MECHANICAL PROPERTIES

OF

BIOPOLYMER FILMS

By

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Thesis submitted to the University of Nottingham

for the degree of Doctor of Philosophy

August 2000

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LIST OF ABBREVIATIONS

χ	Effective pair interaction parameter
ΔF_M	Free energy of mixing
ΔH_M	Enthalpy of mixing in the lattice theory
ΔS_M	Entropy of mixing in the lattice theory
C*	Critical concentration for gelation
C^*_b	Critical concentration for gelation on the binodal
CD	Charge density
CMC	Carboxymethylcellulose
DE	Dextrose equivalent
dwb	Dry weight basis
G	Gradient
F_b	Force at break
G_0	Zero moisture content gradient
G_1	Relative effect of moisture content on the gradient
$G_{plasticiser}$	Relative effect of the plasticiser on the gradient
HPC	Hydroxypropylcellulose
HPMC	Hydroxypropylmethylcellulose
I_{ddb}	Initial drop in the distance at break
PEG	Polyethylene glycol (molecular weight 400)
PG	Propylene glycol
PGA	Propyleneglycolalginate
Rpm	Revolutions per minutes
S_{db}	Slope of the distance at break for increasing plasticiser content at a relative humidity of 44%
S_g	Slope of the gradient for increasing plasticiser content at a relative humidity of 44%
W/w	Weight to weight
μ_i	Chemical potential of i.
η	Viscosity
$\dot{\gamma}$	Shear rate
$[\eta]$	Intrinsic viscosity

ACKNOWLEDGMENT

I would like first to thank John (Prof. John Mitchell) and Sandra (Dr Sandra Hill) for their support, trust and friendship during these three years. I also would like to thank Capsugel for their financial support.

Great thanks to the technical staff that makes life so much easier here in SB. Thanks to Mike, Derrick, Val, and especially Phil. Their effort, efficiency and smile really are invaluable assets.

I would also like to thank Tracey for her great help and participation in the sensory analysis part. Thanks also to Jim Craigon (Biometry) and Dr Ansarifar (Loughborough university, IPTME) for help discussions.

Thanks also to Val P.H. for giving me the chance of knowing about this Ph.D. position.

I would also like to thanks Vasilis V. and Sonia B. for giving me some good times and some hard work in correcting their reports.

I would also like to thanks the friendship of many in SB, in and out of the lab: Imad, Balta, Jean-Francois, Olivier, Cecile, Gaelle, Vanessa, Chris, Gordon, Jules, Antje, Fabienne, Anke, Leonor and many more.

Finally, thanks to the few that believe that may be, I could do it. Thanks to my parents, but most of all, thanks to Anne and Lisa for so much…

ABSTRACT

Hard gelatin capsules have been used for drug delivery for a long time. The current production process takes advantage of the very unusual properties of gelatin: gelation, very low viscosity, film mechanical properties and film solubility. Although the hard gelatin capsules present many advantages compared to other drug delivery systems, their uses are restricted because of the animal origin of the gelatin. A HPMC gelling agent system is currently used for producing animal product free hard capsules. This work examines the possibility of using a different system in a similar production process. The gelling conditions of the mixed system, the potential of various film formers and the mechanical properties of some films are considered.

Gelling agent filler mixed systems were prepared, and the limit concentration of filler that allowed gelation was noted. It was shown that none of the gelling agents would always gel and gelation was never prevented by the maltodextrin (up to a concentration of 14%). The gelation inhibition obtained is likely to be due to phase separation. The charge densities of the various products were also measured. It showed that when there is little charge density difference, gelation is inhibited. Polymer compatibility is increased by increasing the charge density differences. However, an asymmetry is observed. This is explained by the necessary shift of the binodal that would predict prevention of incompatibility.

Many films were cast from various biopolymers. The films were screened via sensory analysis. The process allowed to define terms that discriminate the films. The results showed that cellulose derivatives, alginate and alginate derivative films had sensory analysis scores similar to gelatin. Although none of the starch derivatives had such good scores, some presented some promising results. Alginate and caseinate films were selected for further analysis.

The mechanical properties of gelatin and HPMC films were compared by puncture tests. The results at a relative humidity of 44% are similar. However, the effect of the

moisture content on both films' mechanical properties showed differences. The fracture patterns and polarised microscopy observation were also very different.

Alginate films' mechanical properties were similar to gelatin. However, alginate films are not soluble in acidic environments. The effects of molecular weight on the mechanical properties of cellulose derivatives and alginates films were different. Increasing the calcium content of the alginate sample gave similar results to those obtained by increasing the molecular weight. It is proposed that ultimate deformation occurs through different processes in various films. Alginate/gelatin films are thought to deform through crazing, and the fracture process generates many surfaces (lines). Molecular weight and crosslinking would stabilise the crazes. On the other hand, cellulose derivative would deform through slippage and the energy is dissipated during deformation. This is consistent with the orientation observed after fracture, the lack of new surfaces and the high hydrophobicity of these polymers.

Caseinate films of sodium, potassium, calcium and magnesium were studied. Sodium caseinate presented the best mechanical properties. Glycerol proved to be the best plasticiser. Glyoxal crosslinking or increase in pH did not improve the mechanical properties of these films. Caseinate films are poorer than alginate, HPMC or gelatin films. Caseinate deformation processes might occur through both slippage and crazing owing to the low molecular weight and high hydrogen bonding ability.

Overall, different deformation processes can lead to similar mechanical behaviour. None of the films studied is likely to replace gelatin or HPMC. More complex systems are proposed for further study.

CHAPTER 1. CONTEXT OF THE STUDY

1.1. AIM OF THE STUDY

Hard gelatin capsules (Figure 1-1) have been used to deliver drug formulation for a long time. The first patents appeared more than a century ago and the same principles are still used today. More recently, cellulose derivatives were used in conjunction with a gelling agent in order to prepare hard capsule (Sarkar 1977). Although the capsules produced perform well, they are not yet widely used.

Figure 1-1: Hard gelatin capsule.

The ultimate aim of this study was to find another system able to replace gelatin by one or a combination of polymers in order to produce hard capsules. The successful replacers must be produced on a production line very similar to that already used for gelatin capsule production. Thus, there were some constraints on the choice of material and the method for production.

1.2. INDUSTRIAL PROCESS

The industrial process used for hard gelatin capsule production is summarised in Figure 1-2. The process is illustrated Figure 1-3. The manufacturing process differs depending on the manufacturers, however, the main principles are very similar. Full descriptions of these processes can be found in the literature e.g. Jones (1987), Millender (1991) (Jones 1987).

Firstly, a hot gelatin solution is prepared either directly in hot water or after soaking the gelatin in cold water. To this solution are added the preservatives and the dyes. Removing the bubbles is done by vacuum. The solution contains between 25 and

40% (w/w) of gelatin. Relatively small batches are prepared in order to limit hydrolytic degradation. The temperature (about 45°C), the viscosity (500-1000 mPa.s) and the concentration are controlled and kept constant. These three parameters are dependent on one another and each manufacturer may use slightly different conditions.

The solution is poured into a dish where the capsule will be formed. The dish consists of a circulating bath where temperature and viscosity are continuously monitored and corrected. The loss of water by evaporation creates an increase in viscosity, which is balanced by addition of water. The height level of solution in the dish is also maintained constant.

A bar of stainless steel pins at room temperature is then dipped into the solution. The gelatin solution forms a gel coating the pin due to its lower temperature. The bars are then rotated to ensure an even coating. Viscosity, temperature of the solution, temperature of the pins and dipping rate define the thickness of the film on the surface. The capsule is made of two parts called the body (long part) and the cap. Both parts are prepared separately, usually on two different lines. The pins are then subjected to cool air in order to set the gel further.

The drying of the films is performed in many steps varying in drying rate. Total drying is usually not achieved in order to facilitate the removal of the film from the pins. The pins used are slightly tapered towards the ends in order to prevent the vacuum that would result on removal. After removal, the films are trimmed, checked, printed and packaged. The trimmings are usually recycled.

Figure 1-2: Schematic production process for gelatin capsules.

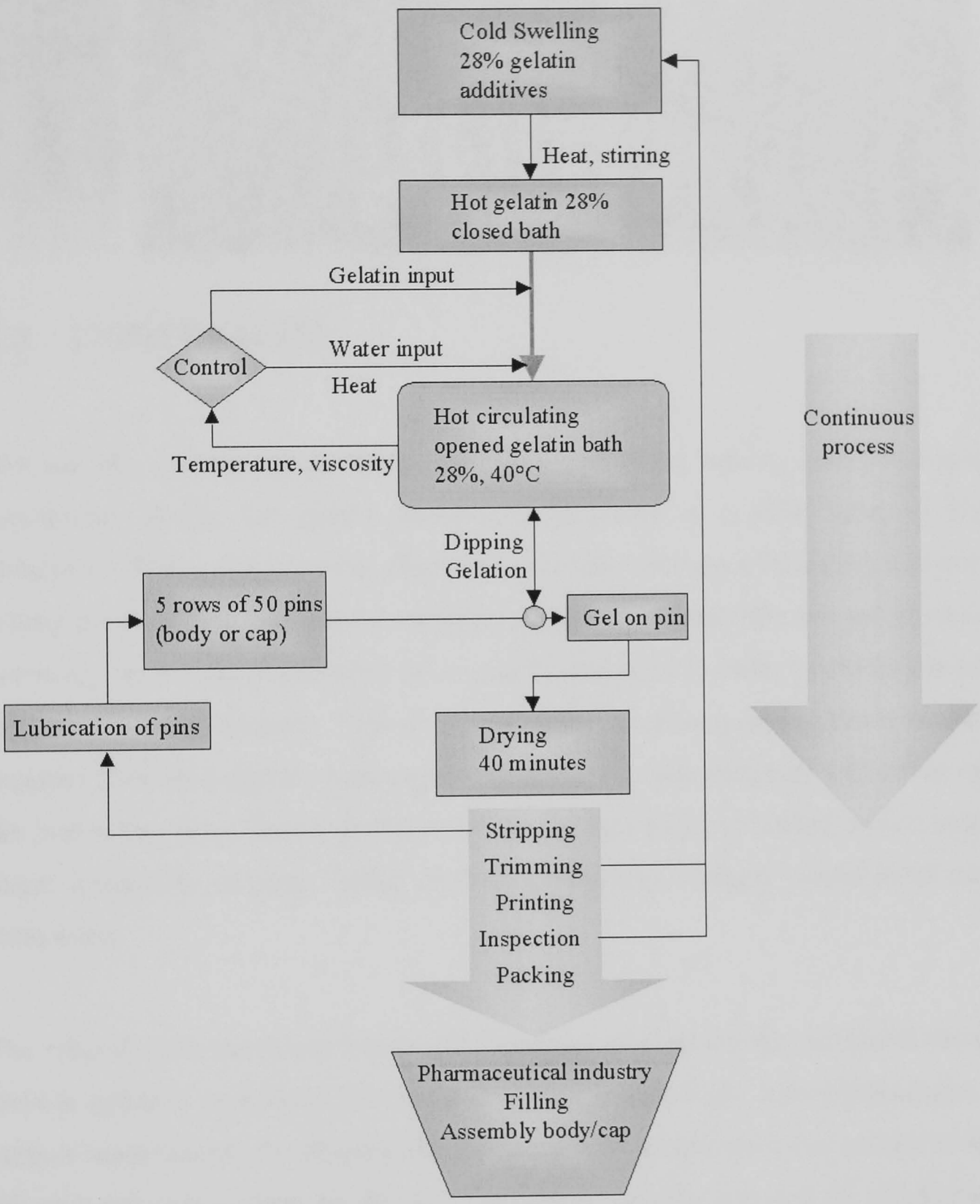
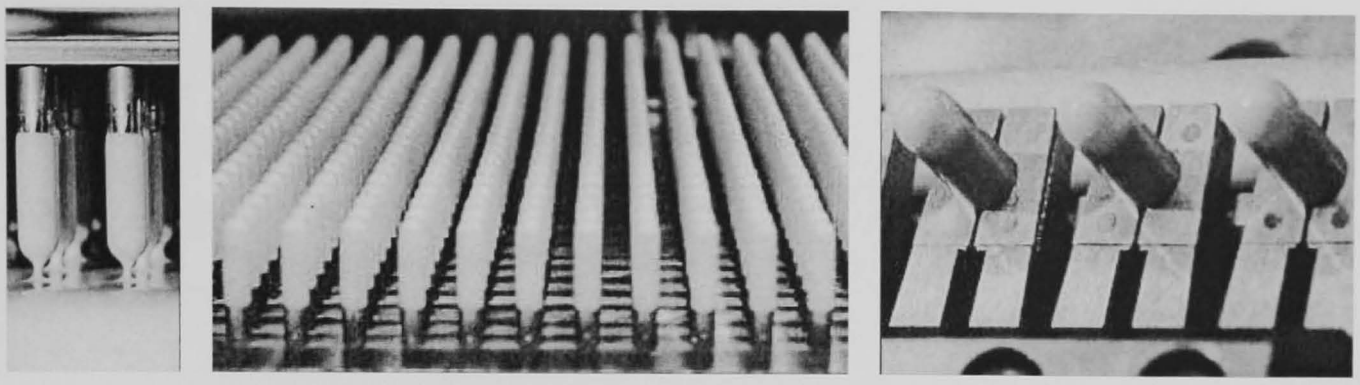


Figure 1-3: Photos illustrating the production process. From left to right: dipping, drying and stripping.



1.3. CONSTRAINTS

The use of a process similar to the one presented above implies some very severe constraints. Firstly the gelatin solution is prepared at a solid level of about 28%(w/w). This is only possible due to the very low viscosity of the gelatin above its setting point. If this high solid content is not achieved, then the amount of wet gel necessary on the pin would have to be greatly increased in order to obtain the same thickness of dried material. This also implies that very long drying times would be required if dilute solutions were used. Using a lower concentration will either force the production to be slowed down or the drying rack to be extended, which implies major industrials changes. Either of these production changes would incur major extra costs.

The second main constraint lies in the formation of a gel on the surface of the pin. Gelatin gelation is a rapid process and occurs at relatively low temperatures. In capsule manufacture, the dipping of a pin at ambient temperature into gelatin at 40°C allows a gel coat to form on the pin. The gelatin quickly sets and the gel holds its shape on the pin. This behaviour would have to be mimicked by a successful replacer system.

The third constraint concerns the mechanical properties of the final capsules. The film must have some non brittle qualities to allow the stripping of the capsule from the pin without breaking. Furthermore, during pharmaceutical usage, very fast processes are used which can generate high stresses on the capsule. Capsules with

poor mechanical properties would break or deform. Finally, the capsule must also resist handling by the consumer (extracting from the pack).

The fourth constraint is that the capsule must dissolve quickly in the stomach. Although there is a large market for colon drug delivery system or slow release systems, most of the drugs must be released quickly. Therefore, the polymer used will have to form films that are solubilised in the stomach after a short time.

The last constraint comes from the nature of the polymer used. Obviously, the polymer must not cause any problem for the patient. Ideally, a food allowed polymer should be used. Other issues such as price or availability must also be considered. Since all the polymers used in this study are currently used by the food industry, potential harm, price and availability will not be discussed further.

Furthermore, non animal products are largely preferred. In fact, the main motivation for this project is to replace the animal product, gelatin, by a non animal product.

The four main constraints are therefore:

- Low viscosity, high solid loading
- Gelation properties
- Mechanical properties
- Solubility

CHAPTER 2. THEORY

2.1. PHASE SEPARATION

2.1.1. Principles

When two homogeneous systems are mixed, they sometimes give rise to phase separation. This phenomenon is also known as incompatibility or demixing and occurs in many simple mixed systems like oil and water. Incompatibility often occurs in polymeric solutions and a brief description of its principles will be given here.

Incompatibility can either lead to macroscopic or microscopic phase separation. Macroscopic phase separation implies that the density of the two phases is different enough to promote complete separation (Kasapis et al. 1993b). Such macroscopic phase separation does not always occur and microscopic structures usually appear for polymeric systems (Brown et al. 1995; Chilvers and Morris 1987; Clark et al. 1983; Clark 1995; Gotlib et al. 1988; Kasapis et al. 1995; Khokhlov and Nyrkova 1992; Kolarik 1994; Kolarik 1996; Mohammed et al. 1998; Morris 1990; Zasypkin, Braudo and Tolstoguzov 1997). Centrifugation is often used in order to fully separate the phases and analyse their compositions.

When microstructures are involved, it is common to observe a continuous phase and an included phase. However, bicontinuous systems with a dominant phase can also be observed (Brown et al. 1995). The microscopic water-water emulsions obtained can scatter visible light so that turbidity is observed (Miles, Morris and Ring 1985; Kasapis et al. 1995). By changing the relative concentrations of the polymers, the included phase can become continuous (Clark et al. 1983). This phase inversion should occur at about 50% phase volume (Brown et al. 1995). The existence of this heterogeneous system leads to changes of the texture properties.

Polymer incompatibility can give rise to three different phase behaviours. Segregative phase separation occurs in a polymer A-polymer B-solvent system when the two polymers are concentrated in two different phases. This is the most common phase separation behaviour. If the polymers attract each other, then associative phase separation can occur and the two polymers are concentrated in one phase. This often occurs in mixtures of oppositely charged polymers (Michon et al. 1995; Piculell, Bergfeldt and Nilsson 1995). In these two cases, the solvent concentration can be different in both phases. Borderline phase separation rarely occurs and corresponds to a case when one polymer concentration is constant in both phases (Piculell et al. 1995).

When gelation of at least one of the compounds occurs, the system can be "frozen" which can prevent the thermodynamic equilibrium being reached. The final system results from a kinetic competition between demixing and gelation. The phase inversion limit could also be shifted by changing the cooling rate of gels (Kasapis et al. 1995). In agarose gelatin mixtures, the phase-separated gels obtained show a complex structure, which lack homogeneity (Clark et al. 1983; Gotlib et al. 1988). Blend laws have been applied to gelling polymers mixtures (Clark et al. 1983; Kasapis et al. 1993a; Kasapis et al. 1995; Kolarik 1994; Kolarik 1996; Mohammed et al. 1998; Morris 1990; Morris 1992) and water partitioning was shown to significantly affect the results (Kasapis et al. 1995; Morris 1992).

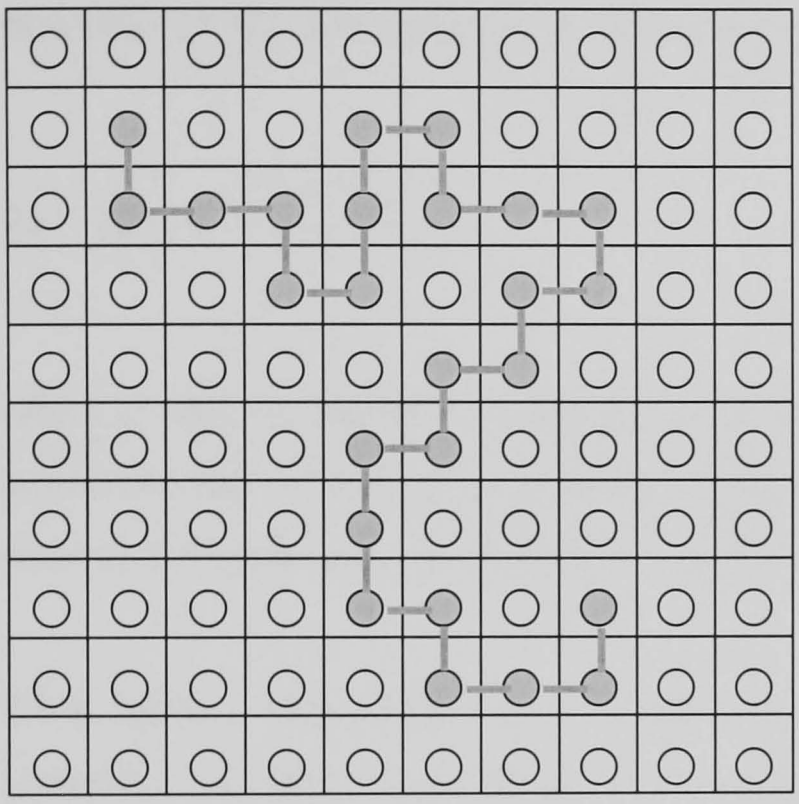
Shearing could also be of importance. The time of shearing during phase separation and gel formation not only promotes smaller inclusions but can also change the nature of the continuous phase (Brown et al. 1995). Sometimes, the included phase can form macroscopic gels if the gel beads are connected (Clark 1995).

2.1.2. Thermodynamics

The basic theory of phase separation was first written for simple mixture using the lattice theory (Flory 1953a; Flory 1953b). The lattice model (Flory-Huggins) is represented in Figure 2-1. Each solvent molecule occupies one site in the lattice whereas only a segment of the polymer molecule occupies one site. The number of

segments per polymer is the molar volume ratio of the polymer and solvent. The greater entropy of mixing is entirely due to the greater number of arrangements in the mixture compared to the two separate solutions.

Figure 2-1: Segments of a chain polymer molecule (grey) located in the liquid lattice. From Flory (Flory 1953b).



The entropy of mixing (ΔS_M) and the enthalpy of mixing (ΔH_M) for binary mixtures are given by

$$\Delta S_M = -k \sum n_i \ln v_i$$

and

$$\Delta H_M = kT \sum_{i<j} n_i v_j \chi_{ij}$$

where k, T, n_i, v_i and χ_{ij} represent respectively the Boltzman constant, the absolute temperature, the number of molecule i, the volume fraction of the compound i and the pair interaction parameter. The free energy of mixing is then given by

$$\Delta F_M = \Delta H_M - T\Delta S_M$$

ΔS_M is always positive which implies that the entropy factor always favours mixing. The incompatibility is therefore driven by the enthalpy. The value of χ_{ij} describes the difference between the interaction of like pair molecules (A-A, B-B) and unlike molecules (A-B). The effective pair interaction parameter was expressed as

$$\chi_{ij} = \frac{z}{kT}\left(w_{ij} - \frac{w_{ii} + w_{jj}}{2}\right)$$

where z and w_{ij} represent respectively the number of nearest neighbours to any lattice site and the energy of interaction between segments of each species i and j situated in neighbouring sites (Piculell et al. 1995).

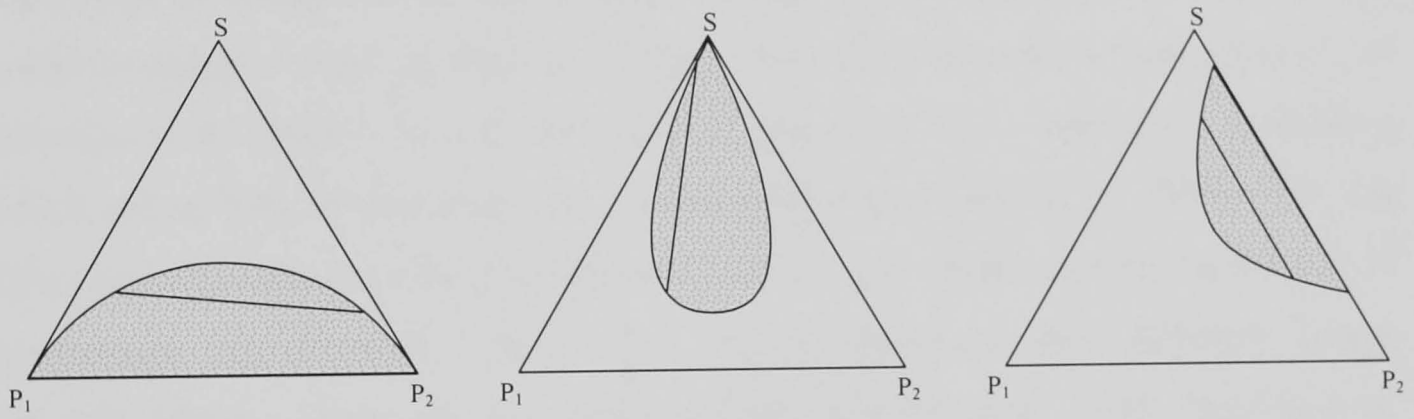
When the free energy of mixing is not negative for a given mixture composition, the systems is incompatible and phase separation occurs leading to at least two phases of differing concentrations. Resolving $\Delta Fm=0$ leads to the binodal curve. The conditions for equilibrium in phase separation systems are then expressed using the chemical potential of each molecular species μ_i. The thermodynamic equilibrium is reached when the chemical potential of each compound in all the n phases is constant ($\mu_{i1}=\mu_{i2}..=\mu_{in}$) (Flory 1953a). Resolving the chemical potential allows the compositions of phases in equilibrium to be expressed.

2.1.3. Phase behaviour of biopolymers

The thermodynamic principles explained earlier allow the determination of the phase diagrams. Many factors influence a phase diagram. The most important are the temperature, the pair interaction parameters χ and the molecular weights of the molecular species. However for biopolymers, because of hydrogen bonding, the solvating layers are large and the degrees of freedom of the water molecules involved are reduced. Nevertheless the basic principles of the Flory-Huggins theory still apply (Gustafsson, Wennerstrom and Tjerneld 1986).

Therefore, on mixing two polymers in water, the various combinations of the χ parameters can lead to three different ternary phase diagrams (Piculell et al. 1995) (Figure 2-2). The phase separations obtained are called segregative, associative and borderline. When a mixture that phase separate is prepared, it is thermodynamically unstable and two phases in equilibrium are generated. The binodal delimits the homogeneous systems from the thermodynamically unstable mixtures. The tie lines link the positions of the two phases in equilibrium. Any mixture whose composition lies on the tie line will demix into the two corresponding phases on the binodal. Respective volume fractions can be expressed using a lever rule (Morris 1990).

Figure 2-2: Schematic ternary phase diagram for segregative (left), associative (middle) and borderline (right) phase separations. The two-phase region is represented by the grey area. S: Solvent, P_i: polymer i (Piculell et al. 1995).



Associative phase separation only occurs when $\chi_{P1\text{-}P2}$ is negative and $\chi_{P\text{-}S}$ are positive. This implies that the different polymers attract each other and that the water is not a perfect solvent. This is common for oppositely charged polymers and the resulting phases are a polymer rich phase and a polymer deficient phase (Piculell et al. 1995; Michon et al. 1995). Borderline phase separation is not very common since it requires that one of polymer concentration is constant in both phases. Closed loop phase diagrams are also considered a result of large $\chi_{P\text{-}S}$ interactions (Zeman and Patterson 1972).

The most common phase separation is the segregative type. For a ternary system, the two phases in equilibrium are called P1 rich phase (P2 deficient phase) and P2 rich phase (P1 deficient phase) respectively. If the tie lines are not horizontal then water

partitioning between the two phases also occurs. Although a large number of biopolymer mixtures have been studied (Antonov et al. 1996; Bourriot, Garnier and Doublier 1999; Chilvers and Morris 1987; Clark et al. 1983; Gotlib et al. 1988; Kalichevsky, Orford and Ring 1986; Kasapis 1996; Michon et al. 1995; Morris 1990; Papageorgiou, Kasapis and Richardson 1994; Sakellariou and Rowe 1995; Sanderson et al. 1987) no theory allows a predictive description of phase behaviour of water soluble polymer mixtures.

The extent of the incompatibility phenomenon is ruled by a few important factors. The molecular weight affects directly the entropy of mixing, and high molecular weight polymers are incompatible at very low concentration (Flory 1953a; Piculell et al. 1991). The ionic strength or salt concentration usually promotes the phase separation of charged polymers (Antonov et al. 1996). The phase separation type could be changed from associative to segregative when the salt concentration varied (Michon et al. 1995). The salt concentration can also be of importance in deciding which phase will be continuous or included (Papageorgiou et al. 1994). The salt effect or ionic strength effects on phase separation are usually due to the entropy of counterions and therefore appear when the two polymers have different charge densities (Perrau, Iliopoulos and Audebert 1989; Piculell et al. 1991; Piculell et al. 1995). Polyelectrolytes show the largest incompatibility zone when the two phases in equilibrium have identical counterions concentrations (Gottschalk, Linse and Piculell 1998; Khokhlov and Nyrkova 1992; Piculell et al. 1995). Hydrogen ions concentration (pH) also affects the phase diagram especially for proteins (Polyakov, Grinberg and Tolstoguzov 1997; Sanderson et al. 1987; Gottschalk et al. 1998; Grinberg and Tolstoguzov 1997; Kasapis 1996; Piculell et al. 1994; Piculell et al. 1995; Sanderson et al. 1987).

Phase separation is also influenced by the chemical structure of the polymers. Block patterns in alginates or distribution of the methyl groups of pectin can affect the phase diagram of alginates (or pectin) gelatin mixtures (Antonov et al. 1996). Proteins with different conformations (i.e. native vs heat denaturated) can also phase separate (Polyakov et al. 1997; Morris 1990).

Usually phase diagrams of biopolymers mixtures are not symmetrical. This asymmetry is often explained by the molecular weight differences (Flory 1953a; Grinberg and Tolstoguzov 1997) but charge density differences (Piculell et al. 1991) or differences in water polymer interaction parameters (Gottschalk et al. 1998) can also lead to this asymmetry.

Weakly charged polyelectrolyte mixtures can form thermodynamically stable microstructures due to a relatively lower entropy loss compared to complete demixing (Khokhlov and Nyrkova 1992).

2.2. POLYMERIC MATERIALS STUDIED

A large number of food products have been used during this project. Only a brief description of the main products used is now given.

2.2.1. Gelatin

2.2.1.1. The gelatin polymers

Gelatin is a cheap polymeric material. It is widely used in food and is probably the main gelling agent in food products. It contains essential amino acids and presents a low melting point.

Gelatin constitutes about 30% of the protein in humans (Ledward 1986; Johnston-Banks 1990). It is industrially extracted from collagen from animal bone and skin. Two extraction methods are used: alkali treatment (lime) and acid treatment that respectively lead to gelatin of type B and A. Mild acid treatments are used for immature collagen from young animals. Mature collagen requires the more aggressive alkaline treatment in order to break the covalent bonds that occur on ageing. Alkaline treatments are slower and the two gelatin types can differ in their properties. The isoelectric point (pI) is the most important difference between type A and B gelatin. Three main gelatin types are obtained:

- Ossein gelatin, type A with a pI from 6.5 to 7.5

- Pigskin gelatin, type A with a pI from 7.5 to 9.5
- Cattle skin or hide, type B with a pI of about 4.9

Limed gelatin has a very low pI (compared to collagen) owing to the hydrolysis of glutamine and asparagine groups during the alkaline treatment (Johnston-Banks 1990). Beside the amino acid composition differences, the various gelatins differ in their molecular weight distributions.

The molecular weight distributions of gelatin samples are very complex. Unlike most protein, they do not exhibit a standard amino acid sequence. However, they are not monodisperse systems and molecular weight distributions of gelatin samples are still studied. The main unit is called the α chain. Its molecular weight is about 95000 g/mole. This unit can be associated in β chains (two α chains covalently bound), γ chains (three α chains), X chains (4 α chains), 1-4 chains (five to eight α chains) or Q chains (up to two hundred α chains). In addition to these structures, there are many derived structures where some amino acids have been lost. These are called A chains (molecular weight of about 86000g/mole) for the α chain derivatives (Johnston-Banks 1990).

The relative proportions of these structures are variable and the various groups of molecular weight have different effects on various properties of the gelatin. For instance setting time, melting temperature and viscosity are influenced by the Q chains content whereas bloom is mainly governed by the α and β chain contents (Johnston-Banks 1990).

The amino acid composition of gelatin is similar to its parent collagen. It is characterised by its very high proportion of glycine (Gly 33%) and imino acids proline (Pro) and hydroxyproline (Hyp) (22%) (Johnston-Banks 1990). The molecule features repeating units (Gly-X-Y) where high proportion of X and Y is one of the imino acids. The terminal regions of the α chains (telopeptide zone) do not present this structure.

2.2.1.2. *Gelatin gel and solution*

Many applications of gelatin take advantage of its gelling properties. Above about 40°C, the gelatin macromolecules in aqueous solution are present in a random coil configuration (RossMurphy 1992). On cooling gelatin solutions, gels are formed. In very dilute systems (<0.1%), the apparent molecular weight of the gelatin molecule almost doubles between 60 and 35°C (Bohidar 1998). Gelation will only occur above a typical concentration threshold of about 0.4-1% (RossMurphy 1992).

The gelation process is very complex. It involves coil helix transition, triple helix formation and aggregation. The triple helices are thought to be similar to collagen triple helical structures. The transition observed here are very slow compared to other gelling systems (agarose, carrageenan) (RossMurphy 1992). It is well known that the gelation process does not stop once the gel has set. Gelatin gels mature with time. Existing linkages are continually reorganising themselves (Ledward 1986) and gelatin gels may never attain equilibrium (Djabourov, Lechaire and Gaill 1993). The amount of helical structure in the gel continually increases (Djabourov et al. 1993). These changes occur so that more and more peptides are in the ordered conformation (RossMurphy 1992). The junction zones are constituted of triple helix aggregates.

The mechanical properties of gelatin gel are often condensed into a measurement of the gelatin quality called the Bloom. The Bloom test was introduced in 1925 in order to compare different gelatin batches. The Bloom strength is measured as ' the weight required to make a 0.5 inches in diameter, flat bottom plunger depress the surface of a gelatin gel 4 mm' (Wainewright 1977). This test is performed on a 6.67% gel matured at 10°C for 18 hours. The Bloom value is measured in gram and is still a standard measurement for gelatin (Wainewright 1977). The Bloom of commercially available gelatin ranges from 50 to 300 (Johnston-Banks 1990).

The viscosity of a gelatin solution above the setting temperature is unusually low and this property is essential for capsule production where high concentrations are

required. Gelatin solutions also have a Newtonian behaviour which differs from most biopolymers of this molecular weight (Wulansari et al. 1998).

2.2.1.3. Applications

Besides its gelling properties, gelatin shows some emulsifying properties, film forming properties and polyelectrolyte characteristics. Gelatin is therefore used for many applications (Wood 1977; Johnston-Banks 1990):

- Jelly desert (dilute gel)
- Elastic gums (concentrated gels with gum arabic)
- Marshmallow (emulsifier, stabiliser)
- Wine production (particle flocculation)
- Tablets, suppositories, plasma expanders
- Microencapsulation
- Adhesives
- Printing
- Hard and soft capsule (pharmaceutical industry)
- Photography

2.2.1.4. Solid gelatin

The relevance of the general studies of gelatin gelation for the capsule production is now discussed. Two major differences occur between the usual gelatin gel and the gel of interest for capsule production. First in capsule production, the initial gel concentration of gelatin used is very high (about 28%) and therefore, the aggregated regions will be small (Stainsby 1977). Second the drying of the films is quick (about 40 min) and therefore the maturation process is very limited.

Capsule production is usually performed with a gelatin blend. The resulting film contains from 10 to 15% of water. Below 5%, the films are too brittle for many applications. It was also shown that when the measurements were performed at a relative humidity of 60% or below, the films dried above 60°C were more brittle than films dried at room temperature (Finch and Jobling 1977). This might be correlated

to the presence of triple helix and the fibril-like arrangements that were observed in the cold dried films but not in the hot dried films (Melia 1983).

2.2.2. Gelling agent

Gelatin is unusual and probably unique in that it can be used at high concentrations without forming highly viscous solutions but will gel on cooling. To match these properties, different polymers have been studied.

The various materials have been classified according to their use in this study, namely potential filler or film former and gelling agent. This classification is based on this current study and product used as filler here can have gelling properties in some conditions (alginate, starches, and cellulose derivatives).

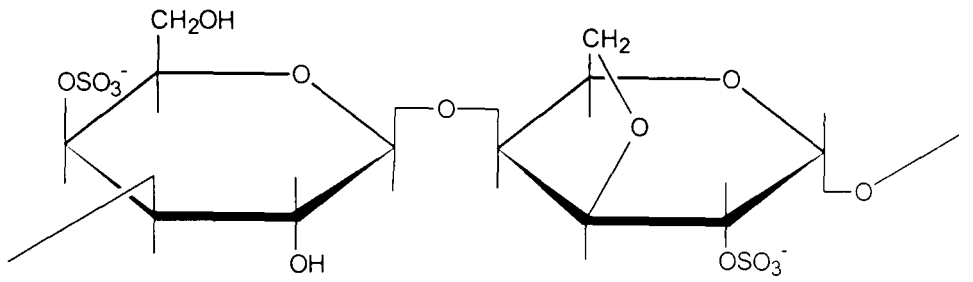
2.2.2.1. Kappa carrageenan

The term carrageenan is used to name a class of galactan polysaccharides that occurs as intercellular matrices in red seaweed (class Rhodophyta). They have been extracted and used in food for centuries.

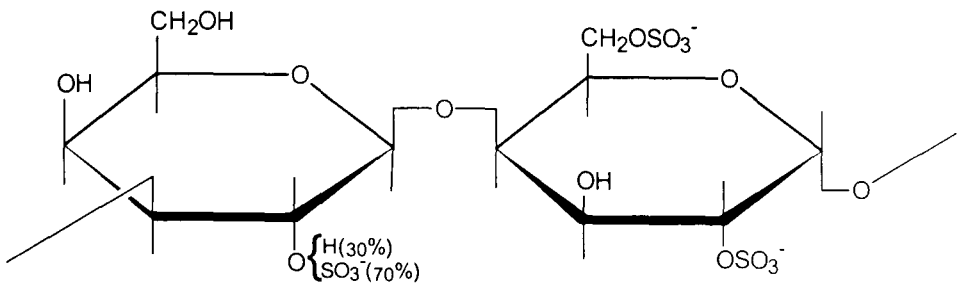
The structure of carrageenan is based on a repeating disaccharide called carrabiose (Stanley 1990). Carrabiose is made of alternating β-1,3- and α-1,4- linked galactose residues. The 1,4-linked residues are commonly present as 3,6-anhydre. The 1,4-linked residue in carrageenan is the D-eniantiomer whereas the L-eniantiomer is present in agar. Carrageenans are highly sulphated and various limit polysaccharides are defined depending on the position and number of sulphate groups. They also differ in the presence or absence of internal 3,6- ether bond. Native carrageenans are in fact mixtures of the limit structures and their hybrids in various proportions (Stanley 1990). The commercially available carrageenans are the kappa, iota and lambda forms. Their basic structures are illustrated in Figure 2-3.

Figure 2-3: Repeating units of limit carrageenans (Glicksman 1982).

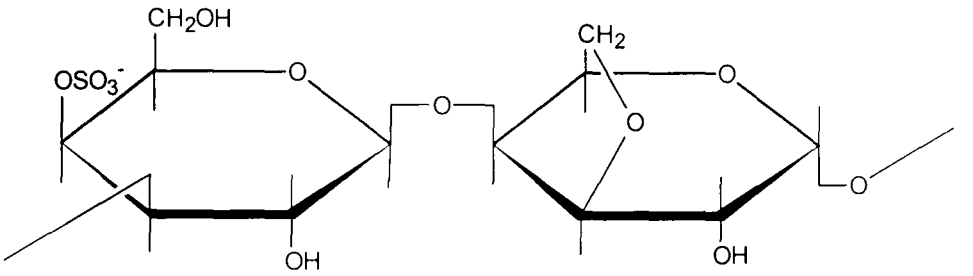
iota carrageenan



lambda carrageenan



kappa carrageenan



Two main groups of carrageenan can be distinguished. Some carrageenans (κ and ι) are able to gel in presence of potassium ions in some conditions. They present a common feature: their 1,3-linked residues can only be sulphated on the carbon C4. λ, ξ and θ carrageenan are not able to form gel. This second group of carrageenan is usually used for its viscous properties (Stanley 1990).

Commercial carrageenans are composed of mixtures of the limit forms and their typical average molecular weights range from 200 000 to 400 000 daltons.

Kappa carrageenans need to be prepared in hot water for complete dissolution. Gelation occurs on cooling in presence of specific counterions (K^+ or Ca^{2+}). On cooling kappa carrageenan, the polymer changes from a random coil conformation to

a double helical structure. If the required counterions are present, these helices aggregate and a gel is formed. The transition temperatures depend on polymer and counterion concentrations. Melting usually occurs at a higher temperature than the setting temperature. Many studies relate the detailed effects of various ions and conditions (Hermansson, Eriksson and Jordansson 1991; Michel, Mestdagh and Axelos 1997; Nishinari 1997; Oakenfull and Morris 1987; Piculell et al. 1997).

Carrageenans were first used to obtain a thickened product when added to milk (Stanley 1990). A very large number of publications on the interaction between carrageenan and milk, milk protein or milk fraction is available in the literature (Bourriot et al. 1999; Drohan et al. 1997; Keogh, Laine and OConnor 1996; Langendorff et al. 1997; Langendorff et al. 1999; Lynch and Mulvihill 1994; Lynch and Mulvihill 1996; Michon et al. 1996; Tziboula and Horne 1999; Xu et al. 1992). Carrageenan κ-casein interactions are very strong and may explain the behaviour of milk carrageenan systems.

Carrageenans are used in many countries. The European Economic Community recognises them as additive (E407). Carrageenans are used widely for their thickening, gelling and stabilising abilities. Many applications imply the presence of milk derivative product and take advantage of the specific interactions mentioned before. They are also used in vegetarian jelly or in the pet food industry.

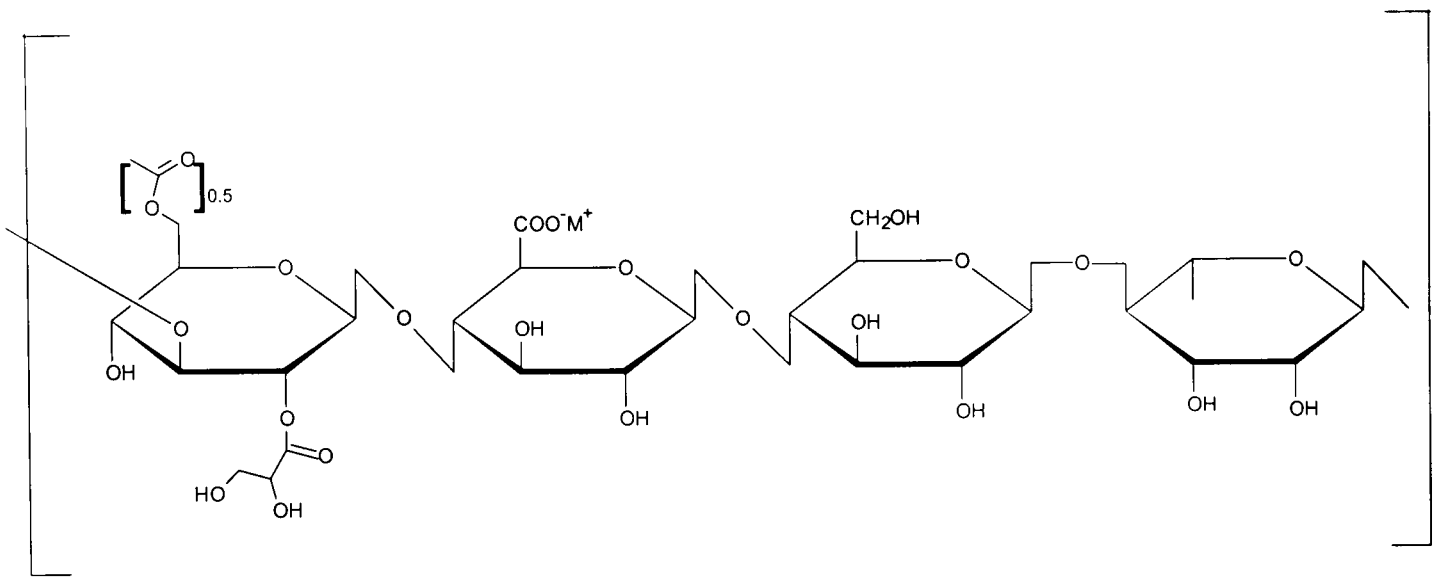
2.2.2.2. Gellan gum

Gellan gum is a recent addition to the polysaccharide market. It was first discovered in 1978 and is produced by *Pseudomonas elodea*. It is now produced commercially by industrial fermentation under controlled conditions. The general information provided here comes mainly from the supplier (Kelco Company 1996b).

