nano-Sizer provides a quick and easy method of defining the particle size of liposomes below 3 μm.

4. A Comparison of the Techniques used to Determine Partition Coefficients between Aqueous Solution and Bulk Organic Solvents.

The mini-AKUFVE apparatus separates mixtures of water and organic solvents primarily by virtue of the difference in their densities. However other physical-chemical properties of the solvents are also important; these include the mutual solubilities and viscosity (see Table 15). Absolute phase separation of the water/cyclohexane system is relatively easy compared to the water/n-octanol system. There is a more significant difference in density between water and cyclohexane, making separation easier. The viscosity of cyclohexane is low so that problems of separation arising from resistance to flow are negligible. Finally the mutual solubilities of water and cyclohexane are low and do not vary with temperature. As a result the absorbance of the aqueous phase is not affected by a change in the amount of dissolved

cyclohexane, when the system is operated over a range of temperature. Although the actual partition coefficients determined for the aqueous/cyclohexane system agree with literature values, the derived enthalpy values (Table 76) are found not to be reproducible and tend to be lower than literature values (see Table 92).

The difference in densities of water and n-octanol is less, and makes separation more difficult. The larger viscosity of n-octanol causes some resistance to flow and this adds to the problem of phase separation. However it is possible to separate water and n-octanol at one particular temperature using the mini-AKUFVE by fine adjustment of the controls.

The physical-chemical properties of the water/n-octanol system change quite significantly with rise in temperature (see Table 15). The solubility of water in octanol increases with rise in temperature, whilst the solubility of octanol in water decreases with temperature. The viscosity of n-octanol decreases dramatically with increase in temperature. All these factors contribute to the fact that the controls of the mini-AKUFVE need to be adjusted to obtain optimum separation conditions at different temperatures. Thus it is not possible to separate water and n-octanol at different temperatures, using the same adjustment of the controls. Although the mini-AKUFVE is not the most suitable method for determining the enthalpy of transfer, it is most suited for continuous extraction of antibiotics from fermentation liquors and of therapeutic agents from plant and animal sources. The temperature of extraction is important, particularly for those molecules with large enthalpies of transfer. Precise temperature control of the mini-AKUFVE apparatus is possible. It is worth

noting that the polar solvent, n-octanol extracts most efficiently at low temperatures. Whilst the converse is true for the non-polar solvent, cyclohexane. The filter probe technique produced accurate and precise thermodynamic data for both the aqueous/n-octanol and aqueous/cyclohexane systems. Since the filter probe system separates mixtures of aqueous and organic solvents through differences in hydrophobicity, it is not affected by the temperature dependence of the physical-chemical properties of the solvents. For the pharmaceutical chemist it provides a quick and easy method of determining the partition coefficient of a drug molecule over a wide range of experimental conditions, namely temperature, different solvents, ionic strength, and pH. (It is possible to study the pH-partitioning profile of a drug molecule using the filter probe and determine its pKa simultaneously - see Appendix).

Although the HPLC method of determining the temperature dependence of partitioning of solute in the aqueous/n-octanol proved unsuccessful, a good correlation between retention time and partition coefficient was produced. Poor resolution of the peaks was due to inadequate coating of the column with n-octanol.

5. The Thermodynamics of Partitioning of Phenolic Compounds between Aqueous Solution and n-Octanol.

The aqueous/n-octanol partitioning system has frequently been used as a model for the biological membrane in the belief it has similar properties to the lipid bilayer (Leo et al., 1971; Kubinyi, 1979). The hydroxyl group of n-octanol provides a hydrogen bonding acceptor group similar to that of the phosphate and acetyl groups of the polar head region of the bilayer, and a

degree of polarity to the molecule. Hydrophobicity is provided by the non-polar octyl group, and weak van der Waals interactions between adjacent octyl groups may occur. Some degree of ordering is found in the n-octanol phase due to hydrogen bonding between octanol molecules, and also between octanol and water molecules (Anderson et al, 1978). n-Octanol comprises (on a mole fraction basis) of 27% water at 25°C (Brodin et al., 1976). The solubility of water in n-octanol increases with rise in temperature (Karl Fischer titration), whilst the solubility of n-octanol in water decreases with temperature (AKUFVE experiments). Thus the composition of the water/n-octanol system varies considerably with change in temperature.

The full thermodynamic parameters for the transfer of phenolic solutes between aqueous solution and n-octanol are shown in Table 87. Good agreement is found with literature values (Table 89). (The numerical values of ΔG and ΔS depend on the choice of units used. Therefore it is important to note the particular unit used, when comparing results from different authors. The ΔH values are independent of choice of unit). Incremental thermodynamic parameters for the transfer of the various substituent groups are given in Table 88.

The large negative free energies for all the solutes studied indicates the greater preference for the lipoidal phase over the aqueous environment. The transfer of a phenol from water to n-octanol occurs in two stages. Initially, disruption of water-phenol hydrogen bonds (positive enthalpy change) is followed immediately by the formation of new water-water hydrogen bonds (negative enthalpy change). Insertion in the lipid phase requires disruption of octanol-octanol interactions and some water-octanol

Table 87 Thermodynamic Parameters for the Transfer of Phenolic Solutes Between Aqueous Solution (.15M NaCl) and n-Octanol.

Phenolic compound	ΔG kJ/mole 22°C	ΔH kJ/mole	ΔS J/mole/K	corr. coef. r	Dominating parameter
phenol	-14.09	-6.4	+26	.996	entropy
p-cresol	-16.55	-7.3	+31	.998	entropy
o-cresol	-16.82	-6.9	+34	.929	entropy
m-cresol	-16.76	-7.6	+31	.992	entropy
p-ethylphenol	-19.01	-9.2	+33	.980	entropy
p-propyl phenol	-21.24	-8.9	+42	.964	entropy
p-bromo phenol	-20.64	-16.0	+16	.997	enthalpy
p-chloro phenol	-19.76	-15.9	+13	.989	enthalpy
p-fluoro phenol	-15.49	-11.1	+14	.996	enthalpy
p-iodo phenol	-22.23	-17.2	+17	.984	enthalpy
p-methoxy phenol	-13.17	-2.3	+37	.926	entropy
p-nitro phenol	-16.39	-17.5	-4	.998	enthalpy
p-hydroxy acetophenone	-13.30	-11.6	+6	.993	enthalpy
resorcinol	-9.94	-15.3	-18	.999	enthalpy
m-methoxy phenol	-14.26	-8.7	+24	.989	enthalpy
m-ethoxy phenol	-16.53	-7.2	+32	.973	entropy
p-cyano phenol	-14.66	-8.0	+23	.994	enthalpy
p-hydroxy benzoic acid, methyl ester	-16.25	-19.4	-11	.997	enthalpy
p-hydroxy benzo - trifluoride	-22.71	-12.7	+34	.994	enthalpy
anisole	-17.21	+0.8	+61	-.728	entropy
p-nitro anisole	-16.71	-1.0	+53	.724	entropy

The partition coefficient, K_d^x, is measured in mole fraction units, and K_d^x at 22°C is taken as theoretical value from linear regression analysis. ΔG and ΔS are determined at 22°C (295.15K).

Table 88 Incremental Thermodynamic Parameters for the Transfer of Substituent Groups Between Aqueous Solution (.15M NaCl) and n-Octanol.

Substituent Group	δΔG kJ/mole	δΔH kJ/mole	δΔS J/mole/K	Π
p-CH_3	-2.46	-0.9	+5	0.436
o-CH_3	-2.73	-0.5	+8	0.484
m-CH_3	-2.67	-1.2	+5	0.473
p-C_2H_5	-4.92	-2.8	+7	0.871
p-C_3H_7	-7.15	-2.5	+16	1.266
p-Br	-6.55	-9.6	-10	1.16
p-Cl	-5.67	-9.5	-13	1.005
p-F	-1.4	-5.1	-12	0.249
p-I	-8.14	-10.8	-9	1.441
p-OCH_3	+0.92	+4.1	+11	-0.163
p-NO_2	-2.30	-11.1	-30	0.495
p-$COCH_3$	+0.79	-5.2	-20	-0.139
m-OH	+4.15	-8.9	-44	-0.734
m-OCH_3	-0.17	-2.3	-2	0.031
m-OC_2H_5	-2.44	-0.8	+6	0.436
p-CN	-0.57	-1.6	-3	0.102
p-CO OCH_3	-2.6	-13	-37	0.382
p-CF_3	-8.62	-6.3	+8	1.526

δΔG, δΔS and Π values taken at 22°C

Table 89 Thermodynamic Parameters for the Transfer of Phenolic Solutes Between Aqueous Solution and n-Octanol - Literature Values.

Phenolic Compound	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mole/K
(1)	(20°C)	(van't Hoff)	
phenol	-8.7	-8.3	+1.4
o-cresol	-11.2	-5.5	+19.4
m-cresol	-11.5	-6.5	+17.0
p-cresol	-11.8	-8.7	+10.6
p-ethyl phenol	-14.5	-11.1	+11.6
p-propyl phenol	-18.0	-14.7	+11.5
p-fluoro phenol	-10.7	-15.3	-15.7
p-chloro phenol	-14.3	-16.9	-8.8
p-bromo phenol	-14.5	-16.8	-7.6
p-iodo phenol	-15.8	-15.4	+1.5
p-nitro phenol	-11.7	-15.6	-13.1
p-hydroxy methyl benzoate	-11.3	-21.8	-36.0
p-methoxy phenol	-8.3	-16.2	+8.5
(2)	(25°C)	(van't Hoff)	
p-chloro phenol	-18.6	-10.5	+27
p-nitro phenol	-16.1	-19.2	-10.3
p-hydroxy benzoic acid	-13.9	-23.0	-30.4
(3)	(25°C)	(van't Hoff)	
p-chloro phenol	-13.4	-15.3	-6.6
p-hydroxy benzoic acid	-8.9	-23.0	-48.0
(4)	(22°C)	(van't Hoff)	
phenol	-8.37	-5.9	+9
(5)	(25°C)	(calorimetry)	
phenol	-7.57	-10.9	+12
(6)	(25°C)	(van't Hoff)	
resorcinol	-4.88	-15.0	+34
m-methoxy phenol	-9.42	-7.9	+5
m-ethoxy phenol	-11.77	-7.20	+15
(7)	(30°C)	(calorimetry)	
m-methoxy phenol	-9.43	-8.03	+4.62
m-ethoxy phenol	-11.85	-6.95	+16.0
(8)	(22°C)	(van't Hoff)	
phenol	-8.9	-5.6	+11
p-cresol	-11.3	-8.8	+8

Table 89 Continued.

References

1. Rogers and Wong (1980), molal units.

2. Dearden and Bresnen (1982), mole fraction units.

3. Dearden and Bresnen (1981), molar units.

4. Korenman (1974, 1980), molar units.

5. Breslauer et al., (1978), molar units.

6. Beezer et al., (1980), molal units.

7. Beezer et al., (1983), molal units.

8. Elson (1978), molar units.

hydrogen bonds (positive enthalpy change) followed by the formation of octanol-phenol hydrogen bonds (negative enthalpy change). Since there is a substantial amount of water dissolved in the n-octanol phase, there may also be the re-formation of water-phenol hydrogen bonds within the lipid phase (negative enthalpy change). All the phenolic solutes studied, except anisole, exhibit negative enthalpies of transfer, indicating that there is an overall increase in the strength and/or the number of molecular interactions. The transfer of the phenolic solutes between aqueous solution and n-octanol, is exothermic in nature. Thus the partition coefficient decreases with rise in temperature. All the phenols, except p-nitro phenol, resorcinol and p-hydroxy benzoic acid, methyl ester, have positive entropy values, indicating that the transfer of the majority of phenols results in an overall increase in entropy or disorder.

The negative free energy term comprises favourable contributions from both the negative enthalpy term and the positive entropy term. Individual entropy and enthalpy contributions to the overall free energy term have been determined for each solute in order to determine the dominating parameter for the transfer process. Results are shown in Table 87. For those solutes whose substituent groups (not hydroxyl group) are capable of increasing the strength of hydrogen bonding, e.g. p-nitro phenol, the transfer process seems to be enthalpically dominated. Conversely, an entropy dominated process is found for those solutes, whose substituent groups reduce hydrogen bonding and/or increase the hydrophobicity of the molecule, e.g. p-alkyl phenols and anisole. The thermodynamics of partitioning for individual phenols will now be discussed.

As the hydrophobicity of the p-alkyl phenols is increased,

due to an increase in the number of methylene groups in the molecule, the negative free energy of transfer is increased as expected. Ortho and meta substitution have increased the free energy of transfer slightly, possibly due to steric hindrance, causing reduced hydrogen bonding in the aqueous phase. The negative enthalpies of transfer for the p-alkyl phenols increase with increase in number of methylene groups on the molecule. The enthalpy of hydrogen bond formation between phenol and a suitable base, in an inert solvent, as determined by calorimetry is reduced by the introduction of an alkyl group to phenol. (Vogel and Drago, 1970; Neerinck and Lamberts, 1966). The incremental changes in enthalpy for the alkyl groups are shown in Table 88. Similar values have been derived for the aqueous/cyclohexane system (Table 91). According to Rogers and Wong (1980) the negative increase in the enthalpy of transfer with p-alkyl group substitution is due to van der Waals forces of attraction between the alkyl group and hydrocarbon chains in the oil. However there is also another possible explanation. The removal of a hydrocarbon molecule (or alkyl group) from water, results in a restructuring of the water molecules and increased hydrogen bonding, leading to a negative enthalpy change, the magnitude of which is proportional to the size of the hydrocarbon moiety. The transfer of the alkyl groups from aqueous solution to n-octanol show an increase in negative enthalpy values going from p-methyl to p-propyl groups (Table 88). Similar values are obtained for the aqueous/cyclohexane system (Table 91). The data of Gill et al (1976) and Arnett et al. (1969) (taken from Tanford, 1980) for the transfer of hydrocarbons and aliphatic alcohols from water to pure liquid show similar trends (Table 93). The presence of the methyl group in the ortho position lowers the

negative enthalpy of transfer slightly, probably due to reduced hydrogen bonding between hydroxyl group of phenol and water molecules, because of steric hindrance. The negative enthalpy of transfer for m-cresol is slightly higher than for p-cresol, and is probably due to experimental error. The increase in entropy for the removal of an alkyl phenol from water to a lipid solvent is characteristic of the hydrophobic effect, and due to an increase in mobility of the phenol in the less ordered n-octanol phase, and a loss of water structure. The magnitude of the entropy increase is dependent on the size of the alkyl group. The small difference in entropy values for the ortho, meta and para cresols is insignificant. The negative free energy values for the halo-phenols increase with the size of the halogen group, due to increased hydrophobicity. The negative enthalpies of transfer increase in the order p-fluoro, p-chloro, p-bromo and p-iodo phenol. The strength of hydrogen bond formation between the halo-phenol and a suitable acceptor increases in the order p-fluoro, p-chloro, p-bromo and p-iodo phenol due to the overall electron withdrawing effect of the halogen (Jones and Watkinson, 1964). (Hydrogen bond donor strength is also shown by the pKa values of the phenols). Increased hydrogen bonding of the p-halo-phenols with n-octanol accounts partly for the larger negative enthalpy values compared to phenol. However by examining the aqueous/cyclohexane thermodynamic data (Tables 90 and 91) there is a decrease in enthalpy for the transfer of the halogen group from water to cyclohexane, due to a re-structuring of water molecules on the removal of the halogen group or due to van der Waals interactions of halogen groups with lipid solvent molecules. In this respect the halogen group behaves like an alkyl group, and the

transfer of such a group results in a negative enthalpy change. There is no significant difference between the entropies of transfer for the p-halo phenols, as might be expected due to increase in size of the halogen group. However the entropy values are smaller with respect to phenol, indicating that there is a smaller increase in disorder. The motion of a halo-phenol in n-octanol will probably be less than that of phenol, because of the stronger hydrogen bonding interactions, and this will introduce a negative entropy contribution to the overall positive entropy of transfer. The introduction of a methoxy group on the para position of the phenol, reduces the free energy of transfer as would be expected from water/octanol π values. The small degree of hydrogen bonding of p-methoxy phenol with n-octanol, due to the electron releasing property of the methoxy group, is shown by the small negative enthalpy of transfer. The entropy of transfer for p-methoxy phenol is larger than that of phenol, indicating a greater increase in disorder on the removal from water. In the aqueous phase there is more structuring of water molecules around p-methoxy phenol than phenol because of the bulky methoxy group. Therefore there is a greater loss in water structure on the removal of p-methoxy phenol to the organic phase, leading to a greater increase in entropy.