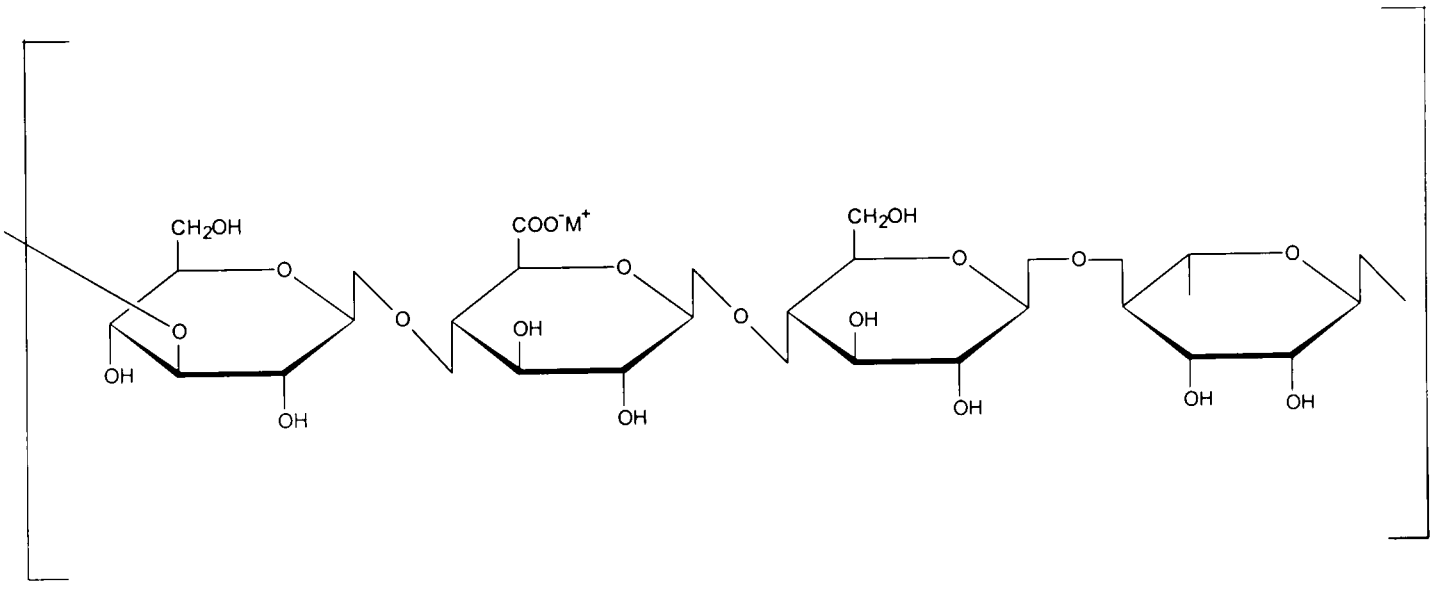
Native gellan gum is constituted of glucose, glucuronic acid and rhamnose in a molar ratio 2:1:1. The linkages are 1,4 within the tetrasaccharide repeating unit and 1,3 between units. The chemical repeating unit is given in Figure 2-4. Two acyl groups (glycerate and acetate) can be present on the gum as substituents. The low acyl form

is obtained when alkaline treatment is used for gum recovery. The molecular weight of low acyl gellan gum is about 500 000 daltons.

Figure 2-4: Repeating unit of gellan gum. (a) Native or high acyl gellan gum.



(b) Low acyl gellan gum.



Gelation occurs on cooling due to the formation of a double helix. As aggregation occurs a gel is formed. Calcium and potassium ions play an important role in stabilising the aggregated structure. The critical concentrations for gelation are very small and concentrations of 0.1-0.5% are often used. The thermal hysteresis observed for carrageenan also occurs here.

Gellan gum is often used in combination with other hydrocolloids (Chilvers and Morris 1987; Miyoshi et al. 1996; Sanderson et al. 1987). Gellan gum can be used in gelled products, fruit filling, batters for chicken or fish, high solids products or toothpaste. It is classed in Europe as a generally permitted additive (E418).

2.2.2.3. Agarose

Agarose (or agaran) is the gelling fraction of agar. Agar is obtained from various species of red seaweed (*Rhodophycea*) where it has a structural role. Agarose is very expensive and little is used. Its structural unit, agarobiose resembles the carrabiose described earlier. However, sulphate groups are absent in agarose. Pyruvate may sometimes be present as a substituent (Selby and Whistler 1993).

Gelation of agar and agarose can occur at very low concentrations. The melting temperature of gels is a function of the concentration and a strong setting temperature-melting temperature hysteresis is observed at high concentrations. The gelling properties of agarose are explained by the formation of a double helix and aggregated domains. Ions are not required for gelation.

Agar is used in microbiology as a culture support because it is low in metabolisable or inhibitory substances. It also supports very well thermal treatment without damage.

Agar is used in food products as a stabiliser or to improve texture. Agarose has little use in the food industry. It is however used in differentiating proteins, enzymes or other high molecular weight compounds in gel-bead filtration or electrophoresis (Selby and Whistler 1993).

2.2.3. Filler and film former

2.2.3.1. Gum arabic

Gum arabic is one of the oldest 'industrial' gums. It was used thousands of years ago for its adhesive properties. It belongs to the group of exudate gums and is harvested from *Acacia* trees. It exudes from wounds and is produced mainly in Africa. The production, price and qualities are very variable depending mainly on climatic changes.

Gum arabic is neutral or slightly acidic. It contains many different sugars. Gum arabic from *Acacia senegal* has: D-galactose (44%) L-arabinose (24%), L-rhamnose (13%), D-glucuronic acid (14.5%). Its molecular weight is about 380000 daltons (Whistler 1993; Williams and Phillips 1999). Different sources can lead to different compositions and molecular weights. Gum arabic also contains protein (2%).

The polysaccharide part in gum arabic is a highly branched molecule. The main backbone is a 1,3 linked β-D galactose chain which is substituted in C6 with branched chains. The overall structure was long thought to be globular. Different fractions were studied and a model where carbohydrate globular structures are attached to a polypeptide chain has been proposed (Williams and Phillips 1999). This structure would explain the emulsifying ability of gum arabic.

The branched structure of gum arabic explains its very low viscosity. Its behaviour is Newtonian up to a concentration of about 40%. pH affects the viscosity but maximum viscosity is almost maintained in the range 2-10.

Gum arabic is mainly used for its emulsifying character owing to the presence of protein covalently bond to the polysaccharide and its low viscosity. Gum arabic is also used in flavour fixation (citrus oil), confectionery (preventing sugar crystallisation), caramel, gums, adhesives and cosmetics.

2.2.3.2. Caseinate

Milk is produced by female mammals for the nutrition of their offspring and contains mainly protein, lactose and fat in water. Protein is present at about 3.5% of bovine milk; 80% of the protein belongs to the casein group.

The term casein is used to define a group of proteins: αs_1, αs_2, β and κ-casein which are present at level of 38, 10, 36 and 13% respectively (Kinsella 1984). The amino acid structure of each casein has been elucidated and major differences were observed. Cystine (disulfide bond) is present in αs_2 and κ casein and the number of phosphorylated groups present on serine residues were respectively 1, 5, 8.5 and 11.5 for κ, β, αs_1 and αs_2. This imparted for difference in charge level and pH sensitivity of the different proteins (Kinsella 1984). Finally, hydrophobic domains away from the charged domains were observed on caseins. This explains the remarkably dipolar and thus surfactant properties of β casein. The molecular weights of the caseins are 23000 for αs caseins, 23900 for β-casein and 19000 for κ-casein. Various amounts of oligosaccharide residues are attached to κ-casein.

In milk, most of the casein is present as colloidal particles called micelles. Casein micelles range in diameters from 50 to 300nm (average 100nm) and have an average molecular weight of about 10^8 g/mol. They consist of casein and ions (calcium and phosphate). Each micelle is composed of submicelles (10-15nm in diameter) and stabilisation of the micellar structure is likely to be due to calcium phosphate and hydrophobic interactions (Fox and Mulvihill 1990). The submicelles are composed of various amounts of each casein type and their position within the micelle depends on this composition. The κ-casein rich submicelles are concentrated on the surface of the micelle.

Caseins are extracted from skimmed milk by isoelectric precipitation using lactic acid (fermentation), mineral acids or by proteolytic coagulation (rennet) (Fox 1989). Whey proteins constitute the remaining milk proteins. Whey is removed from the resulting curd before washing and drying (Mulvihill 1989).

Caseins are not soluble in water unless the pH is increased in some conditions. This leads to the production of caseinates. Sodium, potassium and ammonium caseinates are obtained using the respective bases (NaOH, KOH, NH_3). Calcium caseinate are also produced but a different process is used since they are insoluble (Mulvihill 1989).

Caseinates have little similarity to the native casein micelles in milk (Kinsella 1984). Caseinates are soluble above pH 5.5 (except calcium caseinate). They are heat stable (140°C for 15 minutes at pH 7) (Mulvihill and Fox 1989). Their relatively low viscosity is explained by their molecular weight but viscosity build up can be significant at high concentration (>10%) (Fichtali, vandeVoort and Doyon 1993). The intrinsic viscosities of individual caseins have been measured for various experimental conditions. The results range from 9 to 30ml/g.

Caseins have been used for their adhesive properties for a very long time. Caseins were also used in paints. Nowadays casein and caseinate uses cover a wide spectrum: glue, paper coating, paint, textile fibre, leather industry, rubber product, pet food, beverage stabilisation, baked product, whipped topping, coffee creamer, coating and pharmaceutical products. Extended reviews on casein properties and their uses are available in the literature (Southward 1989; Kinsella 1984).

2.2.3.3. Alginate

Alginate or (alginic acid) is a structural polysaccharide of brown seaweed (*Phaeophyceae*) and alginate type (molecular structure) can vary from species to species. Bacterial alginates have been produced, but not on a commercial scale. Alginate is available in many forms, all accepted as food additives by the European Community: alginic acid (E400), sodium (E401), potassium (E403) and calcium alginate (E404).

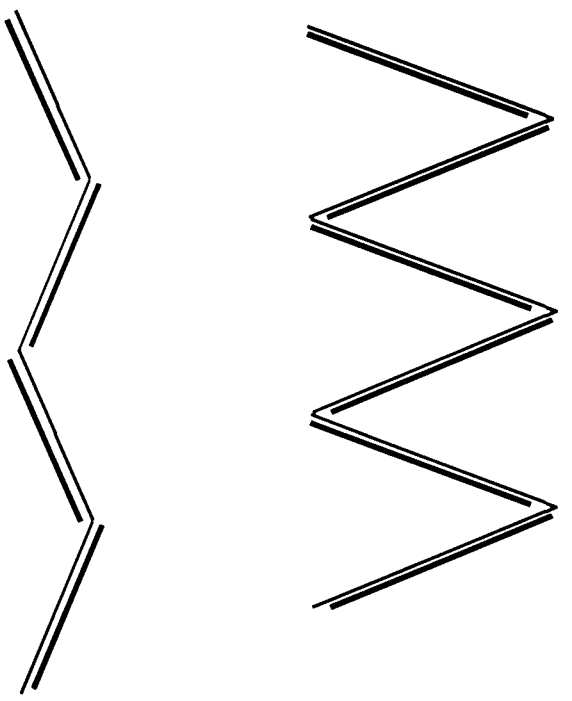
Alginate is composed of D-mannuronic acid (M) and L-guluronic acid (G) linked 1,4 (Figure 2-5). The M residues are linked in the β position whereas the G residues

have a α-1,4 bond. The ratio of both acids and their distribution along the chain can vary.

Figure 2-5: D-Mannuronic acid (left) and L-Guluronic acid (right).

It is usually considered that three types of sequences can occur: MG-block regions where M and G residues approximately alternate, M-block regions and G-block regions. The difference in the linkages between the residues gives distinct geometry for M-block and G-block regions. Polymannuronic acid is often described as a flat ribbon like structure whereas the polyguluronic acid is termed buckled ribbon like structure. Schematic representation is given in Figure 2-6. Percentages of the different block structures determine the gelling properties of the alginates.

Figure 2-6: Schematic representation of conformation of G-blocks and M-Blocks. From Clare (Clare 1993).



Polymannuronic acid
Flat ribbon-like

Polyguluronic acid
Buckled ribbon-like

Alginate salts of divalent ions such as calcium (except magnesium) are insoluble. A controlled release of any divalent ions should lead to the formation of a gel. Calcium gels are the most studied and used. It was shown that the buckled G-block regions of the polymer allowed a specific strong interaction in a model known as egg-box. Alginate containing high level of G-block form stronger gel than high M-block alginate but the latter is more resistant to syneresis (Clare 1993). The M/G ratio is often given as an indication of the gelling properties of alginate. Alginates of various M/G ratio and molecular weights are available.

Calcium release must be well controlled to avoid the formation of precipitate. If not enough calcium is added, viscosity increase is observed but gelation does not occur. Three setting procedures are used. Diffusion setting is based on the diffusion of calcium ions into an alginate gel. This method works well when gel strips are needed. The process can be restricted to surface gelling and is often applied for coating applications. Internal setting of alginate gels is very common. Calcium is released slowly into the solution leading to an even distribution of calcium and slow gelation process. The gel obtained is homogeneous unless the calcium release is too fast. The slow calcium release is obtained either by using an insoluble calcium salt (calcium phosphate) and its late solubility increase (usually by changing the pH) or by using a sequestrant. Finally setting by cooling can also be used if the concentrations of alginate and calcium ions are carefully chosen. Alginate gels do not melt (Clare 1993).

Alginates are used as thickeners, gelling agent, stabilisers and for their film forming properties. They are used in food, pet food, paper, textile or pharmaceutical industry (Clare 1993; Kelco Company 1996a).

2.2.3.4. Propylene glycol alginate

Propylene glycol alginate (E405) is the major alginate derivative. It is approved in many food products. The acid groups of the alginate are partially esterified (40-85%). The reactive agent is propylene oxide leading to two possible ester groups (-CH_2-CHOH-CH_3 or -COH-(CH_2OH)-CH_3). Unlike alginate, propylene glycol

alginate is soluble at low pH. It is however unstable at high pH and depolymerisation occurs (Clare 1993).

Gelation in presence of protein (caseinate, gelatin) occurs at high pH due to the creation of amide bond between the ester group of the propylene glycol alginate and the NH_2 group of the protein (McDowell 1970; Mohamed and Stainsby 1985). The resulting covalently crosslinked gel can be used to improve thermal stability (Clare 1993).

Propylene glycol alginate is mostly used for its emulsifying, stabilizing and foaming properties. It is used in emulsions, baked products and in beer (Clare 1993; Kelco Company 1996a).

2.2.3.5. Cellulose derivatives

Cellulose is the main structural polymer present in terrestrial plants. It is made of linear 1,4-βD glucose units. It is not water-soluble and cellulose as such has few applications in food products. Cellulose has been derivatised and many cellulose derivatives are now available: acetate, ethers, nitrates, carbamate, propionate or sulphate (Reveley 1985). I will concentrate here on the properties and uses of cellulose ethers, which are food allowed.

Derivatisation of cellulose is done after swelling of cellulose in alkaline media. Various types of ether can be obtained:

- Methyl cellulose (MC)
- Hydroxypropyl cellulose (HPC)
- Hydroxyethyl cellulose (HEC)
- Hydroxypropyl methyl cellulose (HPMC, MHPC)
- Hydroxyethyl methyl cellulose (HEMC, MHEC)
- Carboxymethyl cellulose (CMC)

These chemical modifications give the cellulose derivatives different properties. Each derivative is characterised by its molecular weight, degree of substitution and substituent distribution. The maximum degree of substitution is 3 (average number of substituent attached to the glucose repeating unit). For substituents containing hydroxyl groups, the molar substitution can be greater than 3 due to self-etherification. The position of the substituents of various cellulose ethers have been extensively studied (Nehls et al. 1994; Tezuka et al. 1990; Tezuka et al. 1991). When two substituents are added (HPMC or HEMC) the order of addition can affect the substitution pattern and the resulting properties of the cellulose derivative.

Methyl containing cellulose ethers (all cellulose ether except CMC) are often given the generic name of methylcellulose. They all have strong foaming properties and their preparation requires special attention. They are not soluble at high temperature and this allows a good dispersion of the gum. On cooling, dissolution occurs. However, on heating a solution, gelation can occur if molecular weight and concentrations are high enough. This unusual gelation process is likely to be due to the presence of a structured water 'cage' around the polymer. As the temperature increases, the entropy loss is increased and polymer molecules gather together (Haque and Morris 1993; Haque et al. 1993; Desbrieres, Hirrien and Rinaudo 1998; Hirrien et al. 1998). Methylcelluloses are used for their thickening, binding and emulsifying properties in food emulsions (salad dressing). Their unusual gelling properties are their main role in baked products (cakes, doughnuts, cookies...). They are also widely used in non food industries: construction materials, paper, pharmaceutical (coating, binder, film), cosmetics, paints, and textile (Grover 1993).

All cellulose derivatives can be used as thickeners but CMCs are by far the most used (Grover 1993). CMC of high molecular weight and low DS present strong thixotropic behaviour. The viscosity of an undisturbed solution will increase with time but might be returned to its original value after stirring (Feddersen and Thorp 1993). CMC like other cellulose derivatives is a good film former. It is also used in many industrial applications: textile, detergent, food, pharmaceutical, cosmetic and paper.

2.2.3.6. *Starch and derivatives*

Starch is probably the second most important polysaccharide on earth (after cellulose) but definitely the most used by humans (Jane 1995). Many plants produce starch and the resulting starches can have large differences (granule size, amylose content, amylose molecular weight, gelatinisation temperature, viscosity, peak viscosity and tendency to retrograde). Native starch structure is complex and not fully elucidated. The two main polymeric components are amylose (linear 1,4-α-D-glucopyranosyl) and amylopectin (branched structure). The fine structure of amylose and amylopectin are very complex and variable. Starch is the least expensive of all gums and its granular structure and high molecular weight give it very unusual properties (low cold viscosity, gelatinisation and retrogradation). Starch properties are largely documented in the literature.

Starch modification has been performed in order to obtain different properties. A non exhaustive list is given below (BeMiller 1993):

- Chemical reaction
  - Crosslinking (esterification, hemiacetal and acetal formation)
  - Stabilisation (etherification, esterification, oxidation)
  - Graft copolymerization
  - Depolymerisation (acid catalysed, enzyme-catalysed, oxidation followed by alkaline pH)
  - Dextrinisation
- Physical transformation
  - Pregelatinisation
  - Cold water swelling starch
- Genetic control/plant breeding

Derivatisation is performed under alkaline conditions on the whole starch granule. In these conditions, gelatinisation would occur at high degree of substitution and commercial starches usually have degree of substitution in the range 0.001-0.2. Hydroxyethylstarch, hydroxypropylstarch, starch acetate, starch phosphate and

crosslinked starch phosphate diester are produced. These modified starches are less prone to retrogradation and they are often depolymerised (BeMiller 1993).

Oxidised starches are bleached with various oxidants, but the chemical modifications are usually very small. Some of the hydroxyl groups are modified and transformed into carbonyl or carboxyl groups. Again, depolymerisation can occur and resistance to retrogradation is obtained. These starched are more heat and alkali sensitive. They are used in the paper and textile industry.

Depolymerised (thinned) starches are also common. They range from maltodextrin to glucose. Pregelatinised starches dissolve readily without excessive heating.

2.3. RHEOLOGY AND MECHANICAL PROPERTY MEASUREMENTS

2.3.1. Principles of viscosity

The first concept of viscosity was introduced through Newton's postulate $\eta = \sigma / \dot{\gamma}$ in which σ is the shear stress and $\dot{\gamma}$ is the shear rate. Many simple liquids will have a Newtonian behaviour (η independent of $\dot{\gamma}$). However, many polymeric solutions will not behave as ideal Newtonian liquids. Viscosity usually decreases as a function of shear rate (shear thinning) and comparative viscosity measurements must be made cautiously. More complex behaviour can occur: thixotropy, shear thickening, yield stress.

Viscosity of polymeric solutions is a function of molecular structure, molecular weight, solvent, temperature, shear rate and history. At very low concentration and shear rate, viscosity measurements can be related to fundamental hydrodynamic properties of macromolecules.

2.3.2. Intrinsic viscosity measurement via rotational viscosimetry

The intrinsic viscosity of polymer gives an indication of its size and shape (Harding 1997). The intrinsic viscosity is defined as

$$[\eta] = \lim_{c \to 0} \left(\frac{\eta - \eta_s}{c \eta_s} \right)$$

where c is the concentration, η is the viscosity and η_s is the viscosity of the solvent. The units of intrinsic viscosity are ml/g.

The determination of the intrinsic viscosity is usually performed using capillary viscosimetry. Zero shear rate viscosity can also be used to determine the intrinsic viscosity. In order to obtain the intrinsic viscosity, the viscosity of a polymer solution is measured at constant temperature for various concentrations and shear rates. Ideally, the concentrations are low and decreased to infinite dilute systems. Different shear rates are used so that extrapolation to zero shear rate is possible. The following equations are then applied.

Relative viscosity	$\eta_{rel} = \eta / \eta_0$	η_0 : solvent viscosity
Specific viscosity	$\eta_{sp} = \eta_{rel} - 1$	
Reduced viscosity	$\eta_{red} = \eta_{sp} / c$	c : concentration

and the intrinsic viscosity

$$[\eta] = \lim_{c \to 0}(\eta_{red}) = \lim_{c \to 0}(\eta_{sp}/c) = \lim_{c \to 0}((\ln \eta_{rel})/c)$$

Huggins plot (η_{sp}/c versus c) and Kraemer plot ($(\ln\eta_{rel})/c$ versus c) allow the determination of the intrinsic viscosity at zero concentration (Harding 1997). These plots are the most commonly used but other methods are also available. Single point equations for intrinsic viscosity measurement have also been proposed. Solomon's equation is given below (Harding 1997).

$$[\eta] \approx (1/c) \quad [2\eta_{sp} - 2\ln(\eta_{rel})]^{1/2}$$

Molecular weight and intrinsic viscosity are related by the Mark-Houwink equation:

$$[\eta] = K'.M^{a}$$

where both K' and a depend on the polymer conformation and the experimental conditions. The value of a are respectively 0, 0.5-0.8 and 1.8 for sphere, random coil and rigid rod conformation (Harding 1997). Using known values of a and K' from the literature, it is possible to obtain an approximate value for the molecular weight.

2.3.3. Sensory analysis

Sensory analysis is usually used for food testing. However, the principles still apply to any sensory evaluation. Sensory analysis has been used for paper or food texture (Meilgaard, Civille and Carr 1991).

Many sensory analysis techniques are available, the choice of which depends mainly on the type of problem to be solved. Quantitative descriptive analysis relies on the choice by the panellist of a set of terms that describe best the samples to be studied. This technique is very appropriate when a complete description of the sample is necessary, without any external influence.

2.3.4. Large deformation measurements and puncture test

Large deformation measurements are used to characterise the behaviour of product when submitted to stresses. Many tests are available:

- Time dependent tests
  - Creep test (Constant stress, strain as a function of time)
  - Stress relaxation (Constant strain, stress as a function of time)
- Stress-strain tests
  - Compression, tension

- Two, three and four points bend
- Shear

- Empirical tests
  - Impact test
  - Notch test
  - Puncture test
  - Tearing test
  - Fatigue test
  - Friction test

Many tests have been standardised (ASTM). However, standardised tests may not be better than any other test (Nielsen and Landel 1994).

Stress strain tests give direct measurement of fundamental parameters. Yield point, modulus, break strength and strain at break can be obtained for each test. Although fundamental, the results do not always give clear information on the product behaviour in real situations. In fact, results from the three different stress-strain experiments can rank differently a set of products (Julian, Radebaugh and Wisniewski 1988). This makes it difficult to judge the overall quality of a given product.

Puncture tests usually consist of puncturing a material at high speed, and measuring the maximum load. Puncture is a form of impact test where a distance/force curve can be drawn.

All mechanical tests depend on geometry, temperature and speed of deformation. For glassy polymers, speed of deformation (strain rate) and temperature are linked because of the time temperature superimposition principle (Nielsen and Landel 1994).

2.4. FRACTURE MECHANICS

The fracture behaviour of polymer has been extensively studied for synthetic polymers. The polymeric structure encountered (glass, crystal or rubber) will affect the mechanical properties before fracture and the fracture behaviour. In crystal, deformation is due to bending and stretching of the crystalline structures. In glass, the polymers are disordered and bond rotation can occur as well. Uncoiling and slippage could also occur (Kinloch and Young 1983).

Two main phases are involved in fracture: initiation and crack propagation. Before fracture occurs, two phenomenons can appear during the deformation process. First, shear yielding is the ability of translational motion of polymers. Shear yielding corresponds to a plastic deformation and energy dissipation occurs. Secondly crazing or the creation of very small voids bridged with fibrils also occurs. Crazes are usually stabilised by the orientation of the polymer fibrils and the concomitant strain hardening. Crazing usually occurs at imperfections and results in an increase of specimen volume. This differs from shear yielding where volume is kept constant. Crazing would usually lead to early fracture, however, multiple crazing could generate overall yielding and prevent fracture through this toughening process (Kinloch and Young 1983).

The fracture behaviour is difficult to understand since it involves the study of these phenomena (yielding and crazing) in a very localised area namely the crack tip. Although the number of backbone per unit area is correlated to the critical tensile strength, theoretical values are much higher than experimental values showing the importance of crazing and flaws (Vincent 1972).

The study of the fracture of polymer is even more complex when anisotropy is also considered. This is the case for thin structures (films), fibres or drawn materials. It was also shown that without isotropy changes, fracture behaviour could depend on thickness. This was due to a transition from a plane-stress to a plane-strain situation (Kinloch and Young 1983).

Different approaches have been used for the study of fracture behaviour but no generalised theory exists yet. For glassy polymers, crazing seems to be the dominant micromechanism controlling the fracture of brittle materials (Polymethylmetacrylate or Polystyrene) whereas shear yielding may explain the ductile behaviour of polycarbonate. Entanglements of polymeric chain may be of importance in controlling the preferential phenomenon encountered in fracture. It is yet impossible to predict fracture behaviour from the knowledge of the structure (Kinloch and Young 1983).

The effect of molecular weight and molecular weight distribution was reviewed for synthetic polymer (Nunes, Martin and Johnson 1982). Generally, a molecular weight threshold was observed, beyond which the mechanical properties of the materials were not affected by molecular weight changes. The effect of molecular weight on fracture behaviour can be very different depending on the fracture phenomena involved. No general concept can be devised.

2.5. EDIBLE FILMS

A large number of papers and reviews discuss films used by the food industry (Guilbert, Cuq and Gontard 1997; Kester and Fennema 1986; Krochta and Mulder 1997). Two different types of films containing biopolymer are used: edible films (coating or packaging) and biodegradable films (removable packaging). For the biodegradable films, non edible copolymers are often used in conjunction with the biopolymer. For edible films, as in the case of pharmaceutical capsules, all constituents must be food allowed. In both the biodegradable and edible films, various properties are important: mechanical properties, gas permeabilities (oxygen, water and carbon dioxide) and adhesion properties (Krochta and Mulder 1997).

A wide variety of biopolymers has been used to produce films: caseinate and other milk protein (Arvanitoyannis, Psomiadou and Nakayama 1996; Chen 1995; Arvanitoyannis and Biliaderis 1998; Avenabustillos and Krochta 1993; Mezgheni, DAprano and Lacroix 1998; Ressouany, Vachon and Lacroix 1998; Chick and Ustunol 1998), starch (Arvanitoyannis et al. 1994; Arvanitoyannis et al. 1996;

Arvanitoyannis and Biliaderis 1998; Bader and Goritz 1994a; Bader and Goritz 1994b; Coffin and Fishman 1994b; Coffin and Fishman 1994a; Coffin, Fishman and Cooke 1995; Gaudin et al. 1999; Lourdin, Bizot and Colonna 1997; Rindlay et al. 1997), starch derivatives (Arvanitoyannis, Nakayama and Aiba 1998; Fringant, Desbrieres and Rinaudo 1996), gelatin (Arvanitoyannis et al. 1998; Felton et al. 1996; Melia 1983), (Ressouany et al. 1998)pectin (Coffin and Fishman 1994a; Coffin and Fishman 1994b; Coffin et al. 1995; Macleod, Fell and Collett 1997; Wakerly et al. 1996), chitosan (Ichikawa, Mitsumura and Nakajima 1994; RemunanLopez and Bodmeier 1997), alginate (RemunanLopez and Bodmeier 1997).

The effects of plasticisers on the film properties have also been widely studied. Increasing elongation at break was usually observed although antiplasticisation was mentioned at low plasticiser content (Lourdin et al. 1997). Crosslinked films of casein, alginate or chitosan were also studied (Avenabustillos and Krochta 1993; Mezgheni et al. 1998; RemunanLopez and Bodmeier 1997; Ressouany et al. 1998). The effects of UV irradiation and ultrasound on film properties were considered.

The molecular weight effect on permeability properties of edible films was considered for cellulose derivatives (Ayranci, Buyuktas and Cetin 1997). Various authors looked at films containing starch and another biopolymer.

The complexity of the results present in the literature allows little generalization on the effects of various parameters on the film properties. There is little fundamental knowledge for the reasons of the mechanical properties of biopolymers. It is even less possible to predict mechanical properties from structural knowledge.

CHAPTER 3. MATERIALS AND METHODS

3.1. MATERIALS

Most materials used throughout this project were food grade materials. The various suppliers provided little information about the materials. The information supplied concerning the materials used as a potential film former is reported in Tables 3-1 to 3-6. Information about suppliers and batch number are provided in appendix. Materials used for the phase separation study are described in the respective section (3.3.1).

Table 3-1: Gelatin.

Trade Name	Source	Product
A240	Capsugel	Pig skin gelatin 240 bloom strength
B200	Capsugel	Bovine skin gelatin 200 bloom strength
Gelatin	Capsugel	A240 / B200 (50%)

Table 3-2: Alginate derivatives.

Trade Name	Source	Product	Viscosity mPa.s at concentration %
Kelcoloid LVF	Kelco	Propylene Glycol Alginate	1000-1500 at 2%, 25°C
Kelcoloid O	Kelco	Propylene Glycol Alginate	50-175 at 2%, 25°C
Kelcoloid S	Kelco	Propylene Glycol Alginate	50-175 at 2%, 25°C
Manucol ester ERK	Kelco	Propylene Glycol Alginate	90-160 at 1%, 25°C
Manucol LB	Kelco	Alginate	20-100 at 3%
Manucol DH	Kelco	Alginate	40-90 at 1%
Manugel DMB	Kelco	Alginate	200-400 at 1%

Table 3-3: Cellulose derivatives.

Trade Name	Source	Product	Viscosity mPa.s at concentration %
Blanose 7	Hercules	CMC carboxymethyl cellulose	10-25 at 6%, 25°C, spindle 1, 60 rpm
HPMC 606 (Pharmacoat)	Shin-Etsu	HPMC hydroxypropyl methyl cellulose	
Methocel E15	Dow	HPMC hydroxypropyl methyl cellulose	15 at 2%, 25°C
Methocel E50	Dow	HPMC hydroxypropyl methyl cellulose	50 at 2%, 25°C
Methocel E4M	Dow	HPMC hydroxypropyl methyl cellulose	4000 at 2%, 25°C
Klucel EFF	Hercules	HPC hydroxypropyl cellulose	200-600 at 10%, 25°C, spindle 2, 30 rpm
Klucel LF	Hercules	HPC hydroxypropyl cellulose	75-150 at 5%, 25°C, spindle 1, 30 rpm
Klucel MF	Hercules	HPC hydroxypropyl cellulose	4000-6500 at 2%, 25°C, spindle 4, 60 rpm

Table 3-4: Milk products.

Trade Name	Source	Product
Alacid 710	New Zealand Milk Products	Lactic acid casein
Alacid 741	New Zealand Milk Products	Mineral acid casein
Alaren 799	New Zealand Milk Products	Rennet casein
Alanate 380	New Zealand Milk Products	Spray dried calcium caseinate
Alaplex 1180	New Zealand Milk Products	Spray dried milk protein concentrate
Alanate 180	New Zealand Milk Products	Spray dried sodium caseinate
Potassium caseinate	Armor Proteine	Potassium caseinate
Magnesium caseinate	Armor Proteine	Magnesium caseinate

Table 3-5: Starch and derivatives.

Trade Name	Source	Product	Viscosity mPa.s at concentration
Amylogum CLS	Avebe	No information	
C* Avatex	Cerestar	No information	
C* Cream Polartex 06716	Cerestar	Hydroxypropyl distarch phosphate base on waxy maize starch	
C* Cream Polartex 06718	Cerestar	Hydroxypropyl distarch phosphate base on waxy maize starch	
C* Set	Cerestar	No information	
Clinco 460	ADM (Archer Daniels Midland)	Oxidised corn starch	688 at 20%, 40°C,208 rpm (Brookfield viscosity)
Clineo 718	ADM	Hydroxyethyl corn starch	1000 at 25% and 38°C
Colflo 67	National Starch (NS)	Waxy maize based	
Crisp film	NS	Chemically modified high amylose starch	
Crystal Gum S	NS	Tapioca, speciality dextrin	
Dextran	Sigma		
FirmTex	NS	Chemically modified waxy maize starch (E1442)	
Flojel 45	NS	Thin boiling, corn based, gel forming	
Flojel 60	NS	Thin boiling, corn based, gel forming	
Glucidex 2	Roquette	Maltodextrin (dex.equ. 5 max)	100 at 20%, 40°C
Hylon VII	NS	Amylose rich starch	
Instant Clearjel Coarse	NS	Starch ester	
K4484	NS	Speciality dextrin, Tapioca	
LVAWS	Midwest	Low viscosity acetylated wheat starch	
LVHPWS	Midwest	Low viscosity hydroxypropylated wheat starch	
LVOSWS	Midwest	Low viscosity octenyl succinylated wheat starch	
Midsol 35	Midwest	Modified wheat starch	

Trade Name	**Source**	**Product**	**Viscosity mPa.s at concentration**
Miracap	AE Staley	Lipophilic starch substituted with octenyl succinate	100 at 30% 25°C
Nadex 771	NS	Maize	
Nadex 8781	NS	Potato	
National 1900	NS	Thermally processed high amylose starch (cold swelling)	
N-Lite L	NS	No information	100 Bu at 9.5%
N-Lite LP	NS	No information	
N-Tack	NS	No information	200-800 at 30%
Pure Cote 760	GPC (Grain Processing Corporation)	Hydroxypropylated corn starch	
Pure Cote 790	GPC	Hydroxypropylated corn starch	
Stadex90	AE Staley	Dextrin, acid catalysed	1700 at 50%, 25°C
Textra	NS	Tapioca	100-1000 at 5%

Table 3-6: Others.

Trade Name	**Source**	**Product**	**Viscosity mPa.s at concentration %**
Meyproguat 7	Meyhall	Guar gum (degraded)	1000 at 10%
Sunfiber R	Allchem	Guar gum (degraded)	30 at 10%
X98001	Citrus colloids	Pectin	155 at 2%
ExPro	Amylum	Experimental product	
Solpro 500	Amylum		
SWP 050	Amylum	Soluble Wheat Protein (gluten derivative)	
SWP 100	Amylum	Soluble Wheat Protein	
Film forming wheat protein isolate	Midwest	Wheat protein isolate	

3.2. CONFIDENCE INTERVAL

For all measurements performed, standard deviations were calculated. The value of the standard deviation corresponds to the 68% confidence interval of a large population (more than 30). The 95% confidence interval is given by 1.96*standard deviation for large population. However for smaller population, the multiplier increases. Therefore the use of confidence intervals overcomes the differences in sample number. Confidence intervals (95%) are given throughout the text. These values will not reflect the true confidence interval since the data might present non normal distribution (i.e. skewed distribution for the puncture data).

3.3. MIXED GELS

3.3.1. Materials

κ-carrageenan (Satiagel MEO5), agarose (Sigma Type I-A A0169) and high acyl gellan gum (Kelcogel LT100, Kelco) were used as gelling agents. The cosolutes used were gum arabic (Sigma G9752), hydroxypropylmethyl cellulose (HPMC) (Shimatsu grade 606), low viscosity carboxymethyl cellulose (CMC) (Finnfix 2, Metsa), low viscosity sodium alginate (Manucol LB, Kelco, 8.4% w/w sodium, 0.4% w/w calcium) and maltodextrin (Cerestar MD20, DE ~20).

3.3.2. Sample preparation

The solvent was water when agarose was the gelling agent, 0.05M KCl when κ-carrageenan was used, and 0.02M $CaCl_2$ plus 0.005M sodium citrate when gellan gum was present. At this level of $CaCl_2$ the very low viscosity alginate remained soluble. For the carrageenan and agarose systems the polysaccharides were dissolved in the appropriate solvent and heated with stirring and held at 80°C for one hour (carrageenan) and 95°C for 30 minutes (agarose). For gellan gum the temperature of the polysaccharide in water was first raised to 80°C and then the $CaCl_2$ was added. The concentration of gelling agent employed was 0.5g/100ml for κ-carrageenan and

agarose, and 0.3 g/100ml for gellan gum. The cosolute was added during the second half of the heating period. The concentration of cosolute in the final solution was 0, 2%, 4%, 6%, 8%, 10%, 12% and 14% (w/w).

The hot solution was poured into a universal glass bottle (diameter 1.4 cm) covered and allowed to set overnight at room temperature. The pH of all samples were controlled to be pH 6 ±1.0 using a pH meter (CD 620 DIGITAL, WPA).

3.3.3. Gelation assessment

Gelation was assessed by visual examination at room temperature. The tube was gently inverted and the flow properties of the solution examined. Mixtures that did not flow under their own weight within 15 seconds were called gels.

3.4. CHARGE DETERMINATIONS

The determination of the charge density for various polymers was performed using the Mutek charge analyser (Mutek Analytic, Germany) fitted with a 702 SM Titrino unit (Metrohm, Switzerland). Within a cell in the particle charge detector, the macromolecules adsorb on the Teflon surface. The oscillating PTFE probe creates a flow of counterions and hence generates an electric potential. This value is measured and will alter as a polyelectrolyte is added to the solution. The quantity of polyelectrolyte to achieve the zero potential is measured and the charge density of the macromolecule can be calculated.

Poly-Dadmac 0.001N (Poly-diallyl-dimethyl-ammonium-chloride) was used as the cationic polyelectrolyte. The charge density of Poly-Dadmac is virtually pH independent. The volume of the polymeric solution measured was 20ml. The concentrations of the polysaccharides ranged from 0.2g/l for the more charged polymer to 5g/l for the less charged polymer. Addition rates were also varied depending on the polymer.

Three repeats were performed. The charge density CD was calculated as $CD = \frac{[PolyDadmac] \cdot Volume\ for\ zero\ potential}{Mass\ of\ polymer}$ with [Poly-Dadmac]=0.001N.

The value was then corrected for moisture content and expressed in micromole of charge per gram of dried matter (μmol/g).

3.5. SOLUTION PREPARATION

3.5.1. Solutions for film casting

All solutions were prepared in distilled water unless specified otherwise. A double-jacketed flask fitted with a stirring device allowed the preparation of solutions from 0.5 to1.5l. The solutions were made up in hot water (80°C) at a concentration of 7.5% (w/w) (unless specified otherwise). For some of the polymer samples, bubbles and non dissolved matter were present in the solution. Centrifugation at 270g for 10 minutes was performed in order to remove them. For systems involving high viscosity materials, the concentration was adjusted accordingly and is given in the text

All gelatin solutions were left one hour to swell in cold water before heating. The temperature was not raised above 75°C for gelatin solutions.

3.5.2. Caseinate solutions

The same conditions as in 3.5.1 were used. pH adjustment of caseinates films were performed at room temperature using NaOH 1M and monitored using a pH meter (CD 620 DIGITAL, WPA). Acidification was performed by addition of glucono-δ lactone powder and measurement of the pH after 2 minutes.

For caseinates systems with Maillard reactants, the temperature was adjusted to 40°C before addition of the sugar or glyoxal.

3.5.3. Solutions for intrinsic viscosity measurement

One litre was prepared at 80°C in the double-jacketed flask as discussed previously (3.5.1) and cooled to 50°C. The more concentrated solution was prepared first. A known amount of solution was withdrawn for viscosity measurement. Further dilutions were performed by adding the required amount of distilled water in the flask under stirring.

3.5.4. Alginate degradation

Manucol LB was degraded in order to obtain lower molecular weight grades. A better degradation is obtained in presence of ascorbic acid and heat (Smidsrod, Haug and Larsen 1963). The alginate solution was prepared at 7.5% with 0.01mol.l^{-1} ascorbic acid. The solution was heated at 75°C and two samples were taken after one hour 30 minutes (degraded 1) and two hours 15 minutes (degraded 2).

3.5.5. Alginate solution with added calcium

Solution of 5% Manucol LB was prepared and centrifuged at 17000g for one hour. $CaCl_2$ 0.1% (w/w) was prepared in water. The two solutions were mixed in various proportions under rapid stirring. Non gelled mixtures were kept for casting. Three films were cast with added calcium content of 0.5, 2 and 8% (alginate basis).

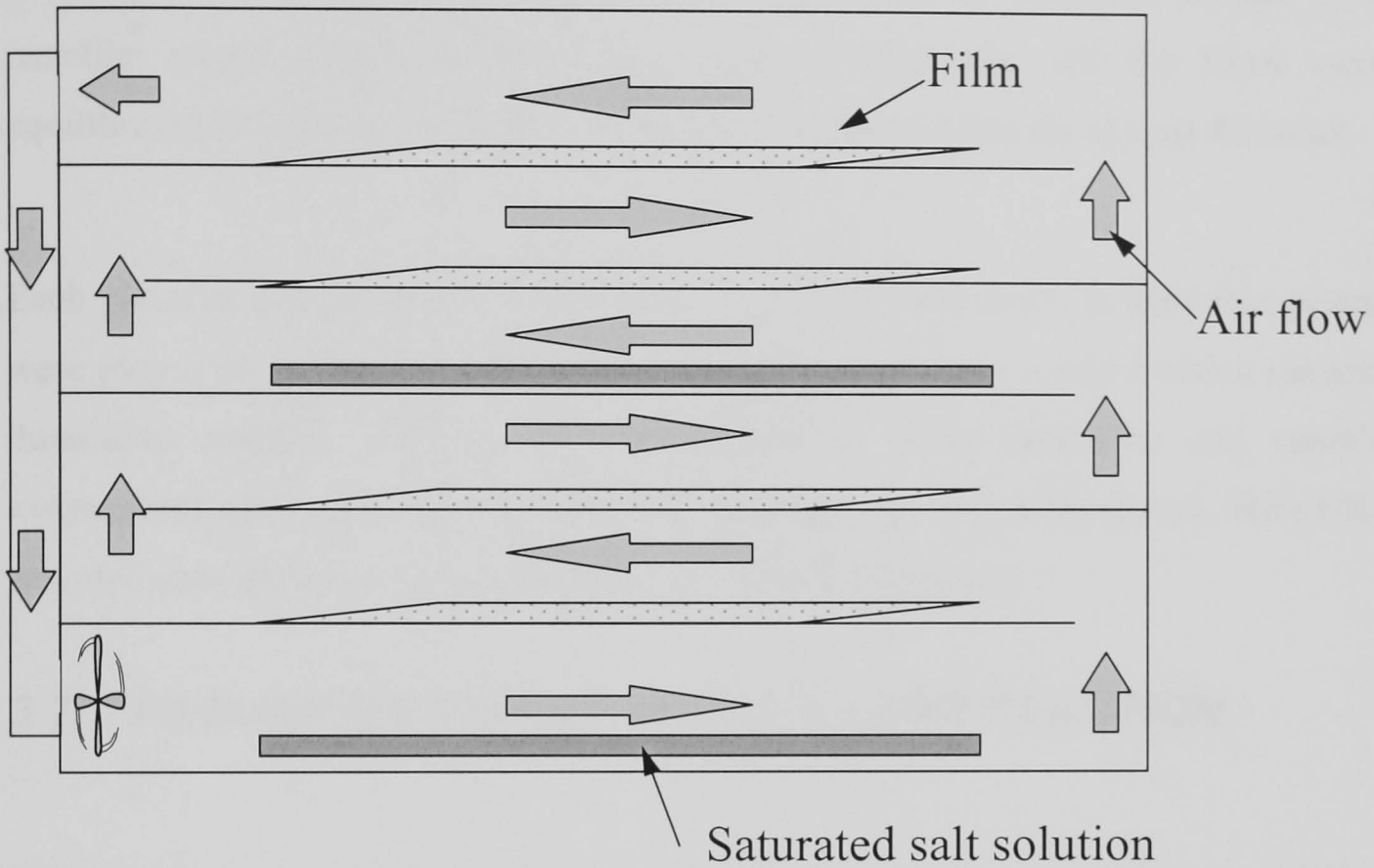
3.6. FILM PREPARATION

3.6.1. Drying

The drying method consisted of using a controlled relative humidity box. A special cabinet has been made for this purpose (Figure 3-1). It consists of a sealed box with circulating air. The airflow between shelves is controlled and the films are placed onto the shelves. The flow passes alternatively above the films and some saturated salt solutions. Any saturated salt solution could be used in order to reach a constant relative humidity. Typically films are dried at a relative humidity of 44% at room

temperature (over K_2CO_3 saturated solution (Nyquist 1983)) (unless specified otherwise).

Figure 3-1: Drying box.



3.6.2. Films for mechanical property measurements

Known volumes of solution were poured into ABS (Acrylobutadiene Styrene copolymer) trays (40x30 cm) so that the film thickness would be of about 100μm (thickness of standard gelatin capsule film). The films were then dried, carefully peeled off and cut into disc of about 46mm in diameter using scissors. The thickness of each disc was measured using a micrometer (Mitutoyo, 1μm resolution). The average over 6 measurements was used. Samples with very large thickness variations were discarded (>10%). Only the films whose average thickness was between 50 and 160μm were used. Samples were stored at a relative humidity of 44% for at least 48 hours before measurement.

3.6.3. Films for sensory analysis

The same casting technique as in 3.6.2 was used. The films were cut in rectangles of 30*10 mm. For each film, the thickness was measured and samples outside the range 70-140 μm were discarded. The samples were sorted by thickness so that each panellist would experience the same range of thickness. All the films were equilibrated at a relative humidity of 44% prior to the analysis for at least 48 hours.

Each panellist had to assess 9 films per session. For each film, at least five pieces were placed in a sealed jam pot (plastic, 30ml). Each pot was marked with a random three-digit number. Films used in both phases (term definition and sample assessment) were given different random numbers. Melinex S (Polyester, HiFi UK) samples were also provided as reference for the flexibility test.

3.7. FILM CROSSLINKING VIA MAILLARD REACTION

Films of caseinate containing various amount of additive were dried at a relative humidity of 44%. The films were then heated at 60°C for 2 hours at a relative humidity of 79% above a KCl saturated solution (Kato et al. 1992). The films were stored in the standard conditions.

3.8. FILM STORAGE

All the films produced were stored at room temperature under controlled relative humidity of 44% in a dark place. Both film surfaces were in contact only with a paper support.

3.9. MOISTURE CONTENT AND SORPTION ISOTHERM MEASUREMENTS

The moisture contents of the films were measured by drying in the oven at 105°C overnight and weighing in the wet and dry states. Three replicates were performed each time and the mean and confidence level were calculated.

For sorption isotherms, different saturated salt solutions were used according to the literature (Nyquist 1983) (Table 3-7).

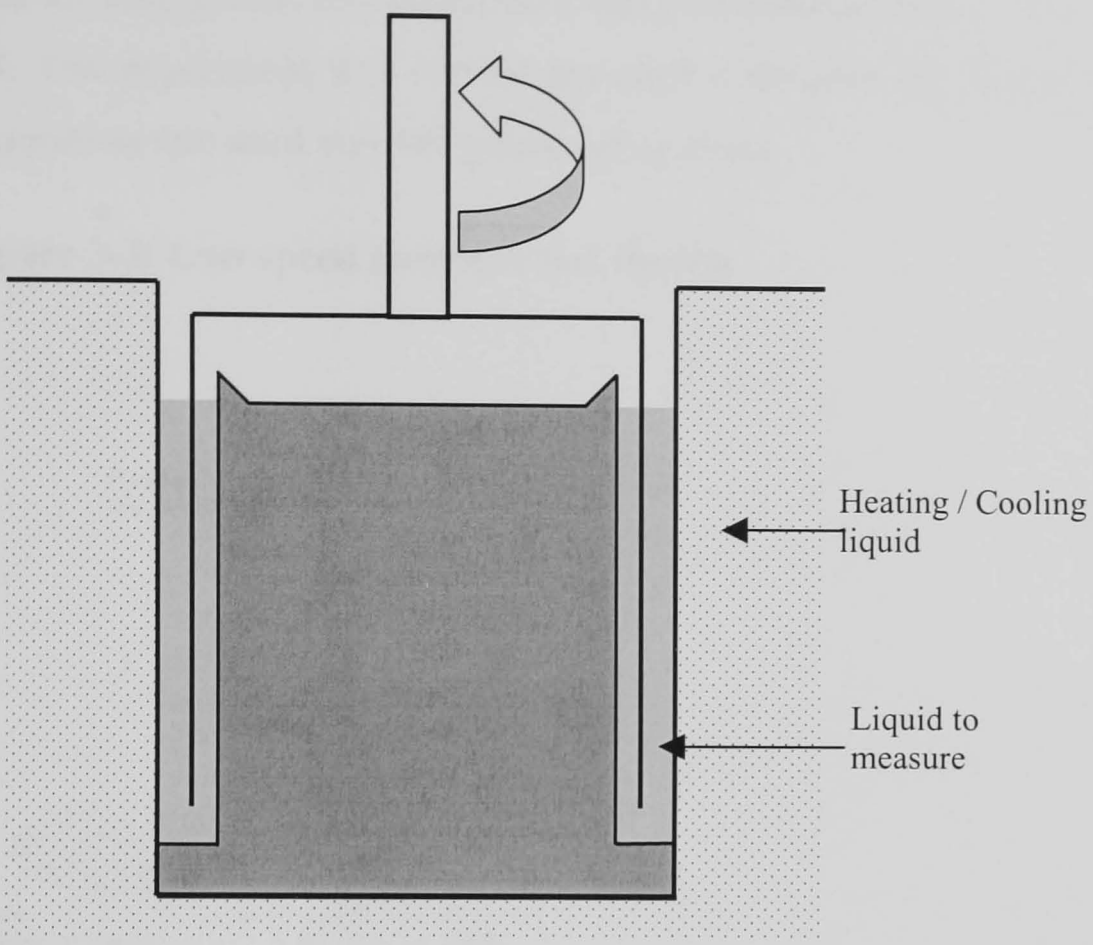
Table 3-7: Saturated salts used for relative humidity control

Salt	Relative humidity (%)
CH_3CO_2K	22.5
K_2CO_3	44
NaBr	59.5
NaCl	75.5
KCl	85.1

3.10. VISCOSITY MEASUREMENTS

A controlled stress rheometer (CS10, Bohlin) was used for measuring the viscosity of the solutions. Constant shear rate was obtained using back control from the attached computer. For most of the samples, the viscosity was measured using a double gap geometry (Figure 3-2), which was the most suitable for the low viscosity systems. The temperature was controlled with a water bath attached to the rheometer. The viscosity measurements have been performed at 50°C unless specified otherwise. Data were then exported and analysed with a PC.

Figure 3-2: Double gap geometry used for viscosity measurement.



3.11. MECHANICAL PROPERTIES OF FILMS: LOW SPEED PUNCTURE TEST

3.11.1. Introduction

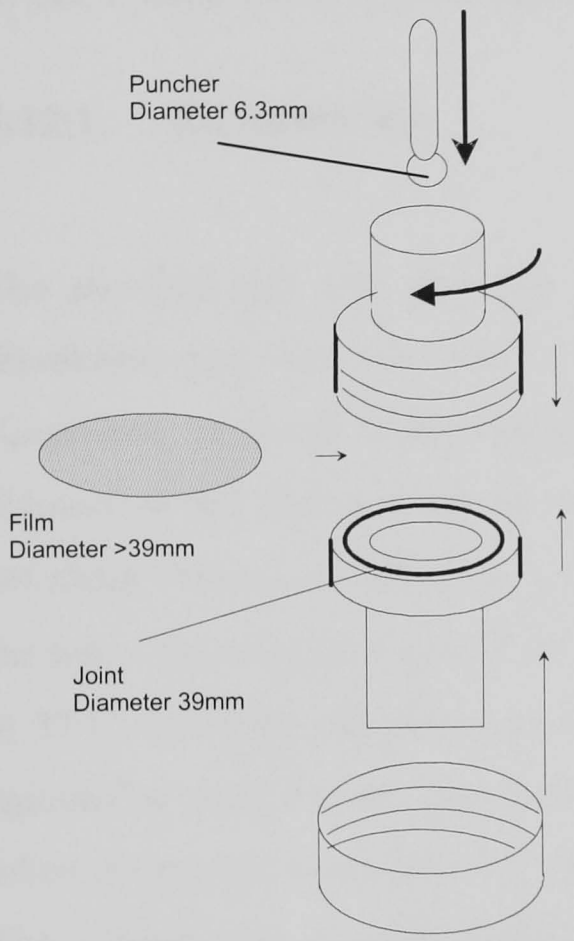
Preliminary experiments showed that tensile tests on rectangular sample give very erratic results for the distance at break. Cutting samples of very different consistency (Gelatin, HPMC, starch, casein…) into dumbbell shapes gave rise to many flaws and even a cutting tool especially made for the purpose could not be used efficiently. Therefore a low speed puncture test was used and is described here.

3.11.2. Method

Samples were cut into circles of more than 39 mm in diameter (3.6.2) and inserted in the homemade device shown in Figure 3-3. The joint diameter was 39 mm and the ball used at the end of the puncher was 6.3 mm of diameter. Lining up of the puncher

and holder was assured using a special homemade base. The test was operated in compression mode with a Texture Analyser TA-HD (Stable Micro System) fitted with a 250kg probe. The experiment was performed at 1mm/s with a trigger force of 1N. The experiment was carried out until a distance of 10mm was reached. The acquisition rate used was 400 points per seconds.

Figure 3-3: Low speed puncture test device.



3.11.3. Analysis

For each measurement about 20 samples were used. They were then ranked by distance at break. Average and confidence interval was measured on the half with the higher distance at break.

It will be shown that thickness affects the gradient G and the force at break F_b linearly (4.3). In order to account for thickness variations between samples, G and F_b were submitted to thickness correction as follows: $G = G/\theta$ and $F_b = F_b/\theta$ where θ

is the average thickness in mm. G was therefore expressed in N/mm^2 and F_b in N/mm.

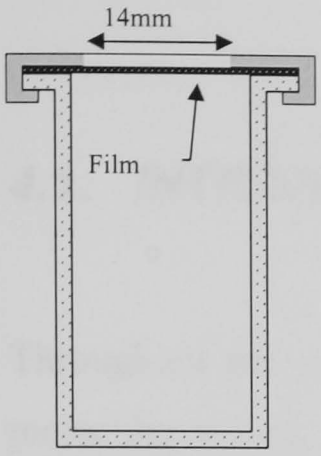
The punctured sample gave various fracture pattern. The number of lines radiating from the fracture was the only parameter measured. The number of lines longer than about 4 mm were counted on the half set defined above.

3.12. PIERCING SOLUBILITY TESTS

3.12.1. Standard test

The standard test was provided by Capsugel (Capsugel, Colmar, France). This dissolution test was shown to be correlated with the standard disintegration test (Capsugel, personal communications, 1998; Ph.Eur. 1998). It consists of a dissolution cell with a 14mm diameter hole (Figure 3-4). A film of known thickness (of about 100μm) was used. A circle of 18mm diameter film was cut and placed on the top of the vial (see Figure 3-4). The vial was immersed into about 900ml of water at 37°C. The film was placed at about 50mm below the surface and the water was agitated at about 50rpm. The time for the film to be pierced was noted as the time when the solvent filled the vial. Ten measurements were performed on each sample. The solvent was changed every ten samples. The piercing time normalised to 100μm, $T_{p,c}$ is given as $T_{p,c} = \left(100/d\right)^2 \cdot T_p$, where d is the thickness in micrometers and T_p is the measured piercing time (Capsugel data).

Figure 3-4: Dissolution vial.



3.12.2. Modified tests

In order to measure the solubility in acidic environment, gastric fluid (without pepsin, Sigma G8285) was also used as dissolution medium. This dissolution media consists of 0.08M HCl and 0.034M NaCl. Finally artificial gastric juice was also used and consisted of 3.2g of pepsin (Sigma, P7125, 600-1800u/mg) added to one litre of gastric fluid.

3.13. MICROSCOPY

Microscopy under polarised light was performed at room temperature using a Leitz Diaplan optical microscope. The films were rotated so that the maximum polarised effect was obtained. Photos were taken using a K1000 Pentax Camera fitted to the microscope.

CHAPTER 4. POLYMER CHARACTERISATION AND BACKGROUND STUDY

4.1. INTRODUCTION

Throughout the project, many polymeric materials were used. For some studies, molecular weight characterisations were necessary. The results are gathered in this chapter and will be used throughout the following chapters.

It was also interesting to study the mechanical properties of the films that are currently used for hard capsule production (gelatin and HPMC). This study provides a comparison tools for the analysis of the mechanical properties of other films.

4.2. VISCOSITY, INTRINSIC VISCOSITY AND MOLECULAR WEIGHT DETERMINATIONS

4.2.1. Introduction

The viscosities of the products of interest were determined only for comparison purposes and to get an idea of the average molecular weight of the polymers studied. Determining the molecular weight or molecular weight distribution of biopolymers can be very long and difficult. The intrinsic viscosity results originate either from Huggins and Kraemer plot or from single point measurements (AbdelAzim et al. 1998; Harding 1997). To obtain molecular weight from viscosimetry measurements requires the Mark-Houwink parameters. These were found in the literature for similar conditions and references are reported in the tables.

4.2.2. Results

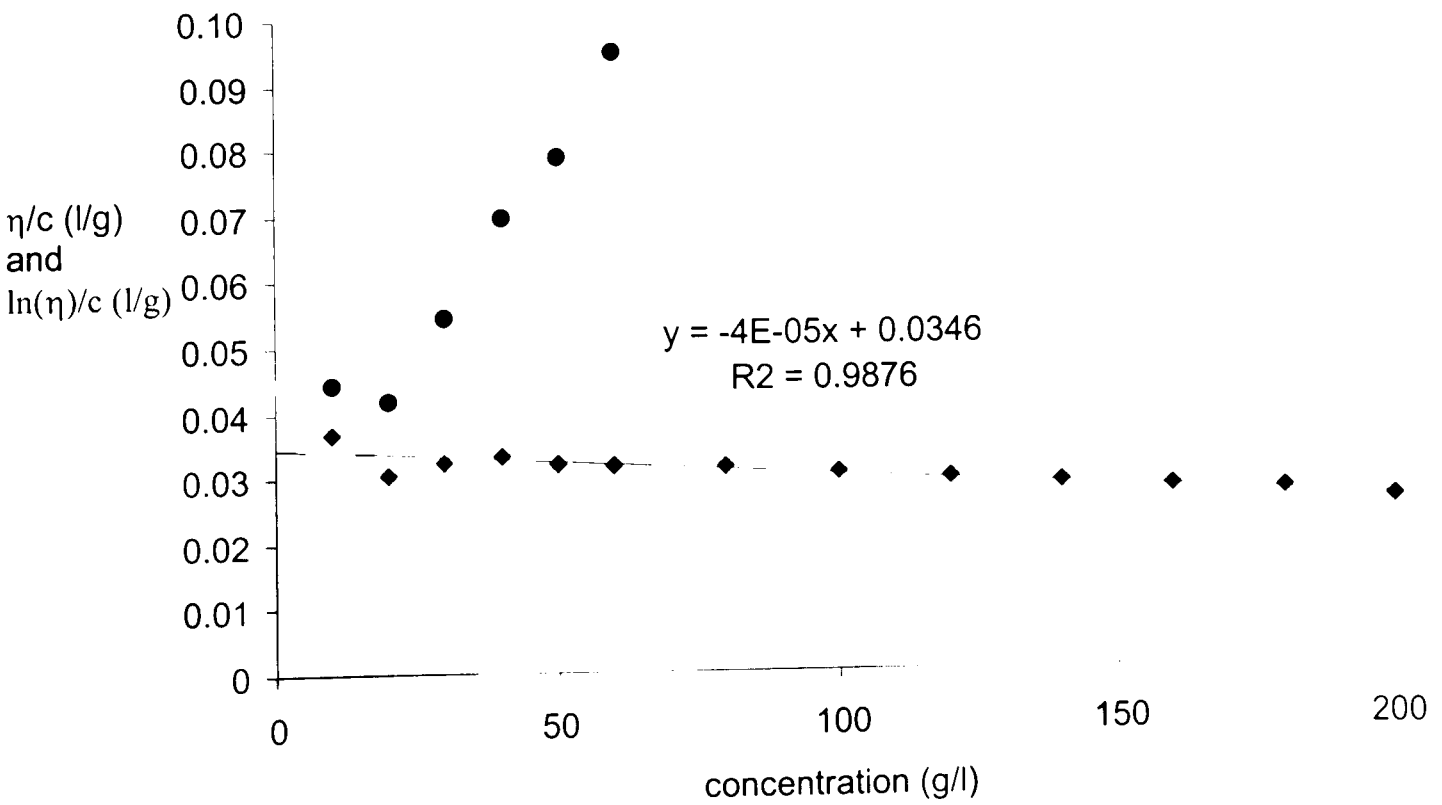
The results used during the studies on the effect of molecular weight on the properties of alginates film (7.6) are gathered in Table 4-1.

Table 4-1: Single points intrinsic viscosity and molecular weight determination for alginates, HPC and HPMC series at 25°C.

Product Type	Source of Mark-Houwink coefficient	Product Name	Intrinsic viscosity(ml/g)	Molecular weight (kDa)
Alginate	Johnson (1997)	Degraded 2	95	48
		Degraded 1	125	62
		Manucol LB	180	90
		Manucol DH	840	420
		Manugel DMB	2422	1211
HPMC	Vazquez (1995)	HPMC 606	114	10
		Methocel E15	204	20
		Methocel E50	511	56
		Methocel E4M	1714	222
HPC	Deduced from Sigma data	Klucel EFF	113	67
		Klucel LF	236	116
		Klucel MF	4467	1037

An example of the Huggins and Kraemer plot is given in Figure 4-1. Data from the Kraemer extrapolation are given thereafter. The results of intrinsic viscosity of charged polymer are however arguable due to the high concentrations used here.

Figure 4-1: Huggins (circles) and Kraemer (diamond) plots for gelatin B200 measured at 50°C.

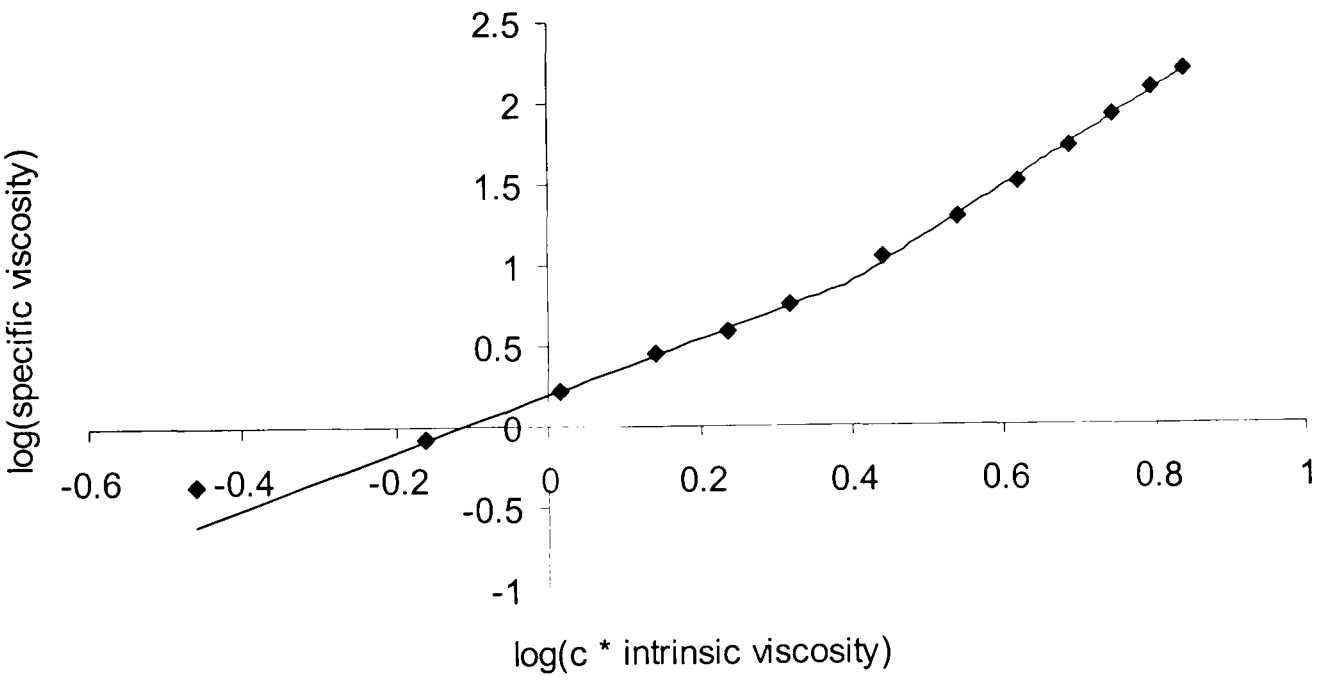


The plot used for determining the coil overlap concentration is shown in Figure 4-2. The fitting is performed with two straight lines that meet at x=k=log(c*.intrinsic viscosity). The fitting was performed using the following equation:

$$y = \left(a + a_2 . \frac{abs(x-k)}{x-k}\right).x + b + a_2 . k . \left(1 - \frac{abs(x-k)}{x-k}\right)$$

where $(a+a_2)$ and $(a-a_2)$ are the slopes below and above the k value, b is the intercept of left straight line and k is defined as above. For the gelatin sample, k=0.396 and c^*=72g/l.

Figure 4-2: Coil overlap measurement of gelatin B200.



The results obtained for various polymers used are given in Table 4-2. For some samples, the coil overlap concentration was not determined because no clear change in slope was observed but only a smooth curvature.

Table 4-2: Viscosity and intrinsic viscosity of various polymers studied obtained via Huggins and Kraemer plots. Data were obtained at 50°C.

	Viscosity at 2% at a shear rate of 20s-1(mPa.s)	Intrinsic viscosity (ml/g)	C* (g/l)	Slope below and above break point	
Glucidex 2	0.8	9.7	66.1	0.73	2.44
Caseinate	1.0	15.0	NM	NM	NM
Gelatin B200	1.3	34.6	72.3	1.74	2.97
HPMC 606 [a]	6.0	93.0	35.1	1.71	2.99
HPC EFF [a]	6.7	100.0	35.4	1.95	3.07
Manucol LB	7.6	123.0[b]	21.8	0.95	3.03
Manucol LB in NaCl 1%	6.3	131.0	22.2	1.59	2.86
Textra	7.6	158.0	5.6	0.94	2.09
PGA2	11.6	165.0	NM	NM	NM
Kelcoloid O	68.0	397.0	2.5	0.67	1.64

[a] Results at 25°C.
[b] Different from Table 4-1 due to temperature and measurement method.
NM Not measurable by method used

4.2.3. Discussion

The results of molecular weight presented here are very rough approximations. Nevertheless, they allow estimation of the correct order of magnitude and are useful for comparing samples.

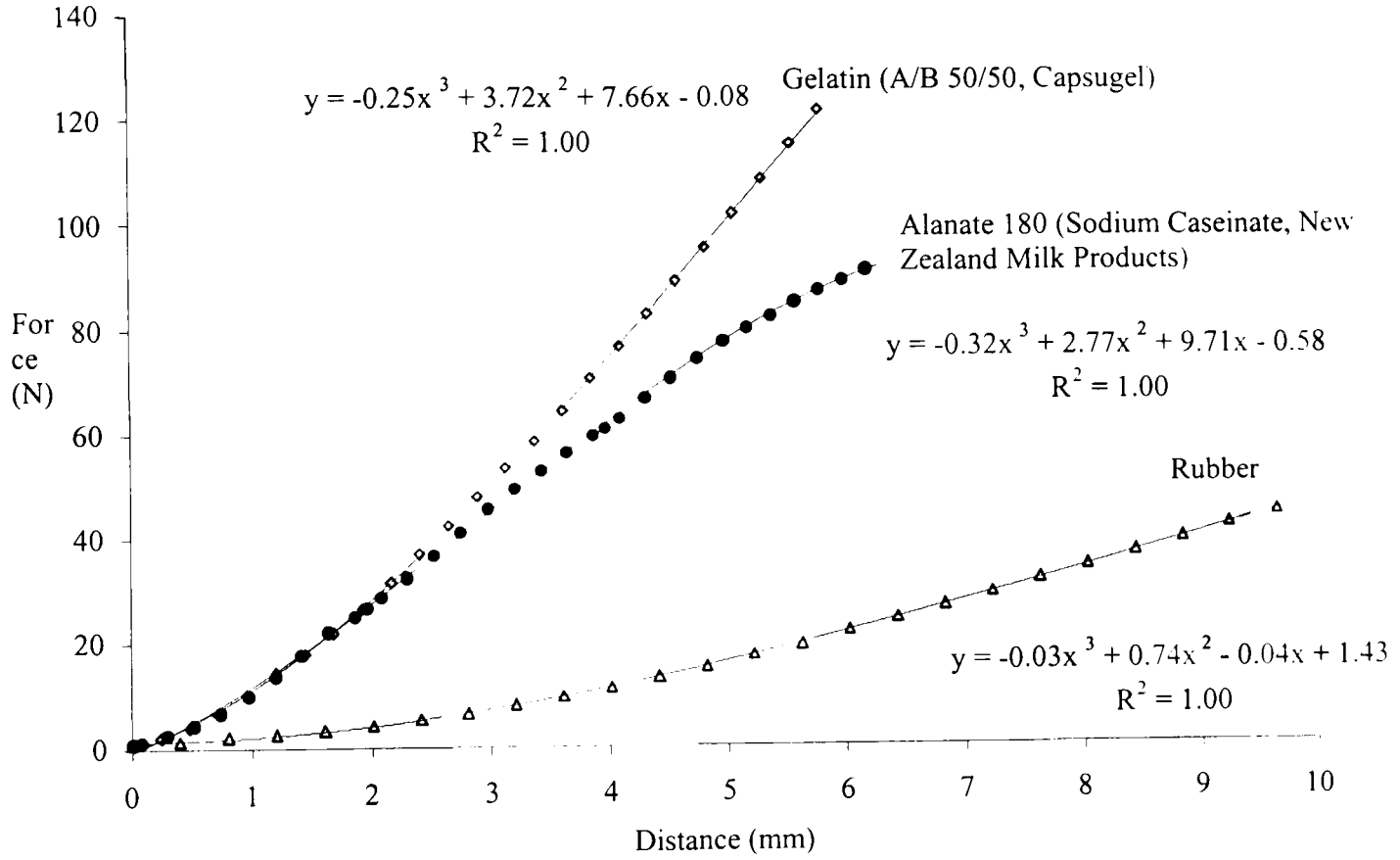
The viscosities and intrinsic viscosity data are useful for comparing samples and their properties in a potential capsule production use. They will be used in the selection of products (6.6).

4.3. EFFECT OF THICKNESS ON PUNCTURE TESTS

The device used defines all the geometry parameters of the samples measured by puncture. The only variable is the thickness of the film. We investigate here how the thickness affects the results obtained by puncture tests.

Similar methods were used previously (Cuq et al. 1996), (Georget, Parker and Smith 1995). Fundamental parameters could only be calculated for very low deformation level (Georget et al. 1995). The curve obtained here could easily be fitted with a third degree polynomial function up to the fracture point. This was true for all films studied (see Figure 4-3).

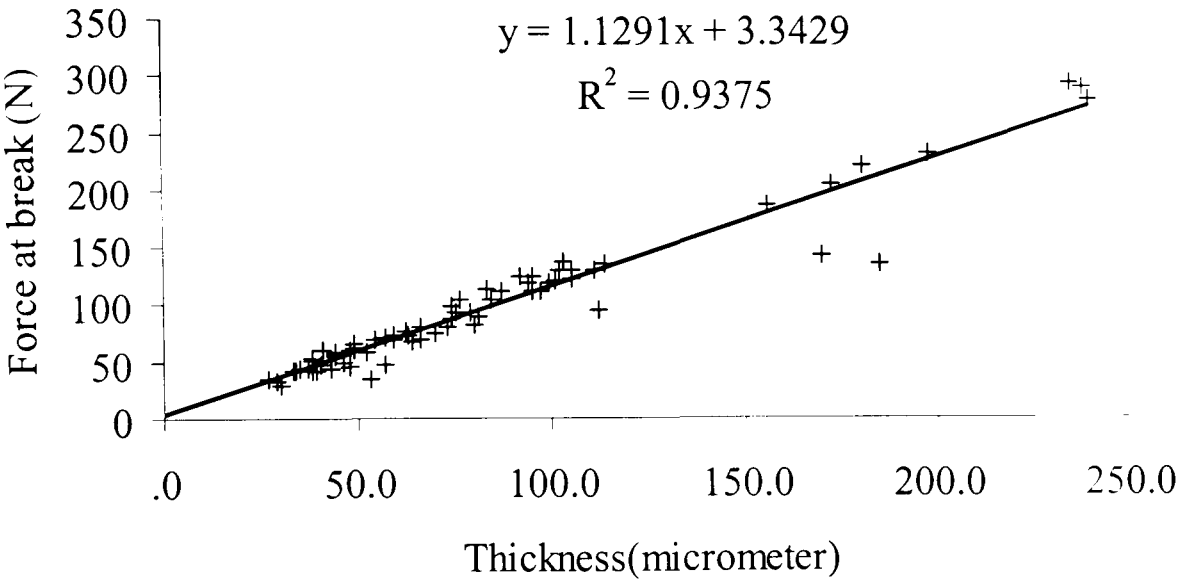
Figure 4-3: Example of low speed puncture tests raw data. (Fitting performed on 500 points minimum. 30 experimental points displayed for clarity).

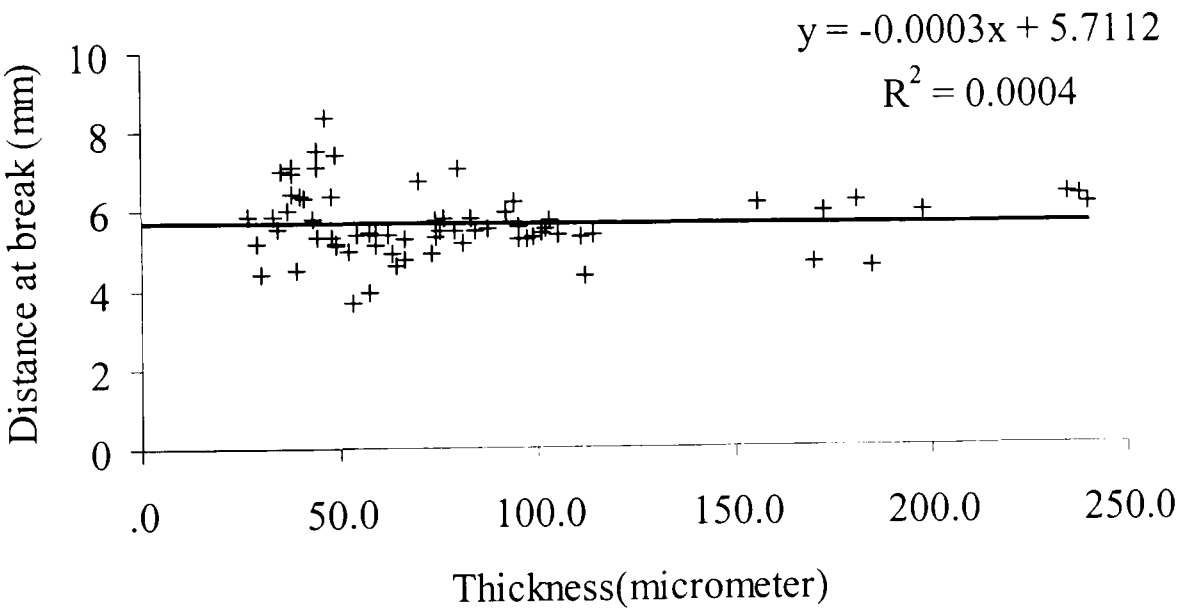


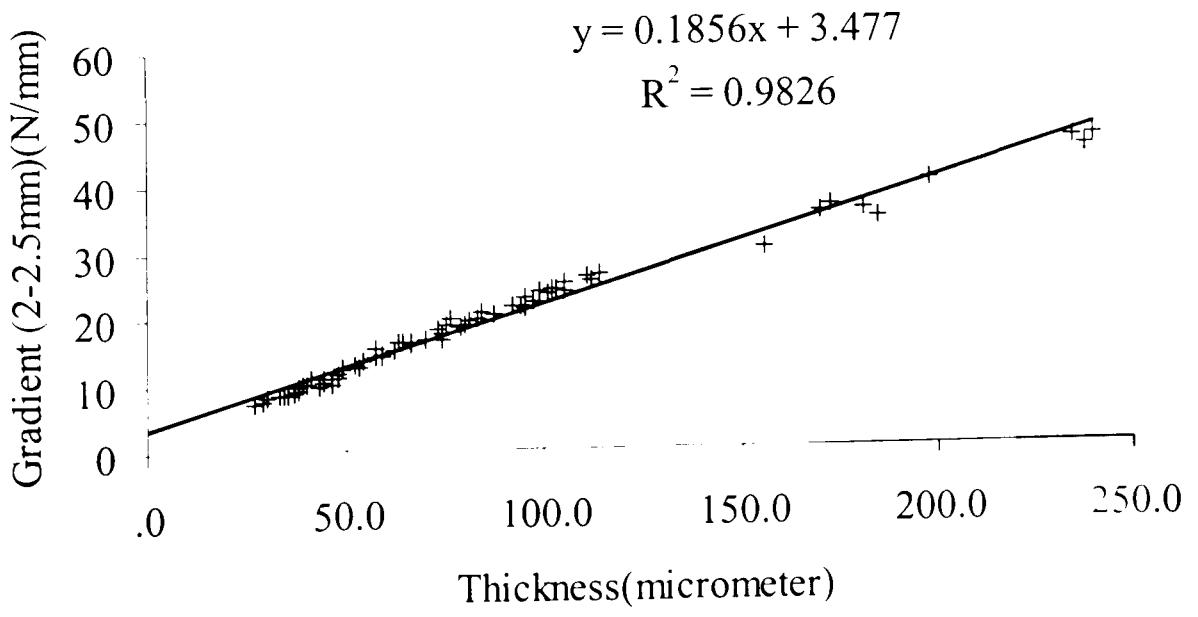
Elongation and puncture strengths were calculated for large deformation puncture tests (Bodmeier and Paeratakul 1993). However, such results are not directly comparable with tensile parameters. Empirical parameters were chosen here. The distance at break d_b, the gradient between 2 and 2.5mm G (N/mm) and the force at break F_b (N) were calculated. Since the thickness of the film was small compared to the disc size, the stress applied to the sample corresponded to a tensile test rather than a bend test. Therefore, it was expected that G and F_b would be linearly dependent on the thickness. Previous work showed that stress at break was thickness dependent (Cuq et al. 1996), (Bodmeier and Paeratakul 1993). However, the distance

at break is independent of the thickness. This was proved on gelatin samples over a wide range of thicknesses (Figure 4-4).

Figure 4-4: Effect of thickness on the mechanical properties measured by a low speed puncture test for gelatin films.







Therefore, in order to account for thickness variations between samples, G and F_b were submitted to thickness correction as follow $G = G/\theta$ and $F_b = F_b/\theta$ where θ is the average thickness in mm. G was therefore expressed in N/mm^2 and F_b in N/mm.

4.4. MECHANICAL PROPERTIES OF GELATIN AND HPMC FILMS

4.4.1. Introduction

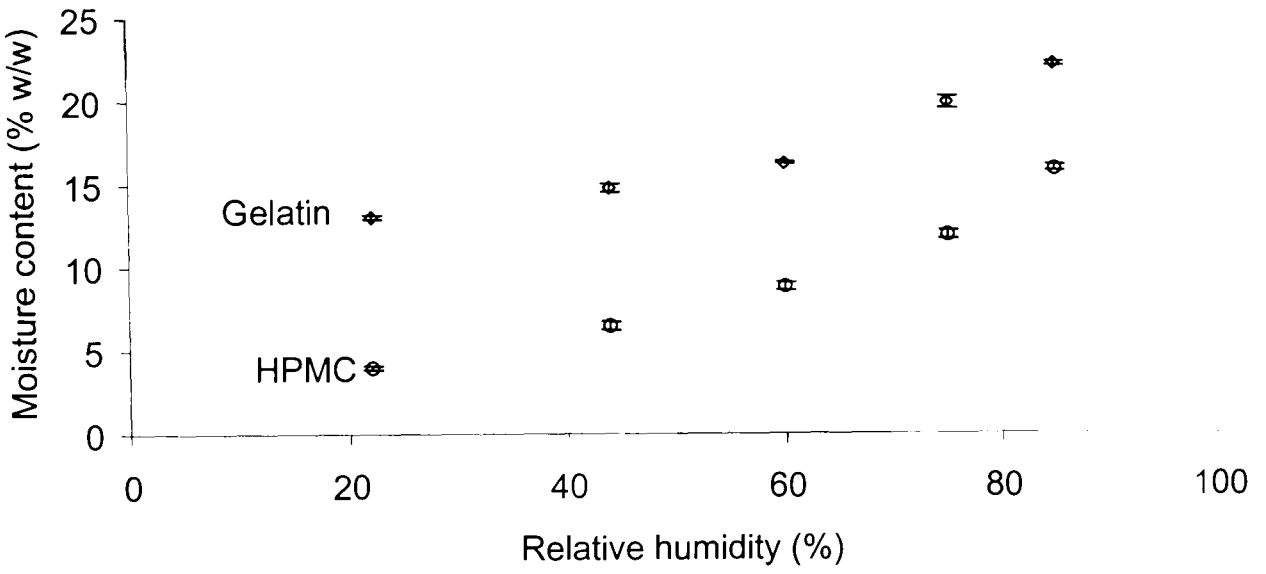
Gelatin and HPMC films are currently used to produce commercial capsules. They can therefore provide standards by which other materials can be compared. The effects of relative humidity and moisture content on the mechanical properties of films made from these materials are studied in this section. Orientation and fracture properties are also considered.

4.4.2. Effect of relative humidity and moisture content on puncture data for gelatin and HPMC films

4.4.2.1. Sorption isotherms

The sorption isotherms of gelatin and HPMC films are given in Figure 4-5. The moisture content of gelatin is higher for the whole relative humidity range studied.

Figure 4-5: Sorption isotherm of gelatin and HPMC films at 25°C.



4.4.2.2. Effect of relative humidity on mechanical properties

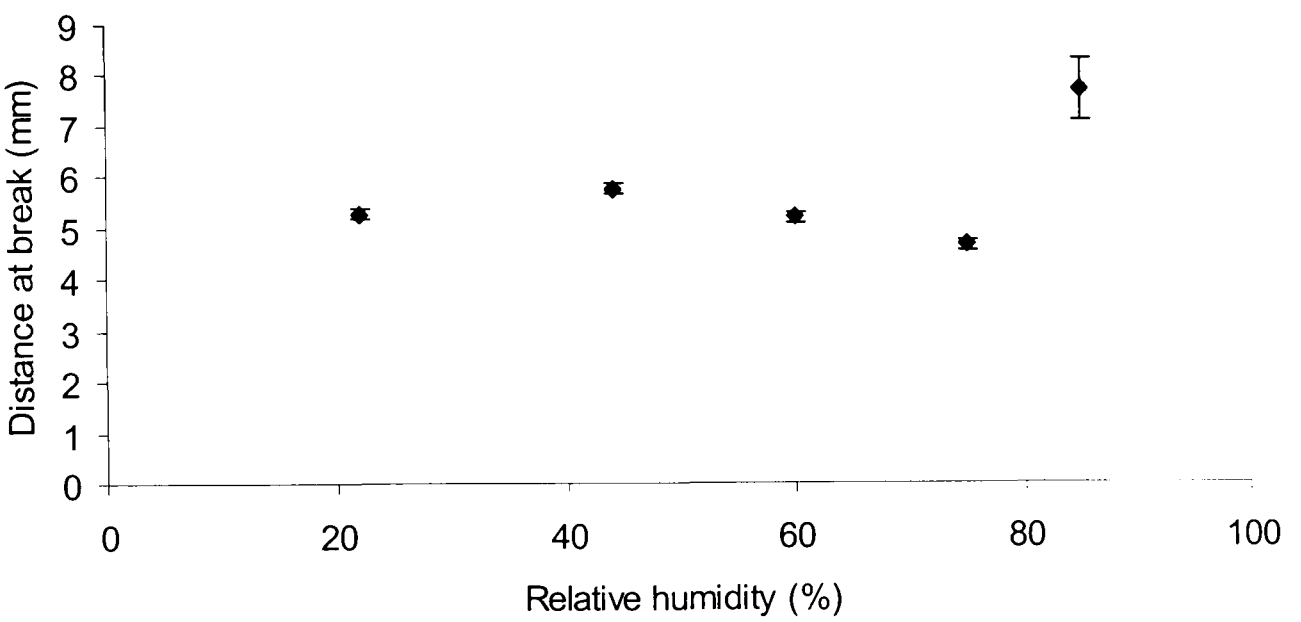
Capsules can be stored in a range of environments. Storage at high relative humidities will affect the moisture content of the samples. It is therefore relevant to store the samples at different relative humidities and determine the changes in behaviour of the films. This will also show the limits of relative humidity for proper storage of gelatin or HPMC films.