The effect of substituting other electron withdrawing groups in the para position on the thermodynamics of partitioning will now be discussed. The free energy of transfer for p-hydroxy acetophenone is less than phenol as would be predicted by the hydrophilic nature of the acetyl group. The negative enthalpy of transfer value for this compound indicates increased hydrogen bonding with n-octanol, compared to water. The hydroxyl group of

p-hydroxy acetophenone would act as both a hydrogen bond donor and acceptor group to the respective hydrogen bond acceptor and donor groups of n-octanol. The acetyl group would also act as hydrogen bond acceptor to the hydrogen bond donor group of n-octanol. The entropy of transfer for p-hydroxy acetophenone is less than for phenol. The increased mobility of the solute when it is removed from water may be reduced due to increased hydrogen bonding with n-octanol. The free energies of transfer for p-cyano phenol, p-hydroxy benzoic acid, methyl ester, p-nitro phenol and p-hydroxy benzotrifluoride are greater than phenol and increase in the order given by their π values. From pKa and sigma values the strength of hydrogen bonding of these phenols with n-octanol would decrease in the order p-nitro phenol, p-cyano-phenol, p-hydroxy benzotrifluoride and p-hydroxy benzoic acid, methyl ester. (pKa values were taken from : Kartum et al., 1961; Barlin and Perrin, 1966; Jencks and Regenstein, 1968; Albert and Serjeant, 1971; Biagi et al., 1975; Serjeant and Dempsey, 1979. Sigma values were taken from : Exner, 1972, 1978; Hine, 1975; Norrington et al., 1975; Hansch and Leo, 1979; Perrin et al., 1981; Schaefer et al., 1981). The enthalpy of transfer for p-cyano phenol is somewhat lower than would be predicted, and a value close to that for p-nitro phenol would be expected. Also the value for p-hydroxy benzoic acid, methyl ester is somewhat higher than expected. It is probable that the nitro and methyl carboxylate groups also take part in bonding, by acting as hydrogen bond acceptor to the n-octanol hydrogen bond donor, as well as increasing the strength of the hydrogen bond between the phenolic hydroxyl group and n-octanol. The negative entropy values for p-nitro phenol and p-hydroxy benzoic acid, methyl ester indicate an

increase in order on the transfer of these molecules. The mobility of these compounds in the lipid phase will be greatly reduced by the strong interactions with n-octanol, and there will be an overall decrease in entropy. The large positive entropy value for p-hydroxy benzo-trifluoride is due to increased structuring of water molecules around the bulky trifluoro-methyl group. On the transfer of this molecule from water to n-octanol there is an overall decrease in water structure and greater motional freedom of the solute in the lipid phase.

Resorcinol and its mono-ethers show interesting partitioning behaviour. The introduction of the m-hydroxy group to the phenol molecule, reduces the negative free energy of transfer due to increased hydrogen bonding in the aqueous phase, whilst m-methoxy and m-ethoxy group substitution increases the negative free energy of transfer, due to an increase in hydrophobicity. The high negative enthalpy of transfer for resorcinol indicates increased hydrogen bonding of both hydroxyl groups with n-octanol. The enthalpy values for m-methoxy and m-ethoxy phenol shows the stronger hydrogen bonding ability of these molecules, compared to phenol, as would be predicted from their pKa values. The negative entropy of transfer for resorcinol indicates an increase in order which is derived from a restriction in mobility in the n-octanol phase due to double hydrogen bonding. The entropy value for m-ethoxy phenol indicates that it causes a larger increase in disorder on transfer from water to n-octanol, compared with m-methoxy phenol due to its size.

Anisole has a larger negative free energy of transfer than phenol due to decreased interactions with the aqueous phase and an

increase in hydrophobicity. The small positive enthalpy change may be due to destruction of very weak interactions between the ether oxygen and water molecules. Alternatively it may be derived from the destruction of octanol-octanol interactions, necessary to transfer the solute into the lipid phase. The relatively large entropy of transfer is due to increased structuring of the water molecules, and greater degree of movement in the n-octanol phase, compared to phenol, since the lipid-solute interactions are very small. p-Nitro anisole has a slightly lower negative free energy of transfer than anisole, because of slightly increased solute-water interactions due to hydrogen bonding between the nitro group and water molecules. The small negative enthalpy value is due to hydrogen bonding between the hydroxyl group of n-octanol and the nitro group. The entropy of transfer is lower than that for anisole due to a slight restriction in motion in the lipid phase, due to hydrogen bonding interactions.

6. The Thermodynamics of Partitioning of Phenolic Compounds between Aqueous Solution and Cyclohexane.

The aqueous/cyclohexane system has distinct advantages over water and n-octanol as a reference system for partitioning studies. The fact that its composition does not change dramatically with temperature, the low water content of the organic phase and relatively low solute-lipid interactions makes interpretation of results each easier. For this reason, it is preferred by many authors for studying the thermodynamics of partitioning (Rytting et al., 1972; Martin, 1979; Kinkel et al., 1981). The derived thermodynamic parameters for partitioning of the phenolic solutes

between water and cyclohexane are given in Table 90, with the incremental values for individual substituent groups given in Table 91. Literature values are given in Table 92 and good agreement is found. Positive and negative free energy values are obtained, indicating that six of the solutes studied preferred the aqueous phase, whilst the remaining fourteen solutes preferred the organic phase. Enthalpy values are positive for all solutes indicating that the transfer process is endothermic in nature, and as the temperature is raised the partition coefficient increases. A positive enthalpy value indicates that there is an overall reduction in the number or strength of molecular interactions. The transfer of a phenolic solute from water to cyclohexane requires the breaking of solute-water bonds (large positive enthalpy change) followed by the re-formation of new hydrogen bonds between water molecules (negative enthalpy change). Insertion of the solute in the lipid phase may require disruption of weak van der Waals interactions between cyclohexane molecules (small positive enthalpy change) and may result in van der Waals interactions between phenol and cyclohexane molecules (small negative enthalpy change). The main contribution to the overall positive enthalpy value for the transfer of a phenolic solute from water to cyclohexane is derived from the destruction of solute-water interactions. The positive entropy values for all the solutes is due to the transfer from the ordered water environment, where translational and rotational motion is greatly reduced, to the organic phase, where structuring is absent, and the solute is free to move unhindered. Also a loss in the structure of water may occur when the hydrophobic moiety of the phenol is removed. Another possible source of increase in

Table 90 Thermodynamic Parameters for the Transfer of Phenolic Solutes Between Aqueous Solution (.15M NaCl) and Cyclohexane.

Phenolic compound	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mole/K	corr coeff. r	Dominating parameter
phenol	+0.55	+19.3	+64	-.990	enthalpy
p-cresol	-3.67	+18.6	+75	-.999	entropy
o-cresol	-4.85	+15.1	+68	-.990	entropy
m-cresol	-3.16	+18.0	+72	-.999	entropy
p-ethyl phenol	-6.92	+17.4	+82	-.990	entropy
p-propyl phenol	-10.64	+13.6	+83	-.996	entropy
p-bromo phenol	-4.59	+14.7	+65	-.993	entropy
p-chloro phenol	-3.55	+16.0	+66	-.999	entropy
p-fluoro phenol	-0.12	+17.9	+61	-.997	entropy
p-iodo phenol	-6.02	+11.8	+60	-.996	entropy
p-methoxy phenol	-0.11	+20.4	+71	-.995	entropy
p-nitro phenol	+3.11	+28.4	+86	-.997	enthalpy
p-hydroxy acetophenone	+6.98	+33.1	+88	-.999	enthalpy
p-hydroxy benzoic acid, methyl ester	+2.81	+26.0	+79	-.995	enthalpy
m-methoxy phenol	+0.44	+16.6	+55	-.999	enthalpy
m-ethoxy phenol	-2.42	+16.4	+64	-.998	entropy
p-cyano phenol	+8.16	+46.8	+131	-.997	enthalpy
p-hydroxy benzo-trifluoride	-4.88	+18.6	+79	-.996	entropy
anisole	-18.16	+0.8	+64	-.931	entropy
p-methyl anisole	-19.24	+0.9	+68	-.648	entropy

The partition coefficient, K_d^x is in mole fraction units, and the value of K_d^x at 22°C is taken from linear regression analysis of data.
ΔG and ΔS are determined at 22°C (295.15K)

Table 91 Incremental Thermodynamic Parameters for the Transfer of Substituent Groups Between Aqueous Solution (.15M NaCl) and Cyclohexane.

Substituent Group	$\delta\Delta G$ kJ/mole	$\delta\Delta H$ kJ/mole	ΔS J/mole/K	Π
p-CH_3	-4.22	-0.7	+11	0.747
o-CH_3	-5.4	-0.42	+4	0.955
m-CH_3	-3.71	-1.3	+8	0.656
p-C_2H_5	-7.47	-1.9	+18	1.322
p-C_3H_7	-11.19	-5.7	+19	1.980
p-Br	-5.14	-4.6	+1	0.909
p-Cl	-4.1	-3.3	+2	0.726
p-F	-0.67	-1.4	-3	0.118
p-I	-6.57	-7.5	-4	1.162
p-OCH_3	-0.66	+1.1	+7	0.116
p-NO_2	+2.56	+9.1	+22	-0.453
p-$COCH_3$	+6.43	+13.8	+24	-1.139
p-$COOCH_3$	+2.26	+6.7	+15	0.047
m-OCH_3	-0.11	-2.7	-9	0.089
m-OC_2H_5	-2.97	-2.9	0	0.331
p-CN	+7.61	+27.5	+67	-1.348
p-CF_3	-5.43	-0.7	+13	0.964

$\delta\Delta G$, $\delta\Delta S$, and Π values taken at 22°C

Table 92 Thermodynamic Parameters for the Transfer of Phenolic Solutes Between Aqueous Solution and Cyclohexane - Literature Values.

Phenolic compound	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mole/K
(1)	(25°C)	(van't Hoff)	
phenol	+0.03	+21.2	+71
p-cresol	-3.29	+21.5	+83
p-ethyl phenol	-6.59	+21.0	+93
p-propyl phenol	-10.34	+20.0	+102
p-methoxy phenol	-1.44	+23.4	+74
p-bromo phenol	-4.23	+17.4	+73
p-chloro phenol	-3.23	+17.2	+78
p-fluoro phenol	+0.04	+22.8	+76
p-iodo phenol	-6.03	+14.9	+70
(2)	(22°C)	(van't Hoff)	
phenol	+3.9	+22.2	+62
p-cresol	+1.3	+25.5	+82
o-cresol	-0.55	+17.0	+59
m-cresol	+1.5	+6.7	+85
p-propyl phenol	-5.9	+10.9	+57
(3)	(22°C)	(van't Hoff)	
phenol	-0.58	+22.6	+77
o-cresol	-5.00	+18.6	+79
m-cresol	-3.65	+18.9	+76
(4)		(van't Hoff)	
o-cresol	-5.3	+15.6	+70
p-cresol	-3.6	+15.3	+63
(5)		(van't Hoff)	
phenol		21.8	
o-cresol		17.2	
m-cresol		19.2	
p-cresol		19.7	
p-chloro phenol		19.6	
		(calorimetry)	
phenol		19.7	
o-cresol		17.6	
m-cresol		20.5	
p-cresol		20.1	
p-chloro anisole		18.4 3.35	

Table 92 Continued

Phenolic Compound	ΔG kJ/mole	ΔH kJ/mole	ΔS J/mole/K
(6)	(25°C)	(van't Hoff)	
phenol	+4.2	+17.2	+42
p-cresol	1.26	+17.2	+50
p-ethyl phenol	-1.68	+16.6	+63
p-propyl phenol	-5.46	+14.7	+67
b-butyl phenol	-9.66	+12.2	+71
p-methoxy phenol	+6.9	+18.9	+43

References

1. Anderson et al., (1983), mole fraction units.

2. Elson (1978), molar units (calculated).

3. Elson (1978), mole fraction units.

4. Dearden and Bresnen (1982), mole fraction units.

5. Huyskens and Tack (1975).

6. Crugman (1971), molar units.

Table 93 Incremental Enthalpy Values for the Transfer of Methylene and Alkyl Groups of Hydrocarbons from Water to Pure Liquid Hydrocarbon at 25°C (Gill et al., 1976).

Group	δΔH cal/mole
CH_2	-800
C_2H_4	-1,700
C_3H_6	-2,000
C_4H_8	-2,500
CH_3	+90
C_2H_5	-20
C_3H_8	-50

Incremental Enthalpy Values for the transfer of Methylene Groups of Aliphatic Alcohols from Water to Pure Liquid at 25°C (Arnett et al., 1969).