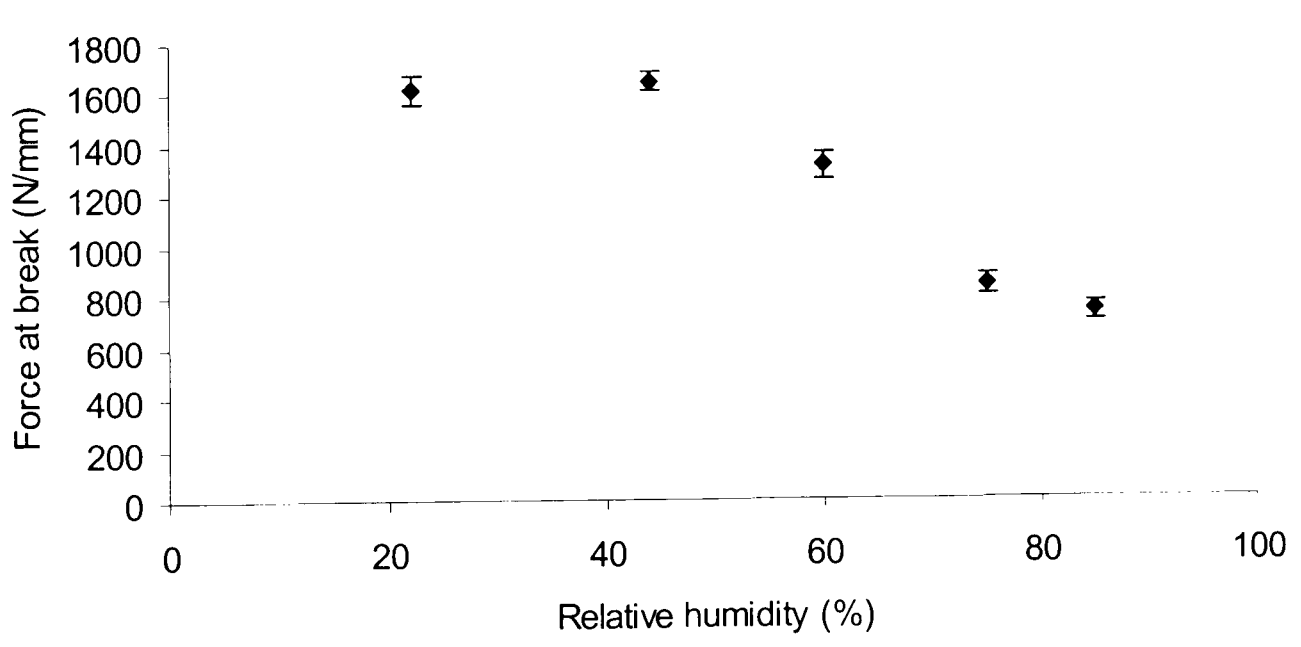
4.4.2.2.1. Gelatin

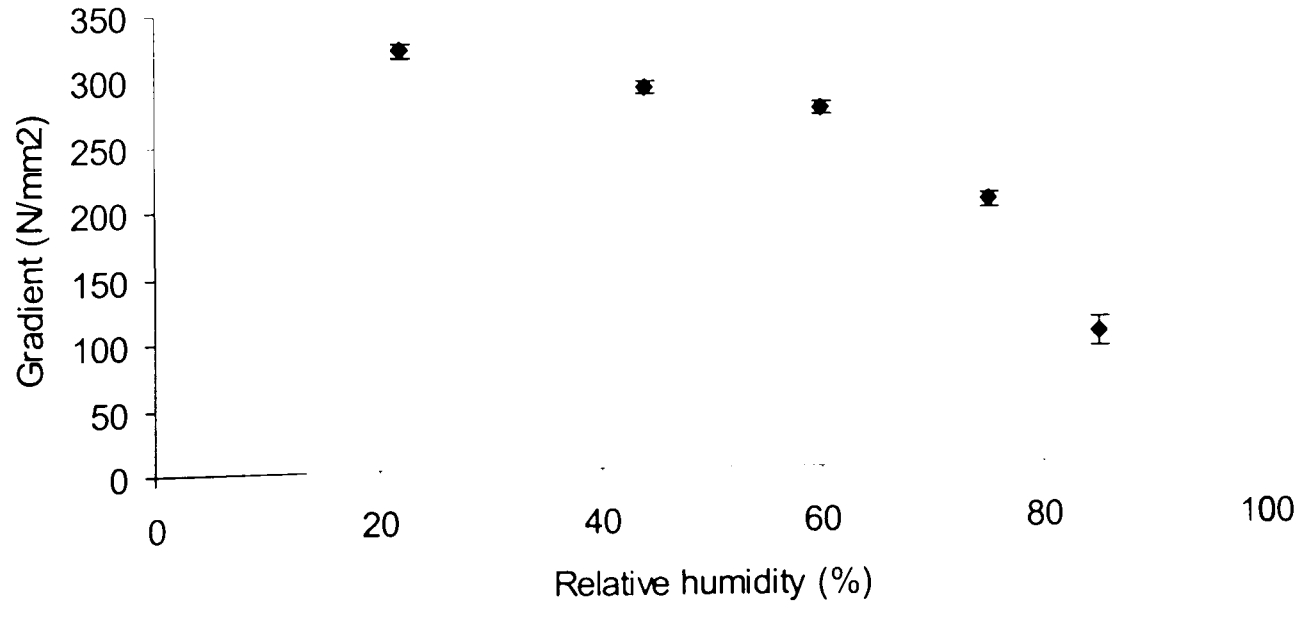
The effects of relative humidity on gelatin film properties are shown in Figure 4-6. The distance at break shows a large increase at very high relative humidity. A local maximum is observed at about 44% of relative humidity. This was also observed in tensile measurements of films made from gelatin of various origins (Melia 1983).

Above a relative humidity of 60%, the gradient is decreasing sharply, showing the strong effect of the increasing moisture content. This forbids the use of gelatin films in 'wet' environments. The force at break combined the two effects discussed before and is stable until a relative humidity of 44-60% is attained.

Figure 4-6: Effect of relative humidity on mechanical properties of gelatin films. Each point represent the average of the 10 values with the highest distance at break from a set of 20 discs cut from one film. The 95% confidence interval is also shown for the 10 values.







4.4.2.2.2. HPMC

For HPMC, increasing the relative humidity produces decreases in both gradient and distance at break (Figure 4-7). This differs from the results of other authors (RemunanLopez and Bodmeier 1996) where the elongation increased at high relative humidities. However, the HPMC grade used in that study was Methocel E50 (Dow), which has a molecular weight much higher than HPMC 606 (Table 4-1).

Unlike gelatin, no decrease in the distance at break is observed at low relative humidity. This might explain the less brittle behaviour of HPMC films compared to gelatin when used at low relative humidity (Capsugel personal communications).

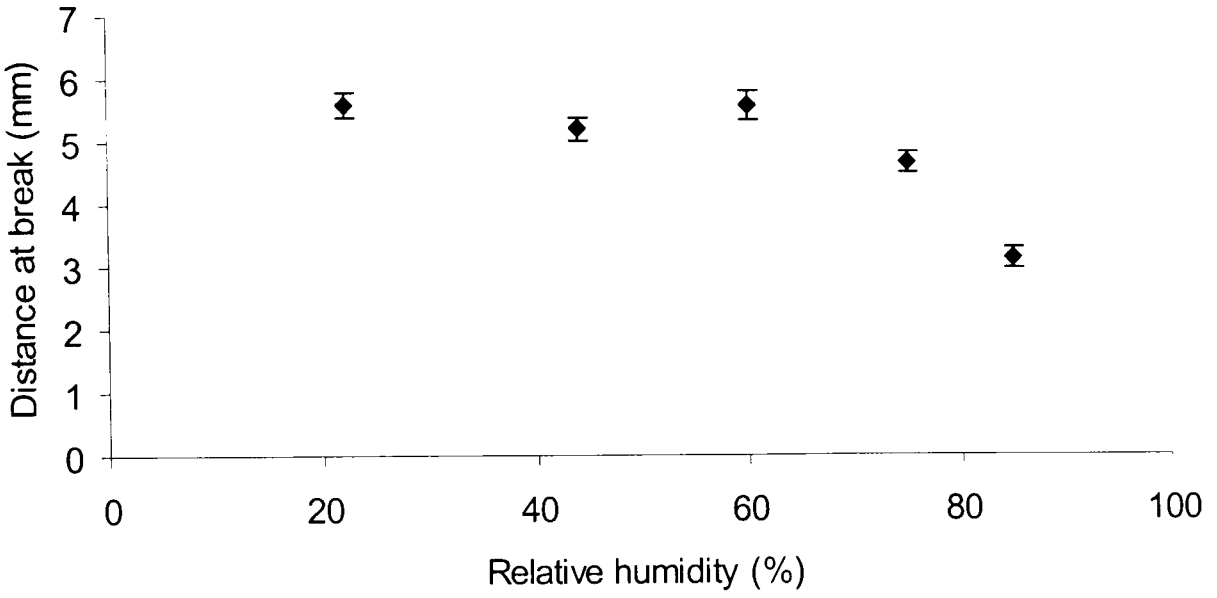
4.4.2.3. Effect of moisture content on mechanical properties

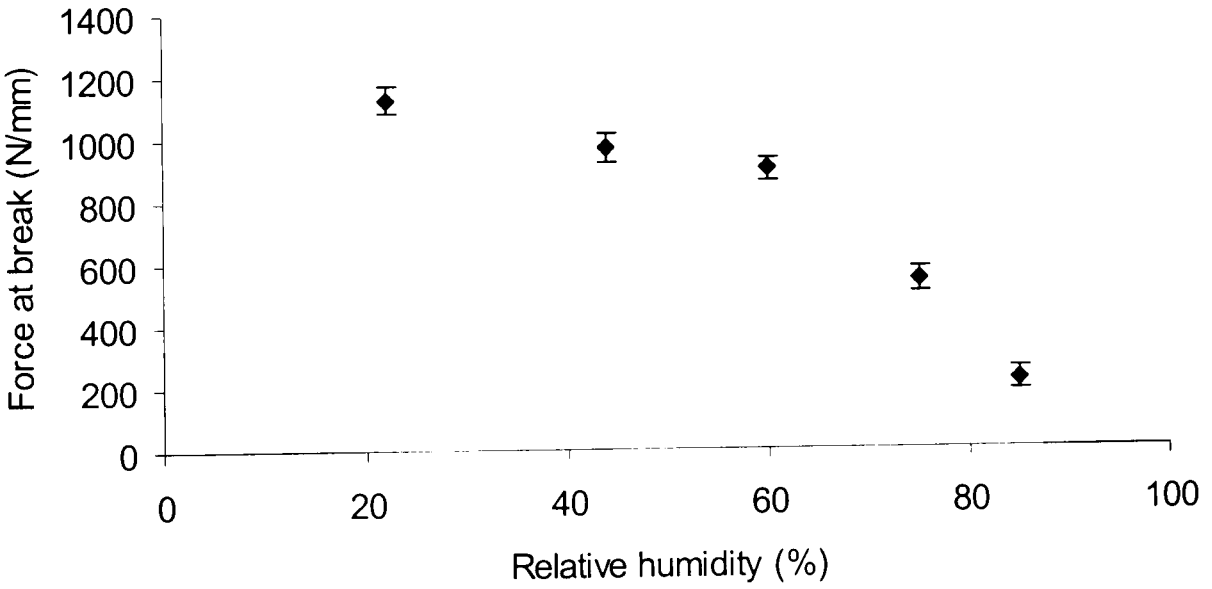
Within this section, the changes in the mechanical properties of films at different moisture contents are reported.

4.4.2.3.1. Gelatin

Gelatin films present a sharp increase of the distance at break when used above a moisture content of about 20% (Figure 4-8). The decrease observed for the gradient is highly correlated to the moisture content increase. Up to a moisture content of about 20%, gradient and moisture content are linearly dependent (Figure 4-8). The same properties were observed by Melia (1983) on various gelatin films. Two parameters are used to compare the effect of moisture content on gradient of various films. We can define the zero moisture content gradient (G_0 or intercept is 543 N/mm^2 for gelatin) and the relative effect of the moisture content on the gradient (G_1 or slope divided by G_0 =16.43/543 *100=3.10%). The zero moisture content gradient is the theoretical gradient in absence of any water. This is not attainable practically but allows comparison of the intrinsic properties of the samples. The relative effect of moisture content on the gradient is an indication of how much plasticising effect the water has on the gradient.

Figure 4-7: Effect of relative humidity on mechanical properties of HPMC films (average and 95% confidence interval as defined in Figure 4-6).





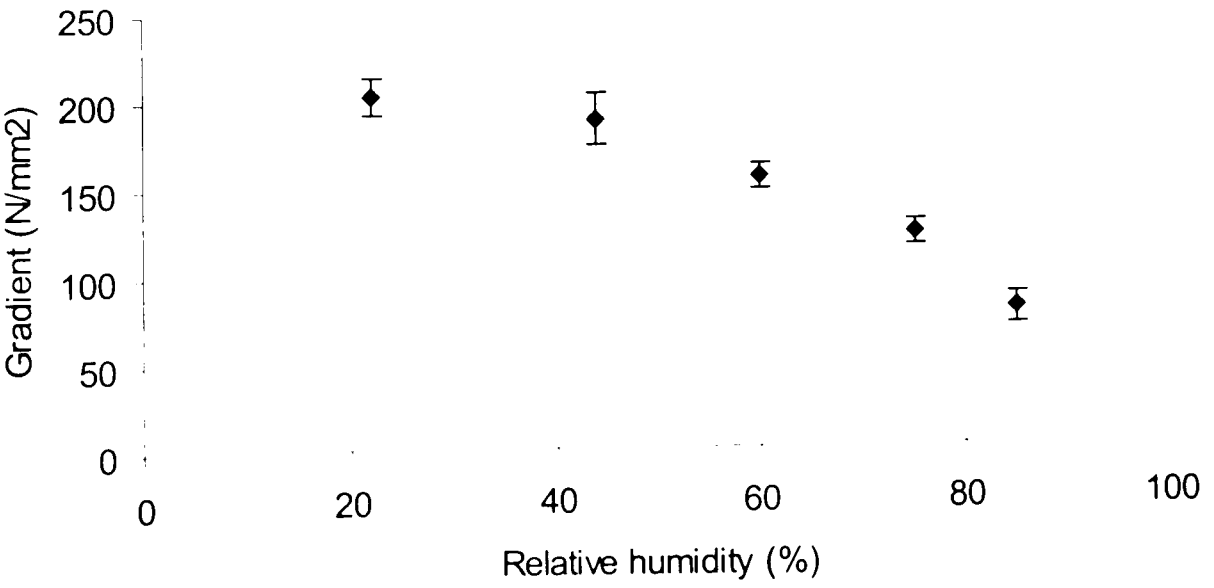
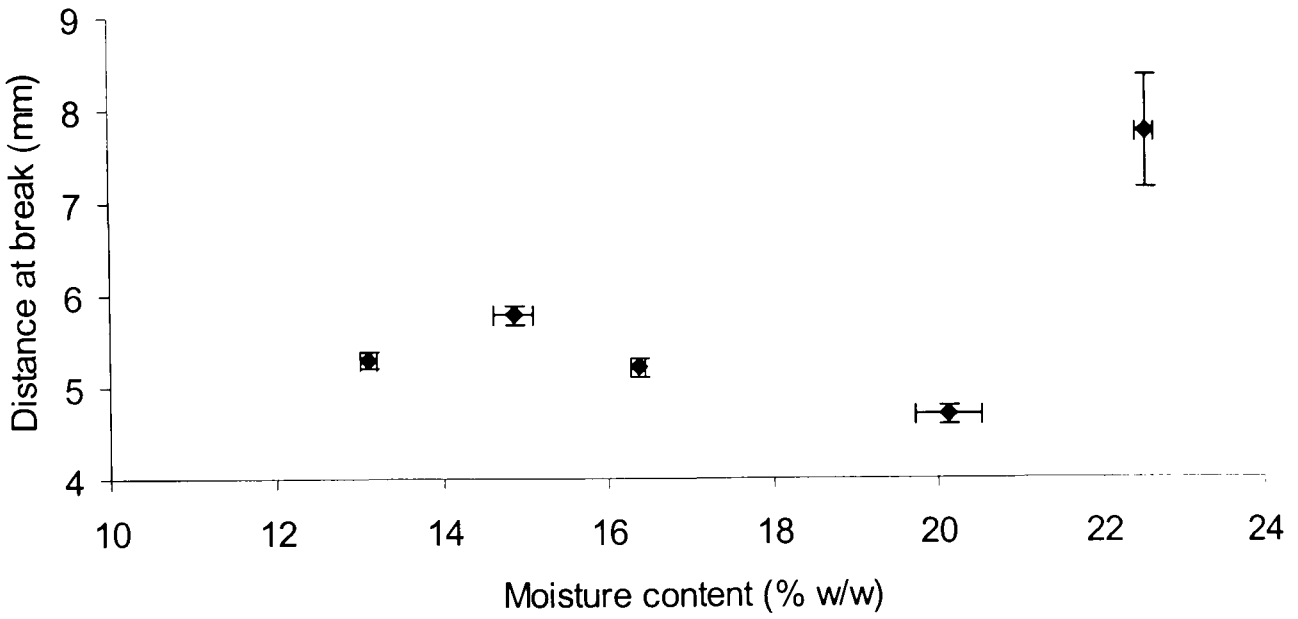
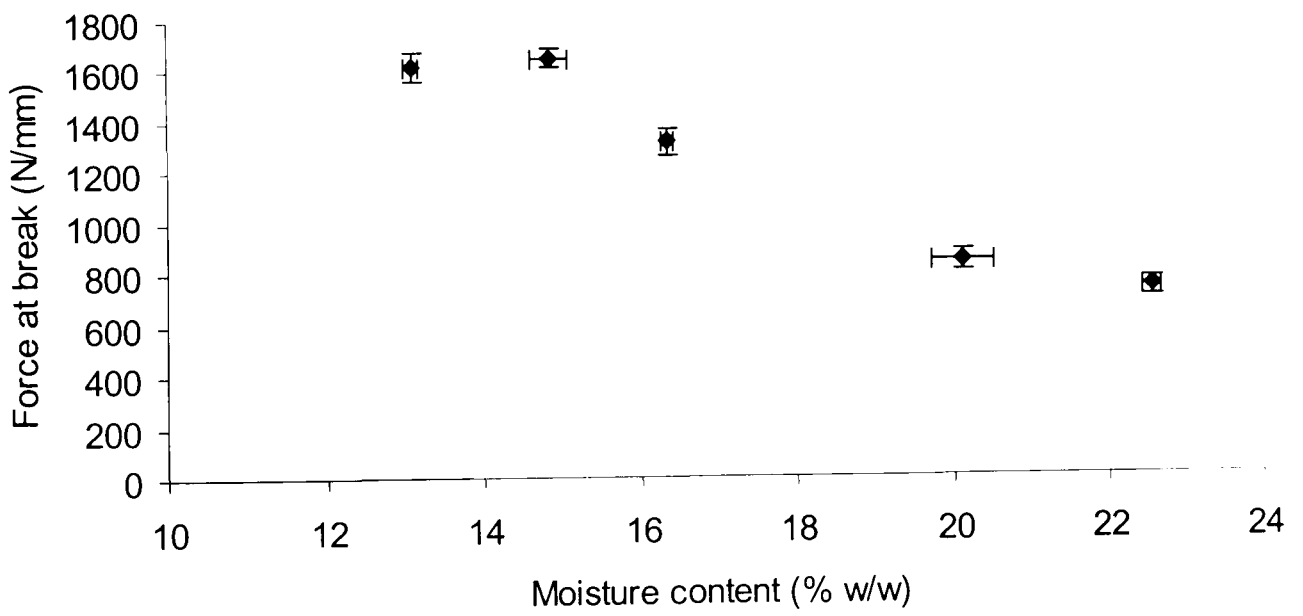


Figure 4-8: Effect of moisture content on mechanical properties of gelatin films (average and 95% confidence interval as defined in Figure 4-6).





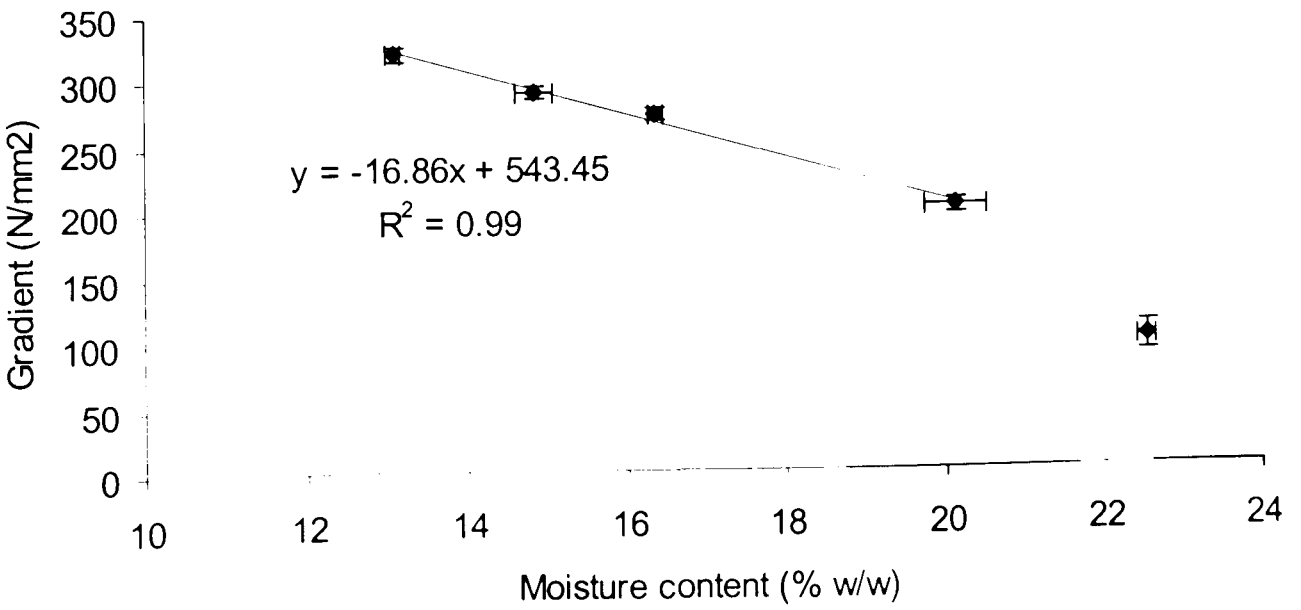
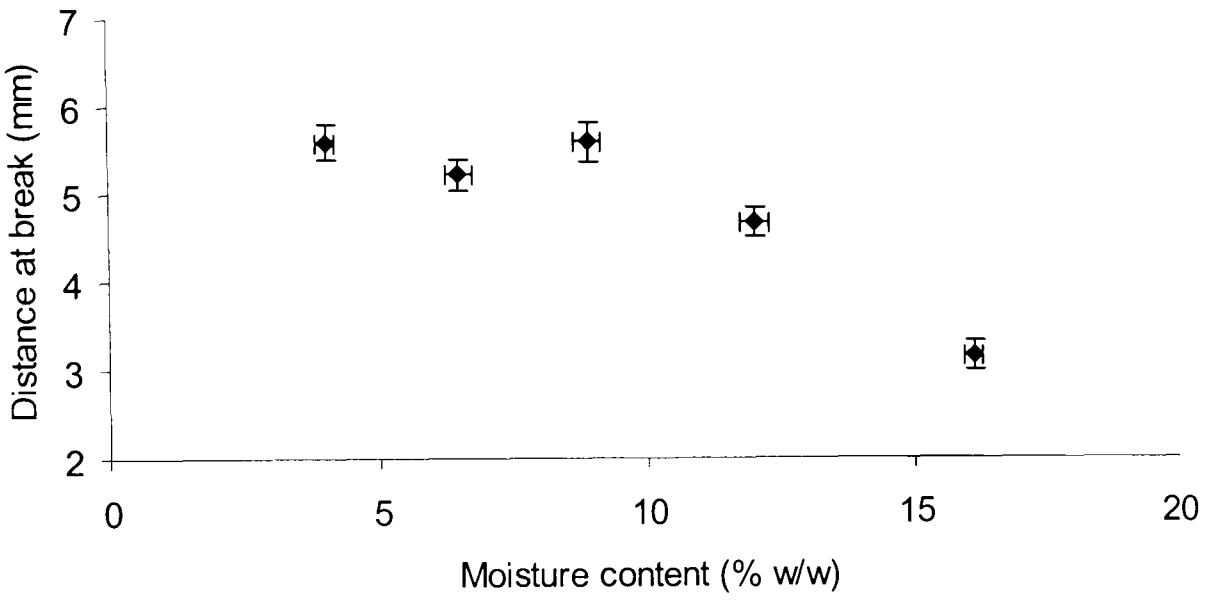
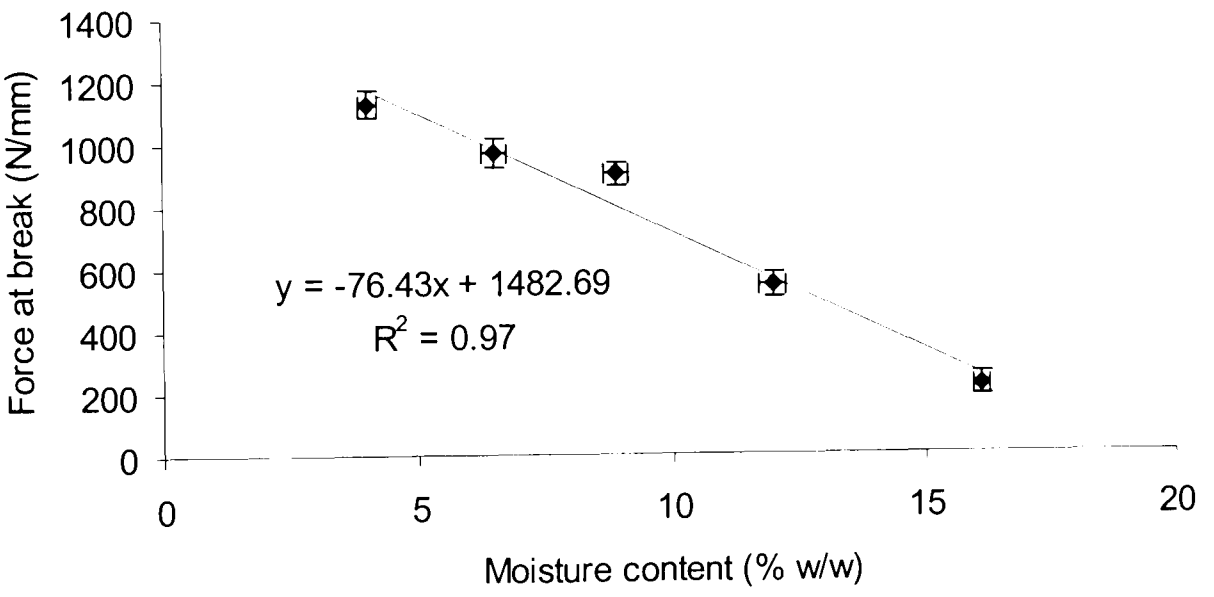
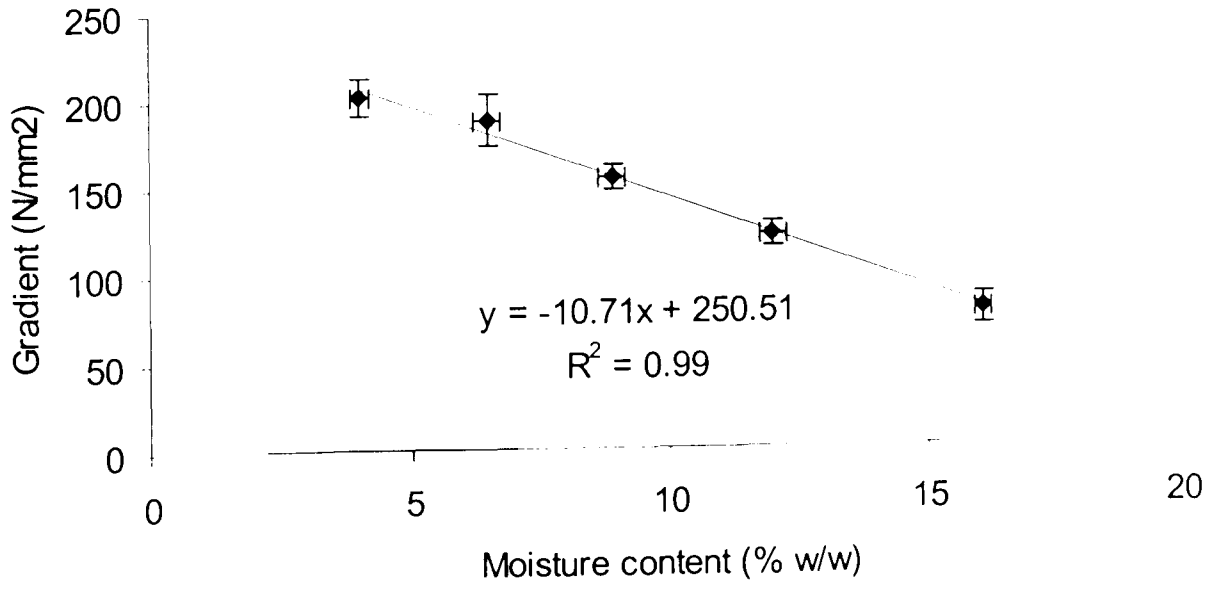


Figure 4-9: Effect of moisture content on mechanical properties of HPMC films (average and 95% confidence interval as defined in Figure 4-6).







4.4.2.3.2. HPMC

The results for HPMC are presented in Figure 4-9. The distance at break decreases as the moisture content exceeds 10%. A linear correlation was again observed between the gradient and moisture content. The parameters G_0 and G_1 were 251 N/mm^2 and 4.27% for the HPMC samples.

4.4.2.4. Effect of moisture content on fracture

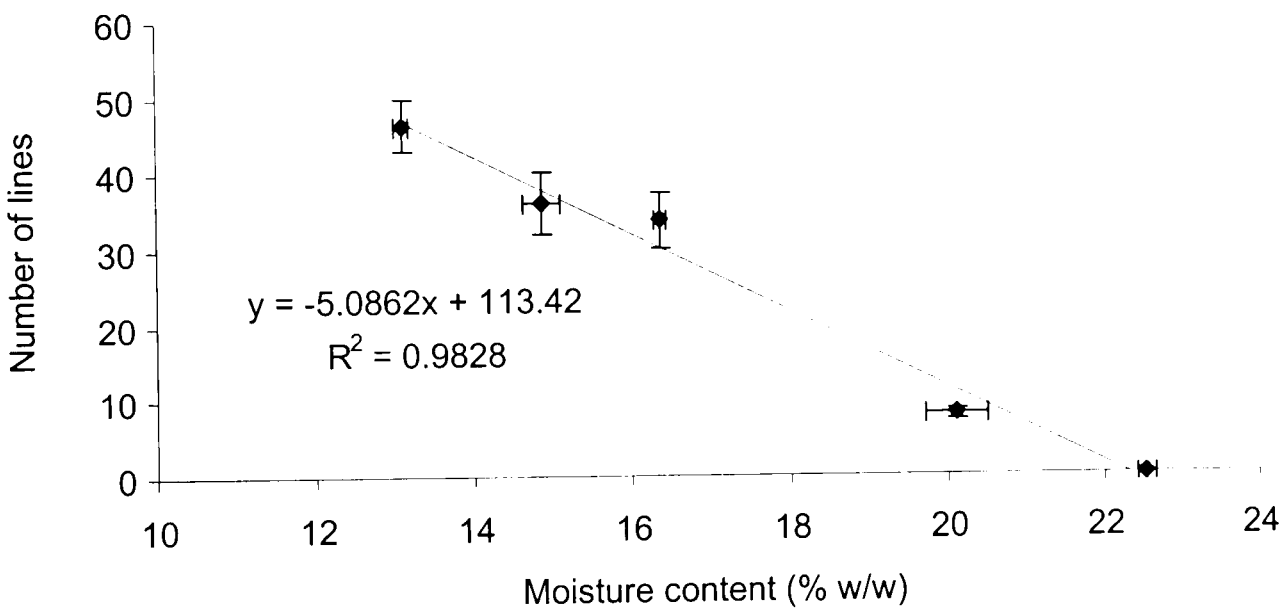
4.4.2.4.1. Gelatin

Figure 4-10: Gelatin film samples stored at different relative humidities and used in the puncture tests. The relative humidity is denoted on the film as % (i.e. RH22 indicates a film stored at a relative humidity of 22%).

The fractured samples were collected for further observations. Some samples are shown in Figure 4-10. It can be seen that the number of lines radiating out from the puncture point varies from sample to sample. The number of lines was counted for each set and is shown as a function of the moisture content in Figure 4-11.

The number of lines observed correlates with the moisture content (Figure 4-11). The large variation in the number of lines indicates a large effect of the water on the fracture properties of the gelatin films.

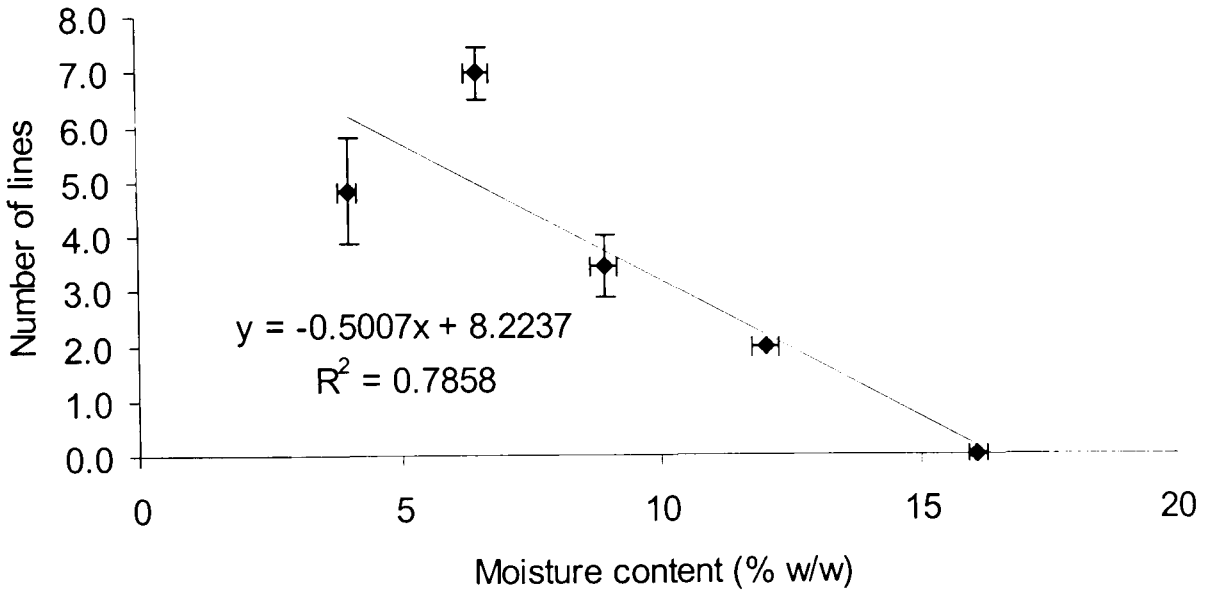
Figure 4-11: Effect of moisture content on the number of lines observed after puncture of gelatin films.



4.4.2.4.2. HPMC

HPMC films also show an increase in the number of lines on decreasing the moisture content (Figure 4-12). However the number of lines generated on puncture is much lower than for gelatin and the relative error on the measurement is large.

Figure 4-12: Effect of moisture content on the number of lines observed after puncture of HPMC films.



4.4.3. Surface orientation

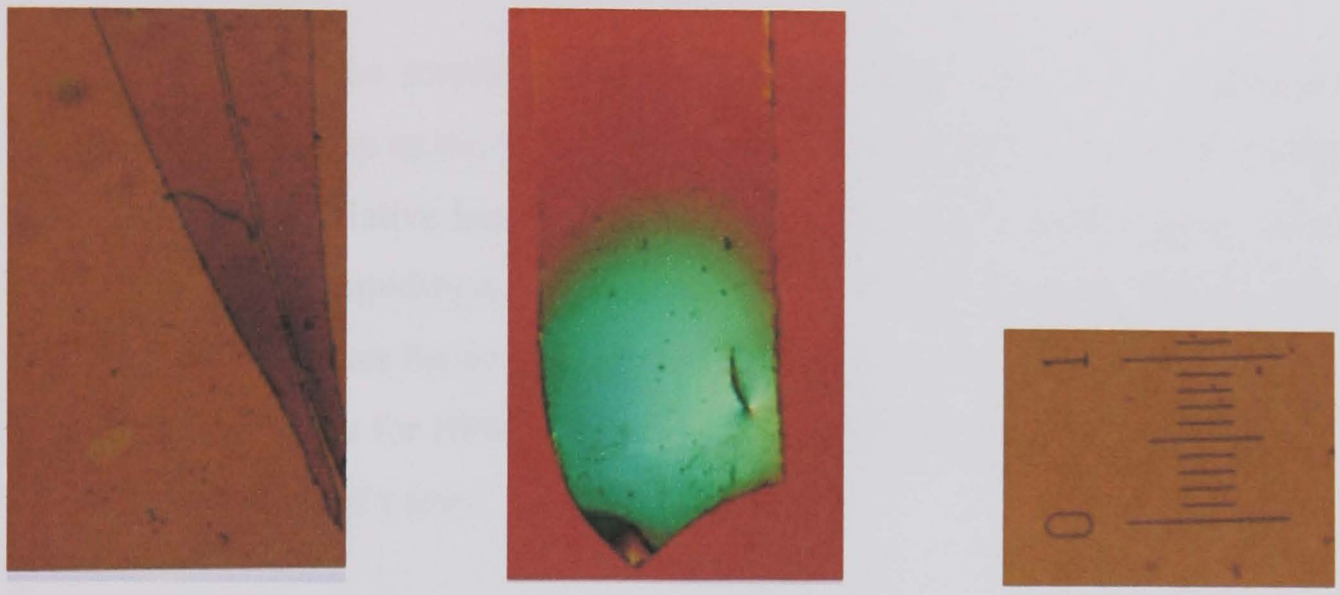
Cast films always present a certain degree of orientation of the molecular segment parallel to the surface but below a certain depth no ordering can be found (Pavlov et al. 1999). Using birefringence, it was shown that the optical anisotropic surface layer did not exceed 0.14mm for dextran films (Pavlov et al. 1999). For gelatin films, this layer is at least 0.5mm (Pavlov et al., 2000). Preliminary studies showed higher anisotropic intensity for cellulose derivatives than for gelatin indicating a stronger alignment of the segments parallel to the surface (Pavlov, personal communication).

4.4.4. Microscopy observation

When the samples were observed under optical microscopy using polarised light, a strong effect was observed. Pictures of gelatin and HPMC films around the central puncture point are shown in Figure 4-13. The polarisation effect is clearly visible around the centre for HPMC. This implies a very strong molecular orientation, which is absent away from the central region. This orientation is also observed for gelatin films but to a lesser extent. Radial orientation of the molecules during puncture was expected due to deformation. However, the observation made here shows that some

orientational order is permanently created. It is difficult to quantify orientation using such a simplistic approach but the intensity of colouration when viewing HPMC under polarised light was much stronger than for gelatin. This might indicate a stronger orientational effect.

Figure 4-13: Samples around the puncture centre observed under polarised light. From left to right: gelatin, HPMC and scale (mm).



4.4.5. Discussion

All the films were dried and stored at a relative humidity of 44%. This relative humidity is within the range advised by the hard gelatin capsule manufacturers. We will use 44% as the standard relative humidity in the following studies. At this relative humidity, the distance at break of gelatin is slightly higher than the distance at break for HPMC. The difference in gradient is even bigger (Table 4-3).

For the relative humidities studied (22 to 85%) the moisture contents of gelatin and HPMC ranges respectively from 13% to 23% and from 4% to 16%. The difference between the two polymers can be explained in terms of the hydrophobicity of the HPMC substituent groups. The HPMC grade used here has a methoxyl content of 28-30% and a hydroxypropyl group content of 7-12%. Very little self-substitution on hydroxypropyl groups should occur in most HPMC (Tezuka et al. 1991). Assuming no substitution occurs, the degree of substitution can be calculated from the average

contents given above. We found that about 0.8 of the hydroxyl groups of the three available in cellulose will not be substituted. Methoxyl groups present a degree of substitution of 1.9 whereas hydroxypropyl groups only have a degree of substitution of 0.26. Methoxyl groups are unable to provide hydrogen bonding which explains the high hydrophobicity of these products and hence the difference in the sorption isotherm compared with gelatin.

In the first part of the sorption isotherm, both polymers show little change in their mechanical properties as the water content alters. This implies that they can be used within a range of relative humidity with predictable structural behaviour. However, beyond a relative humidity of 60%, the mechanical properties are strongly affected. The gradient decreases for both polymers whereas the distance at break increases for gelatin and decreases for HPMC. This is the first indication that both polymeric films are of a very different nature.

When the mechanical properties are plotted against moisture content, the behaviour is consistent with those described above. However, the main new feature is the strong correlation between moisture content and gradient. The values obtained for G_0 and G_1 are gathered in Table 4-3. The zero moisture content gradient is much higher for gelatin than for HPMC. The effect of the relative humidity on the gradient of both polymers seemed more important for gelatin (320 to 100 N/mm^2) than for HPMC (200 to 77 N/mm^2) but this is only due to the sorption isotherm differences. As seen in Table 4-3, the relative effect of moisture content on the gradient is in fact more important for HPMC. This difference indicates that water plasticisation is more important in the case of the HPMC than in the case of gelatin.

Table 4-3: Comparative results of gradient properties for gelatin and HPMC films.

Film	Gradient at a relative humidity of 44% (N/mm^2)	Zero moisture content gradient G_0 (N/mm^2)	Relative effect of the moisture content on the gradient G_1 (%)
Gelatin	291	543	3.10
HPMC	188	251	4.27

Another important difference between the two films was the number of lines after fracture. Gelatin films present a large number of lines compared to HPMC film. This difference may indicate more intrinsic differences in the various phenomena involved in deformation and fracture. It is also an indication that the gelatin sample stored a large amount of energy that is allowing the formation of the new surfaces (Kinloch and Young 1983) whereas for HPMC films, the energy is likely to be dissipated during the deformation process.

The differences observed between the two film formers along with the differences in molecular structure and molecular weight indicate that the molecular organisations of the films are different. Gelatin films present crosslink and junction zones. These junction zones are important for the film mechanical properties. Films dried above the setting temperature of gelatin were known to be very brittle (Finch and Jobling 1977). The presence of crosslinks will limit slippage therefore uncoiling, stretching, rotation and crazing are likely to be the phenomenon involved in the deformation process. The gelatin α chains are of about 95000 Da which imply that entanglements between gelatin chain are very likely. Entanglements in the film will act as more anchor points and reinforce the crosslink density of the film.

HPMC (grade 606) on the other hand is of relatively small size (degree of polymerisation of about 50) and the cellulose backbone is rather rigid. This makes the presence of entanglements unlikely. The result would be a film with aligned cellulose chains, which will slip during deformation and align further. The results from optical anisotropy also support the idea of alignment of macromolecular segments in cellulose derivative films. The water is unable to bond strongly with HPMC and therefore slippage would be increased as the moisture content increases. This model would explain the differences in G_1 and G_0. These models are discussed more extensively in the context of the alginate studies in Chapter 7.

CHAPTER 5. INFLUENCE OF PHASE SEPARATION ON THE GELATION PROPERTIES OF POLYSACCHARIDES MIXTURES

5.1. INTRODUCTION

The production process used for capsule formation involves the quick gelation of the hot liquid gelatin onto a pin dipped into the gelatin solution. The controlled gelation allows the control of the shape of the capsule and also its weight.

The materials to be tested for their film forming properties are unlikely to fulfil all the criteria of suitable mechanical properties, low viscosity and gelation required in capsule production. Mixing a gelling agent at low concentration and a non gelling filler (film former) at high concentration could solve this problem. However, polymer mixtures tend to phase separate and the gelling properties could be lost due to the presence of the filler.

In this chapter, work to ascertain that biopolymers with different charge levels are less likely to phase separate and therefore more likely to allow gelation than polymers with similar charge level will be discussed.

5.2. CHARGES ON THE POLYMERS STUDIED

5.2.1. Conditions

The method was described earlier (3.4). The various conditions used for the charge determination are given in Table 5-1.

Table 5-1: Experimental conditions used for charge determination

Compound	Concentration (g/l)	Addition rate of Poly-Dadmac solution (ml/min)
Agarose	0.4	0.12
Kappa carrageenan	0.2	1.50
Gellan Gum	0.4	1.50
Maltodextrin	5.0	0.03
HPMC	1.0	0.03
Gum Arabic	0.4	1.50
CMC	0.2	1.50
Manucol LB (Alginate)	0.2	1.50

5.2.2. Results

The measurements of the potential as a function of the volume of Poly-Dadmac (Poly-diallyl-dimethyl-ammonium-chloride) added are shown in Figure 5-1. The volumes needed for zero potential were recorded and the calculation of the charge density performed as described earlier (Section 3.4).

The results obtained from the charge analysis were corrected for moisture content and are gathered in Table 5-2. The experimental results showed averages with confidence interval between 1% and 5%.

Figure 5-1: Charge density measurement. The curves represent triplicate runs on 8 polymers: 1-alginate, 2-CMC, 3-kappa carrageenan, 4-gellan gum, 5-gum arabic, 6-agarose, 7-HPMC and 8-maltodextrin.

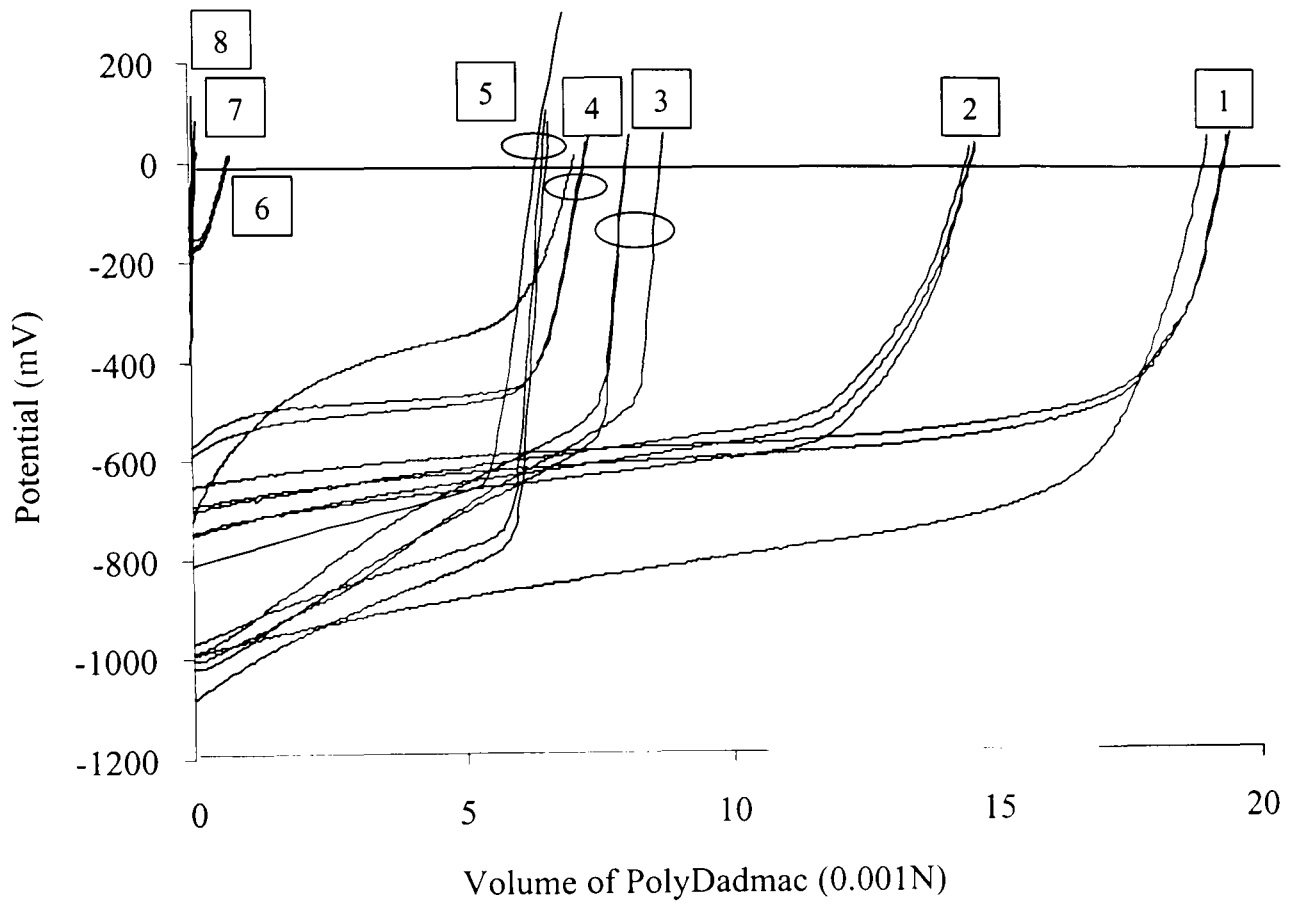


Table 5-2: Charge density of dry biopolymer

	Charge density (μmole/g)	Confidence interval (μmole/g)
Alginate (Manucol LB)	5298	69
CMC	4231	17
kappa carrageenan	1789	33
Gellan gum	1167	59
Gum Arabic	1064	19
Agarose	97	3
HPMC	6	0
Maltodextrin MD20	1	0

Sodium alginate consists of sodium mannuronate and sodium guluronate unit. Both repeating units have the same molecular weight and charge density, which allows the calculation of the theoretical charge density. The experimental value 5298 μmol g is in reasonable agreement with the theoretical value (5051 μmol/g).

The analysis provided by the CMC supplier (Metsa) gives an average degree of substitution of 0.85. This allows the calculation of the theoretical repeating unit molecular weight as 230=162+80*0.85 and the theoretical charge density 0.85/230=3695μmol/g. The charge density found experimentally corresponds to a degree of substitution of about 1. The difference observed is likely to be either due to bad specification analysis or overestimation in charge density measurement.

The theoretical charge density of high acyl gellan gum as described in Figure 2-4 with sodium counterions is of 1288 μmol/g. Theoretical values of charge densities for HPMC and maltodextrin are 0 μmole/g. In the case of the gum arabic, most of the charge is due to glucuronic acid residues. However, gum arabic contains about 2% of protein and hence charge level will depend on the pH. The technique used only measures the net charge present on the polymer. Therefore, the charge density of the gum arabic could be underestimated.

The values for the other polymers can not be calculated theoretically due to their complex structure.

5.3. MIXTURES GELATION PROPERTIES

Mixtures of polymers have been prepared as described before (3.3.2). The limit concentration that allow gelation are described and discussed here.

5.3.1. Results

The data in Figure 5-2 where a low viscosity CMC and alginate were used show that agarose can gel these systems up to the highest concentration of alginate or CMC used. However, kappa carrageenan (Figure 5-3) and gellan (Figure 5-4) fail to form a gel at this concentration. Similarly, high levels of HPMC prevented gelation of agarose but kappa carrageenan and gellan gum gelations were unaffected. In the case of gum arabic, only the gellan gum gelation was prevented. Maltodextrin did not prevent any gelling agent used from setting.

Figure 5-2: Maximum levels of cosolute in the range investigated (0-14%) allowing gelation of agarose at 0.5%. Cosolutes: low viscosity CMC (cmc), low viscosity alginate (alg), low viscosity HPMC (hpmc), maltodextrin (DE 20) (malt), gum arabic (gum ar).

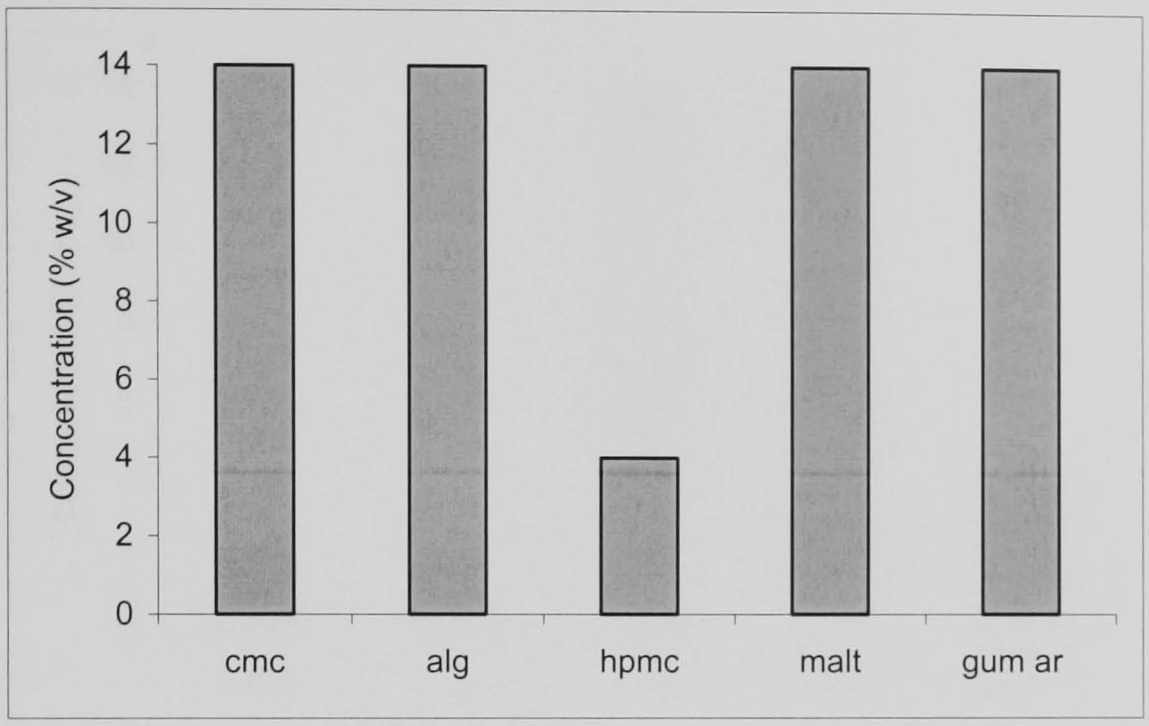


Figure 5-3: Maximum levels of cosolute in the range investigated (0-14%) allowing gelation of kappa carrageenan at 0.5%. Cosolutes: low viscosity CMC (cmc), low viscosity alginate (alg), low viscosity HPMC (hpmc), maltodextrin (DE 20) (malt), gum arabic (gum ar).

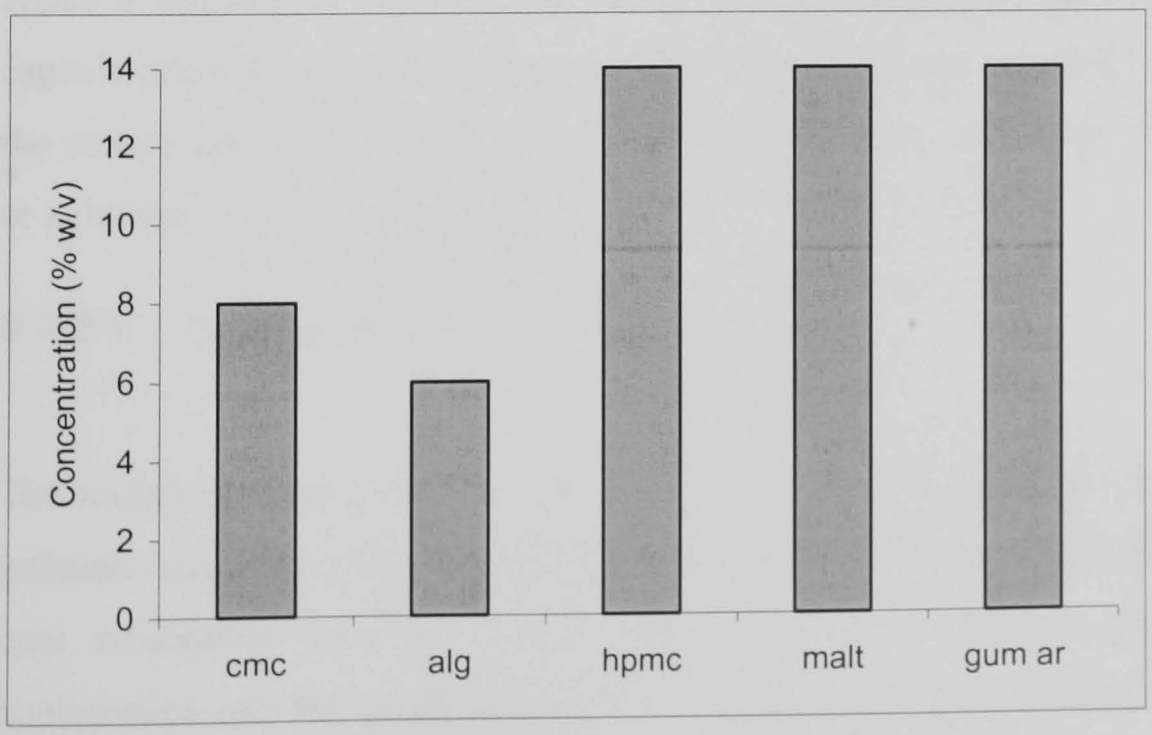
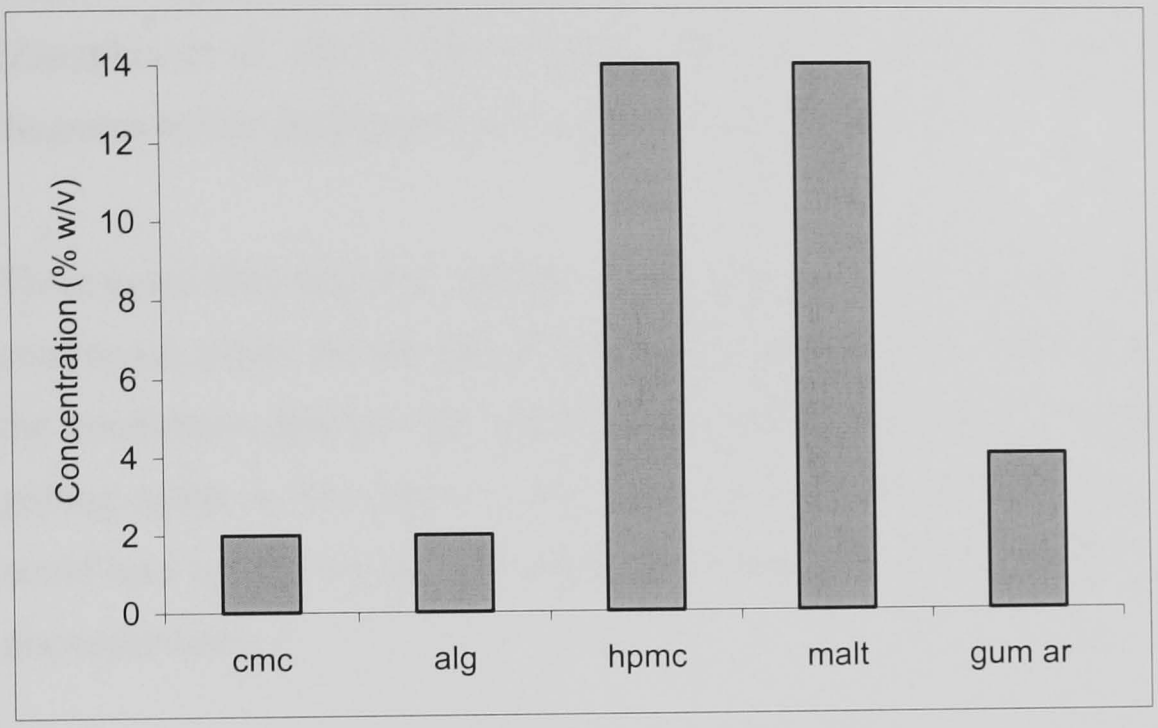


Figure 5-4: Maximum levels of cosolute in the range investigated (0-14%) allowing gelation of low acyl gellan gum at 0.3%. Cosolutes: low viscosity CMC (cmc), low viscosity alginate (alg), low viscosity HPMC (hpmc), maltodextrin (DE 20) (malt), gum arabic (gum ar).



5.3.2. Discussion

In the experimental data, phase separation was not assessed but only the ability to create a self-supporting gel. The reason for using this criterion lies in the gelatin capsule process itself. The levels and viscosities of the co-solutes used here allowed the assumption that self-supporting systems were gels rather than viscous solutions or solutions with high yield stress.

5.3.2.1. Limiting gelling conditions

The understanding of the effects of the presence of the filler on the gelling agent gelation requires preliminary understanding of how gelation could be prevented. The case considered here is of a mixture that is continuously mixed, preventing coalescence and the generation of two clear macroscopic phases prior to gelation. This would be the case in a real process of capsule production.

In the absence of specific chemical interactions, the limiting condition that allows gelation is that the concentration of the gelling agent in the continuous phase is sufficiently high. For a pure polymer water system, this limiting concentration is called the critical concentration c^*. When a second polymer is added, in the absence of phase separation, gelation below c^* can occur due to excluded volume effect (Zasypkin et al. 1997). This explains that the c^* line is not vertical in the phase diagrams below the binodal in Figure 5-5 and Figure 5-6.

Three cases obey the limit condition for gelation if phase separation occurs. First, the continuous phase can be the gelling agent rich phase. Second, despite the fact that the continuous phase is the gelling agent deficient phase, the concentration of the gelling agent in this phase is still high enough. Finally, interconnected gel beads could lead to macroscopic gel properties (Clark 1995). This unlikely case will not be discussed here.

Simplified phase diagrams considering gelation and phase separation can be drawn (Zasypkin et al. 1997). The rectilinear diameter is a line linking all the midpoints of the tie lines. Phase inversion is supposed to happen in the region of this rectilinear diameter, where both phase volumes are similar. We will therefore assume here that the rectilinear diameter delimits which phase is continuous. The critical point is defined as the intersection between the binodal and the rectilinear diameter (Bourriot et al. 1999). C^*_b is defined here as the limit concentration for gelation on the binodal curve.

Depending on the relative positions of c^*_b and the critical point, two schematic phase diagram are proposed (Figure 5-5 and Figure 5-6).

Figure 5-5: Schematic phase diagram with c^*_b above the critical point (The grey area delimits the gelling mixtures). Adapted from (Zasypkin et al. 1997).

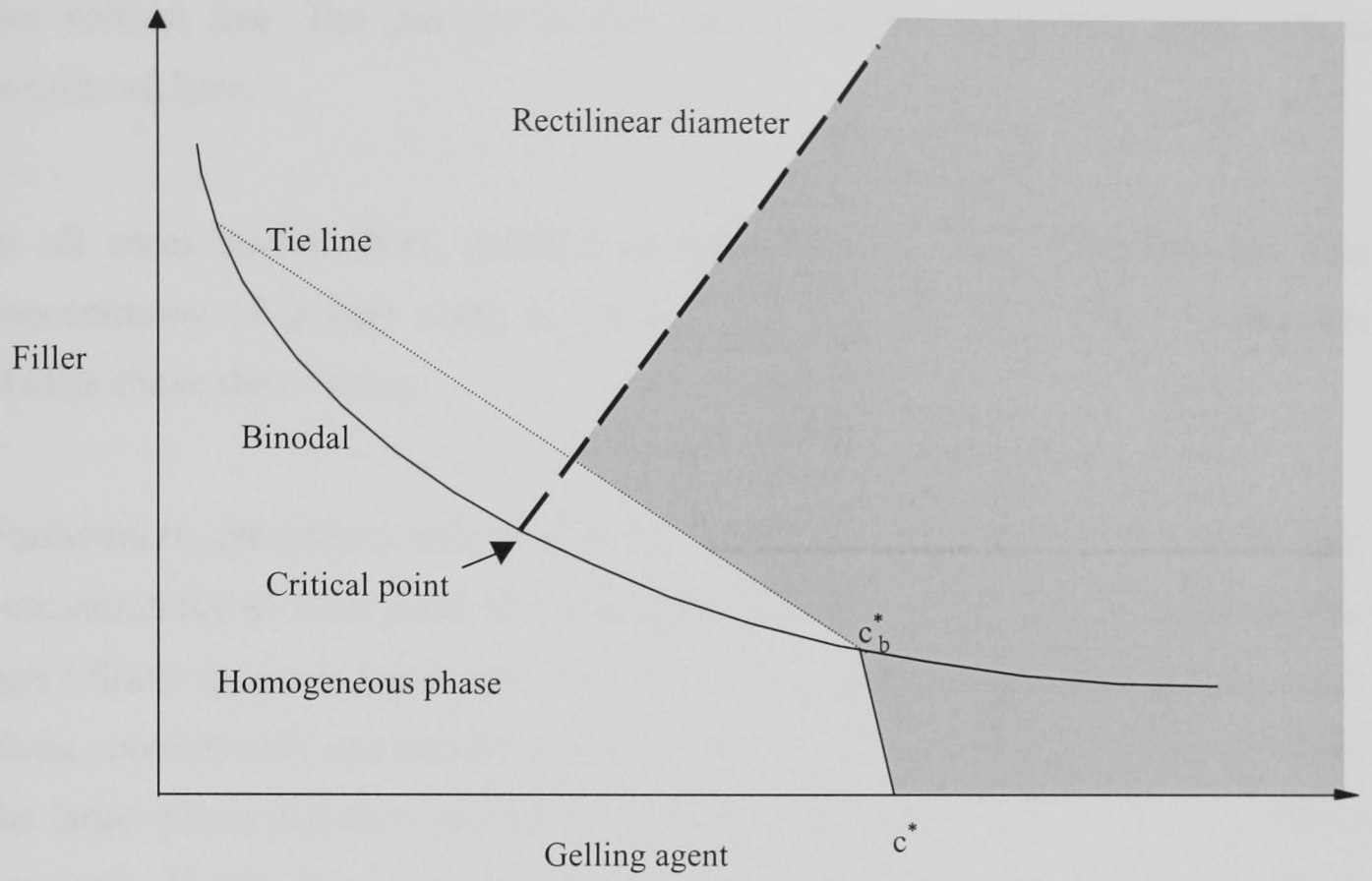
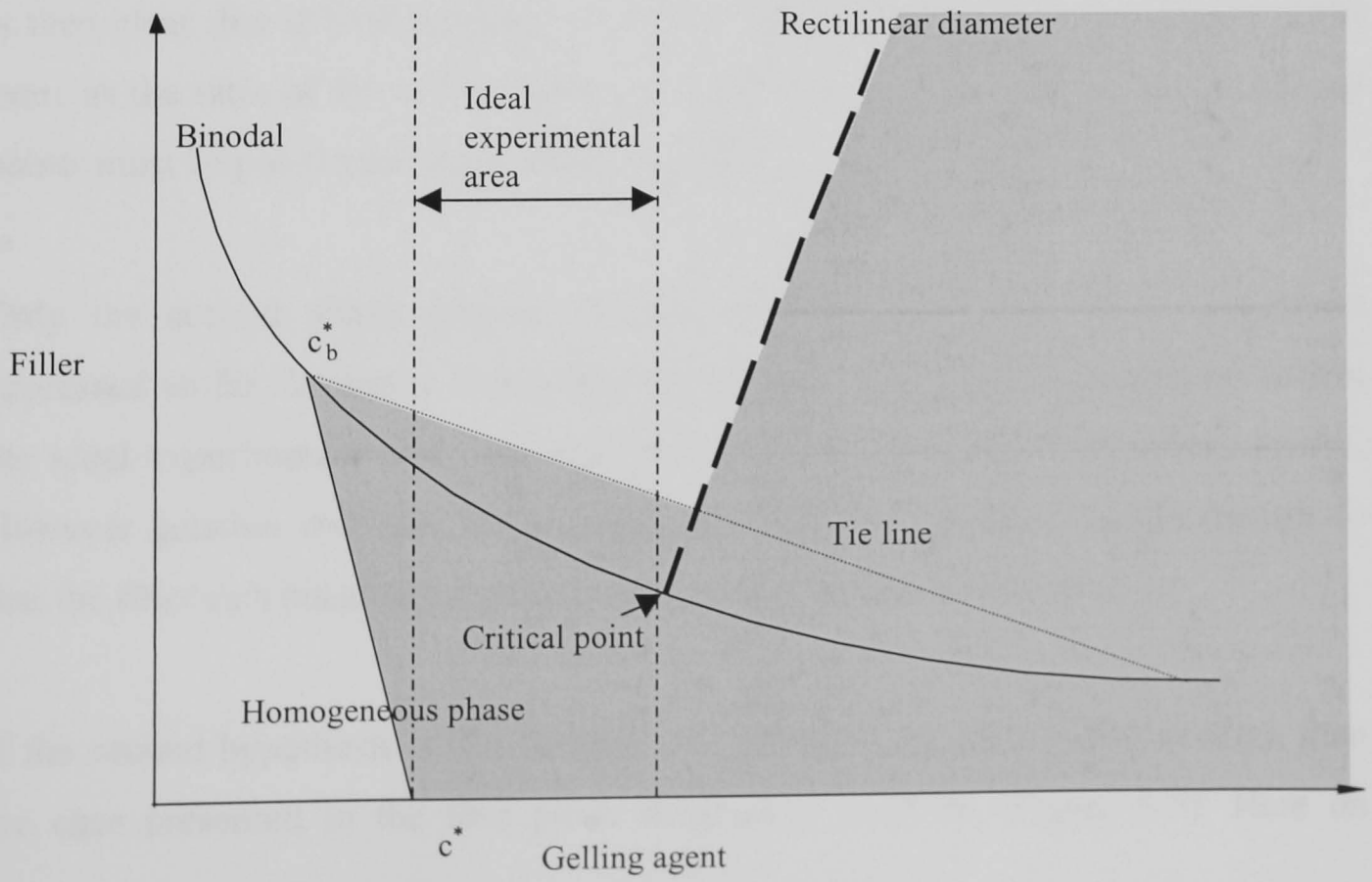


Figure 5-6: Schematic phase diagram with c^*_b below the critical point. (The grey area delimits the gelling mixtures). Adapted from (Zasypkin et al. 1997).



In order to assess more exactly the situation encountered here, we shall consider the positions of the experimental points on the phase diagrams. Since a single gelling agent concentration was used for a given system, the experimental points will lie on one vertical line. The position of this line relative to the gelling agent axis is considered here.

In all cases studied here, gelation occurred without filler. Therefore the total concentration of gelling agent in the mixtures (and the experimental points) are always above the c^* value.

Furthermore, the concentrations of gelling agent used here are much lower than the concentrations of filler used. Consequently, the ratio of the concentration (gelling agent/filler) is much lower than one. If complete phase separation occurs, (each phase contains only one polymeric species and water) then the filler phase should be the larger phase and therefore be continuous. This is true if two more assumptions are made. Firstly, the molar volumes of each polymer are similar. This hypothesis implies that the same amount of each polymer is needed to fill a lattice site, which seems reasonable since molecular structures are relatively similar. Secondly, limited or no water partitioning occurs (the water polymer ratio of both phases are equal). It is then clear that if both hypotheses are verified, the ratio of the two phases is the same as the ratio of the concentrations (i.e. lower than one). Then the experimental points must be positioned below the critical point.

Only the second phase diagram (Figure 5-6) can fulfil the two requirements expressed so far. It clearly shows that by increasing the filler concentration within the ideal experimental area, one reaches the binodal and phase separation occurs. However gelation will still happen until the filler concentration is high enough so that the filler rich phase has a gelling agent concentration lower than c^*_b.

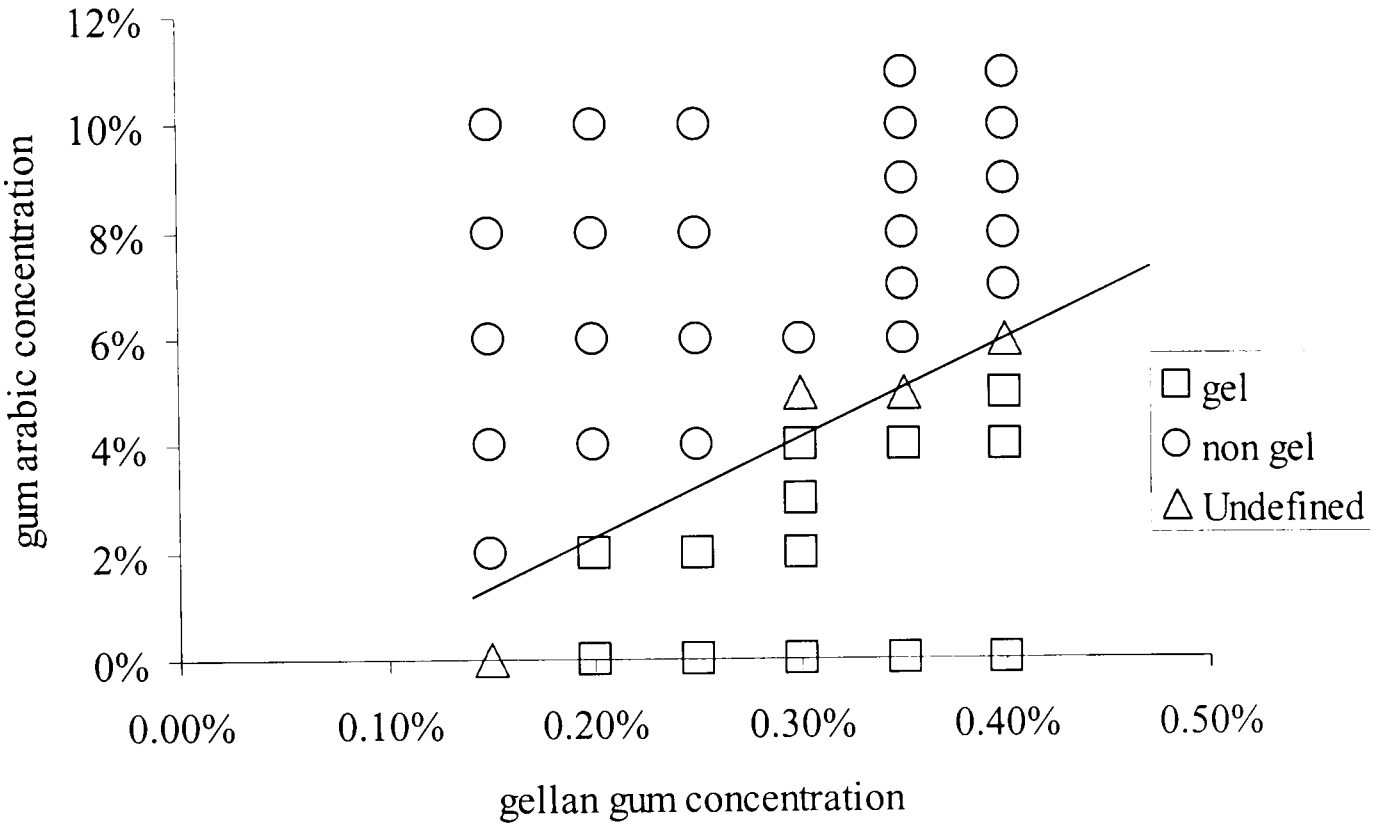
If the second hypothesis is not verified (i.e. strong water partitioning occurs), then the case presented in the first phase diagram is possible (Figure 5-5). Here on

increasing the filler concentration, gelation would be prevented when the phase inversion occurs.

It is not surprising that the binodal is not the limit for gelation of gelling agent filler mixture in both cases. Indeed, a mixture with concentrations just above the binodal will demix into two phases A and B. The first phase, A will have almost the same concentrations as the mixture and the second phase, B can have very different concentrations. Since the original mixture has a concentration above c^* (hence c^*_b), the phase A is very likely to present a concentration above c^*_b. Furthermore, the phase A will have a phase volume much higher than the phase B which imply that the phase A is more likely to be the continuous phase. This fulfils the two requirements for gelation.

Using the same experimental procedures as for 5.3.1, a complete gelation diagram was constructed for gellan gum and gum arabic mixtures (Figure 5-7). It shows that on increasing the gellan gum concentration, gelation is possible at higher gum arabic level. This is again in contradiction with the idea that the binodal defines the limit for gelation. The direction of the delimiting line between gelling and non gelling areas is not compatible with a tie-line. Therefore in the gellan gum gum arabic mixture, phase inversion is preventing gelation. This implies that in this case, strong water partitioning occurs. Indeed the concentration ratio on this delimiting line is in the range 10-15.

Figure 5-7: Gelation diagram of gellan gum and gum arabic mixtures. The line is drawn to emphasise the separation between the gelling and non gelling areas.



It has been shown here that although phase separation will affect gelation of polymer mixtures, knowing the position of the binodal does not allow the prediction of the limit for gelation. Phase inversion limit and c^*_b tie line will delimit the area of gelation.

5.3.2.2. Effect of molecular weight differences

Before considering the influence of the charge density, the molecular weight of the polymers studied should be discussed. For a perfect comparison of the effect of the charge density on the gelation of biopolymer mixtures, polymers of identical molar volumes (and therefore similar molecular weights) and χ parameters should be used. These two conditions could only hold if the same polymer couple was used. The methods for obtaining the molecular weights of the polymers used here were described earlier (4.2 page 51). Approximate molecular weights are given in Table 5-3.

Table 5-3: Approximate molecular weights of polymer used in the phase separation study.

Polymer	Molecular weight (kg/mol or kDa)	Source
Gellan Gum	1000-2000	(Kang and Pettitt 1993)
Kappa carrageenan	100-500	(Glicksman 1982)
Agarose	120	(Glicksman 1982)
Gum arabic	380	(Whistler 1993)
Alginate	65.3	Intrinsic viscosity
CMC	22	Viscosity
HPMC	8.1	Intrinsic viscosity
Maltodextrin	0.9	DE value

The contribution of a molecular species i to the entropy of mixing is proportional to the number of molecule i ($\Delta S_M = -k\sum n_i \ln v_i$) and therefore inversely proportional to its molecular weight. Since the contribution of other species (i.e. solvent) is not affected, high molecular weight compounds have very little effect on the overall entropy of mixing. Therefore, above a certain molecular weight threshold, changes in molecular weight will not affect significantly the phase diagram of mixed systems.

It is reasonable to use the solvent as the elementary size (molecular weight of 18g/mol). Apart from the maltodextrin sample (molecular weight ratio of 50) all the other compounds have very large molecular weight compared to the solvent (ratio from 450 to 110 000). Therefore their contributions to the overall entropy of mixing are relatively small, and changes in their molecular weights should not strongly affect the phase diagram.

5.3.2.3. Effect of charge density

Although the binodal does not define the limit for preventing gelation, its shift towards high polymer concentration will increase the compatibility area and therefore ensure gelation. The theoretical position of the binodal curve is rather difficult to determine since it assumes the knowledge of each interaction parameter

(χ_{P1-P2}, χ_{P1-S} and χ_{P2-S}) and the relative molar volume of the polymers and solvent. Already shown in the previous sections is, that except for maltodextrin, molecular weight differences should not affect the results significantly.

Quantitative prediction of the phase diagram of mixtures of neutral and slightly charged biopolymer systems have been performed (Khokhlov and Nyrkova 1992). The principle used was the simple reduction in effective molecular weight due to the counterions. The molecular weight N was replaced by N/(1+f.N), (f.N) being the number of charge per molecule. This approach showed the increase of compatibility that slightly charged biopolymer could present. However, the charge level studied was low (f<<1) and mixed polyelectrolyte systems were not considered.

Piculell et al (Piculell et al. 1994) calculated theoretical phase diagrams of highly charged polyelectrolytes using the same principle. The reduction of the apparent molecular weight only applies if the counterion concentration is different in separating phases. They showed that similarly charged polyelectrolytes would phase separate more easily than polyelectrolytes with different charge density. However, it is important to remember that this approach does not take into account the variation in interaction parameter that would be observed if the charge densities of the polymer were changed.

The principle of increased compatibility due to counterion entropy for polyelectrolyte systems (polyelectrolyte-polymer-water or polyelectrolyte-polyelectrolyte-water) is solely due to the presence of the concentration gradient of the ions between the phases in equilibrium. This gradient can disappear in the presence of salts or when the concentration of polyelectrolyte and water in each phase is appropriate (Piculell et al. 1991).

If complete phase separation occurred and no water partitioning was possible, then the charge density difference between the polyelectrolyte would be the main driving force toward compatibility. The charge density results and limiting gelling concentrations are gathered in Table 5-4.

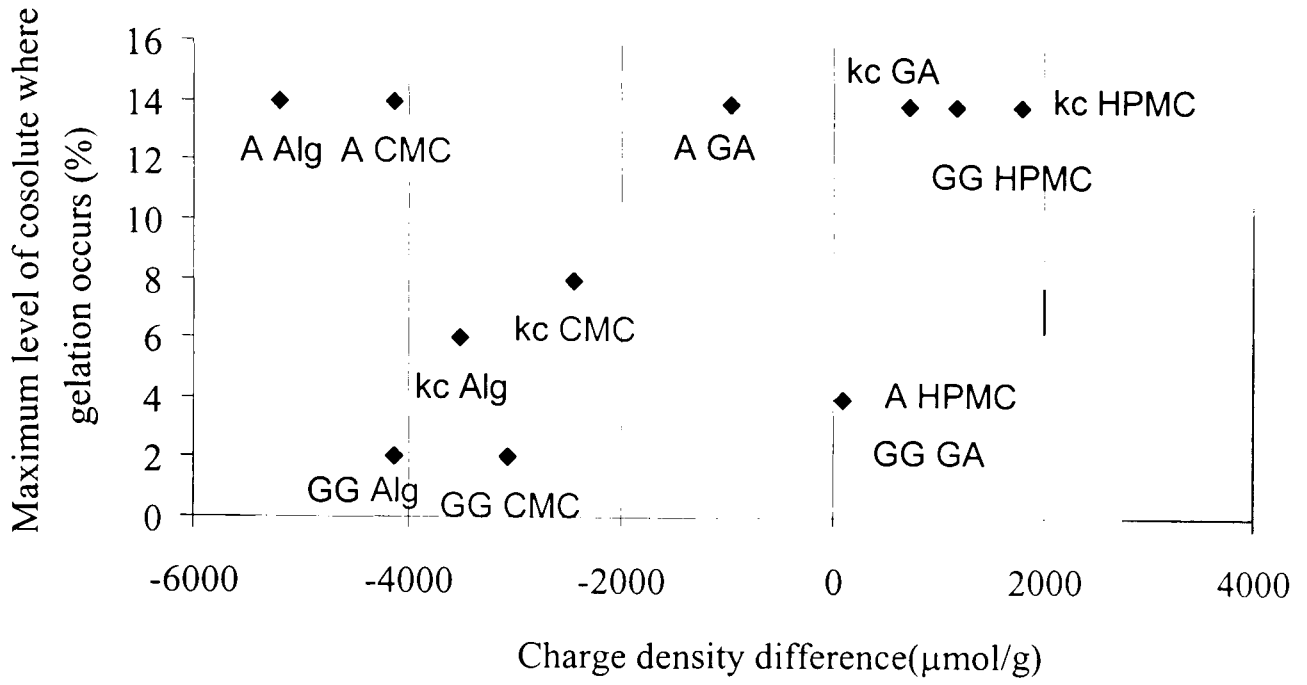
Table 5-4: Charge density difference and gelling properties of polysaccharide mixtures. (charge density difference = gelling agent charge density – filler charge density)

Polymer Gelling	Filler	Charge density (μmol/g) Gelling	Filler	Charge density difference (μmol/g)	Max. filler level for gelation (%)
Gellan gum	Maltodextrin	1167	1	1166	14
kappa carrageenan		1789	1	1788	14
Agarose		97	1	96	14
Gellan gum	HPMC	1167	6	1161	14
kappa carrageenan		1789	6	1783	14
Agarose		97	6	90	4
Gellan gum	Gum arabic	1167	1064	103	4
kappa carrageenan		1789	1064	725	14
agarose		97	1064	-968	14
Gellan gum	Alginate	1167	5298	-4131	2
kappa carrageenan		1789	5298	-3509	6
agarose		97	5298	-5202	14
Gellan gum	CMC	1167	4231	-3064	2
kappa carrageenan		1789	4231	-2442	8
agarose		97	4231	-4135	14

The maltodextrin does not inhibit the gelation of any gelling agent. This was expected since the molecular weight of the maltodextrin is very low and therefore the entropy loss that would occur during phase separation would be too large. This is true in these conditions; however, phase separation would occur at very high concentrations of maltodextrin polymer mixtures.

The plot in Figure 5-8 shows the influence of the charge density difference on gelation inhibition level. It can easily be seen that the plot is not symmetrical. This implies that depending on whether the filler or the gelling agent is the more charged polymer, the gelling ability of the system will be different.