Group	δΔH cal/mole
CH_2	-10
C_2H_4	-180
C_3H_6	-560

entropy may be that the 'clusters' of water molecules may have greater motional freedom once the structuring influence of the phenolic solute has been removed. The overall process, either entropy or enthalpy dominated, depends upon the type of substituent in the molecule. Generally, for those solutes whose substituents increase solute-water interactions (e.g. p-nitro and p-cyano groups) either by increasing the strength of hydrogen bonding of the hydroxyl group of the phenol or participate in hydrogen bonding themselves, the transfer process is enthalpically controlled, whilst for those solutes whose substituents reduce solute-water interactions due to the hydrophobic effect, (e.g. p-alkyl, p-trifluoro methyl groups) partitioning is entropy controlled.

Individual results will now be discussed. The positive free energy of transfer for phenol shows that it prefers the aqueous phase over the organic environment. The p-alkyl phenols have negative free energy values showing a greater tendency to partition into the organic phase. The hydrophobicity of the solute has been increased due to methylene group contribution, solute-water interactions have been reduced, and the solute now prefers the organic phase. Substitution of the methyl group in the ortho position has increased the free energy of transfer, with respect to p-cresol, and therefore preference for the organic phase. The close proximity of the methyl group to the hydroxyl group reduces hydrogen bonding, and therefore solute-water interactions, by steric hindrance. m-Cresol has a slightly lower negative free energy term than p-cresol, indicating that preference for the lipid solvent has been diminished.

The positive enthalpy of transfer for phenol is derived

mainly from the disruption of solute-water bonds. The introduction of an alkyl group into the para position decreases the positive enthalpy of transfer. The decrease in enthalpy is proportional to size of alkyl group. The incremental enthalpy values (Table 91) for the alkyl groups are similar to those for the water/octanol system (Table 88). The decrease in enthalpy, found on the introduction of an alkyl group, is due to a negative enthalpy contribution derived from increased hydrogen bonding on the removal of alkyl groups from water and possible van der Waals interactions with cyclohexane molecules. The negative enthalpy contribution increases with size of alkyl group. The enthalpy of transfer for o-cresol is slightly smaller than for m- and p-cresol, due to reduced hydrogen bonding between the hydroxyl group of phenol and water, because of steric hindrance. For the p-alkyl phenols the positive entropy of transfer increases with size of group, as would be expected from the hydrophobic effect. The transfer of the alkyl phenol from water to cyclohexane results in a decrease in the structure of water and a greater mobility of the phenol in the structureless organic phase. The entropy of transfer for o-cresol is less than for p-cresol, since the presence of this molecule causes less structuring of water molecules around it.

The negative free energies for the halo-phenols increase in the order p-fluoro, p-chloro, p-bromo and p-iodo phenol as the size of the para substituent is increased. The increase in the hydrophobicity of the molecule increases the preference for the organic phase. Since the p-iodo phenol-water hydrogen bond is stronger than p-fluoro phenol-water hydrogen bond, it might be expected that the enthalpy of transfer for p-iodo phenol would be

greater than that of p-fluoro phenol, because stronger hydrogen bonds would have to be broken. However the reverse is true. This is due to a negative enthalpy contribution derived either from increased hydrogen bond formation between water molecules on the removal of the halogen groups from water, or from van der Waals interactions between the halogen groups and cyclohexane molecules. The magnitude of the negative enthalpy contribution is proportional to size of halogen group. In this respect the halogen atom behaves like an alkyl group. This effect was also seen in the water/n-octanol system. There is little difference between entropy values for the halo-phenols. It might be expected that the entropy value would increase with size of group. However since the derived entropy of transfer is the least accurate of the thermodynamic parameters, any trend may be masked by experimental error.

The introduction of the p-methoxy group into the phenol molecule, does not seem to have appreciably altered the thermodynamic parameters of transfer. The small negative free energy of transfer indicates that the hydrophobicity of the molecule has been increased and it now prefers the organic phase. Since the presence of the para substituted methoxy group reduces the strength of hydrogen bonding of the hydroxyl group with water molecules, the enthalpy of transfer might be expected to be slightly lower than for phenol. In fact it is found to be slightly higher. However the difference is within experimental error. The entropy of transfer for p-methoxy phenol is slightly larger than that for phenol indicating a slightly larger increase in disorder on transfer. This is due to a decrease in structuring of water

molecules and an increase in motional freedom of p-methoxy phenol on transfer.

Substitution with the electron withdrawing p-nitro, p-acetyl, p-cyano and p-methyl carboxylate groups has a considerable effect on the partitioning characteristics. The positive free energy of transfer increases in the order p-hydroxy benzoic acid, methyl ester, p-nitro phenol, p-hydroxy acetophenone and p-cyano phenol. This is due to increased molecular interactions with water and a greater tendency to remain in the aqueous phase. The high enthalpy values indicate the large amount of energy required to break the strong molecular interactions between the solutes and water molecules. The strength of hydrogen bonding increases in the order p-hydroxy benzoic acid, methyl ester, p-nitro phenol, p-hydroxy acetophenone and p-cyano phenol and corresponds to the increase in free energy terms. The entropy of transfer for these phenols is increased, with respect to phenol, due to increased molecular interactions with the aqueous phase. These phenols are more strongly bound in the aqueous phase through increased hydrogen bonding of the hydroxyl group and probably the other polar group with water molecules, and when it is transferred it undergoes a greater degree of motional freedom in the organic phase.

Substitution of phenol with p-trifluoro methane has increased the hydrophobicity of the molecule, and consequently the free energy term has become more negative. The enthalpy of transfer of p-hydroxy benzo-trifluoride is slightly lower than expected, since the strongly electron withdrawing properties of the substituted group might have increased the strength of the hydrogen bonds with water. However the increased hydrophobicity of the molecule would

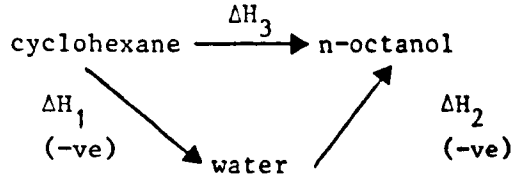
tend to reduce solute-water interactions, and tend to drive the solute into the organic phase. Another reason may be that a negative enthalpy contribution from van der Waals interactions with cyclohexane molecules or increased hydrogen bonding of water molecules on the removal of the phenol may have reduced the positive enthalpy term. The larger entropy of transfer compared to phenol, is due to the bulky trifluoro-methane group producing greater structuring of water molecules and leading to a larger hydrophobic effect.

Although m-methoxy phenol has a slightly positive free energy term, it favours the organic phase slightly more than phenol. Substitution with a m- ethoxy group has produced a negative free energy term, indicating a preference for the organic phase due to increased hydrophobicity. The smaller enthalpies of transfer for m-methoxy and m-ethoxy phenol may be due to a negative enthalpy contribution derived from van der Waals interactions of the methoxy or ethoxy group with cyclohexane molecules and/or a restructuring of the water molecules on the removal of the solutes to cyclohexane. The entropy values show no significant changes.

Anisole and p-methyl anisole show large negative free energy values indicating a much greater preference for the organic phase, due to reduced solute-water interactions and increased hydrophobicity. The small positive enthalpy values indicate negligible interactions with water molecules. The removal of anisole and p-methyl anisole from water shows the same increase in disorder as when phenol is removed.

7. The Enthalpy of Transfer of Phenolic Compounds between Cyclohexane and n-Octanol.

Since it is assumed that solute-solvent interactions in cyclohexane are negligible, it is useful to study the thermodynamics of transfer of solutes between cyclohexane and n-octanol, as only the interactions with the polar solvent will be revealed. The enthalpy of transfer of the phenolic compounds between cyclohexane and n-octanol can be derived from the thermodynamic data using the following sequence :



This is really a statement of Hess's Law of Constant Heat Summation, which states that the overall heat change in a chemical reaction (or process) is independent on the route taken. The derived enthalpy values are given in Table 94. Whilst the overall accuracy of this data is questionable, nonetheless interesting trends are observed. The enthalpies of transfer for the o-, m- and p-methyl phenols are very similar, indicating that the enthalpic interactions with n-octanol are very similar. The higher negative enthalpy values for the halo-phenols indicate the stronger hydrogen bonding with n-octanol, compared with phenol. Those phenols with strong electron withdrawing groups, which are capable of hydrogen bonding with octanol, i.e. p-nitro phenol, p-hydroxy benzoic acid, methyl ester and p-cyano phenol, have much higher negative enthalpies of transfer, indicating much increased hydrogen bonding on transfer. The enthalpy value for p-hydroxy benzotrifluoride indicates stronger hydrogen bonding with n-octanol than does phenol, but it is lower than other strongly

Table 94 Enthalpies of Transfer of Phenolic Compounds Between Cyclohexane and n-Octanol.

Phenolic Compounds	ΔH kJ/mole
phenol	-25.7*
p-cresol	-25.9
o-cresol	-22.0
m-cresol	-25.6
p-ethyl phenol	-26.6
p-propyl phenol	-22.5
p-bromo phenol	-30.7
p-chloro phenol	-31.9
p-fluoro phenol	-29.0
p-iodo phenol	-29.0
p-methoxy phenol	-22.7
p-nitro phenol	-45.9
p-hydroxy acetophenone	-44.7
p-hydroxy benzoic acid, methyl ester	-45.4
p-cyano phenol	-54.8
p-hydroxy benzo-trifluoride	-31.7
m-methoxy phenol	-25.7
m-ethoxy phenol	-23.6
anisole	0

(* Breslauer et al (1978) obtained a value of 27.3kJ/mole for the enthalpy of transfer of phenol between octane and n-octanol using calorimetry.)

hydrogen bonding phenols because the trifluoro methyl group is not capable of hydrogen bonding interactions with n-octanol. The zero enthalpy value for anisole indicates negligible interactions with n-octanol. The transfer of anisole from cyclohexane to n-octanol may involve a small enthalpy change due to disruption of van der Waals interactions with cyclohexane molecules, but this may be cancelled by a small negative enthalpy change on the formation of van der Waals interactions with n-octanol. The relationship between the enthalpy of transfer between cyclohexane and n-octanol and the sigma minus values (σ^-) and pKa values for the phenols has been investigated.

Figure 57 shows the relationship of the enthalpy of transfer, between cyclohexane and n-octanol, and the sigma minus values for the phenols. The equation of the line is as follows :

$$\Delta H = -20.48\ \sigma^- - 26.25,$$

$$r = -.930;\quad n = 17$$

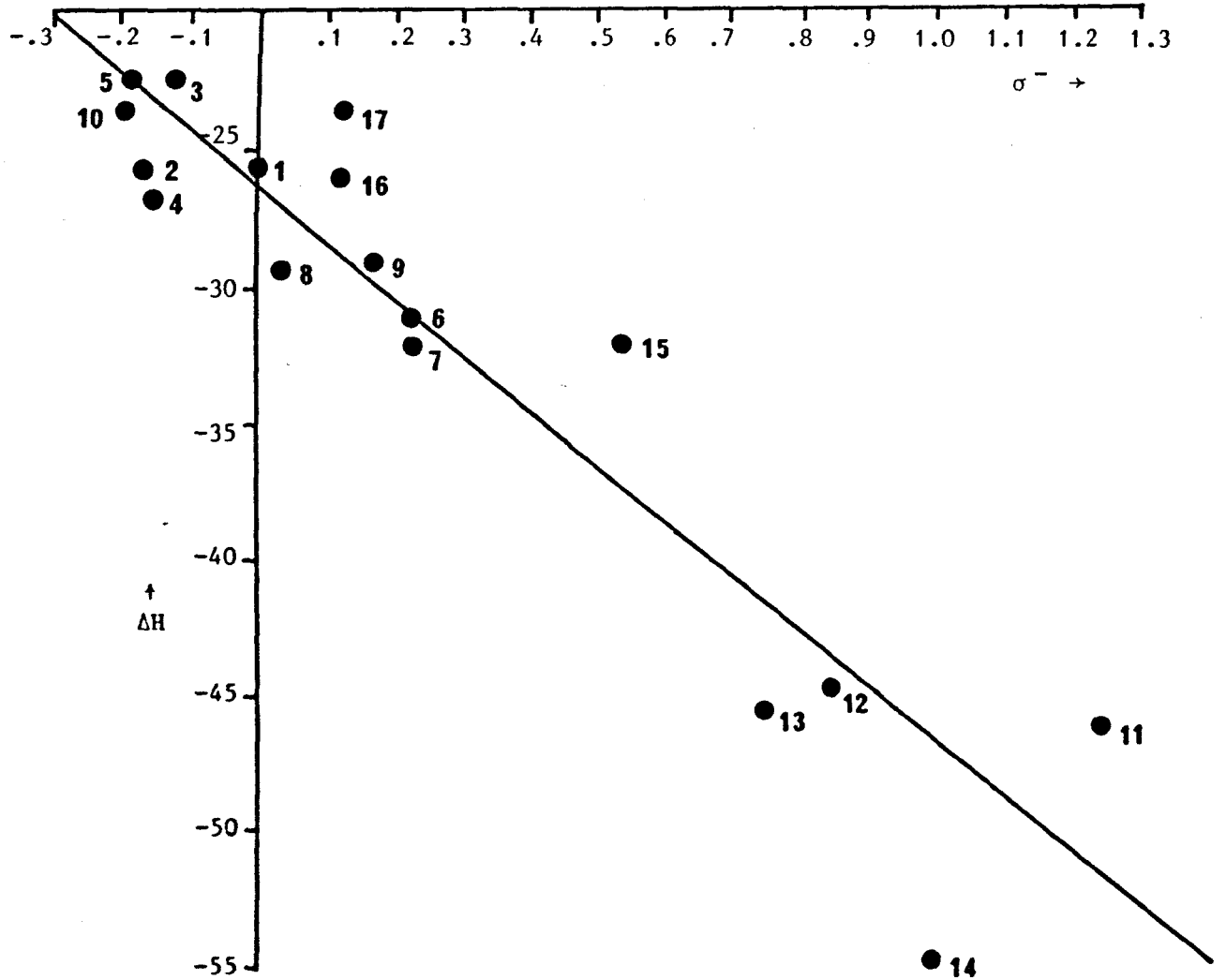
Figure 58 shows the relationship of the enthalpy of transfer between cyclohexane and n-octanol and the pKa values for the phenols. The equation of the line is as follows :

$$\Delta H = 9.604\ pKa - 121.71,$$

$$r = +.921;\quad n = 17$$

Excellent correlations are found in both cases.