Figure 5-8: Effect of absolute charge density difference (gelling agent charge density– filler charge density) on maximum level of cosolute allowing gelation (A:agarose, kc:kappa carrageenan, GG:gellan gum, Alg: alginate, GA:gum arabic, CMC:carboxymethylcellulose, HPMC:hydroxypropylmethylcellulose).



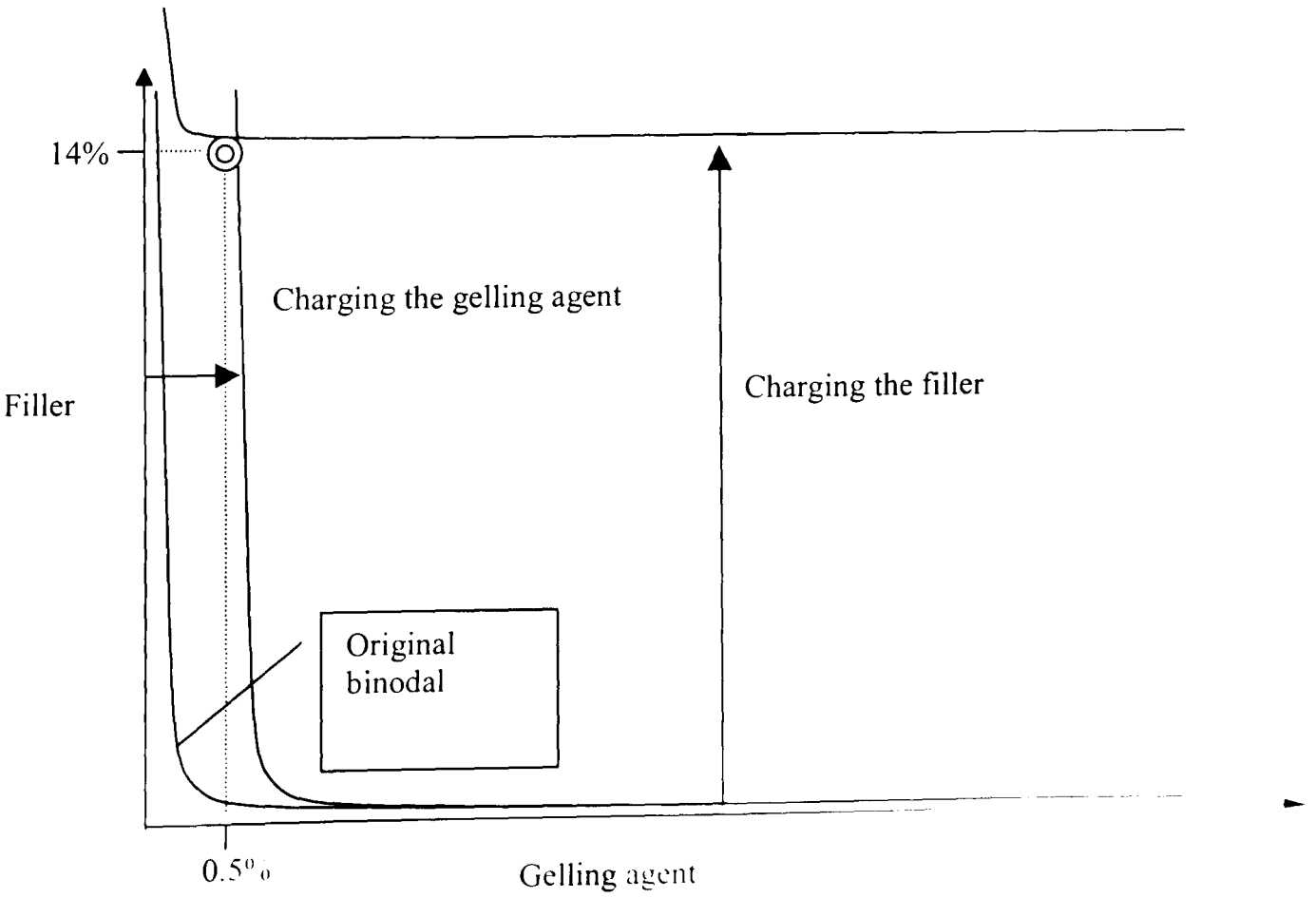
At very high charge density difference, gelation always occurs whereas at very low charge density difference, phase separation prevents gelation. However, for gellan gum (or kappa carrageenan) system in presence of alginate (or CMC), gelation is prevented at relatively low filler concentration. Furthermore, the agarose gum arabic system can gel although its charge density difference is lower than the two previous systems.

The results obtained at very high or very low charge density difference are easy to explain in term of entropy of counterions. For the same reasons, when only one of the polymers is completely uncharged (agarose or HPMC), little charge density difference is necessary to allow gelation (HPMC and kappa carrageenan or agarose gum arabic systems).

If we compare the systems kappa carrageenan gum arabic and kappa carrageenan alginate, the results are very different. Whereas the gum arabic does not prevent

kappa carrageenan gelation (up to a concentration of 14% gum arabic), gelation in presence of 6% alginate is inhibited. This is in contradiction with charge density differences, which are respectively 725 for gum arabic kappa carrageenan mixtures and -3509 for alginate kappa carrageenan mixtures. The explanation for this disagreement might be found in the phase diagram changes occurring if charges are added to filler and gelling agent. When the charge density is increased on one polymer, then the binodal is pushed away from the other polymer axis (Piculell et al. 1994). This results from an apparent decrease in molecular weight. If the charge density of the filler was increased, then the binodal would be shifted away from the gelling agent axis. In order to allow gelation the binodal should be shifted up to 14% filler. However, if the charge density of the gelling agent was increased, a shift up to 0.5% (0.3% for gellan gum) would be enough to allow gelation (Figure 5-9). This difference between the filler and gelling agent charge density effects might explain why kappa carrageenan behaves differently in presence of gum arabic and alginate.

Figure 5-9: Schematic phase diagram: necessary binodal shift to allow gelation of mixed systems (for simplification purposes, the binodal is considered as the gelation limit).



We have considered so far two simplistic hypotheses: complete phase separation and no water partitioning. Obviously, this does not happen and both polymers are present to a certain extent in both phases. Furthermore, the overall concentration of polymer in each phase can be different. In order to prevent gelation, the rich gelling agent phase must generally be included (Figure 5-5 and Figure 5-6). When the charge densities of both polymers are different, the equilibrium in counterion concentrations can be obtained via two processes. Firstly, water partitioning can dilute the more concentrated phase. Secondly, if the filler is more charged than the gelling agent, an increase of the filler concentration in the gelling agent rich phase will balance the counterion concentrations. This could also explain the difference observed in gum arabic-kappa carrageenan and alginate-kappa carrageenan systems.

The analysis made so far assumed similar behaviour for all polymers. Indeed, interaction parameters between systems are likely to be very different and would give different phase behaviour for the different systems. For example, water was considered a poor solvent for HPMC (Jumel et al. 1996). It was also proposed that polymers with different shapes (rod and random coil) would phase separate even without energetic interaction($\chi_{p1\text{-}p2}=0$) when little solvent was present (Khokhlov and Nyrkova 1992). We have indeed here polymers with very different structures rigid rod (HPMC), random coil (alginate, CMC) or compact spheres (gum arabic) which should add to the complexity of the study.

Furthermore, although the thermodynamic process should ultimately be attained, kinetically driven situations can be observed. This is obviously the case once gelation of one of the polymer has occurred. Moreover, if one of the phases is becoming viscous during the phase separation process, it will be more difficult for the system to reach thermodynamic equilibrium. In the gum arabic gellan gum mixture, if no water partitioning occurred, then the total polymer concentration in each phase would have been equal which implies that for the more concentrated samples, concentration of about 15% was obtained. Since the viscosity of gum arabic and gellan gum are very different, the gellan gum rich phase would behave like a gel

as its concentration increased. This could explain the large water partitioning observed in gum arabic gellan gum systems.

5.4. CONCLUSION

It is known that charge density differences influence greatly the phase diagram of polymer mixtures (Gottschalk et al. 1998; Kramarenko and Khokhlov 1998; Piculell et al. 1991). This originates from the entropy of the counterions. However, the effect on gelation of a gelling agent filler mixture is not always straightforward. The limit condition for gelation is likely to be either the 'inversion line' or the c^*_b tie line rather than the binodal itself. However, shift of the binodal will also shift these limits.

Very low molecular weight fillers did not prevent gelation of any gelling agent used. It was shown that if one of the polymers is fully uncharged (agarose, HPMC) then very little charge density on the other polymer will allow gelation (up to 14% filler).

When both polymers carry charges, the system is more complex. If small charge density difference is observed then the system is incompatible and gelation is prevented (agarose and HPMC, gellan gum and gum arabic). If the charge densities are very different then gelation is not prevented. However, the charge density difference threshold for gelation is lower when the gelling agent is the more charged polymer. This can be explained since the necessary shift of the binodal for gelation (up to a concentration of 14% of filler) is less important when the gelling agent is the more charged polymer. Furthermore, when the filler is more charged than the gelling agent, increased concentration of the filler in the included gelling agent rich phase will promote phase separation. Overall, this data gives some support to the hypothesis outlined in the introduction.

It can be concluded that the different phase behaviour of mixtures of polyelectrolytes and non polyelectrolytes described by Piculell and co-workers (Piculell et al. 1991) provide a basis for predicting phase separation phenomenon in biopolymer mixtures.

The application of these phase behaviour to the gelation of a filler gelling agent system led to two basics rules for allowing gelation.

1. One polymer is uncharged, the other is slightly to highly charged.

2. The charge density of the gelling agent is higher than the charge density of the filler.

CHAPTER 6. SELECTION OF FILMS BY SENSORY ANALYSIS

6.1. INTRODUCTION

The question should be asked: "Why use sensory analysis for testing films, when the mechanical properties can be easily observed using more classical measurement techniques such as tensile tests, puncture tests, impact tests or dynamic measurements?" There are many reasons to choose this less usual approach.

Firstly, at the beginning of this work, people were using terms such as 'brittle', 'hard' or 'soft' but it was difficult to find a consensus in determining what they really meant, hence how to assess these properties. Using a sensory analysis approach, it would be possible to define these terms independently of prior scientific knowledge and define ways to assess the corresponding properties.

The use of sensory analysis was also motivated by the fact that differentiation between various films, such as gelatin or starch based films, using texture analysis was not easy, yet they were clearly different when handled. Therefore, sensory analysis would provide a straightforward screening tool in order to select the samples of interest.

A very large number of films were prepared and sensory analysis presented the advantage of being a quick assessing technique compared to more classical mechanical properties measurements.

Finally, the use of sensory analysis via a panel of people without prior knowledge of the aim of the study or scientific background should allow the exploitation of all the sensory differences present within the set of samples.

6.2. TERM DEFINITION STAGE

The first part of the sensory analysis was to define a number of sensory terms that allow the discrimination of samples. A panel of seven people was formed. These people were selected according to their ability in quantifying sensory parameters.

6.2.1. Conditions

In order to define the terms, six rectangular samples (30*10*0.1mm) of different characteristics were provided to the panel. Each sample was numbered and labelled with a three-digit number. These samples were selected so that they should cover as much as possible the range of mechanical properties for the whole set of films (Table 6-1).

Table 6-1: Samples used for the term definition phase

Number	Film
067	Gelatin
110	Meyproguat 7
948	Klucel EFF
054	Manucol LB
986	Textra
100	Clinco 460

In order to avoid any bias, none of the panellist knew about the purpose of the study. A panellist leader, without knowledge about the samples, asked the panellists to write the terms that would define the behaviour of the film for each of the samples in isolation (not in a comparative way). This phase was performed individually.

6.2.2. Initial terms

After one session, a very large number of terms were gathered (Table 6-2). Most of these terms expressed the same properties. During a second session, a discussion between the panellists allowed the number of useful terms to be reduced to very few. Two 'creasability' terms were later discarded because they would describe the behaviour of the films in conditions not realistic to their proposed use.

Table 6-2: Initial terms proposed by the panellist.

Bendy	Hard	Slow bend twist resilient
Brittle	Heavy	Smooth
Creased	Medium thick	Snapped when pulled
Delicate	Moderately soft short pieces	Snaps into pieces
Durable	No breaks	Snaps when bent slightly
Easily bent	No memory	Soft
Easily broken	No split with pressure on crease	Soft fabric like
Fairly thick	No twist	Split when folded
Fast bend/twist fractures	Not shattered on tearing	Splits when twisted
Fast bending	Paper like	Strong
Firm	Plastic like	Tears easily
Flexible	Pliable	Thick
Flexible in all directions	Resilient	Thin
Flexible when bent slowly	Resistant to bending	Tore not shattered
Foldable	Robust	Tore slightly on twisting
Folded easily	Rough on one surface	Tore slowly
Fractures on fast twists	Scrunchable	Very brittle
Fractures on vigorous bending	Shattered on twisting small bits	Very flexible
Fragile	Shatters	Would not bend
Friable	Short lengths flexible	
Glazed	Silky	
Good memory		

6.2.3. Selected terms and definitions

Following these first phases, four terms were kept: brittleness, brittleness flick test, memory and flexibility. The panel also provided a full description of how these terms are measured.

6.2.3.1. Assessment method

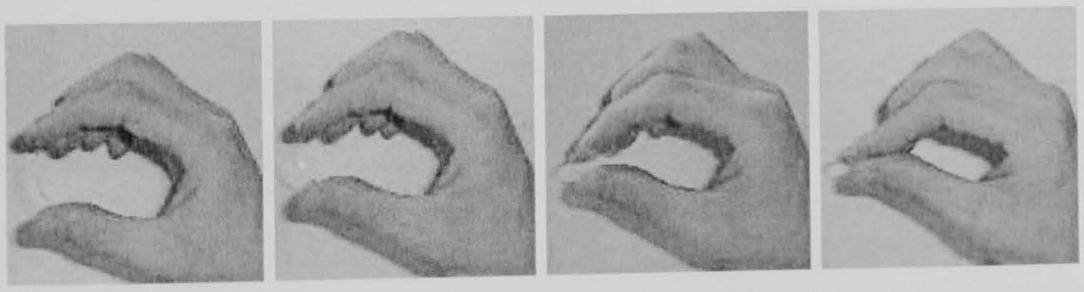
During the term definition phase, the panellist became very aware of how handling the films could affect their mechanical properties, especially through moisture transfer. Therefore, minimum handling of the film prior to assessment was ensured. Hands would be regularly dried using paper towels.

6.2.3.2. Brittleness

The Brittleness was defined as "*The ease with which the sample snaps when bent slowly*".

Method: 'The sample is held at the very edges between thumb and first finger. It is bent slowly by bringing the ends together, folding downwards. The movement is only completed once and no force is applied to the bend. For shiny films, they are held shiny side up. The films should be of a reasonable and consistent length and size. The score given on the scale (0-9) relates to the distance that the film can be bent, as shown in the diagram.'

Figure 6-1: Diagram indicating the measurement of brittleness.



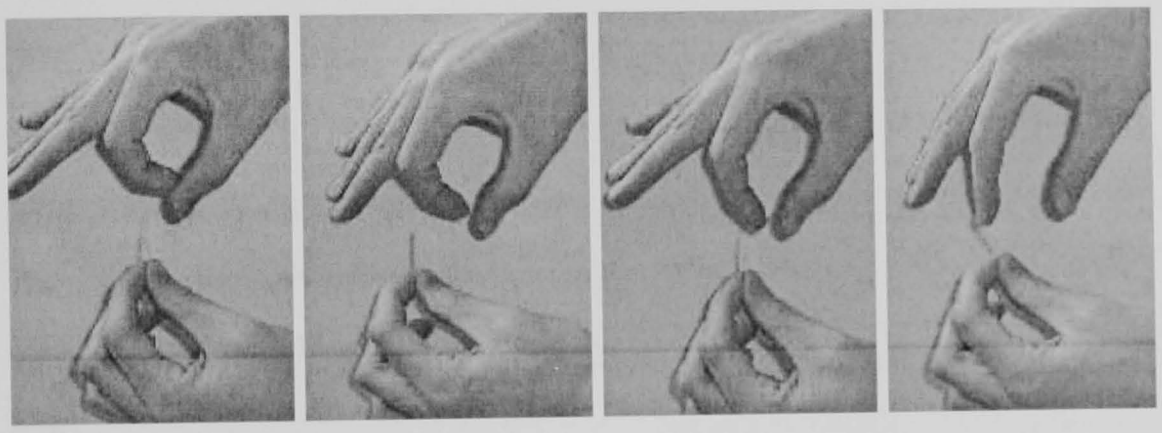
0 3 6 9

6.2.3.3. Brittleness Flick Test

The Brittleness Flick Test (or flick test) was defined as "*The ease with which the sample snaps when flicked*".

Method: 'Hold one edge of the sample with one hand between thumb and first finger, with the shiny side towards you. Flick the opposite edge of the film with the first finger with the maximum amount of force in the flick. Flick five times in succession counting the number of flicks as follows – One and two and three etc. This provides a slight pause between each flick so the film can stop moving. The flick test score is measured by the number of flicks survived before breaking. If the film breaks on the first flick it has survived none and is given a score = 0, if it breaks on the third flick, it has survived two and is given a score of 2, if it survives all five flicks it is given the maximum score of 5.'

Figure 6-2: Diagram indicating the measurement of brittleness via flick-test.

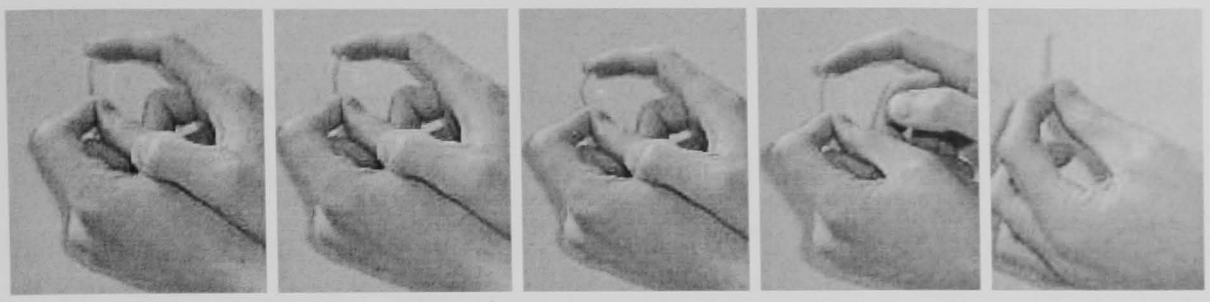


6.2.3.4. Memory

The Memory was defined as "*The ability of the film to return to its original position after being bent*".

Method: 'The film should be held vertically between the thumb and first finger of one hand. Using the first finger of the opposite hand bend the film to 90 degrees. The film should be held in the bent position for 5 seconds, counting 1 and 2 and 3 and 4 and 5. The film should then be released and the memory assessed as the degree of bending that remains after 5 seconds, counted as before (scale 0-5).'

Figure 6-3: Diagram indicating the measurement of memory.



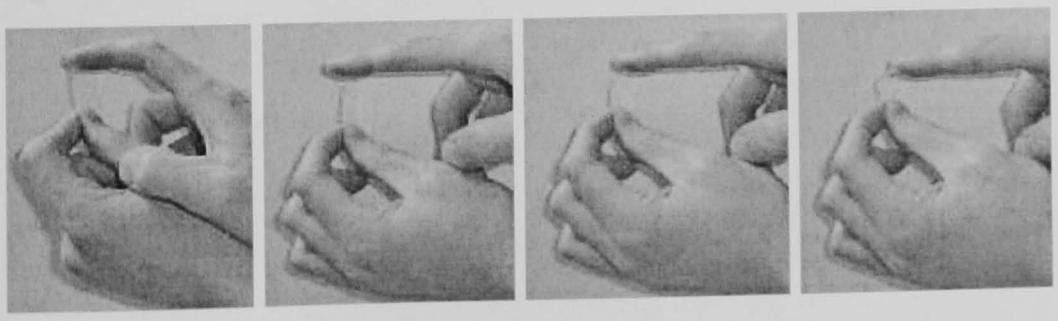
6.2.3.5. Flexibility

The Flexibility was defined as "*The force applied from fingers to bend slightly the film*".

The panellists expressed their concern in performing correct measurements of flexibility and requested a reference sample. One polyester sample (Melinex 100, HiFi Industrial Film Ltd) was used as a reference sample and given a score 9 for this test.

Method: 'The film should be held tight between the thumb and first finger at one end. Using the first finger of the opposite hand, a force is applied in the horizontal plane. Only small vertical movement is allowed (0.5cm). The force is measured on a scale 0-9.'

Figure 6-4: Diagram indicating the measurement of flexibility.



6.3. PRELIMINARY RESULTS OF CASTING

All the products described in materials and methods were cast. The resulting films are briefly described in Table 6-3. Most of the products used had some film forming properties. However, many were too fragile or brittle to be characterised. Therefore, a selection was necessary in order to keep the more interesting films only.

Table 6-3: Films produced by casting.

Trade Name	Product	Concentration	Comments on films
Alacid 710	Acid casein	10%	Powder
Alacid 741	Acid casein	10%	Powder
Alanate 180	Sodium caseinate	10%	Transparent yellowish solution. Clear film
Alanate 380	Calcium caseinate	10%	White solution. Clear film
Alaplex 1180	Milk protein concentrate	10%	White solution. Clear film
Alaren 799	Rennet casein	10%	Powder slightly film forming
Amylogum CLS	NIG*	7.5%	Long scales* on ABS. Not easy to remove.
Blanose 7	CMC	7.50%	Good film.
C* Avatex	NIG	7.50%	Good film. Slightly brittle.
C* Cream Polartex 06716*	Distarch Phosphate	5%	Too viscous. Good film, weak and slightly brittle. Few pieces.
C* Cream Polartex 06718*	Distarch Phosphate	5%	Too viscous. Good film, weak and slightly brittle. Few pieces.
C* Set	NIG	7.50%	Film a bit brittle. Cracks on removal. Good film.
Clinco 460	Oxidised starch	7.50%	Good film on ABS
Clineo 718	Hydroxyethyl starch	7.50%	Bits on drying (no scales). Good film. Feels elastic like gelatin. Might be a bit brittle.
Colflo 67	Waxy maize starch	3.75%	Not Removable, Bit brittle.
Crisp film	High amylose modified starch	7.50%	Thin. Cracks in small bits.
Crystal Gum S	Tapioca dextrin	7.50%	Very thin. Many scales
Dextran	Dextran	7.50%	Very thin. Very sticky.
ExPro	NIG (protein)	7.5%	Scales are formed. Very weak and very brittle film. Feels sticky.
Film forming wheat protein Isolate	Wheat protein Isolate	7.5%	Very sticky. Too soft.

FirmTex	Modified waxy maize starch	3.75%	Viscous. Good film. Brittle on quick bending.
Flojel 45	Thin boiling corn starch	7.50%	Bit viscous. Big bits can be detached but it mostly breaks on removal. Good film.
Flojel 60	Thin boiling corn starch	7.50%	Cracks on drying. Quite sticky but big bits can be detached. Non brittle
Gelatin	Gelatin blend (A/B 50/50)	7.5%	Good film.
Glucidex 2	Maltodextrin	7.50%	Breaks in big to medium scales. Not too brittle (unlike crystal gum).
HPMC 606	HPMC	7.5%	Good film.
Hylon VII	Amylose rice starch	7.50%	Scales form on drying. High level of shrinkage.
Instant Clearjel Coarse	Starch ester	3.75%	Viscous. Bend a bit. Nice film. Clear.
K4484	Tapioca dextrin	7.50%	Scales.
Kelcoloid LVF	PGA	2.00%	Viscous. Very bubbly. Good film but particles.
Kelcoloid O	PGA	4.50%	Viscous. Good film
Klucel EFF	HPC	7.50%	Soft but good film
Klucel LF	HPC	7.50%	Dispersion was too rough. Fine dispersion was obtained by reheating a cooled solution. Fine film
Klucel MF	HPC	7.50%	Dispersion as LF. Nice film
LVAWS	Acetylated wheat starch	7.50%	Sticky. Not too brittle.
LVHPWS	Hydroxypropylated wheat starch	7.50%	Bent and not too brittle.
LVOSWS	Octenyl succinylated wheat starch	7.50%	Pieces. Not too brittle.
Manucol ester ERK	PGA	3.75%	Viscous. Nice film
Manucol LB	Alginate	7.50%	Good film.
Meyproguat 7	Degraded guar gum	7.50%	Brittle. Rough surface.
Midsol 35	Modified wheat starch	7.50%	Good film.
Miracap	Octenyl succinylated starch	7.50%	Long scales. Very brittle. Yellowish.
Nadex 771	Maize starch	7.5%	Very small scales.
Nadex 8781	Potato starch	7.5%	Very small scales.
National 1900	Col swelling high amylose starch	5%	Difficult to dissolve. Cracks on drying.

N-Lite L	NIG	7.50%	Good film.
N-Lite LP	NIG	7.50%	Good film.
N-Tack	NIG	7.50%	Very brittle.
Pure Cote 760	Hydroxypropylated corn starch	7.50%	Good film.
Pure Cote 790	Hydroxypropylated corn starch	7.50%	Good film.
SOLPRO	NIG	7.5%	Long scales. Very brittle
Stadex 90	Dextrin	7.50%	Long scales. Very brittle.
Sunfiber	Degraded guar gum	7.50%	Scales.
SWP050	Soluble wheat protein	7.5%	Scales. Very brittle film.
SWP100	Soluble wheat protein	7.5%	Scales. Very brittle film.
Textra	Tapioca starch	7.50%	Good film, soft.
X98001	Pectin	5%	Very fragile

*NIG: No information given

*Scales: Film has broken into pieces of various dimensions (5mm to 100 mm) during drying

6.4. PRESELECTION

From the set of samples given above, the most promising products were selected. The criteria chosen are as follows.

1. The film should be cast from a solution, which was preparable with a concentration of at least 7.5% in water at 50°C. This is motivated by the necessity of obtaining a high solid content in capsule production.
2. The film should not form scales on drying (if only a few bits are found the product is accepted).
3. The film should not be too fragile. Some films were so fragile and brittle that they could not be cut at a relative humidity of 44%.
4. The film should be removable from the tray used for casting (ABS) at a relative humidity of 44%.
5. For series of products (see for example Flojel 45 and Flojel 60), only one of the films is selected.

Two exceptions to the rules were Kelcoloid O and Glucidex 2. Kelcoloid O was too viscous but presented very promising mechanical properties. Glucidex 2

(maltodextrin) formed small scales on drying but offered the advantage of being a very simple starch derivative with a very low viscosity.

6.5. RESULTS

6.5.1. Samples

Thirty-three samples were prepared for the sensory analysis. Four sessions were necessary for completing the assessment of all the samples. The samples used are gathered in Table 6-4. Gelatin samples were present in duplicate in each session.

Table 6-4: Samples analysed by sensory analysis.

Session	Label	Compound	Session	Label	Compound
B	290	Blanose 7 (CMC)	B	765	Gelatin TiO2 10%
B	119	C* Avatex (NIG*)	D	938	Gelatin TiO2 10%
C	059	C* Set (NIG)	A	443	Gelatin TiO2 5%
D	376	Alanate 180 (Sodium caseinate)	A	856	Glucidex 2 (maltodextrin)
B	720	Clinco 460 (oxidised starch)	B	241	HPMC 606
C	620	Clineo 718 (Hydroxyethyl starch)	C	958	Kelcoloid O (PGA)
A	435	Flojel 45 (Thin boiling corn starch)	D	953	Klucel EFF (HPC)
A	654	Gelatin (Blend A/B 50/50)	C	164	Klucel EFF
A	483	Gelatin	C	701	LVHPWS (hydroxypropylated starch)
B	801	Gelatin	D	615	Manucol LB (alginate)
B	412	Gelatin	C	603	Manucol LB
C	535	Gelatin	B	575	Meyproguat 7 (Degraded guar gum)
C	964	Gelatin	A	387	Midsol 35 (modified wheat starch)
D	674	Gelatin	A	599	N-Lite L (NIG)
D	981	Gelatin	C	860	Pure Cote 760 (Hydroxypropylated corn starch)
B	193	Gelatin Glycerol 15%	A	199	Textra (Tapioca starch)
A	592	Gelatin Glycerol 30%			

*NIG: No information given

6.5.2. Brittleness

It was observed that the brittleness (measured by bending) was a poor discriminating parameter. As shown on Figure 6-5, most of the samples had a very high score. Furthermore, any sample with a bad brittleness score would also give a bad flick score (Figure 6-6). This is however not reversible. Samples with bad flick score can present good brittleness score. The ultimate aim is to select samples with sensory

scores close to the gelatin scores. Thus, using only the flick test results for discriminating purposes simplifies the analysis without the risk of discarding good samples. The brittleness results were therefore not studied any further.

Figure 6-5: Average brittleness score and 95% confidence interval (sample name, session and label are given).

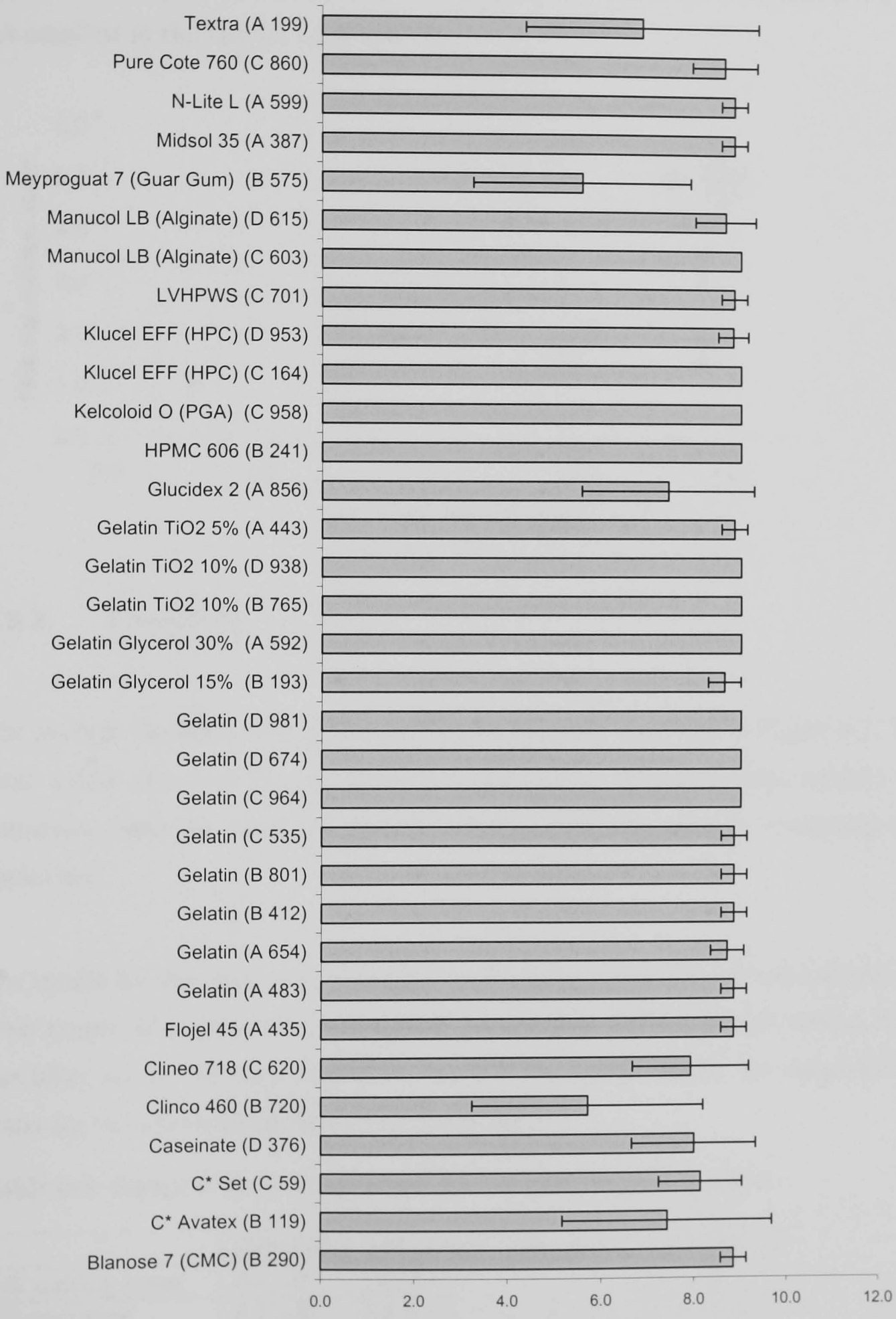
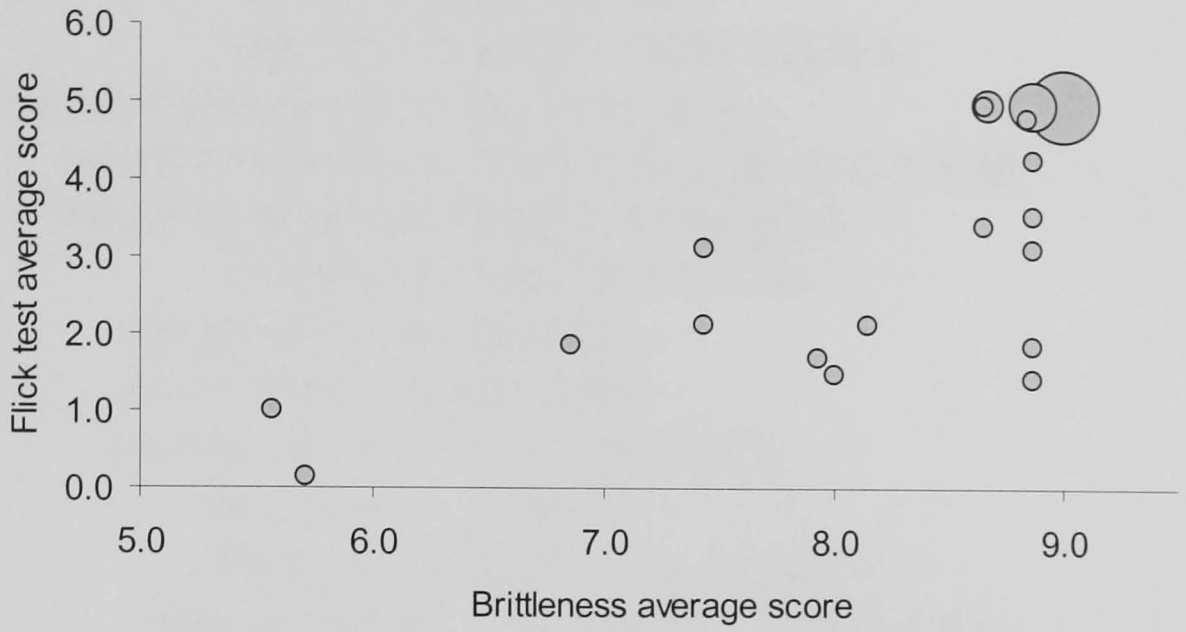


Figure 6-6: Relation between average brittleness and flick test scores. The bubble sizes represent the number of samples at each point (respectively from the smallest to the largest 1,2,5 and 10).



6.5.3. Flexibility

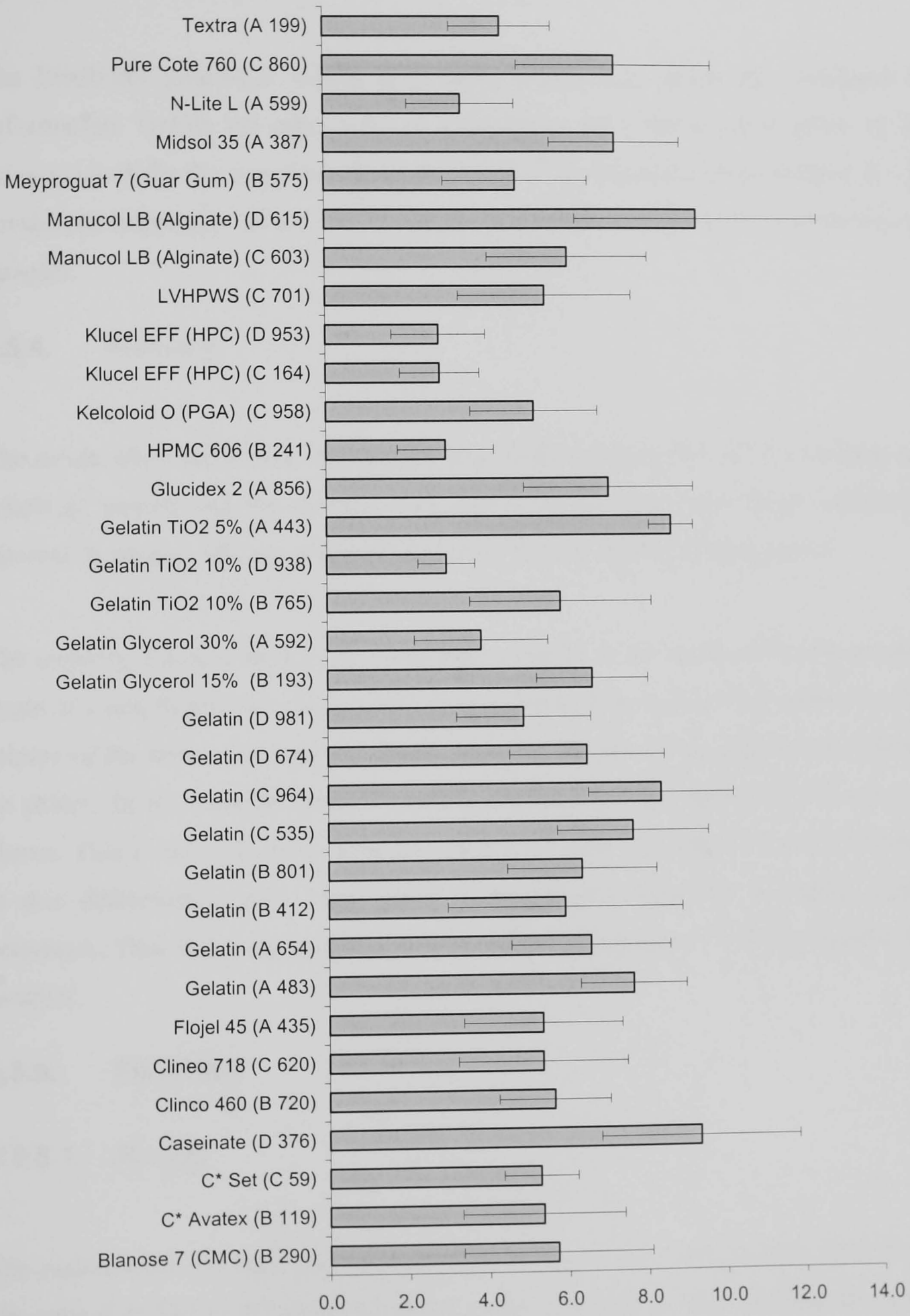
The average flexibility scores and confidence intervals are given in Figure 6-7. The large values observed for the confidence interval on all the samples confirm the comments from the panellists on the difficulty they expressed in measuring this parameter.

The results for the eight gelatin samples varied from 4.9 to 8.4. For comparison, the other ranges of average scores for gelatin are given in Table 6-5. It is clear that the flexibility results are very unreliable, and that any sample within the range 4.9-8.4 could not be considered different from the gelatin.

Table 6-5: Range of the average score for the eight gelatin samples

	Brittleness	Flick test	Memory	Flexibility
Full scoring range	0-----9	0-----5	0-----5	0-----9
Gelatin range	8.7---9	4.3---5	4.8---5	4.9---8.4

Figure 6-7: Average flexibility score and 95% confidence interval (sample name, session and label are given).



The thickness of the films were recorded during the sample preparation. Attempts to apply a correction via the thickness values proved impossible. It was also checked that the differences between gelatin samples were not due to session effects.

The flexibility parameter would give some information about the resistance to deformation. Unlike the other sensory parameters used, the score is given by an assessment of the force applied rather than a visual examination of the sample. It was considered impossible to use the flexibility as a reliable criterion for discriminating samples.

6.5.4. Memory

The results observed for the memory test are given in Figure 6-8. All the samples are relatively similar and the samples with low memory score have large confidence interval. It appears that the memory test has very little discriminating power.

The memory test described by the panellists is similar to the application of a bending strain to a sample and the observation of the remains of its deformation state after the release of the stress. For a purely elastic material, the remaining deformation should be absent. In the samples used, the results indicate that the materials behaviour is elastic. This is not surprising since the strain levels are very small (about 0.4%) and at this deformation level most glassy material would present a highly elastic behaviour. Thus it is not possible to use the memory parameter for discriminating the samples.

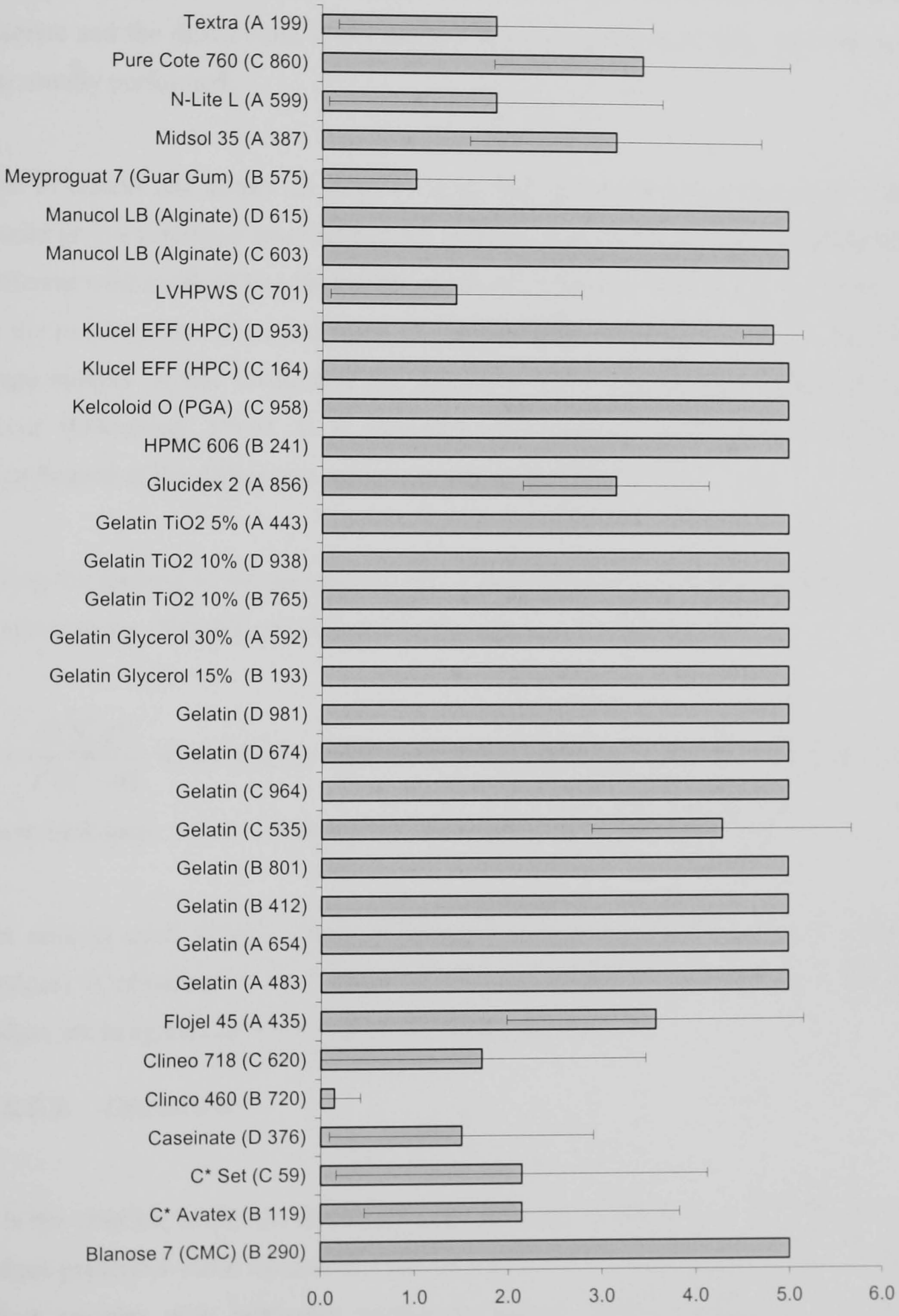
6.5.5. Flick test

6.5.5.1. Results

The results from the flick test are presented in Figure 6-9. The average score from the gelatin C 535 is 4.3 compare to the usual value of 5. This resulted from the average of six scores of 5 and one score of 0. This last score is likely to be due to a sample with a defect such as a notch.