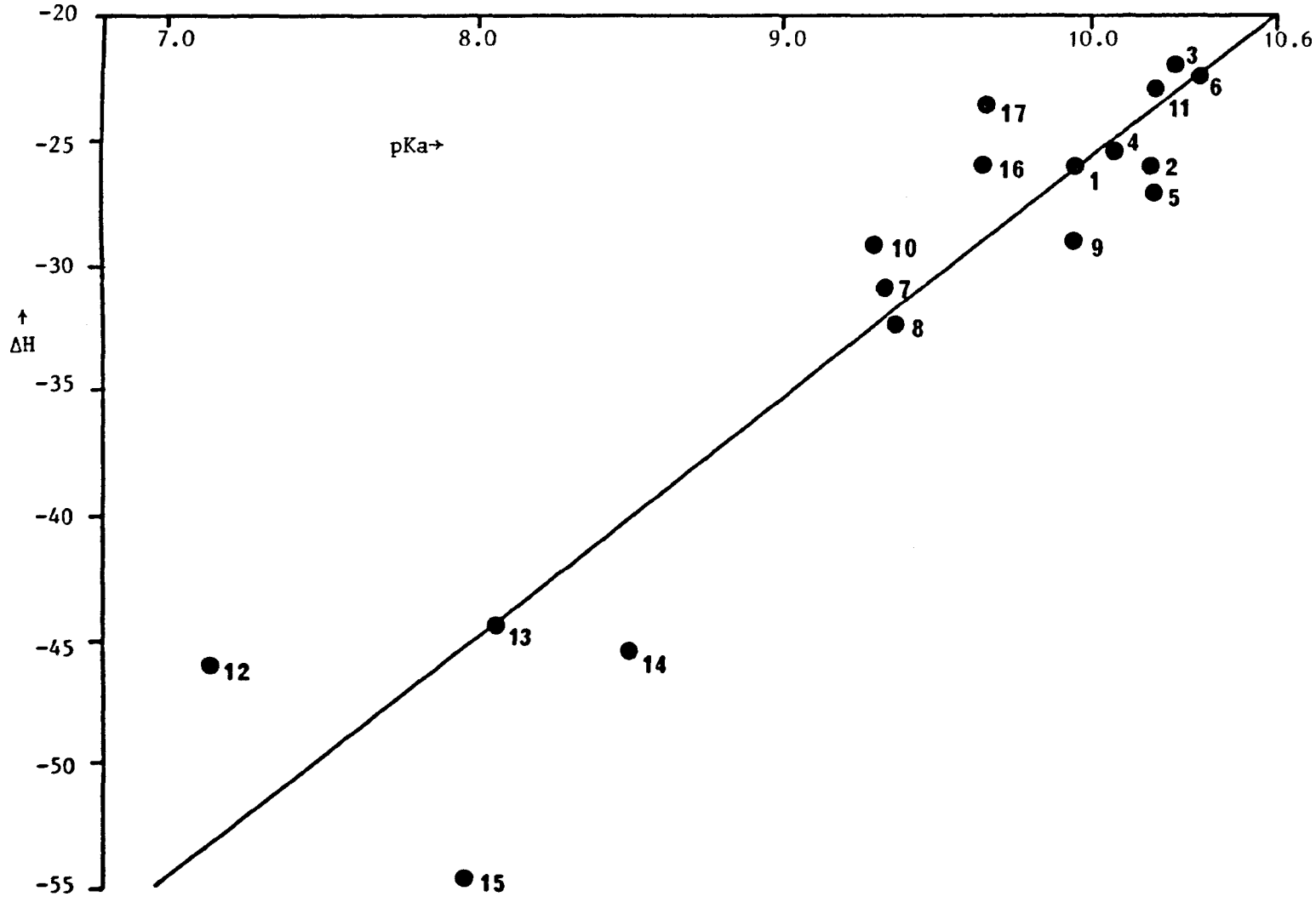
Figure 57 ΔH (cyclohexane → n-octanol) versus σ^- for phenols.



Key to Figure 57

Number	Phenol
1	phenol
2	p-cresol
3	o-cresol
4	p-ethyl phenol
5	p-propyl phenol
6	p-bromo phenol
7	p-chloro phenol
8	p-fluoro phenol
9	p-iodo phenol
10	p-methoxy phenol
11	p-nitro phenol
12	p-hydroxy acetophenone
13	p-hydroxy benzoic acid, methyl ester
14	p-cyano phenol
15	p-hydroxy benzo-trifluoride
16	m-methoxy phenol
17	m-ethoxy phenol

Figure 58 ΔH (cyclohexane → n-octanol) versus pKa for phenols.



Key to Figure 58

Number	Phenol
1	phenol
2	p-cresol
3	o-cresol
4	m-cresol
5	p-ethyl phenol
6	p-propyl phenol
7	p-bromo phenol
8	p-chloro phenol
9	p-fluoro phenol
10	p-iodo phenol
11	p-methoxy phenol
12	p-nitro phenol
13	p-hydroxy acetophenone
14	p-hydroxy benzoic acid, methyl ester
15	p-cyano phenol
16	m-methoxy phenol
17	m-ethoxy phenol

8. A Discussion of the Use of the van't Hoff Method to Determine the Enthalpy of Transfer.

When deriving the enthalpy of transfer by the van't Hoff method it is assumed that the enthalpy term is temperature independent over the temperature range studied. Much criticism has been made of determining the van't Hoff enthalpy of transfer for solutes in the aqueous/n-octanol system, since its composition changes significantly with temperature, and it is argued that the enthalpy term will no longer be temperature independent (Kinkel et al., 1981). However close examination of the plots of $\ln K_d^x$ versus $\frac{1}{T}$ for phenols in the aqueous/n-octanol system show excellent linearity, good correlation and little evidence of curvature. Similar results are obtained for the water/cyclohexane system. This suggests that over the temperature range studied, approximately 30°C, the enthalpy of transfer is temperature independent. Calorimetrically determined enthalpies of transfer for phenols in the aqueous/n-octanol and aqueous/cyclohexane systems are given in Tables 89 and 92 respectively. The calorimetrically derived enthalpy of transfer for phenol between water and n-octanol (Breslauer et al., 1978) is slightly larger than the van't Hoff enthalpy of transfer (Table 87). However the recently determined calorimetric enthalpies of transfer of m-methoxy and m-ethoxy phenol between water and n-octanol, by Beezer et al., 1983 (Table 89), agree exactly with the van't Hoff enthalpies of transfer for these compounds (Table 87). Huyskens and Tack (1975) derived calorimetric enthalpies of transfer of phenols between aqueous solution and cyclohexane (Table 92) and these agree favourably with the van't Hoff enthalpies (Table 90). When a larger temperature range is used,

50°C, there is evidence of curvature in plots of $\ln K_d^x$ versus $\frac{1}{T}$. There is also curvature in plots of $\ln K_d^m$ versus $\frac{1}{T}$ for both water/n-octanol and water/cyclohexane systems, although correlation is still good. The use of the mole fraction unit takes into account the change in density and molar volume of the solvents with temperature and this serves to reduce the variation in enthalpy due to change in composition with temperature. The plots of $\ln K_d$ versus $\frac{1}{T}$ for solutes in the aqueous/liposome system show less linearity than for those in the aqueous/bulk organic solvent systems. However this is due more to inaccuracies in partition coefficient measurement, rather than the temperature dependence of the enthalpy term, since the temperature range used is narrow (17°C).

8. An Investigation of Enthalpy-Entropy Compensation.

According to Lumry and Rajender (1970) an enthalpy-entropy compensation phenomenon for a chemical reaction or process appears to be a thermodynamic manifestation of the structure forming and structure breaking properties of water solutions. If an enthalpy-entropy compensation pattern is observed when the thermodynamics of partitioning of a series of related compounds, e.g. substituted phenols, between aqueous solution and an organic phase is studied, then it is reasonable to assume that the same mechanism is involved in the transfer process. Krug et al., (1976a and 1976b) have recommended that for thermodynamic quantities derived from van't Hoff relationships, regressions between these quantities should be carried out in ΔG_{Thm} - ΔH_{Thm} coordinates (where Thm refers to the harmonic mean temperature of the experiments). Linear enthalpy-

entropy compensation can arise as statistical artefacts, since the enthalpy and entropy terms are derived from the same set of data. However the use of Thm values minimizes any statistical bias in the subsequent analysis. Using 25°C as the harmonic mean temperature, the partitioning of the phenolic compounds in the aqueous/n-octanol and aqueous/cyclohexane systems is examined for enthalpy-entropy compensation. Figure 59 gives the relationship for the aqueous/n-octanol system and Figure 60 the relationship for the aqueous/cyclohexane system. It would appear that enthalpy-entropy compensation is not present in the aqueous/-n-octanol system, whilst it is present in the aqueous/cyclohexane system. Similar results were found by Kinkel et al. (1981). However closer examination of the free energy-enthalpy relationship for the aqueous/n-octanol system shows that there is a correlation between ΔG and ΔH for structurally related phenols. The alkyl phenols (1) seem to exhibit enthalpy-entropy compensation in the transfer from the aqueous phase to n-octanol and a good correlation between ΔG and ΔH is obtained.

$$\Delta G = 1.983\ \Delta H - 2.193\ ;\ r = .890;\ n = 8$$

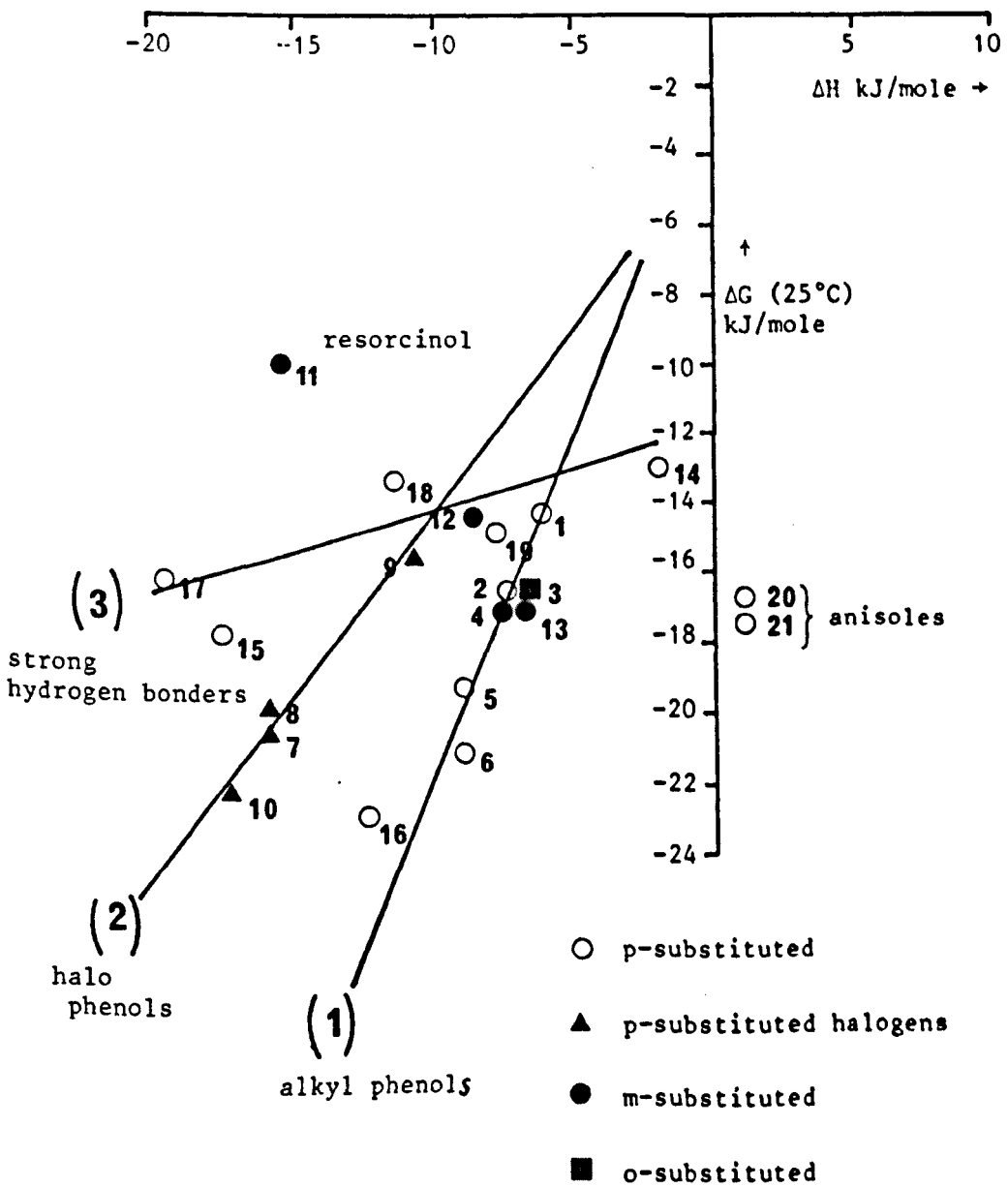
The alkyl group has the effect of increasing the hydrophobicity of the molecule and the transfer of these phenols is mainly governed by hydrophobic interactions, although hydrogen bonding in both phases will be present. The second correlation is found for the p-halo-phenols (2).

$$\Delta G = 1.05\ \Delta H - 3.72;\ r = .987;\ n = 4$$

It is reasonable to assume that the halo-phenols will be transferred by the same mechanism. The halogen molecule increases the hydrophobicity of the molecule due to its size, and hydrogen bonding

Figure 59

Relationship between free energy and enthalpy of transfer for the water/octanol system.

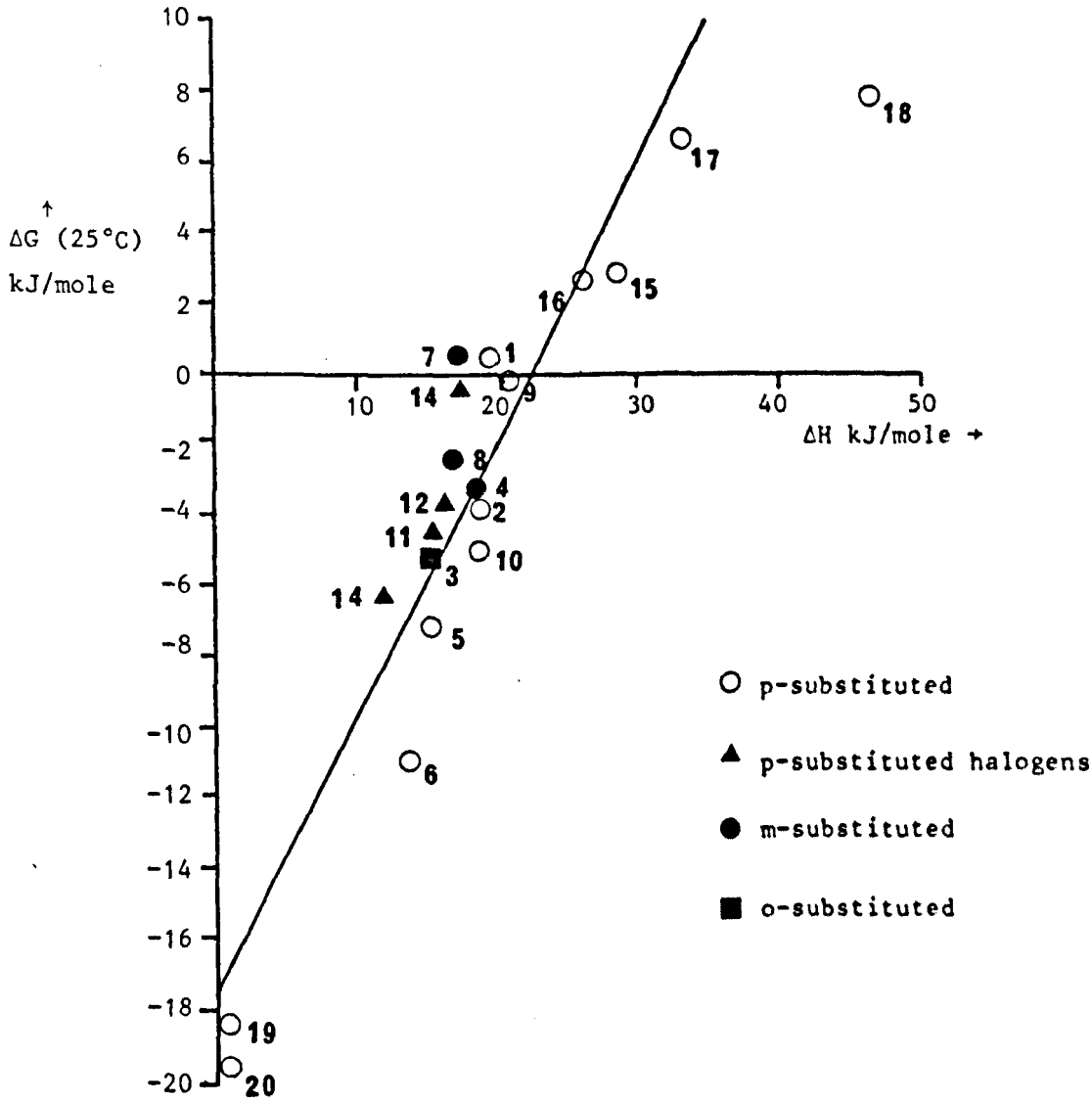


Key to Figure 59

1. phenol
2. p-cresol
3. o-cresol
4. m-cresol
5. p-ethyl phenol
6. p-propyl phenol
7. p-bromo phenol
8. p-chloro phenol
9. p-fluoro phenol
10. p-iodo phenol
11. resorcinol
12. m-methoxy phenol
13. m-ethoxy phenol
14. p-methoxy phenol
15. p-nitro phenol
16. p-hydroxy benzotrifluoride
17. p-hydroxy benzoic acid, methyl ester
18. p-hydroxy acetophenone
19. p-cyano phenol
20. anisole
21. p-nitro anisole

Figure 60

Relationship between free energy and enthalpy of transfer for the water/cyclohexane system.



Key to Figure 60

1. phenol
2. p-cresol
3. o-cresol
4. m-cresol
5. p-ethyl phenol
6. p-propyl phenol
7. m-methoxy phenol
8. m-ethoxy phenol
9. p-methoxy phenol
10. p-hydroxy benzotrifluoride
11. p-bromo phenol
12. p-chloro phenol
13. p-fluoro phenol
14. p-iodo phenol
15. p-nitro phenol
16. p-hydroxy benzoic acid, methyl ester
17. p-hydroxy acetophenone
18. p-cyano phenol
19. anisole
20. p-methyl anisole

with n-octanol is increased due to its hydrogen bond strengthening effect. A third correlation (3) is found for those phenols whose substituents increase the strength of the phenolic hydrogen and can also act as hydrogen bond acceptors. These solutes are p-nitro phenol, p-hydroxy benzoic acid, methyl ester, p-hydroxy acetophenone and p-cyano phenol.

$$\Delta G = .22\ \Delta H - 12.11;\ r = .743;\ n = 4.$$

The mechanism involved in the transfer of these compounds receives a major contribution from increased hydrogen bonding with n-octanol. The transfer of anisole and p-methyl anisole from water to n-octanol would be governed by hydrophobic interactions, and hydrogen bonding interactions with either phase would be negligible. The result for resorcinol is difficult to explain. The complex nature of n-octanol and the many different mechanisms involved in the transfer of the phenolic compounds makes the study of enthalpy-entropy compensation in this system difficult.

The transfer of the substituted phenols from aqueous solution to cyclohexane, involves the same basic mechanism; i.e. the breaking of solute-water interactions, followed by a strong hydrophobic interaction and possibly weak van der Waals interactions between the phenol molecules and the cyclohexane molecules. Therefore it is most likely that an enthalpy-entropy compensation effect would exist for this system. The correlation between ΔG and ΔH is very good for all compounds, except p-cyano phenol

$$\Delta G = .798\ \Delta H - 17.73;\ r = .933;\ n = 19.$$

An investigation of enthalpy-entropy compensation for the partitioning of substituted phenols into DMPC liposomes (below the phase transition temperature) is difficult because of the wide

variation in the free energy and enthalpy values. However if the free energy and enthalpy values for the solutes in each liposome experiment are compared to the free energy and enthalpy of transfer for p-cresol (the control) which are given a zero value, then a linear relationship is found between the free energy and enthalpy of transfer terms, as in Figure 61. For the substituted phenols the relationship between free energy and enthalpy of transfer is as follows :

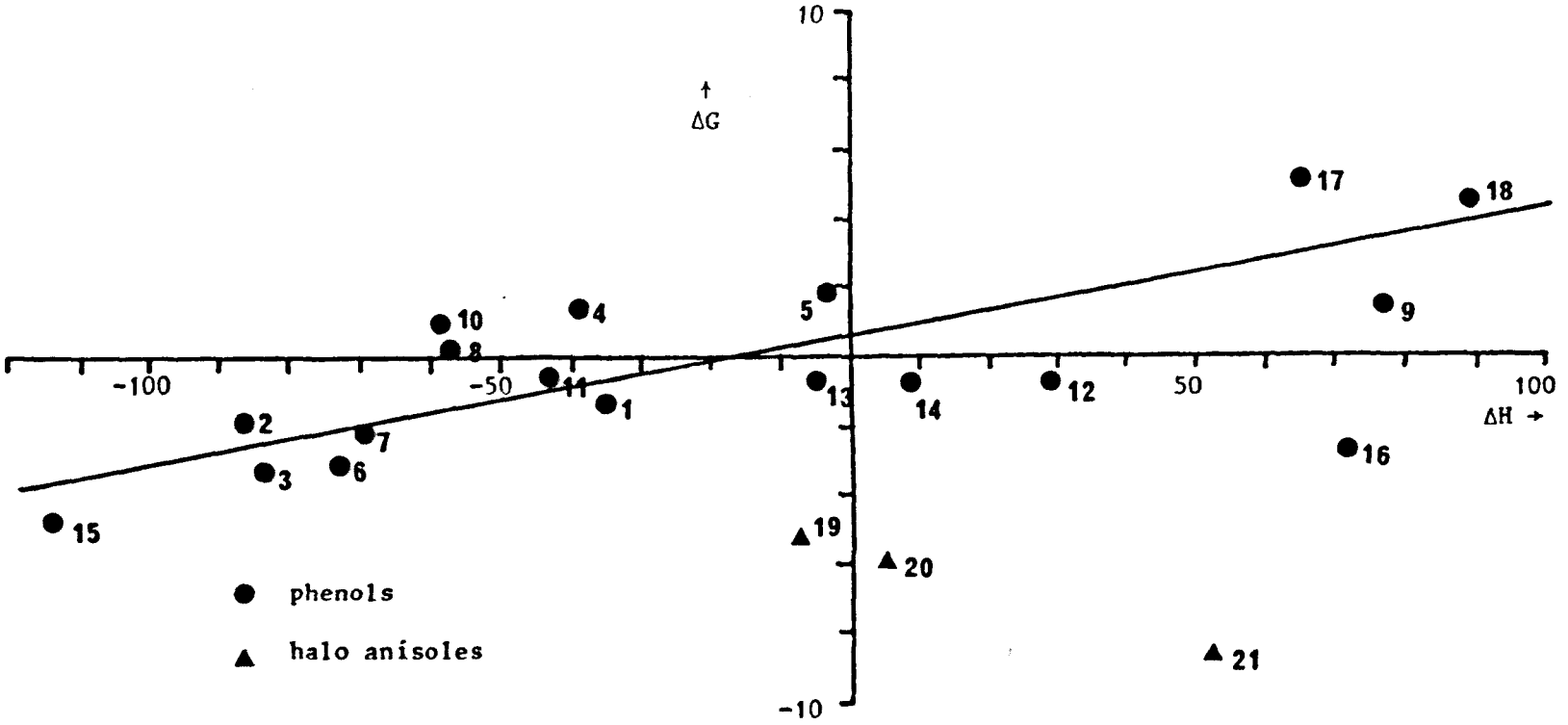
$$\Delta G = .035\ \Delta H + 0.78\ ;$$

$$r = .810,\quad n = 17$$

This seems to suggest that there is indeed an enthalpy-entropy compensation effect for the transfer of substituted phenols between aqueous solution and DMPC liposomes (below the phase transition temperature). This would imply that the phenols are transferred by the same mechanism. It is interesting to note that the p-halo anisoles show a different trend from the substituted phenols. Since these compounds do not hydrogen bond, to any significant extent, in either the aqueous or liposomal phase, the transfer mechanism for these compounds is mainly due to hydrophobic interactions, whereas for the substituted phenols hydrogen bonding interactions are important as well. It is difficult to explain why there should be one mechanism for the transfer of substituted phenols between aqueous solution and DMPC liposomes (below T_c), yet several mechanisms exist for the transfer of the same substituted phenols between aqueous solution and n-octanol.

Figure 61

Relationship between the free energy and enthalpy of transfer for phenolic compounds distributed between aqueous solution and DMPC liposomes.



Key to Figure 61

Number	Phenolic Compound
1	p-ethyl phenol
2	p-propyl phenol
3	p-isopropyl phenol
4	o-cresol
5	m-cresol
6	p-bromo phenol
7	p-chloro phenol
8	p-fluoro phenol
9	p-methoxy phenol
10	p-nitro phenol
11	resorcinol
12	m-methoxy phenol
13	m-ethoxy phenol
14	p-cyano phenol
15	p-hydroxy benzotrifluoride
16	p-hydroxy benzoic acid, methyl ester
17	p-hydroxy acetanilide
18	p-hydroxy acetophenone
19	p-bromo anisole
20	p-chloro anisole
21	p-fluoro anisole

10. A Comparison of the Thermodynamics of Partitioning of Phenolic Compounds in the Aqueous/Liposome, Aqueous/n-Octanol and Aqueous/Cyclohexane Systems.

Values of the partition coefficients of related solutes between water and various bulk organic solvents usually show a systematic relationship (Collander, 1949) which may be described by :

$$\text{Log } K_y = a.\text{Log } K_x + b$$

for a solute in solvent y compared with solvent x. The slope, a, is indicative of the similarity of the solvent environment in each case with respect to the solute, and is referred as a selectivity constant (Diamond and Katz, 1974). Partitioning of phenolic solutes in the aqueous/liposome system is compared with partitioning in the aqueous/n-octanol and aqueous/cyclohexane systems respectively. The liposome bilayer is taken as solvent y, whilst the bulk organic solvent is solvent x. Plots of $\text{Log } K_{d\ \text{lecithin}}$ versus $\text{Log } K_{d\ \text{octanol}}$ and $\text{Log } K_{d\ \text{lecithin}}$ versus $\text{Log } K_{d\ \text{cyclohexane}}$ are shown in Figures 62 and 63 respectively. (Partition coefficients are measured at 22°C and given in molal units).

The relationship between $\text{Log } K_{d\ \text{lecithin}}$ and $\text{Log } K_{d\ \text{octanol}}$ is as follows :

$$\text{Log } K_{d\ \text{lecithin}} = 0.786\ \text{Log } K_{d\ \text{octanol}} - 0.05\ ;$$

$r = .832,\ n = 16$ (excluding 11 and 18)

The relationship between $\text{Log } K_{d\ \text{lecithin}}$ and $\text{Log } K_{d\ \text{cyclohexane}}$ is as follows :