Figure 6-8: Average memory score and 95% confidence interval (sample name, session and label are given).

Figure 6-9: Average flick test score and 95% confidence interval (sample name, session and label are given).



6.5.5.2. Statistical significance

The average scores shown in Figure 6-9 showed large differences. An analysis of variance cannot be used here because the data are not parametric: the points are discrete and the distribution is not normal. For comparing such data, tests on ranks are usually performed.

The Friedman test allows an ANOVA type test on a table of ranked results. This would give a Friedman test value of 91, which means that samples are significantly different with a risk of less than 0.1%. However, a large number of ties are observed in the ranking. This is logical since 33 samples were scored from 0 to 5. This very large number of ties invalidates the Friedman test, which supposes that few ties occur (O'Mahony 1986). It is therefore not possible to measure a statistical significance of the differences within the whole set of data.

Using the ranking of the samples scores, it is possible to calculate the coefficient of concordance c (Kendall coefficient of concordance). c is defined by

$c = \frac{12\sum d^2}{j^2(n^3 - n)}$ where d is the difference between the rank total of a sample and the mean rank total, n is the number of samples and j the number of judges.

On ranking each judge's score, a measure of the agreement between assessors (judges) is obtained. A coefficient of 0.48 was obtained. This indicates that the judges are in agreement with a risk lower than 1%.

6.5.5.3. Discussion

It is not possible to discuss statistically the differences within the set. However, the judges presented some agreement. The ultimate aim of the sensory analysis was to select samples with behaviour similar to gelatin. Although there is a lack of statistical evidence it is still possible to detect the samples similar to gelatin.

Some of the samples measured by flick tests gave the same average value than the gelatin (maximum score of 5). These samples are adulterated gelatins (with TiO_2 or glycerol), the cellulose derivatives (HPMC, HPC, CMC) and the alginate and derivatives (Manucol LB and Kelcoloid O). The other samples are listed in Table 6-6.

Table 6-6: Average flick score of samples with score less than 5.

Compound	Flick average score	Confidence interval
Flojel 45 (A 435) (thin boiling corn starch)	3.6	1.6
Pure Cote 760 (C 860) (Hydroxypropylated corn starch)	3.4	1.6
Midsol 35 (A 387) (modified wheat starch)	3.1	1.6
Glucidex 2 (A 856) (maltodextrin)	3.1	1.0
C* Set (C 59) (NIG*)	2.1	2.0
C* Avatex (B 119) (NIG)	2.1	1.7
Textra (A 199) (Tapioca starch)	1.9	1.7
N-Lite L (A 599) (NIG)	1.9	1.8
Clineo 718 (C 620) (Hydroxyethyl starch)	1.7	1.7
Caseinate (D 376)	1.5	1.4
LVHPWS (C 701) (hydroxypropylated starch)	1.4	1.3
Meyproguat 7 (B 575) (Degraded Guar Gum)	1.0	1.0
Clinco 460 (B 720) (oxidised starch)	0.1	0.3

6.6. CONCLUSION

The use of sensory analysis for assessing film properties has not been well documented in the past. It was very useful for describing terms and tests that can be used in the future. Of the four tests, the flexibility parameter was very unreliable. The memory and brittleness tests had very little discriminating power. The flick test was the most useful in separating the gelatin samples from the other samples. The use of the flick score allowed the classification of the various samples measured by sensory analysis. Some samples had scores very similar to gelatin. The judges were in significant agreement.

The ultimate aim was to choose samples with promising characteristics. The choice of the polymeric materials to be studied further was made in conjunction with the sponsor. The criterions included the sensory analysis results, the viscosity but also other considerations (e.g. sample already studied by the sponsor).

The obvious candidates were the cellulose derivatives and the alginate and derivatives. HPMC is already used in capsule production and the use of other cellulose derivatives would not be very innovative. Alginate derivative (PGA) were not studied further due to their very high viscosity (intrinsic viscosity of 397 ml/g). Lower viscosity propylene glycol alginate (PGA2) was not as promising. The low viscosity alginate sample was very promising. Although viscosity and dissolution might become problems, they were chosen for further studies.

Among the other types of product, none had an average flick score of 5. Some starch derivatives might be interesting for further studies (Flojel, Pure Cote, Midsol, Glucidex). Some of these samples are studied by the sponsor.

Although the caseinate sample (Alanate 180) had a very low flick score, it was chosen for further studies because of its very low viscosity. Its protein nature would also allow more possibility for modifications (pH, ions, chemical reaction).

CHAPTER 7. ALGINATE FILMS

7.1. INTRODUCTION

Amongst the samples used for sensory analysis, alginate was very promising. The study of the mechanical properties of alginate films in various conditions was therefore carried out.

7.2. EFFECT OF IMPURITY LEVEL

The alginate used in the study (Manucol LB) was food grade alginate without further purification. It was noticed that the solutions obtained were brown and unclear (opaque). It was also observed that alginate films prepared from these solutions had a slightly rough surface. The presence of small particles would lead to such effects and would also change the mechanical properties of the resulting films. Using centrifugation, such particles could be removed and the supernatant can be used.

7.2.1. Puncture tests

Various centrifugation times were used for the purification. Centrifugation at 17000g of a 5% alginate solution was performed before casting. The results from the puncture tests are shown in Figure 7-1. Up to one hour of centrifugation, the film properties are strongly affected. The distance at break increases linearly with the centrifugation time. T-test with a risk of 5% showed no difference for the distance at break between 60 and 100 minutes of centrifugation. The same observations were made for the force at break. This was expected since at constant gradient the force at break is proportional to the distance at break.

For the gradient graph (Figure 7-1), no clear pattern was observed. The very bad confidence interval observed on the non centrifuged sample is explained by the

difficulty in obtaining the gradient (distance from 2 to 2.5mm) when fracture occurs at about 2.5mm. T-tests results for all gradients are shown in Table 7-1. Although some samples are different, the data are difficult to interpret. The gradient seems to reach a minimum for a centrifugation time of 60 minutes.

Table 7-1: T-tests results with a risk of less than 5% for the gradient measured by puncture tests on alginate films obtained from solution centrifuged for different times. D indicates samples that are different.

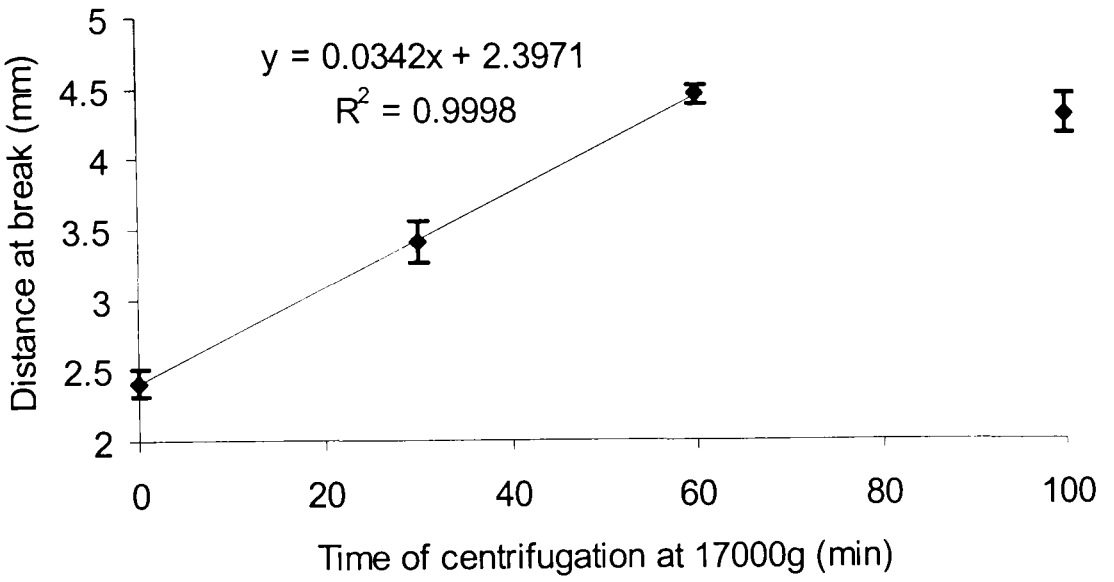
	0 min	30 min	60 min	100 min
0 min			D	
30 min			D	D
60 min				D
100 min				

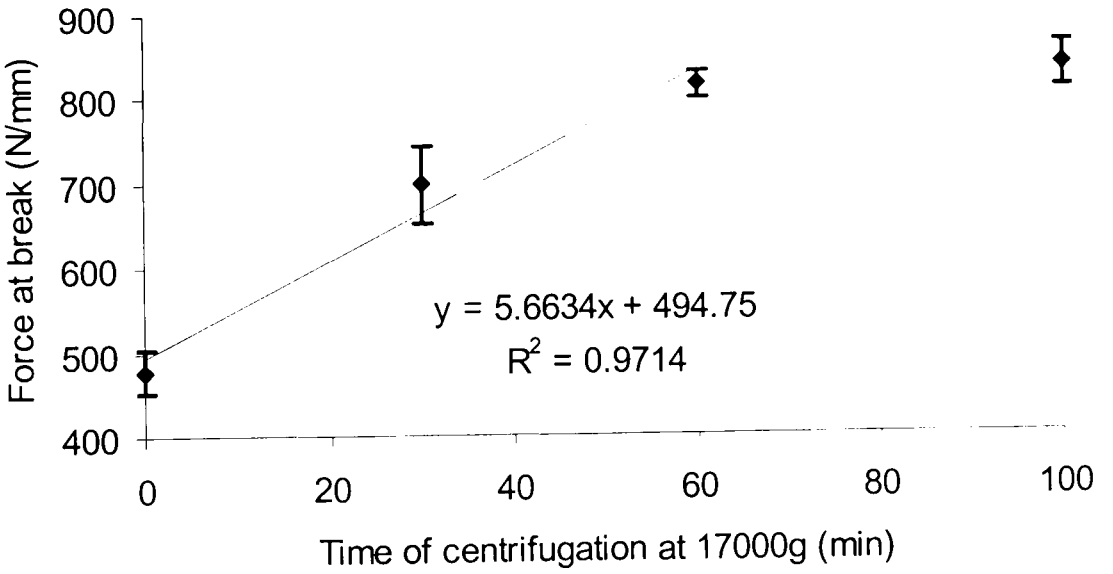
7.2.2. Particle size analysis

The assumption that particles were removed from the alginate solutions by centrifugation was checked using particle size analysis. Centrifuged and non centrifuged solutions were used for the comparison of particle size distribution.

The particle size distribution is obtained in volume percentage. Because the refractive index of the particles themselves is unknown, the calculations that lead to the determination of the sizes (diameters or volumes) are biased. Nevertheless, as seen in Figure 7-2, the shift of the distribution towards smaller particles is obvious. Since the particles present in the centrifuged samples were present in the original sample, it was possible to calculate the minimum amount in volume, of particle discarded during centrifugation. We found that at least 95% of the original volume of particle was discarded by centrifugation.

Figure 7-1: Effect of centrifugation time on mechanical properties measured by puncture test for alginate films (average and 95% confidence interval as defined in Figure 4-6).





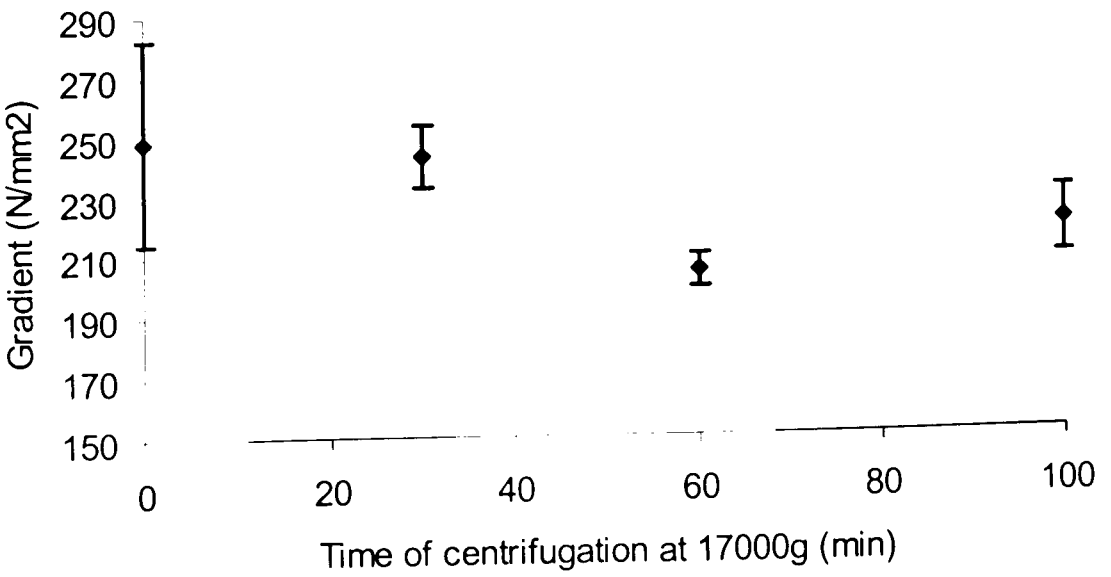
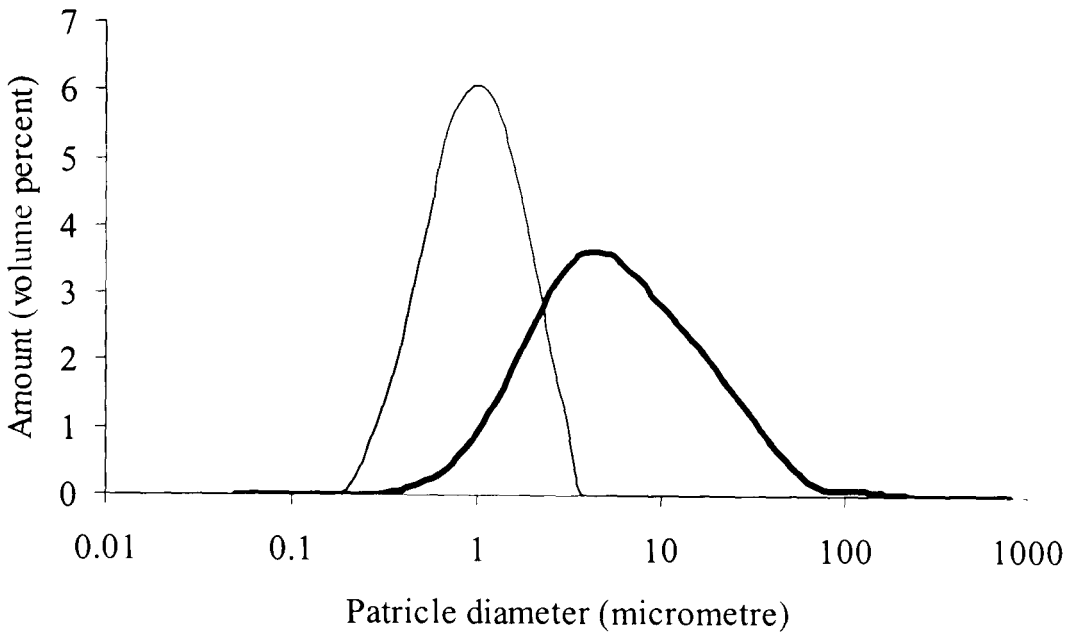


Figure 7-2: Particle size distribution for raw (thick line) and centrifuged (thin line) alginate solutions (one hour, 17000g, 4°C).



7.2.3. Discussion

The project is based on using real food products readily available and preferably economically viable. The results obtained here with food grade alginate show the problems that might be encountered and the necessary purification steps for improving the mechanical properties.

The effect of centrifugation on the particle size distribution is obvious. As the density of the particles is slightly higher than the solvent density, sedimentation occurs. Bigger particles are removed first and the resulting solution is mostly depleted in the bigger particles. The solution has therefore fewer particles (total amount in volume) and on average, the particles are smaller.

The possible decrease in gradient with decreasing particle size and volume could be explained using the blending laws. The isostress model would be applied here since the particles (filler) are likely to be harder than the surrounding matrix. This leads to the weight average of the compliance of each phase. It is indeed expected that the material will then show a greater resistance to deformation at low deformation and hence a higher gradient when particles are present. Such models are commonly applied for synthetic polymer blends (Kolarik 1994; Kolarik 1996) and biopolymer gels (Morris 1992).

Flaws are known to be a major source of fracture initiation (Kolarik 1994). The increase of the distance at break with decreasing particle content is therefore not surprising. Both the quantities of particles and the size of the particles can influence the fracture limit or distance at break. The more particles, the more likely a particle is to be the source of crack initiation and propagation. The particle size is also important since the larger particles will generate locally very high stresses, which are likely to originate fracture.

This shows that the adhesion between the particles and the polymer is rather bad leading to early fracture. If good adhesion was present, then the filler should have an extremely low distance at break. This was shown for thermoplastic blends (Kolarik 1994).

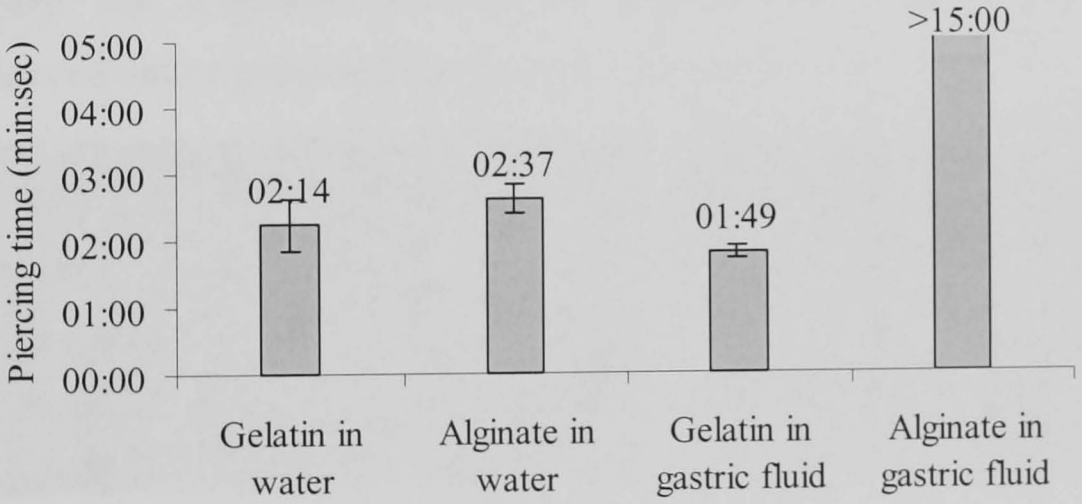
When notches or flaws are inserted into a material, the fracture properties vary with the flaw size above a minimum size. This minimum value corresponds to the inherent flaw size. The inherent flaw size is the size of the flaws in the materials under stress before fracture and might correspond to the larger crazes present prior fracture (Kinloch and Young 1983). When bad adhesion between filler and matrix occurs, filler particles can be considered as flaws. It could then be argued that inherent flaw sizes in alginate films are between one and ten micrometer. We must nevertheless be cautious because the amount of particles present in centrifuged and raw samples are very different. However, introducing known amount of particles with known particles size distribution might give an insight into inherent flaw size and fracture properties of biopolymer films.

In order to get rid of the particles present in the alginate films, all subsequent alginate samples will be centrifuged at 17000 g for one hour.

7.3. SOLUBILITY OF ALGINATE FILMS

The solubility of alginate films was measured using the piercing test. The results are presented in Figure 7-3. The two samples in water are not significantly different ($p<0.05$) but the gelatin piercing time is shorter in gastric fluid than in water. Alginate piercing time in gastric fluid is higher than that of gelatin. This was expected since alginate solution precipitate in an acid environment. This proves that alginate can not be used as such for gelatin replacement since the capsule might not dissolve at all in the stomach. However, it could have some potential use for late drug delivery if the 'stomach treatment' is not irreversible.

Figure 7-3: Piercing time of gelatin and alginate film in water and gastric fluid.



7.4. MICROSCOPY OBSERVATION

The alginate films produced were slightly brown and not fully transparent. It was noticed that unlike gelatin and HPMC films, no effect on polarised light was obtained from most of the punctured films. This could not be attributed to the colour of the film since when gelatin films were placed under an alginate film, the colour was still observable (Figure 7-4). It was also observed that under tension, alginate films showed clear polarised effect indicating the internal orientation of the film structures during deformation (Figure 7-5). This gives strong evidence that radial

orientation is kept to a lesser extent in alginate films than it is in HPMC or gelatin films.

Figure 7-4: Films after puncture observed under polarised light. Alginate without polarised effect (A) above gelatin (G), gelatin and scale (mm).

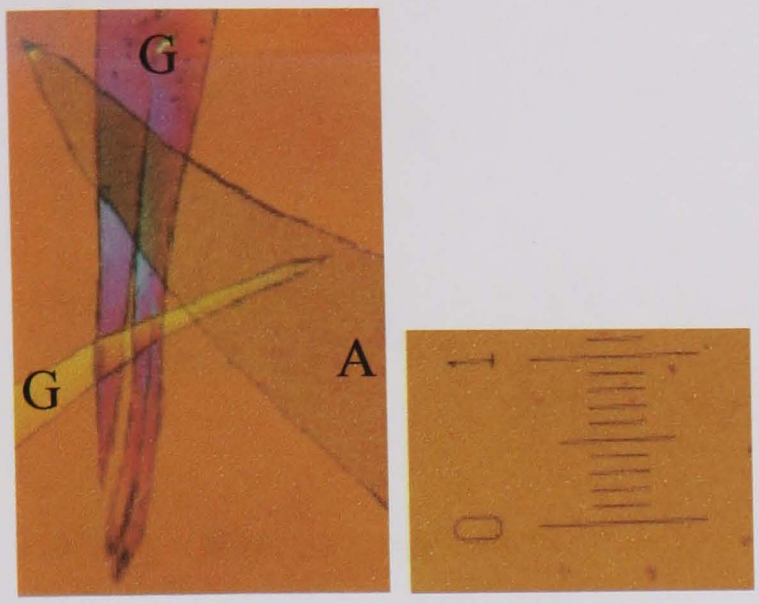
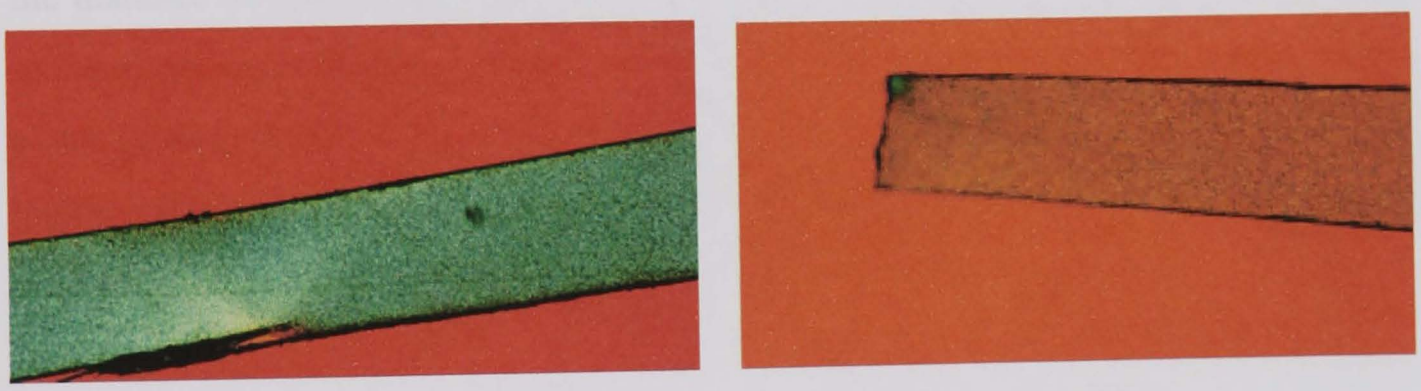


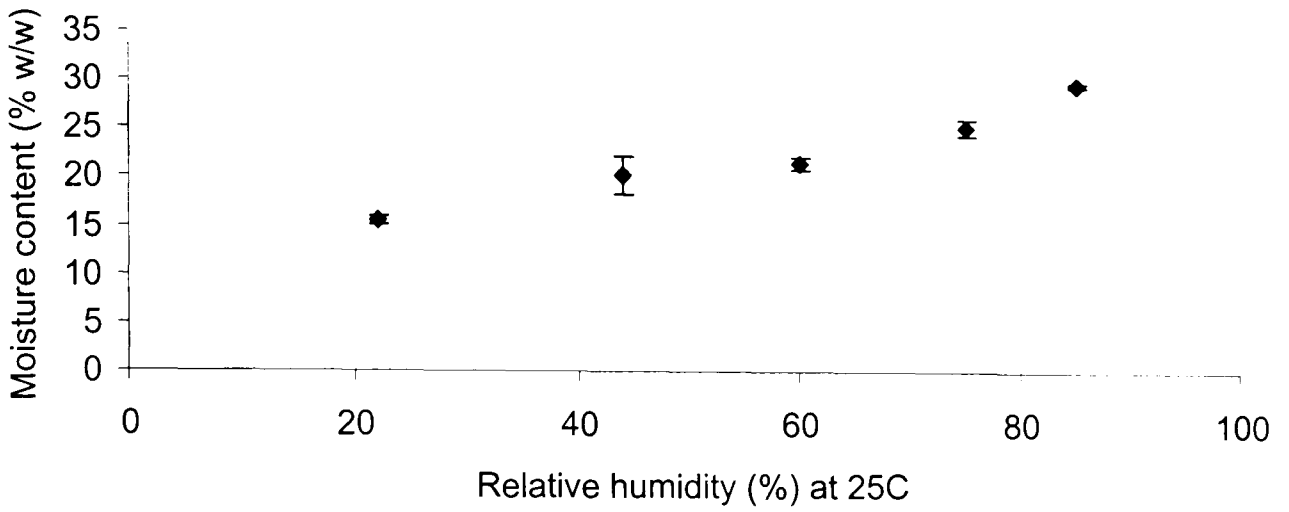
Figure 7-5: Alginate films during tension (left) and after fracture (right) observed under polarised light (scale as above).



7.5. RELATIVE HUMIDITY AND MOISTURE CONTENT

Alginate films were stored at five relative humidities and the moisture contents and mechanical properties were measured. The sorption isotherm is given in Figure 7-6. Alginate moisture content is higher than gelatin and HPMC for all relative humidities studied.

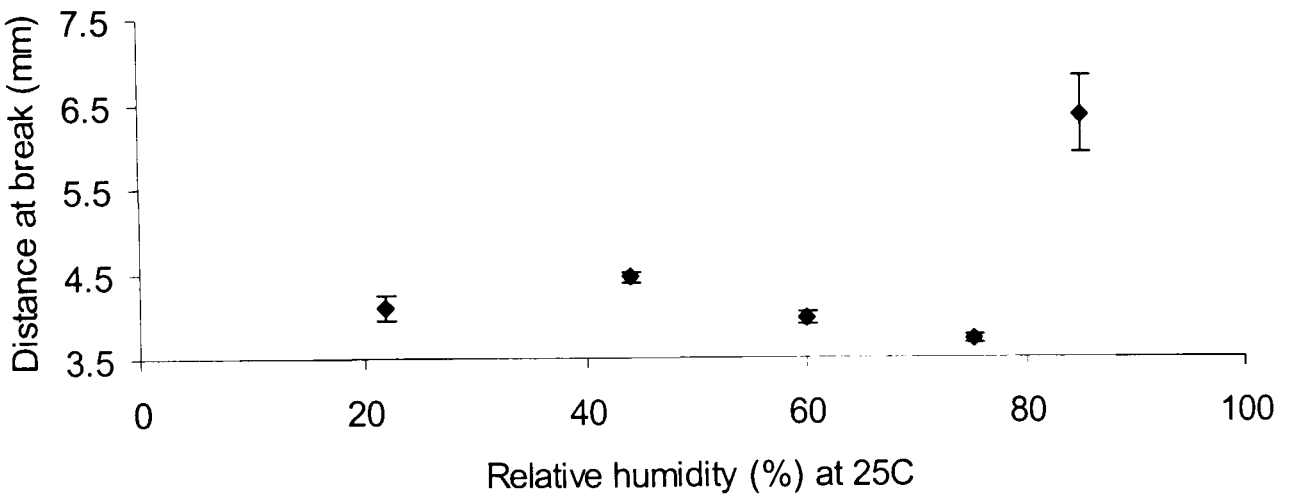
Figure 7-6: Sorption isotherm of alginate (Manucol LB) film.

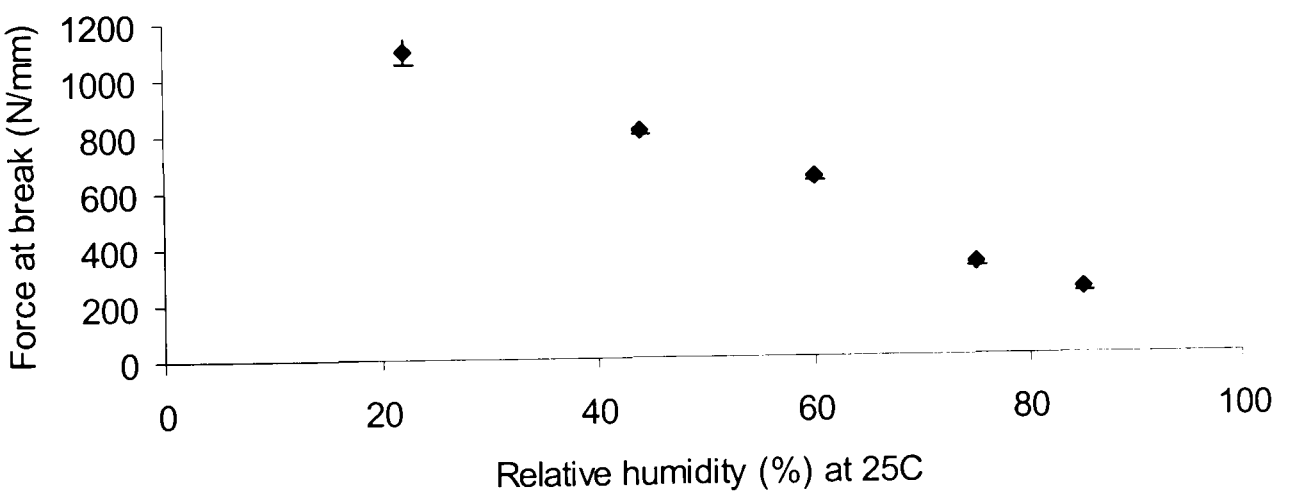


7.5.1. Relative humidity

By altering the environmental conditions, the water activity and the moisture contents of the films were modified. The mechanical properties of these films were measured. The results obtained are shown in Figure 7-7. At high relative humidity, the distance at break increases sharply. Gradient and force at break are significantly reduced on increasing the relative humidity. This is likely to be due to the large water uptake resulting in a greater plasticisation. It is clear that alginate films are highly affected by relative humidity and that like gelatin films, they must only be used in controlled relative humidity environment.

Figure 7-7: Effect of relative humidity on the mechanical properties of alginate films (average and 95% confidence interval as defined in Figure 4-6).





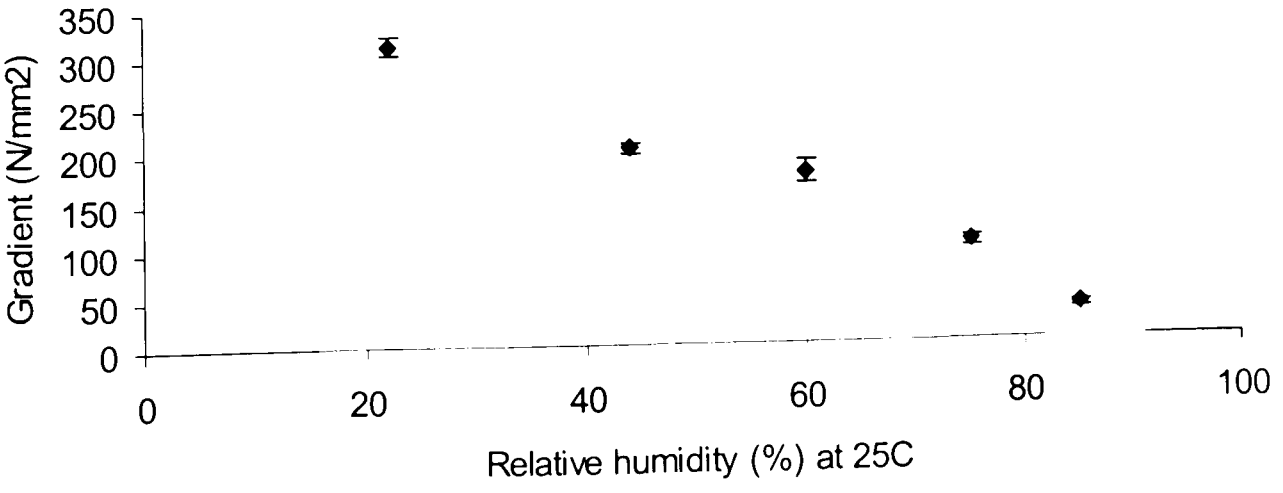
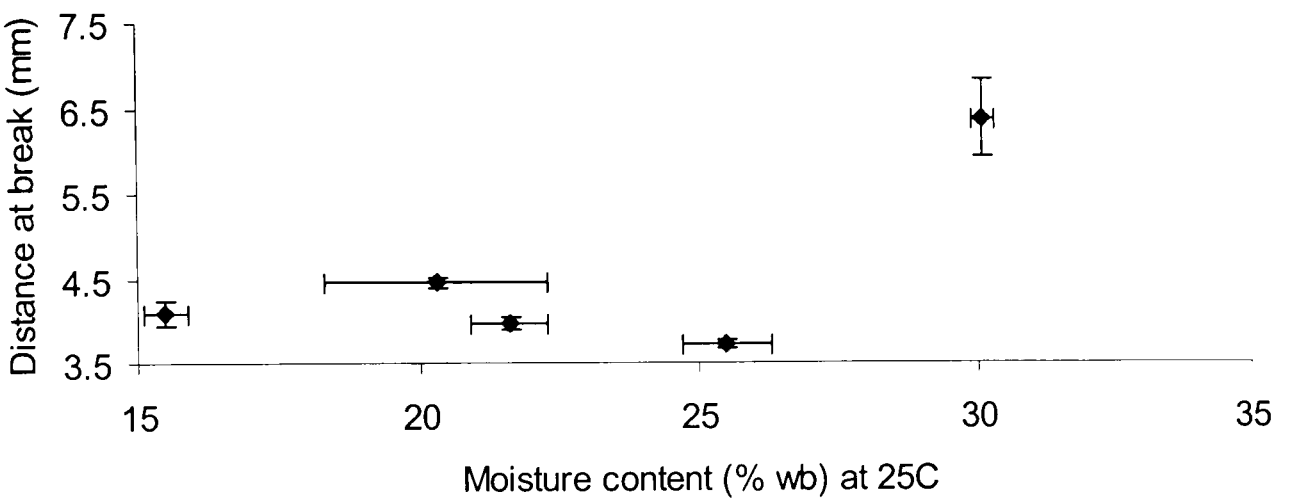
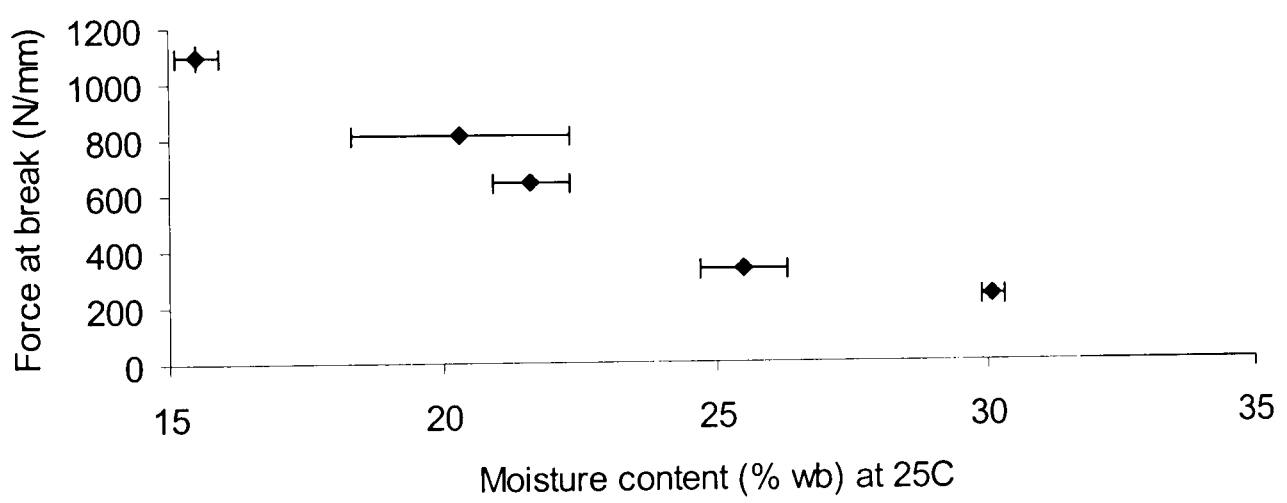
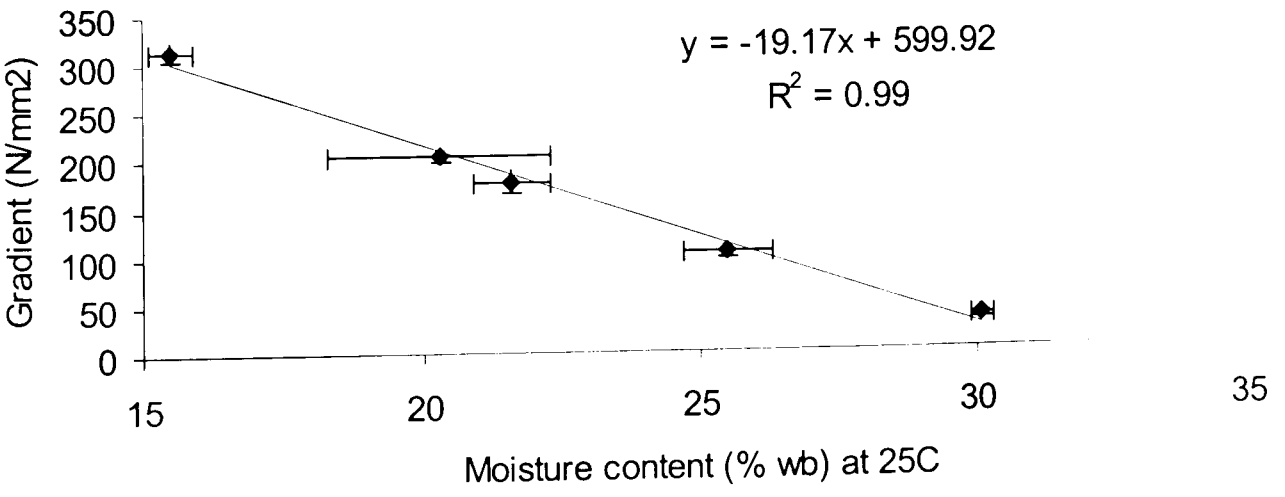


Figure 7-8: Effect of moisture content on distance at break for alginate films (average and 95% confidence interval as defined in Figure 4-6).







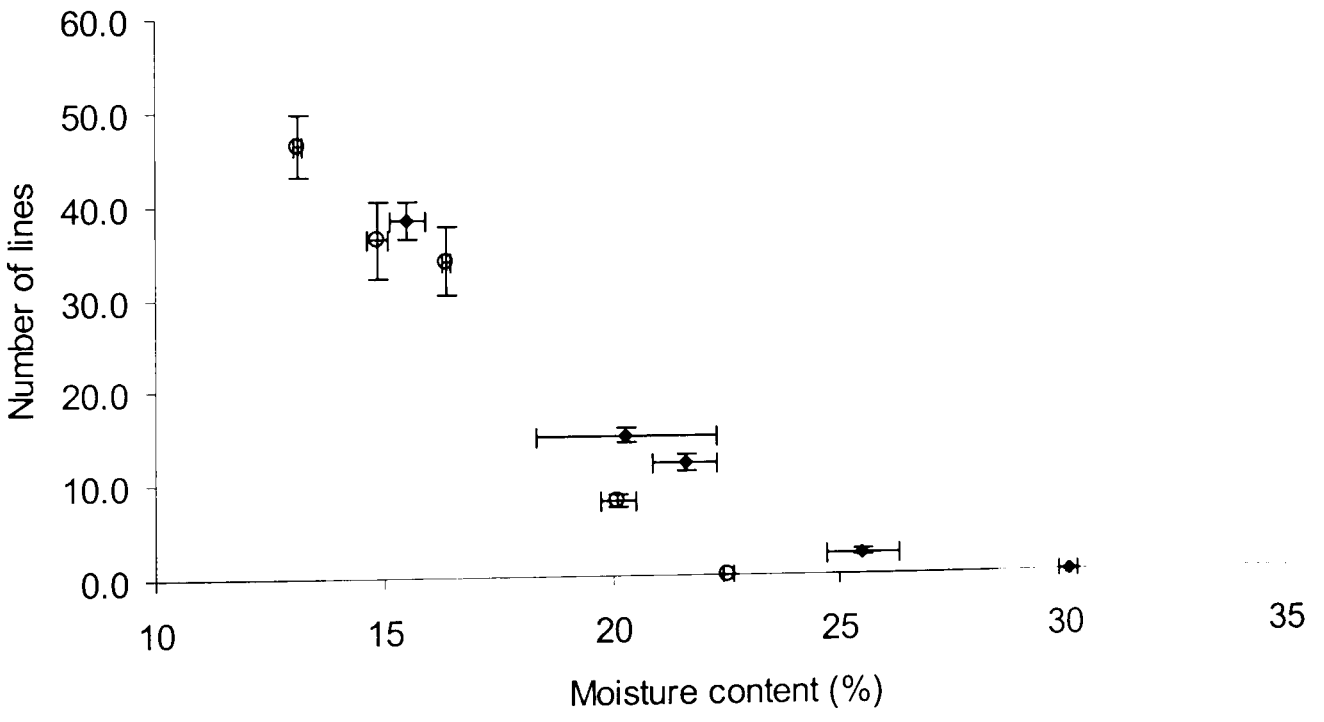
7.5.2. Moisture content

If relative humidity studies are relevant for understanding the behaviour of the films in real conditions, the moisture content is the factor that should be correlated to mechanical property changes. The effect of moisture content on the mechanical properties obtained by puncture on alginate films is shown in Figure 7-8. The distance at break increases at large moisture content and the gradient varies linearly with the moisture content. Zero moisture content gradient (G_0) and the relative effect of the moisture content on the gradient (G_1) are respectively 600N/mm^2 and 3.20%.