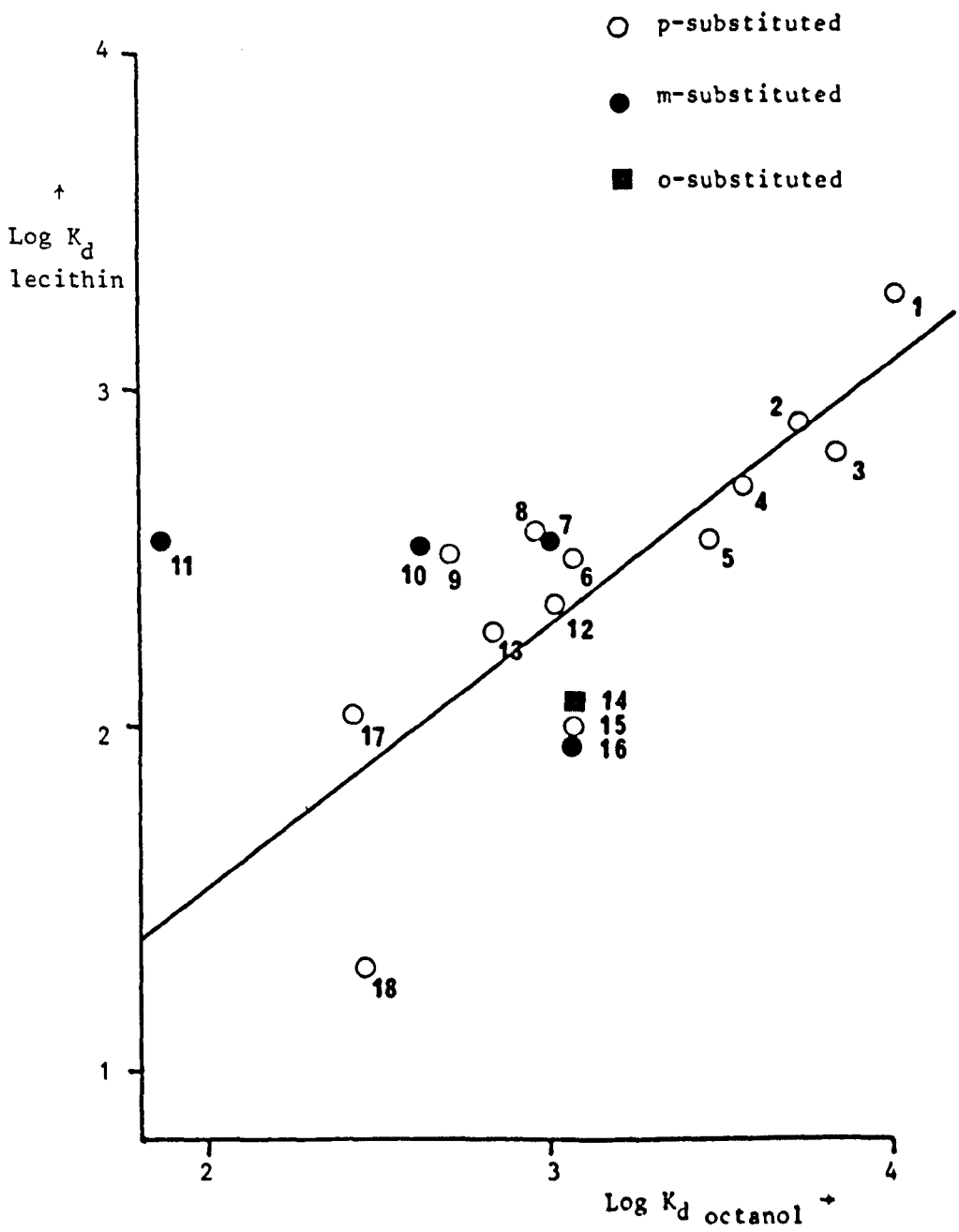
$$\text{Log } K_{d\ \text{lecithin}} = 0.238\ \text{Log } K_{d\ \text{cyclohexane}} + 2.376;$$

$r = .555;\ n = 16$

Figure 62

Log K_d lecithin (22°C) versus Log K_d octanol (22°C)

(molal units at 22°C).

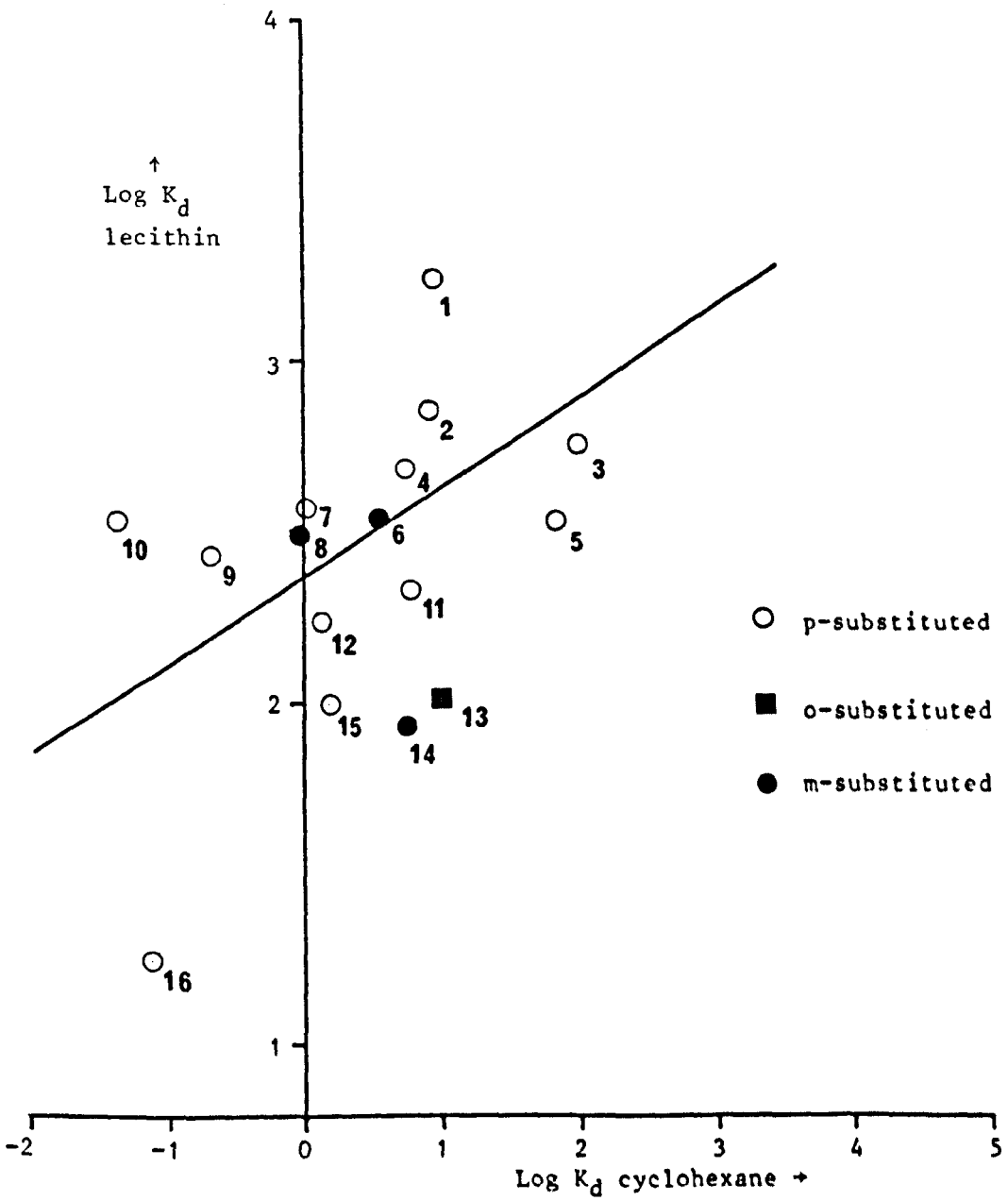


Key to Figure 62

1	p-hydroxy benzotrifluoride
2	p-bromo phenol
3	p-propyl phenol
4	p-chloro phenol
5	p-ethyl phenol
6	p-nitro phenol
7	m-ethoxy phenol
8	p-hydroxy benzoic acid, methyl ester
9	p-cyano phenol
10	m-methoxy phenol
11	resorcinol
12	p-cresol
13	p-fluoro phenol
14	o-cresol
15	p-nitro anisole
16	m-cresol
17	p-methoxy phenol
18	p-hydroxy acetophenone

Figure 63

Log K_d lecithin versus Log K_d cyclohexane (molal units at 22°C).



Key to Figure 63

1	p-hydroxy benzotrifluoride
2	p-bromo phenol
3	p-propyl phenol
4	p-chloro phenol
5	p-ethyl phenol
6	m-ethoxy phenol
7	p-hydroxy benzoic acid, methyl ester
8	m-methoxy phenol
9	p-nitro phenol
10	p-cyano phenol
11	p-cresol
12	p-fluoro phenol
13	o-cresol
14	m-cresol
15	p-methoxy phenol
16	p-hydroxy acetophenone

Obviously there is a better correlation between partitioning in the aqueous/liposome and the aqueous/n-octanol systems than in the aqueous/liposome and aqueous/cyclohexane systems. The values of the partition coefficients in the aqueous/octanol system more closely resemble in both absolute and relative terms the values in the aqueous/liposome system, than do the aqueous/cyclohexane partition coefficients. The selectivity constant, s, of 0.79 for the $K_{d\ liposome}/K_{d\ octanol}$ correlation, compared with a selectivity constant of 0.24 for the $K_{d\ liposome}/K_{d\ cyclohexane}$ relationship indicates that the interior of the liposome bilayer is more similar to n-octanol than cyclohexane. The overall character of the liposome bilayer is somewhat less hydrophobic than n-octanol, and much less hydrophobic than cyclohexane. Similar conclusions were made by Diamond and Katz (1974).

The distribution behaviour of the phenols in the water/n-octanol system is compared with partitioning in the aqueous/cyclohexane system in Figure 64. There is no obvious correlation between $\text{Log } K^x_{d\ octanol}$ and $\text{Log } K^x_{d\ cyclohexane}$ for all types of phenolic compounds. However there are definite trends for structurally related solutes. There is a linear relationship between $\text{Log } K_{d\ octanol}$ and $\text{Log } K_{d\ cyclohexane}$ for the alkyl phenols (ortho, meta and para), m- and p-methoxy phenols and m-ethoxy phenol (slope 1).

The equation of the relationship is as follows :

$$\text{Log } K^x_{d\ octanol} = 0.664\ \text{Log } K^x_{d\ cyclohexane} + 2.516;$$

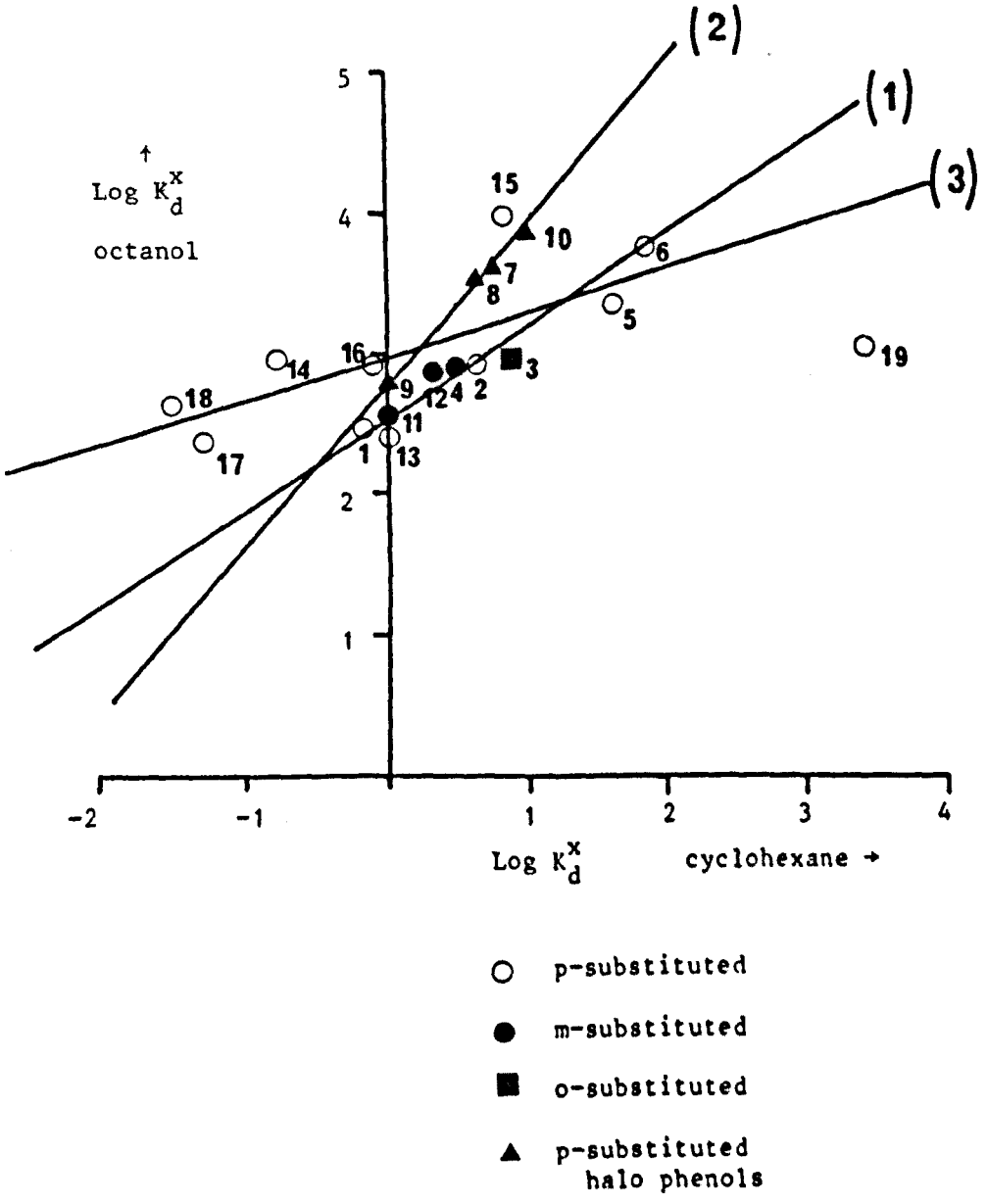
$$r = .975,\ n = 9$$

These substituents tend to reduce the strength of the hydrogen bonding of the phenol, through an electron releasing effect, and at the same time increase the hydrophobicity due to their size.

Figure 64

Log K_d^x _____ octanol versus

Log K_d^x _____ cyclohexane (mole fraction units at 22°C).



Key to Figure 64

1 phenol

2 p-cresol

3 o-cresol

4 m-cresol

5 p-ethyl phenol

6 p-propyl phenol

7 p-bromo phenol

8 p-chloro phenol

9 p-fluoro phenol

10 p-iodo phenol

11 m-methoxy phenol

12 m-ethoxy phenol

13 p-methoxy phenol

14 p-nitro phenol

15 p-hydroxy benzo-trifluoride

16 p-hydroxy benzoic acid, methyl ester

17 p-hydroxy acetophenone

18 p-cyano phenol

19 anisole

There is a very good correlation between the partition coefficients of the p-halo phenols in the aqueous/n-octanol and aqueous/cyclohexane systems (slope 2). The relationship is as follows :

$$\text{Log } K_{d\ \text{octanol}}^{x} = 1.143 (\text{Log } K_{d\ \text{cyclohexane}}^{x}) + 2.734$$

$$r = .998; \quad n = 4$$

The much larger aqueous/n-octanol partition coefficients reflect the increased hydrogen bonding of the p-halo phenols in the n-octanol phase. The third correlation is for those phenols whose substituent groups increase the strength of hydrogen bonding of the hydroxyl group of phenol and also take part in hydrogen bonding themselves (slope 3).

The relationship between the partition coefficients for these solutes in each phase is as follows :

$$\text{Log } K_{d\ \text{octanol}}^{x} = .311 (\text{Log } K_{d\ \text{cyclohexane}}^{x}) + 2.977$$

$$r = .675; \quad n = 4$$

The negative water/cyclohexane partition coefficients of p-hydroxy acetophenone, p-hydroxy benzoic acid, methyl ester, p-nitro phenol and p-cyano phenol reflects the increased solute-water interactions. The positive water/cyclohexane partition coefficient for p-hydroxy benzotrifluoride reflects the increased hydrophobicity of the phenol due to the tri-fluoro methyl group.

The partition coefficient of anisole in the water/n-octanol and water/cyclohexane systems is almost the same. Anisole is removed from water through hydrophobic interactions and the same mechanism is responsible in both the aqueous/n-octanol and aqueous/cyclohexane systems. Hydrogen bonding of anisole to the hydroxyl group of n-octanol is insignificant.

The correlation between Log K_d^x octanol and Log K_d^x cyclohexane can be improved by including a hydrogen bonding parameter with the Log K_d^x cyclohexane term. Figure 65 shows the relationship between Log K_d^x octanol and Log K_d^x cyclohexane plus the hydrogen bonding parameter I_H of Seiler (1974a). Figure 66 shows the correlation between Log K_d^x octanol and Log K_d^x cyclohexane plus the hydrogen bonding parameter h_D of Higuchi et al. (1969).

The equations for these relationships are as follows :

(1) Log K_d^x octanol = 0.88 (Log K_d^x cyclohexane + $\sum I_H$) + 0.21

r = .881, n = 17

(does not include solute number 18, p-cyano phenol)

(2) Log K_d^x octanol = 0.772 (log K_d^x cyclohexane + h_D) + 2.526

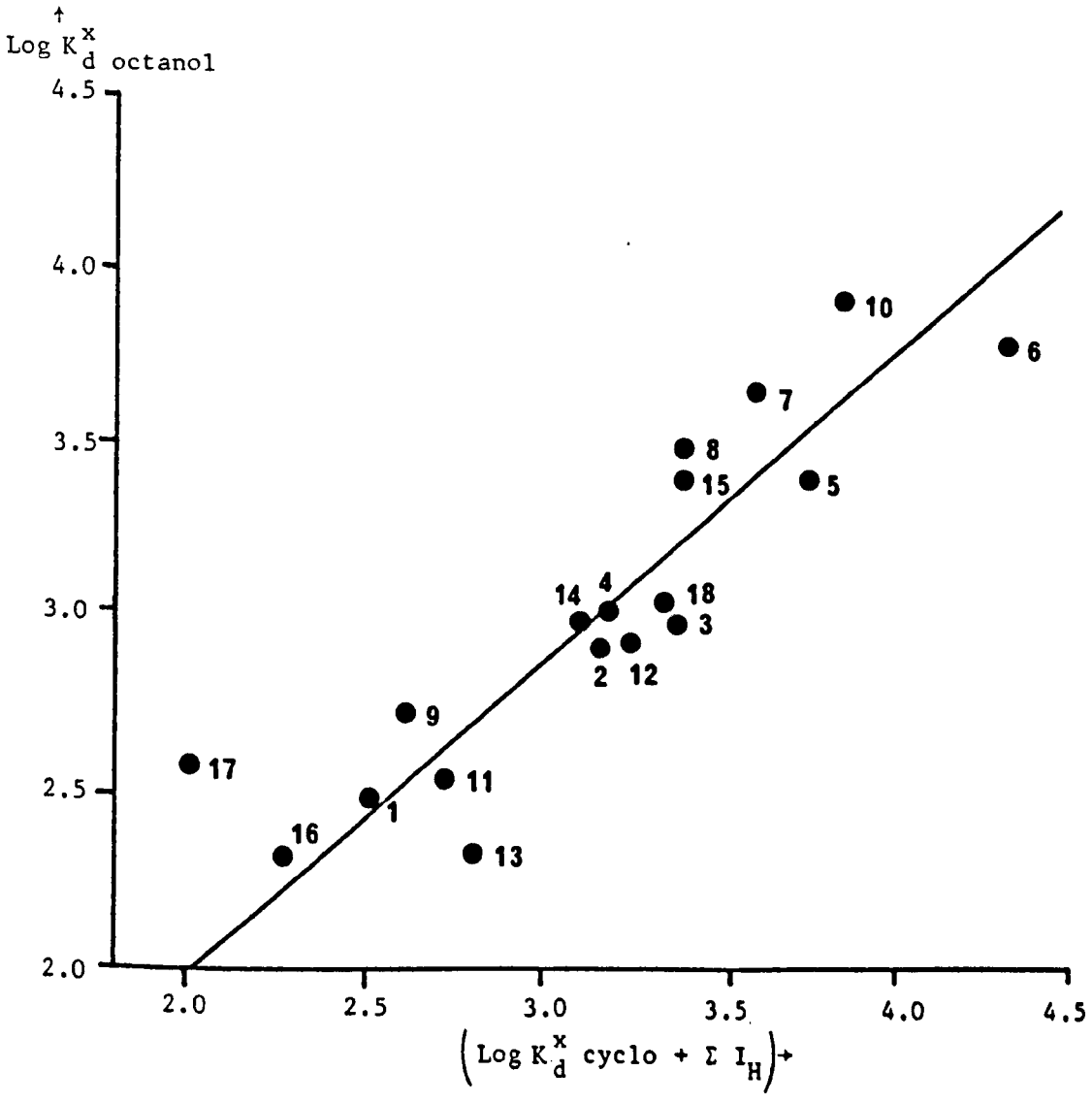
r = .954; n = 8

(includes solutes numbered 1-8, whose substituents do not hydrogen bond).

There is a linear relationship between Log K_d octanol and Log K_d cyclohexane plus $\sum I_H$, for all substituted phenols, because I_H takes into account not only the hydrogen bonding characteristics of the hydroxyl group but also of the substituent group on each particular phenol. Figure 66 shows two linear relationships between Log K_d^x octanol and Log K_d^x cyclohexane plus h_D for the substituted phenols. Slope (1) includes those phenols whose substituents cannot take part in hydrogen bonding, whilst slope (2) includes the double hydrogen bonding phenols. The hydrogen bonding parameter h_D does not take into account the hydrogen bonding ability of the substituent group itself.

Figure 65

Log K_d^x octanol versus (Log K_d^x cyclohexane + ΣI_H).

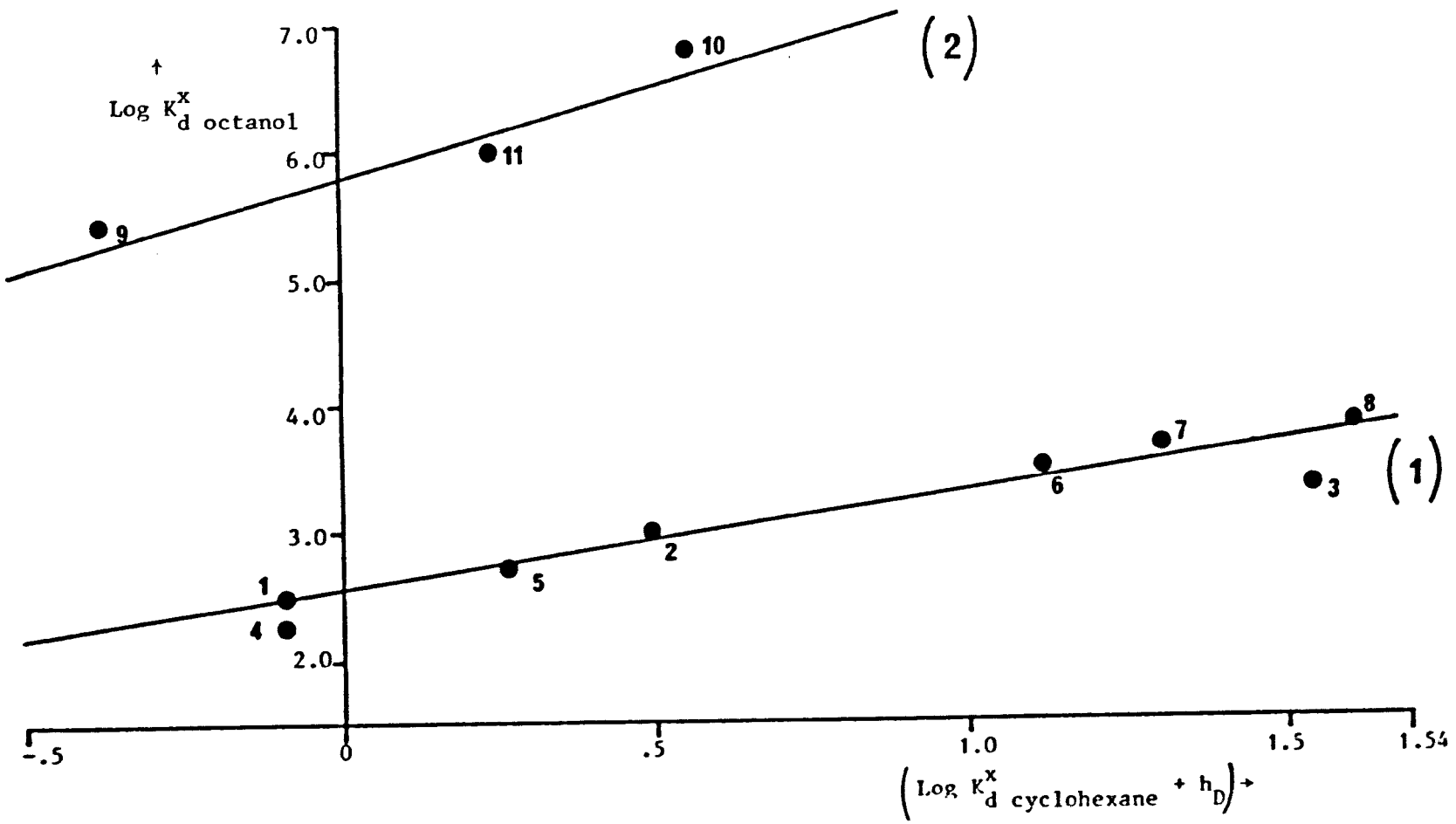


Key to Figure 65

Number	Solute
1	phenol
2	p-cresol
3	o-cresol
4	m-cresol
5	p-ethyl phenol
6	p-propyl phenol
7	p-bromo phenol
8	p-chloro phenol
9	p-fluoro phenol
10	p-iodo phenol
11	m-methoxy phenol
12	m-ethoxy phenol
13	p-methoxy phenol
14	p-nitro phenol
15	p-hydroxy benzoic acid, methyl ester
16	p-hydroxy acetophenone
17	p-cyano phenol
18	anisole

Figure 66

Log K_d^x octanol versus (Log K_d^x cyclohexane + h_D).



Key to Figure 66

Number	Solute
1	phenol
2	p-cresol
3	p-ethyl phenol
4	p-methoxy phenol
5	p-fluoro phenol
6	p-chloro phenol
7	p-bromo phenol
8	p-iodo phenol
9	p-hydroxy acetophenone
10	p-nitro phenol
11	p-cyano phenol

(11) A Correlation of Partitioning Data for the Phenolic Compounds with Biological Data.

The partitioning behaviour of the phenolic compounds in the three aqueous/model membrane systems is compared with partitioning into red blood cell ghosts and is shown in Figure 67. The aqueous/red blood cell ghost partition coefficients for the phenols are taken from Roth and Seeman (1972). Excellent correlations are found for aqueous/red blood cell and aqueous/n-octanol systems, and for the aqueous/red blood cell and aqueous/DMPC liposome systems. The correlation between partitioning in the aqueous/red blood cell system and the aqueous/cyclohexane system is not as good however. The equations for the correlations are as follows :

(1) $\text{Log } K_{d\ RBC} = 1.295 \text{ Log } K_{d\ liposome} - 1.861,$

$n = 6, \quad r = .969$

(2) $\text{Log } K_{d\ RBC} = 0.843 \text{ Log } K_{d\ octanol} - 1.273,$

$n = 7, \quad r = .988$

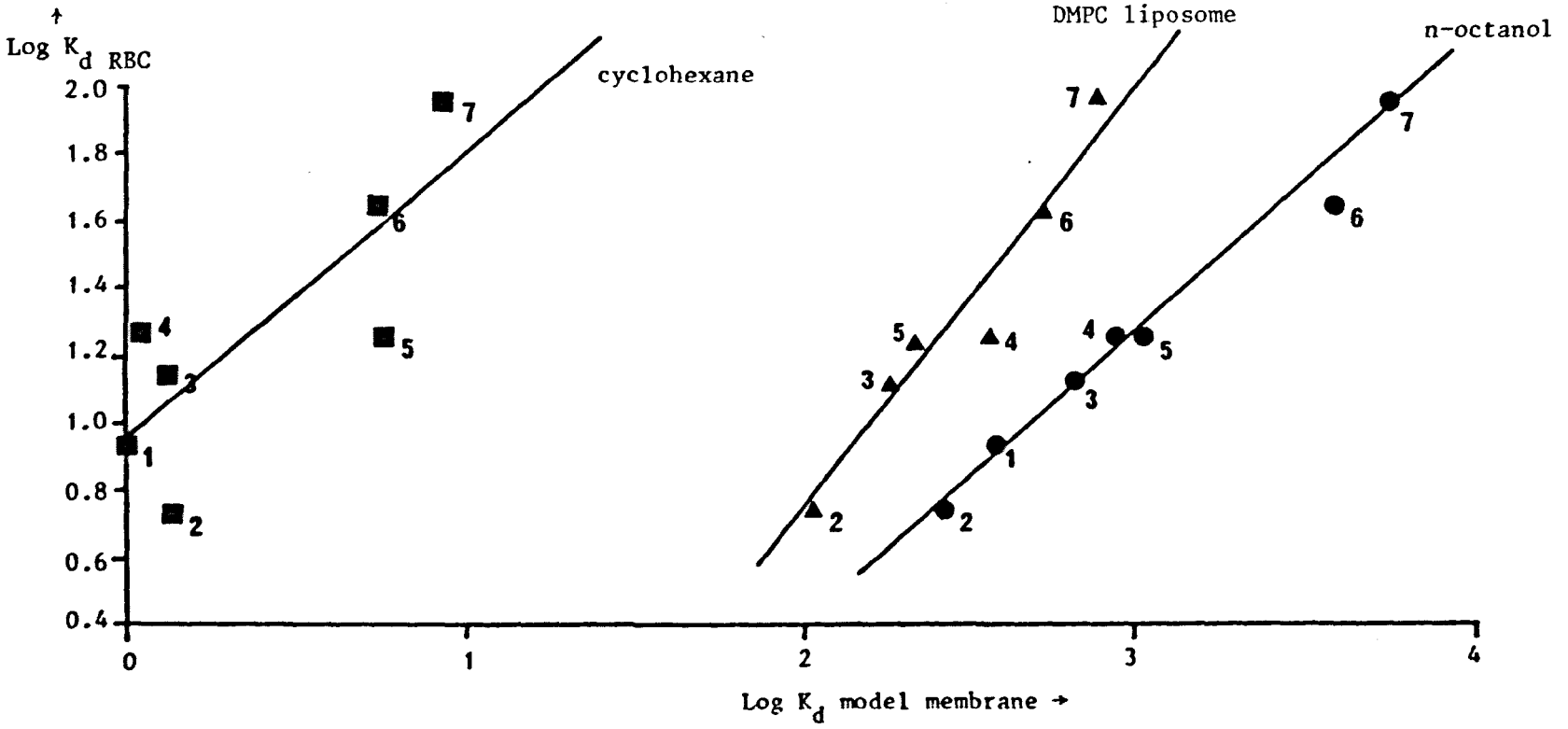
(3) $\text{Log } K_{d\ RBC} = 0.863 \text{ Log } K_{d\ cyclohexane} + 0.93,$

$n = 7, \quad r = .813$

It is interesting to compare the biological activity or interaction of the substituted phenols (taken from literature values) with their partitioning in the aqueous/model membrane systems under study. A brief comparison was made and the results are shown in Table 95. The equation for the particular correlation is shown, together with the reference and type of biological interaction. For those systems studied, the correlation between the aqueous/n-octanol partition coefficients and biological activity is better, than the same correlation between the aqueous/cyclohexane partition coefficients

Figure 67

Relationship between Log K_d R.B.C. and Log K_d model membrane for substituted phenols (Data for red blood cells taken from Roth and Seemen, 1972).



Key to Figure 67

Solute Number	Phenol
1	phenol
2	p-methoxy phenol
3	p-fluoro phenol
4	p-hydroxy benzoic acid, methyl ester
5	p-cresol
6	p-chloro phenol
7	p-bromo phenol

Table 95 *Relationship Between the Biological Activity of Substituted Phenols (Taken from Literature Values) and Partitioning in the Aqueous/Model Membrane Systems.*

Relationship	n	r	Reference	Type of Interaction
(1)				
$\text{Log } \frac{1}{C} = 0.754 \text{ Log } K_d^x \text{ octanol } -0.659$	6	.952	Lien et al., (1968)	Anti-bacterial action against Staph. aureus, where C is the molar concentration of drug which prevents growth.
$\text{Log } \frac{1}{C} = 0.867 \text{ Log } K_d^x \text{ cyclohexane } +1.139$	6	.909		
(2)				
Log ΔH 50% = -1.273 Log Kd liposome +0.332	6	-.921	Roth and Seemen (1971)	Anti-haemolysis activity where ΔH 50% is the concentration which reduces osmotic haemolysis of red blood cell by 50%.
Log ΔH 50% = -.952 Log Kd octanol +0.170	7	-.971		
Log ΔH 50% = -1.103 Log Kd cyclohexane -2.267	7	-.904		
(3)				
$\text{Log } \frac{1}{C} = .995 \text{ Log } K_d^x \text{ octanol } -0.573$	7	.974	Biagi et al., (1975)	Anti-bacterial action against Staph. aureus, where C is the concentration which prevents growth.
$\text{Log } \frac{1}{C} = .673 \text{ Log } K_d^x \text{ cyclohexane } +2.136$	7	.840		
(4)				
$\text{Log } \frac{1}{C} = .497 \text{ Log } K_d^x \text{ octanol } +1.13$	8	.875	Biagi et al., (1975)	Acute toxicity in mice, where C is the LD50.
$\text{Log } \frac{1}{C} = .373 \text{ Log } K_d^x \text{ cyclohexane } +2.459$	8	.774		

Table 95 Continued

Relationship	n	r	Reference	Type of Interaction
(5)				
$\text{Log } \frac{1}{C} = 1.129 \text{ Log } k_d^x \text{ octanol } -1.20$	7	.927	Biagi et al., (1975)	Haemolytic activity, where C is the molar concentration of phenol provoking a 50% haemolysis of red blood cells
$\text{Log } \frac{1}{C} + .737 \text{ Log } K_d^x \text{ cyclohexane } +1.88$	7	.773		
(6)				
$\text{Log } P = 1.118 \text{ Log } K_d^x \text{ octanol } -3.028$	10	.868	Roberts et al., (1977)	Permeability of human epidermis, where P is the permeability coefficient of the phenol across the epidermis
$\text{Log } P = 0.417 \text{ Log } K_d^x \text{ cyclohexane } +0.255$	9	.868		

and biological activity. This would sum to suggest that it is preferable to correlate biological activity with aqueous/n-octanol partition coefficients rather than aqueous/cyclohexane partition coefficients, when investigating structure activity relationships. It must be stated however that a much larger number of correlations with literature values must be carried out before any definite conclusions can be drawn.

(12) The Temperature Dependence of Partitioning.

It is obvious from this study that the distribution behaviour of substituted phenols, varies with temperature. The actual temperature dependence of partitioning depends upon the enthalpy of transfer for the phenol between aqueous solution and the particular lipoidal phase. Therefore it would be unwise to measure the partition coefficient of a phenol at for example 22°C, and then relate this value to a biological interaction at the body temperature of 37°C. Also it would be unwise to determine π values at one temperature and use these values to calculate the partition coefficient of a substituted compound at a different temperature.

Chapter Eight

CONCLUSIONS AND FUTURE WORK

CONCLUSIONS

The basic principles of equilibrium thermodynamics have been successfully applied to the distribution behaviour of structurally related solutes between aqueous solution and various lipoidal phases. This has led to a greater understanding of the molecular interactions and mechanisms involved in the transfer of solute molecules and their various substituent groups from aqueous solution to lipophilic solvents. The introduction of the various functional groups into the phenol molecule, dramatically alters not only the partitioning behaviour of the parent compound, but also the molecular interactions and mechanisms involved in the transfer process. The thermodynamic analysis of partitioning can be seen as a much more rigorous treatment than the simplistic Hansch treatment. The effect of functional groups on the overall distribution behaviour (free energy of transfer) can be assigned to changes in molecular interactions (enthalpy of transfer) and/or structural changes (entropy of transfer). The presence of a particular type of group on the molecule can not only affect the properties of another group, but also contributes to the overall properties of the molecule as well. For example, the introduction of a para substituted ethyl group into the parent phenol molecule, reduces the potential hydrogen bonding strength of the hydroxyl group, and at the same time increases the overall hydrophobicity of the molecule. The effect of a particular functional group depends not only on its chemical structure, but also on its position on the molecule.

A comparison of the thermodynamics of partitioning of the

substituted phenols between aqueous solution and the three model membrane systems shows that there is no ideal bulk organic solvent which resembles the biological membrane in every aspect. The thermodynamic data for the aqueous/liposome system, shows that solute-lipid interactions are important in the transfer process. Partitioning into the liposome bilayer is entropy driven for most solutes, and the large positive entropy values are derived mainly from disruption of the bilayer, with a small contribution from the removal of the solute from water. The positive enthalpy of transfer for most solutes is largely due to the disruption of molecular interactions within the bilayer, whilst the negative enthalpy values reflect hydrogen bonding of the phenols with the polar head groups. Solute-lipid interactions are also important in the partitioning of solutes in the water-n-octanol system, and this is reflected in the negative enthalpies of transfer for this system, which result from increased hydrogen bonding of the phenols with n-octanol. The positive entropy of transfer for the majority of solutes is derived from the increase in motion of the solute and the decrease in structure of the water molecules on the removal of the solute from water. The transfer of solutes between aqueous solution and n-octanol is either entropy or enthalpy controlled depending on the type of substituents used. The large negative free energies of transfer for both the aqueous/liposome and aqueous/n-octanol systems reflect the greater preference for the lipoidal phase, aided by solute-lipid interactions. Close agreement is found between partitioning in the aqueous/liposome system and partitioning in the aqueous/n-octanol system.

Solute-lipid interactions are less important in the transfer

of phenols between aqueous solution and cyclohexane. The transfer process is entropy driven, and this is derived from the hydrophobic interaction between the particular phenol and water molecules. The positive enthalpies of transfer are derived from the disruption of solute-water interactions. Generally there is a diminished preference for the organic phase, and this is indicated by the low negative and positive free energy of transfer values. Poor correlation is found between partitioning in the aqueous/cyclohexane and aqueous/liposomes systems.

Finally there are two main conclusions that can be drawn. Firstly, it would seem desirable to correlate biological activity with partition coefficients measured in the water/n-octanol system, since these show better correlation with partitioning into the liposome bilayer. However from a thermodynamic point of view, it is preferable to study the temperature dependence of partitioning of solutes in the aqueous/cyclohexane system since the molecular interactions and structural changes involved in the transfer process are easier to interpret.

FUTURE WORK

FUTURE WORK

The exact location and specific interactions of a solute partitioned into the liposome bilayer cannot be determined from partition studies alone. Techniques such as differential scanning calorimetry, fluorescent probes, X-ray and neutron diffraction studies, and spin label studies must be used in conjunction with partition studies.

The study of the thermodynamics of partitioning of substituted phenols into membrane systems might be extended to include red blood cell ghosts and reconstituted cell membranes. Previous studies suffer from the limitation that they have not studied in depth the effect of the many varied functional groups.

Although the van't Hoff method of determining the enthalpy of transfer produces reliable data, it suffers from the disadvantage that it involves a differentiation step and this can propagate errors. Therefore it is desirable to determine the enthalpy change directly, by micro-calorimetry. (This is particularly true when investigating the phenomenon of enthalpy-entropy compensation, where it is desirable to obtain the enthalpy value by a different method from the free energy term to remove any complications due to statistical artefacts). In the past this technique has suffered from practical problems. However recent work by Riebesehl and Tomlinson (1981, 1983) and Beezer et al (1983), using flow and batch calorimetry respectively, has produced reliable data on the transfer of solutes between aqueous solution and bulk organic solvents. Future workers in this field will strive to improve the instrumentation and operation of micro-calorimeters to produce accurate and precise calorimetrically determined enthalpies of transfer.

APPENDIX

APPENDIX

A pH-Distribution Study of Ranitidine Hydrochloride in an Aqueous Buffer/n-Octanol System using the Filter Probe Method.

It is possible to study the pH-distribution behaviour of an ionisable drug molecule in a suitable aqueous buffer/solvent system, and simultaneously determine its pKa value using the filter probe technique.

The pKa of a drug molecule is related to its partition coefficient (K_d) and distribution coefficient (D) by the following equation :

$$pKa = \text{Log } (\frac{Kd}{D} - 1) + pH$$

The distribution coefficient is equal to the partition coefficient when there is zero ionisation of the drug molecule. Thus, if the distribution behaviour of the drug molecule is determined over a range of pH then its pKa value can be determined.

Materials

n-Octanol - Fisons' special for partition coefficient measurement.

The following buffers were used :

pH 6.8 - 8.04, 0.01 M disodium hydrogen phosphate + sodium dihydrogen phosphate

pH 8 - 10, 0.01 M ammonium acetate + ammonia solution to pH,

pH 10.35, 0.01 M sodium carbonate.

Method

The filter probe system employed was identical to that used

to study the temperature dependence of partitioning of the phenolic compounds (see Experimental Section). The distribution coefficient was determined between buffered solutions, of varying pH, and n-octanol at 22°C. Solute concentration in the aqueous phase was measured by monitoring at the λ_{max} of 316 nm.

Results

The results are given in Table I and the pH-distribution profile is given in Figure I. The mean pKa value was 8.30 at 22°C. The actual measured pKa value (as determined by Glaxo Group Research Limited) was found to be 8.30 at 22°C.

Conclusion

The filter probe system provides a reliable method for the simultaneous determination of the partition coefficient and pKa of an ionisable drug molecule.

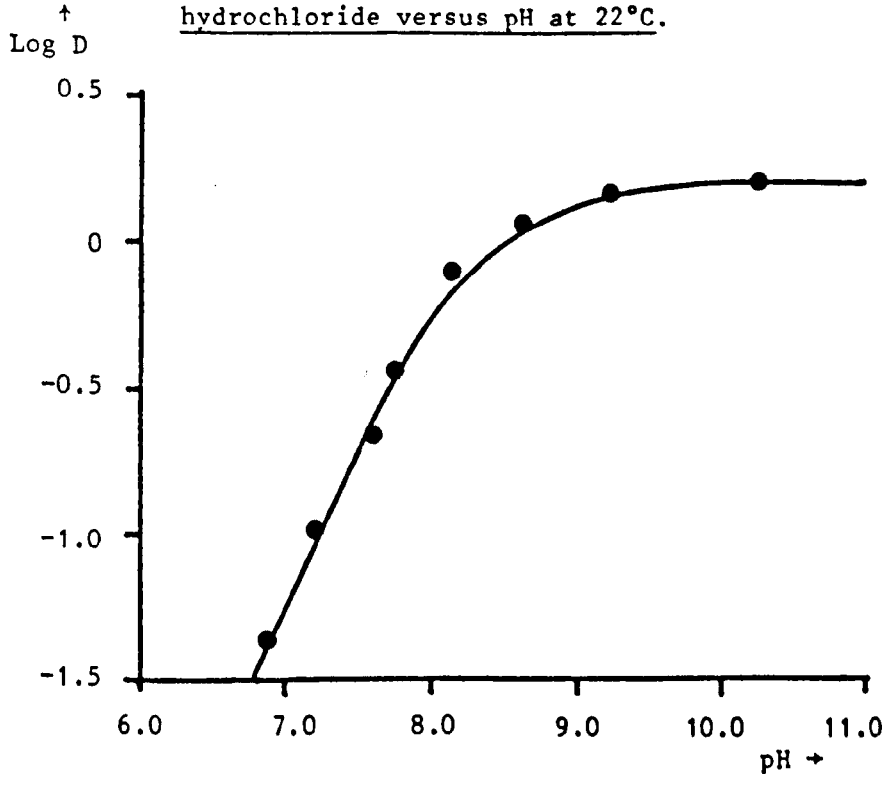
Table I

The Distribution Coefficient of Ranitidine Hydrochloride versus pH at 22°C.

pH	D	Log (Kd/D-1)	pKa
6.82	0.045	+1.545	8.36
7.15	0.105	+1.160	8.31
7.45	0.227	+0.789	8.23
7.73	0.368	+0.533	8.26
8.07	0.617	+0.213	8.28
8.60	1.121	-0.346	8.25
9.20	1.422	-0.843	8.35
		Mean	8.30

Figure I

Distribution coefficient of ranitidine hydrochloride versus pH at 22°C.



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