7.5.3. Fracture behaviour

The number of lines measured after puncture was very dependent on the moisture content of alginate films and slightly higher than for gelatin films at similar moisture content (Figure 7-9).

Figure 7-9: Effect of moisture content on number of fracture lines for alginate films (diamond) compared to gelatin (circle).



7.5.4. Discussion

The changes in mechanical properties of alginate films observed on varying the moisture content behave similarly to gelatin films. The distance at break increases at large moisture content and G_1 values are similar for both polymers (3.20% and 3.10%). Furthermore, the number of lines reaches high values at low moisture content. Although the absolute values do not match, the effect of relative humidity and moisture content are rather similar indicating some similarity in the behaviour of both films. This will be discussed later in this chapter.

7.6. MOLECULAR WEIGHT

Alginate samples are available at various viscosities hence molecular weights. The effect of the molecular weight on the mechanical properties of the films was considered here.

7.6.1. Alginate degradation

Alginate depolymerisation was followed by rotational viscosimetry at $10s^{-1}$. The viscosity decreased with time following an exponential decay (Figure 7-10). The samples selected were then cast for film mechanical property analysis. The viscosities of the samples are given in Table 7-2.

Figure 7-10: Viscosity changes measured at a shear rate of $10s^{-1}$ during the degradation of alginate (7.5%) at 75°C.

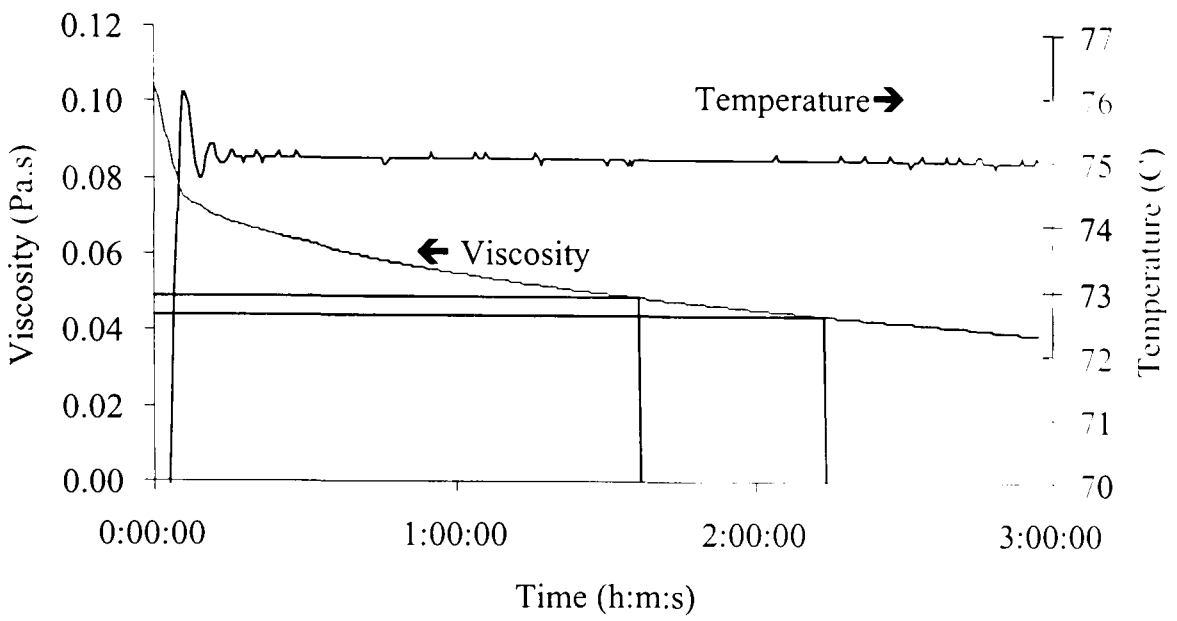


Table 7-2: Viscosity of 7.5% alginate samples during degradation

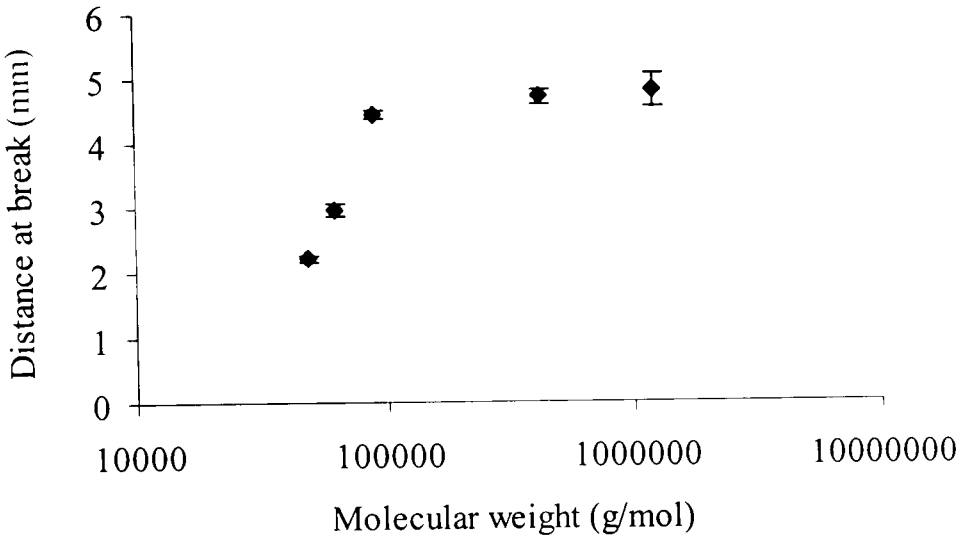
Sample	Degradation time (h:m:s)	Viscosity at 75°C, 7.5% measured at $10s^{-1}$ (mPa.s)
Manucol LB	0	72.2
Degraded 1	1:30:00	48.9
Degraded 2	2:15:00	29.9

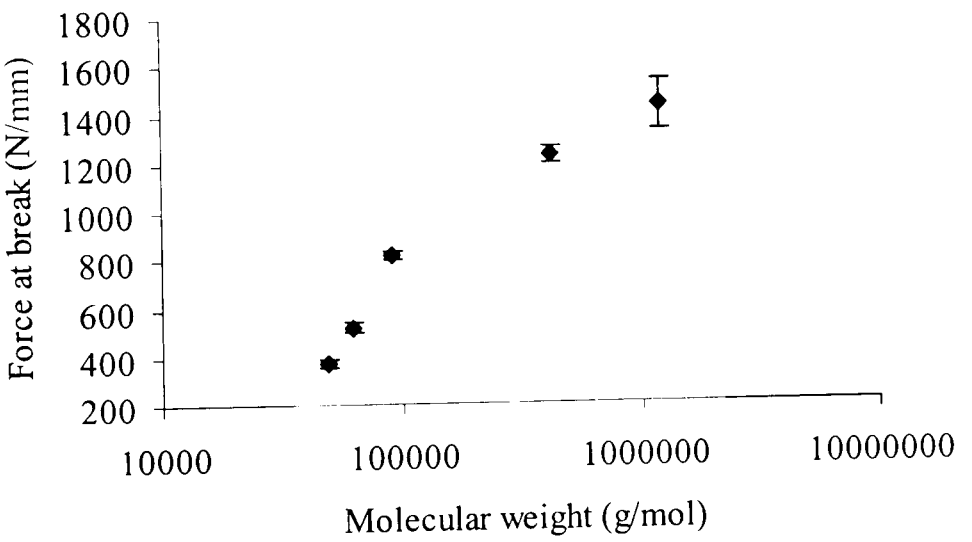
7.6.2. Mechanical properties

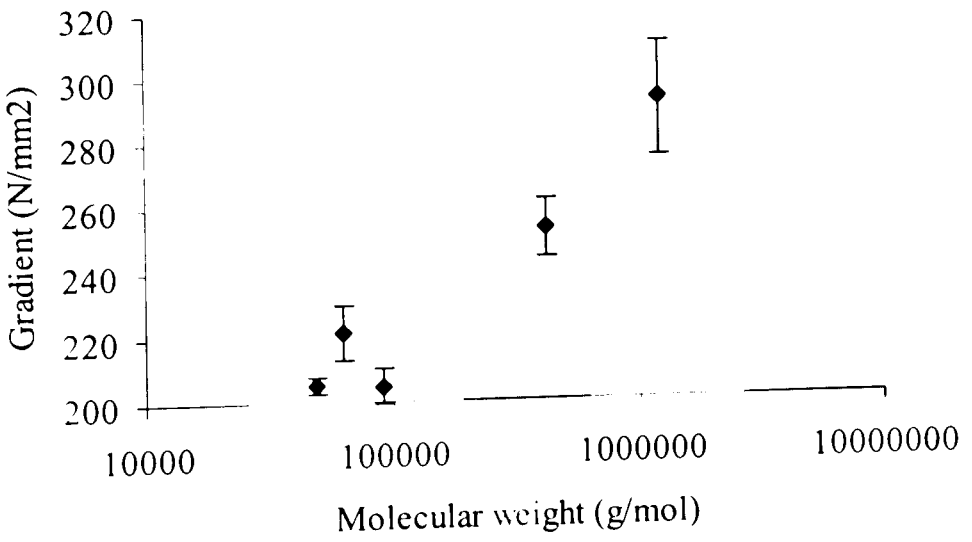
As discussed earlier (4.2.3), the intrinsic viscosity and molecular weight used here are approximate. Nevertheless, the order of magnitude for the molecular weight is likely to be correct. The mechanical properties are plotted against the molecular weight of various alginates (Figure 7-11). The use of a logarithmic scale further reduces the possible errors.

The distance at break increases with increasing molecular weight until a certain threshold. Beyond this value, the increase is very small. The gradient increases linearly with the molecular weight and the force at break represents a combination of two previous variables.

Figure 7-11: Effect of molecular weight on the mechanical properties obtained by puncture on alginate films at a relative humidity of 44% (the gradient before break was used here for Degraded 2 sample because fracture occurred before 2.5mm) (average and 95% confidence interval as defined in Figure 4-6).







The number of lines was counted for these samples (Figure 7-12). A clear effect was observed. Also a high correlation was obtained between the number of lines and the gradient (Figure 7-13).

Figure 7-12: Effect of molecular weight of alginate on the number of lines obtained from puncture tests.

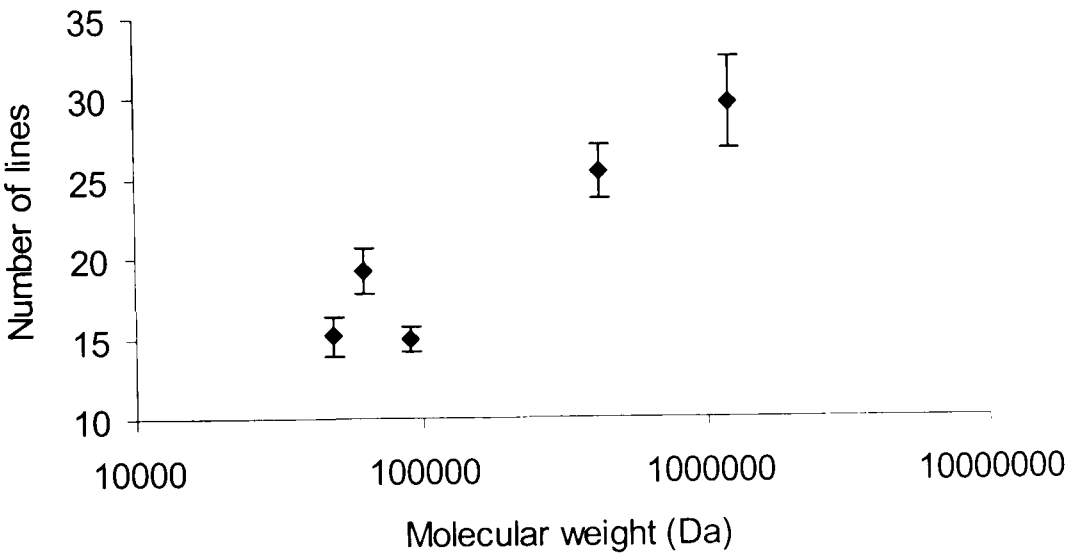
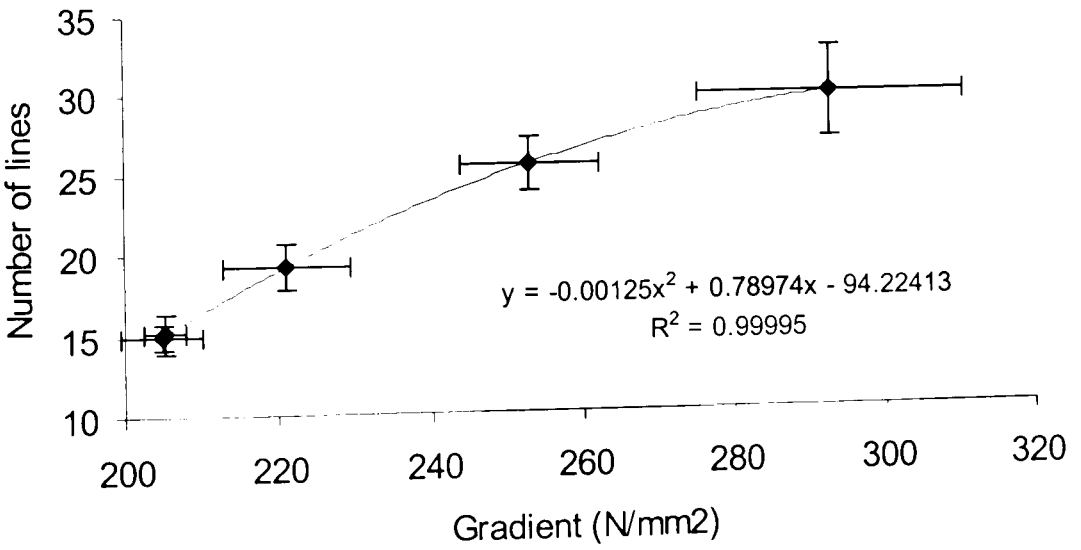


Figure 7-13: Effect of gradient of alginate of different molecular weights on the number of lines obtained from puncture tests.



7.6.3. Comparison with cellulose derivatives

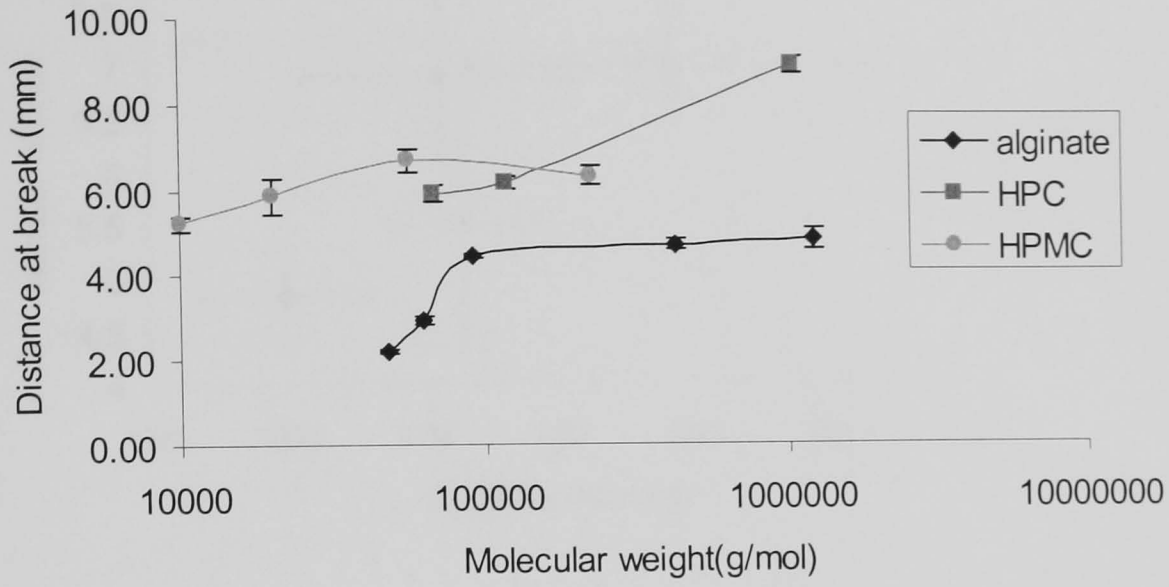
The same experiments were performed with cellulose derivatives HPMC and HPC. The results are shown in Figure 7-14. Both cellulose derivatives behave differently in terms of distance at break. HPMC seems to present a threshold value whereas the distance at break for HPC increases up to the higher molecular weight studied.

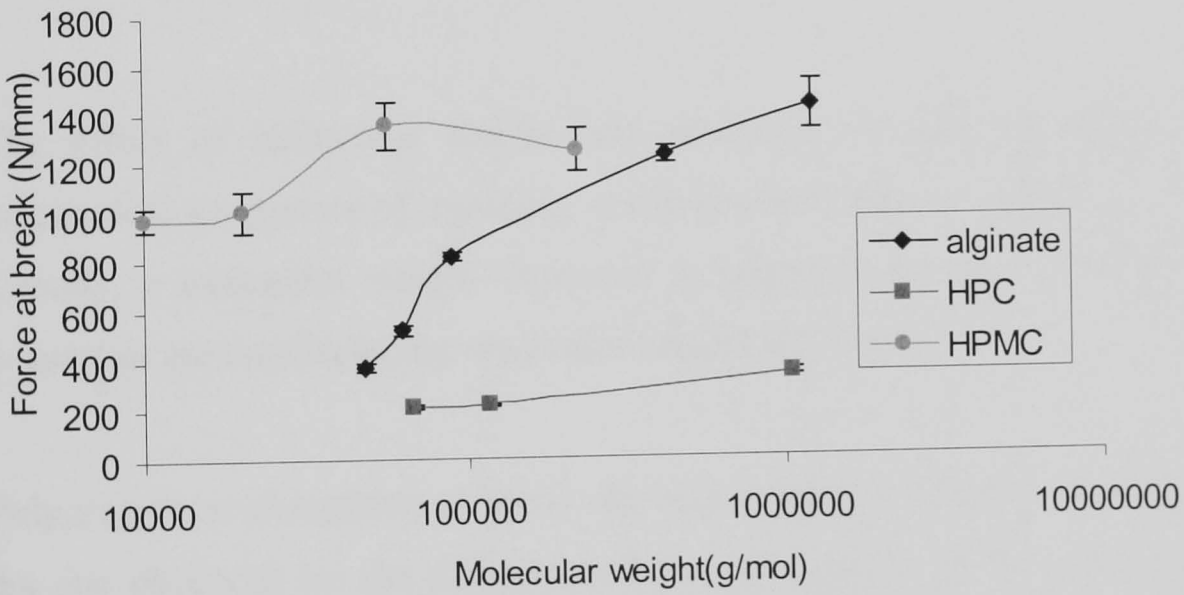
The gradient of the HPMC is almost independent of the molecular weight. For HPC, the high molecular weight sample presents a much lower gradient than the two others do. Both behaviours differ significantly from the alginate samples.

The results from HPMC resemble the data obtained for HPMC (RemunanLopez and Bodmeier 1996) or methycellulose (Park et al. 1993) using tensile tests. However, Park et al. (1993) found that HPC presented a maximum for the elongation at break.

The force at break increases with molecular weight for all samples. The correlation observed for alginates between the number of lines and the gradient (Figure 7-13) might still exist for HPMC samples but the range of gradient covered and the noise in the data makes this point arguable (Figure 7-15).

Figure 7-14: Effect of molecular weight on mechanical properties for various biopolymer films (arrows indicate which axis is used) (average and 95% confidence interval as defined in Figure 4-6).





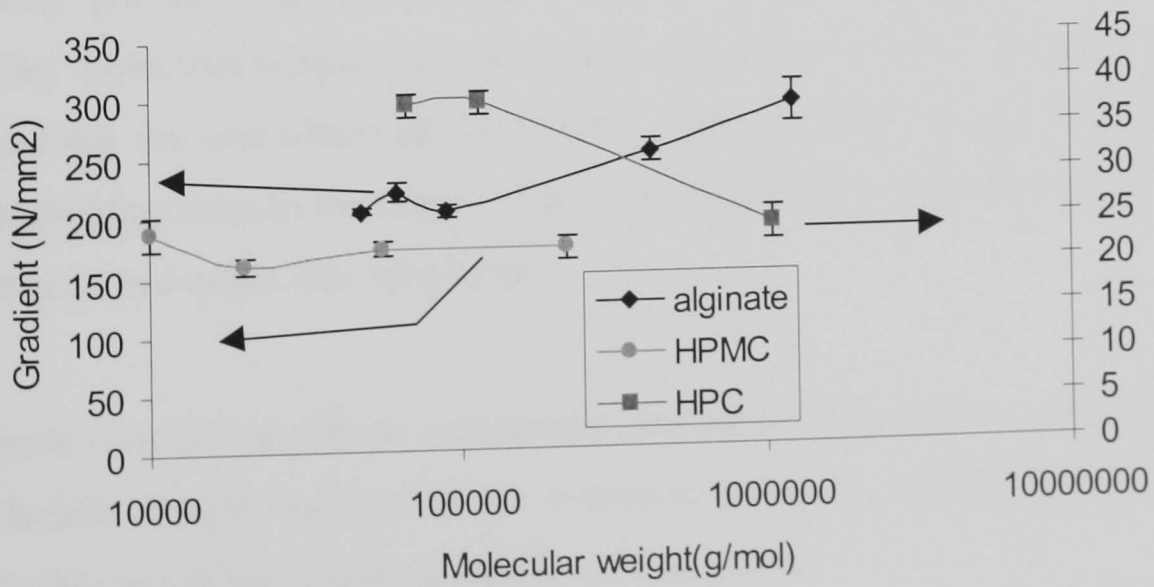
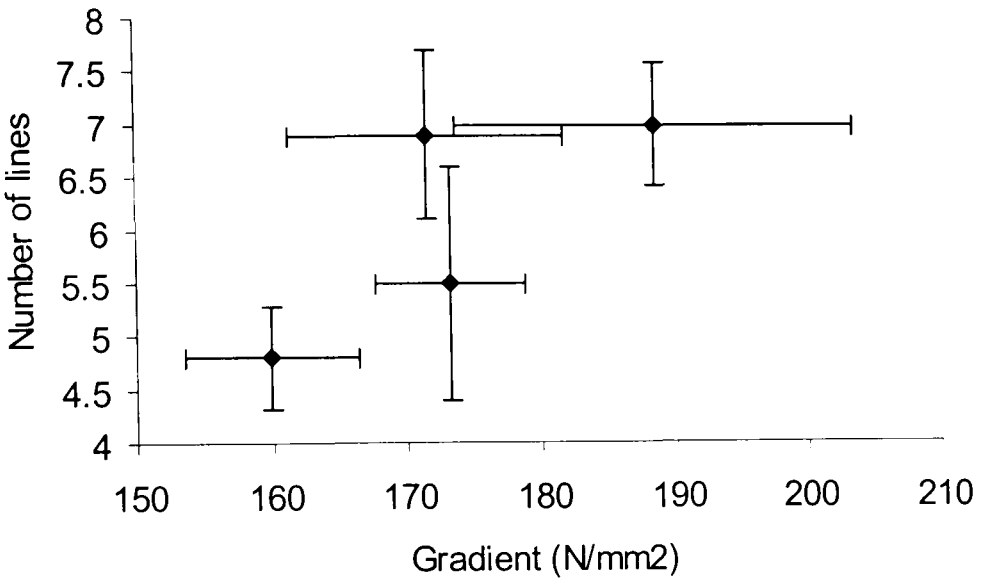


Figure 7-15: Effect of gradient on the number of lines for HPMC samples of different molecular weights.



7.6.4. Discussion

The effect of molecular weight and molecular weight distribution on various mechanical properties of synthetic polymers has been reviewed by Nunes (1982). Usually, a molecular weight threshold is observed beyond which the mechanical properties are unaffected by molecular weight increases.

Polypropylene elongation at break showed a threshold molecular weight similar to the one observed for the distance at break of alginate. In the case of HDPE (High density polyethylene), a maximum for the ultimate elongation is observed, and further molecular weight increase leads to the decrease of this parameter. This effect resembles the one observed for HPMC. Limited chain slippage and limited chain straightening (due to the large entanglement density) at high molecular weight were proposed to explain this behaviour.

Tensile moduli have been extensively studied as functions of both draw ratio and molecular weight distribution for synthetic polymers. In the following discussion, only the case of non drawn materials will be given.

PMMA (polymethylmethacrylate) samples of various molecular weights show little difference in their modulus in the glassy state. However, the glass transition temperature increased with the molecular weight. For polystyrene and polyurethane, low strain moduli were also independent of molecular weight whereas at high strain, the moduli were a function of molecular weight and molecular weight distribution. Decrease in modulus was observed for PVC (Polyvinylchloride) on increasing the molecular weight. This was explained as a secondary effect due to structural changes resulting from the creation of a two-phase system (crystalline and amorphous). Overall, increasing the molecular weight leads to increases of large strain moduli.

For HPC and HPMC, such increases are not observed. It can be hypothesised that the very large molecular weight of HPC prevents proper alignment of the chains leading to a decrease of the gradient. However, the films were prepared from diluted solutions and alignment during drying should be possible. A block structure for HPC could also explain these behaviours. The high molecular weight HPC could form very large packed areas or 'junction zones' areas due to their high molecular weight. These blocks could align on drying. The only slippage possible during elongation would be between the HPC blocks rather than the HPC molecules explaining the lower gradient observed. The distance needed to separate two blocks would be proportional to molecular weight and increase of the distance at break would then be observed.

For alginates, the results resemble more closely synthetic polymer behaviour. This might easily be explained on the basis of increasing entanglement density (or anchor point through junction zones via residual calcium). When the molecular weight is high enough, entanglement prevents early break and chain stretching prevents fracture. At higher entanglement density, stretching is harder and the gradient increases. The increase of the number of lines with the molecular weight support the idea that with increasing entanglement density, more energy is stored during deformation leading to formation of more fractured surface.

The observation made with polarised light show the radial orientation around the crack centre. As the distance from the centre increases, the stress experienced by the

film is decreasing and therefore the radial orientation during puncture decreases. It is expected that under strain, the molecules must stretch, uncoil or slip so that the deformation takes place (Kinloch and Young 1983). Stretching and uncoiling should be reversible but slippage might deform the sample permanently. For cellulose and gelatin, it was shown that remains of this phenomenon were present in the fractured sample. For alginate, the absence of visible polarisation effect implies that uncoiling and stretching are the main events occurring. This is understandable on the basis of the junction zones and the high hydrogen bonding ability of the alginate which both would limit slippage.

The two models presented here are highly speculative. However, the differences in behaviour are consistent with the differences in molecular structure. Cellulose derivatives are more hydrophobic allowing less hydrogen bonding and more slippage. Furthermore, high alignment and less entanglement are more likely in the stiff cellulose derivative products than in alginates.

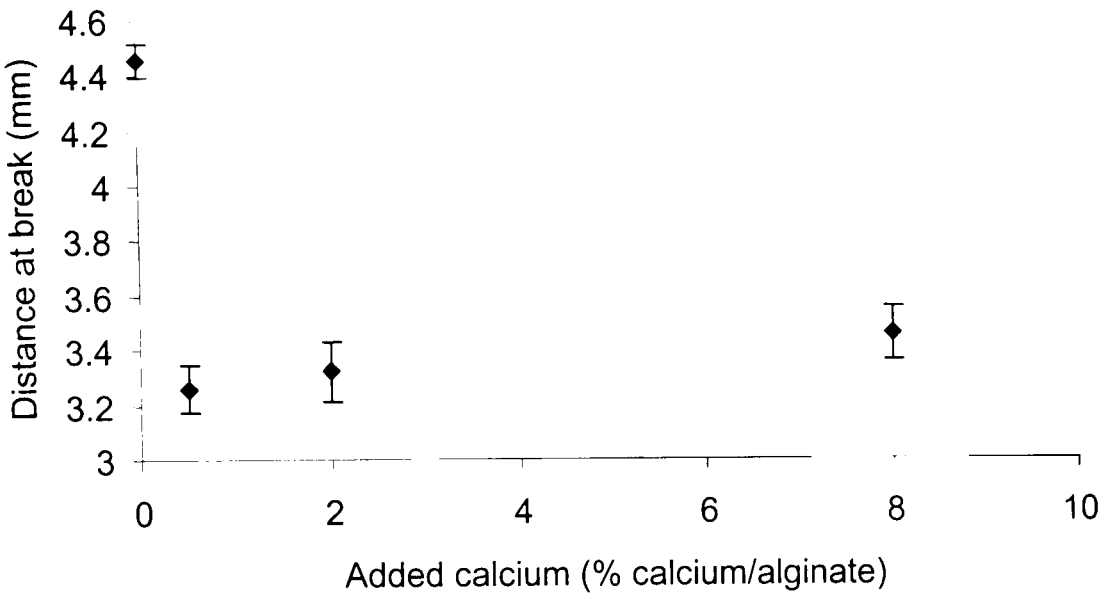
7.7. ADDED CALCIUM

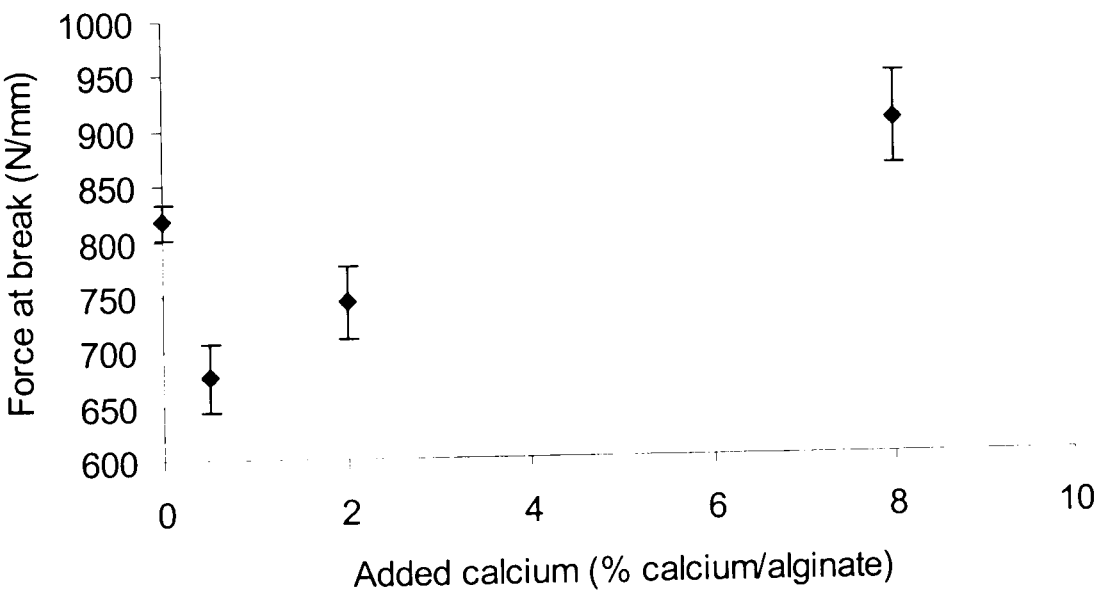
Alginate solutions gel in presence of calcium. The effect of the addition of calcium on the mechanical properties of this non gelling grade of alginate is studied.

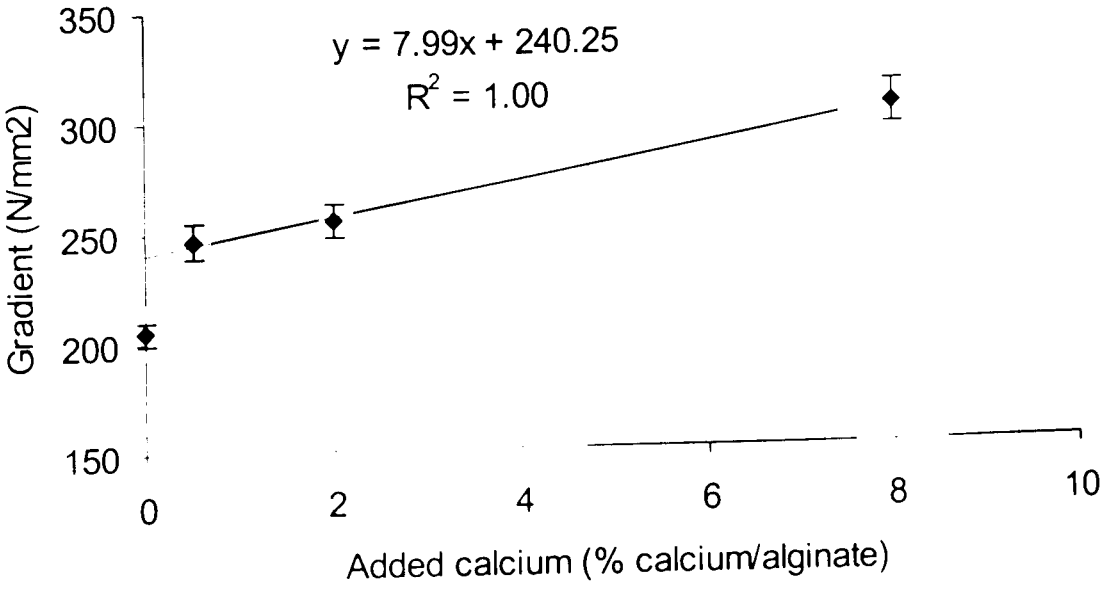
7.7.1. Results

The effects of adding calcium on the mechanical properties of alginate films are given in Figure 7-16. The distance at break of all samples with added calcium is lower than in the original sample. A slight increase of the distance at break is observed for the set of supplemented samples with increasing calcium content. The gradient increases sharply at very low level of calcium compared to the original sample. On increasing the calcium content further, the gradient increases steadily. The force at break shows a net increase on increasing the added calcium.

Figure 7-16: Effect of added calcium on alginate films mechanical properties (average and 95% confidence interval as defined in Figure 4-6).







7.7.2. Discussion

The effect of the added calcium on the distance at break is rather unexpected. The initial decrease is more likely to be due to the presence of heterogeneity in the samples. Indeed on mixing the calcium and alginate solutions, it is likely that junction zones are formed at the interface, before active mixing occurs. This would lead to the presence of 'hard' gelled particles in the solution hence in the film. This would explain the original decrease in the distance at break. Beyond this point, the slight increase in distance at break could be explained by the presence of more anchor points in the film.

The increase of gradient is consistent with the idea that the crosslink (junction zones) that form on drying help to built a resistance to deformation. The initial large increase might indicate that the original sample dramatically lacks junction zones. Another explanation could come from the previously discussed presence of hard particles. According to the blending rules, this would increase the overall gradient of the system.

Both distance at break and gradient behaviours resemble the behaviour observed on increasing the molecular weight of alginate. A 'gradient equivalence' between added calcium and molecular weight can be deduced since both are linearly related to gradient ($\log(MW) = 5.31 + 0.133(\%Ca)$). Increasing crosslink density or the entanglements density either by increasing the calcium content or increasing the molecular weight lead to similar behaviour: increase in gradient and slight increase in the distance at break.

7.8. COMPARISON WITH GELATIN AND HPMC FILMS

A summary of the various results measured for a relative humidity of 44% and over the range of relative humidity is given in Table 7-3. The distance at break of alginate films is significantly lower than the distance at break of both gelatin and HPMC at a relative humidity of 44%. However, the gradient is higher than the gradient of

HPMC. The zero moisture content gradient of alginates is the highest showing an intrinsically stronger material. Gelatin and alginate films behave in a similar way. The sorption isotherms are close and the moisture content affects the mechanical properties similarly. However, the microscopy observation showed differences in deformation behaviour. The force at break and distance at break of alginate and HPMC match the data obtained by Remunan-Lopez (1996) for similar molecular weights.

Table 7-3: Comparative results for alginate, HPMC and gelatin films.

		Gelatin	HPMC	Alginate
Data for a relative humidity of 44%	Distance at break (mm)	5.78	5.22	4.46
	Gradient (N/mm^2)	291	188	205
	Force at break (N/mm)	1656	976	817
	Number of lines	36	7	15
Relative humidity independent data	G_0 (N/mm^2)	543	251	600
	G_1 (%)	3.10	4.27	3.20

7.9. DISCUSSION

The use of alginate film for replacement of gelatin capsule is impossible due to its insoluble character. Nevertheless, late delivery (colon delivery) or special coated systems could be devised in order to take advantage of the good mechanical properties of this polysaccharide. Furthermore the choice a particular source of alginate with stronger gelling abilities should allow the use of a slightly lower molecular weight, therefore a higher concentration.

It was shown that the presence of particles could substantially influence the mechanical properties. Particles are the sources of stress concentration in the sample that lead to early break. In polystyrene, impurities have a large effect on crazing leading to early fracture. Polystyrene fracture in this case presented a craze controlled fracture (Nunes et al. 1982).

Many observations showed that similarities existed between gelatin and alginates films. This would indicate similar deformation behaviour. However, differences were observed in polarised microscopy.

Two limit models were presented for explaining the difference in mechanical properties. Firstly, the entangled network is proposed for alginates. The chains are long enough to present entanglements. The molecular weight will substantially affect entanglement density and gradient. This was consistent with the effect of molecular weight or added calcium. The deformation is due to uncoiling, stretching and ultimately crazing. No slippage is expected between the macromolecules, which would explain the absence of polarised effect in the fractured sample. The slight increase of distance at break with molecular weight could be explained by the enhanced stabilisation of crazes for higher molecular weight. Such effect was observed in polystyrene (Nunes et al. 1982). In this model, the energy would be stored in the sample up to fracture and restored by the creation of new surfaces in the fracture samples (lines). The presence of strong hydrogen bonding between the macromolecular chains would also support this model.

Secondly, the slippage model is proposed for cellulose derivatives. Here slippage between molecules is possible due to the lack of entanglements and hydrogen bonding. The distance at break increases with molecular weight due to possibility of extensive slippage but the gradient is almost unaffected. However, the relative effect of the moisture content on the gradient is more important than in the first model because beyond the dilution effect, easier slippage is allowed. Crazing is unlikely since no fibrillar structure would stabilise the crazes and shear yielding is the main deformation process. Large orientational effect was observed around the centre of the sample. In this model, most of the energy is dissipated.

Any real sample would combine both behaviours to a certain extent. The results and similarities suggest the alginate is mostly an entangled networked film whereas cellulose derivatives fulfil the slippage model.

Gelatin generally behaves like alginate, however, the orientational effect observed indicates a certain degree of slippage. This is in contradiction with the number of lines observed at a relative humidity of 44%. However, at the same moisture content alginate films presented more lines. Furthermore, the number of lines should be

proportional to both the energy stored and the surface free energy, which will change from polymer to polymer.

CHAPTER 8. CASEINATE FILMS

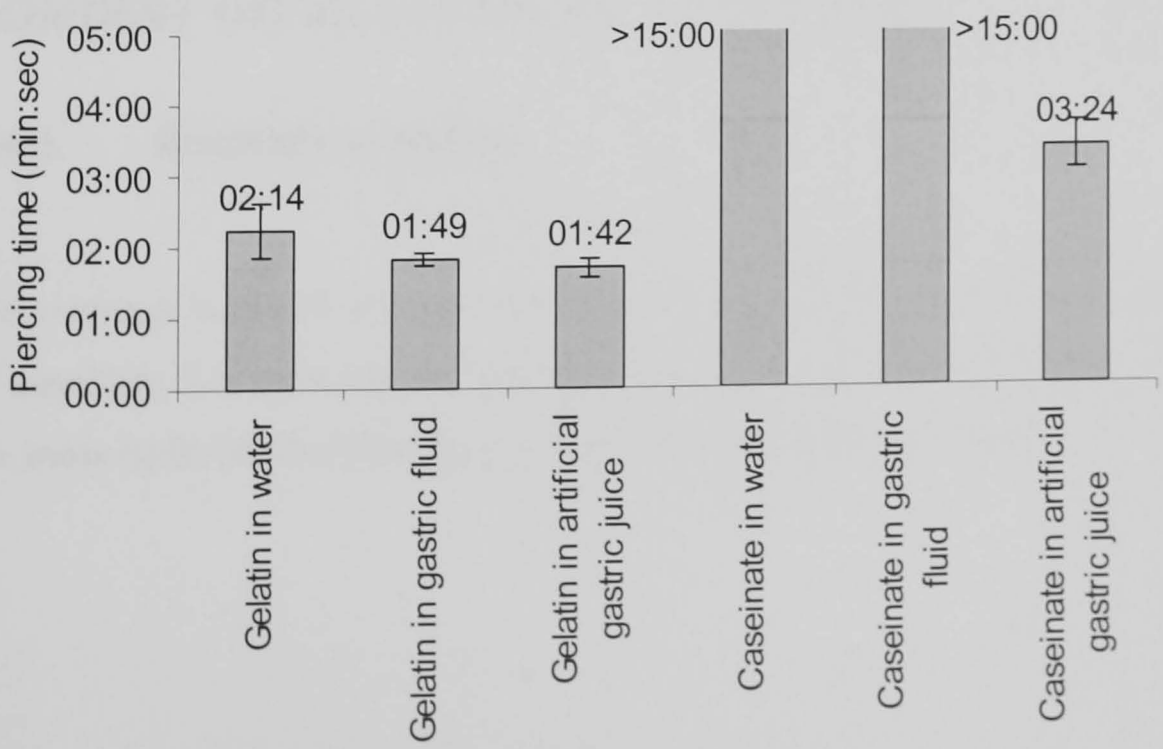
8.1. INTRODUCTION

Although the sensory analysis results were not very promising, caseinates were selected for further studies. This choice was stimulated by the very low viscosity of the product, its low price and because a vast range of conditions could modify the film properties of a protein.

8.2. SOLUBILITY OF CASEINATE FILM

The solubility of caseinate films was measured using the piercing test. The results are given in Figure 8-1. The presence of pepsin in the media does not affect significantly the piercing time of gelatin films. For caseinates, the films were insoluble both in water and gastric fluid (within 15 minutes). The presence of pepsin is necessary to obtain a quick dissolution of the caseinate films.

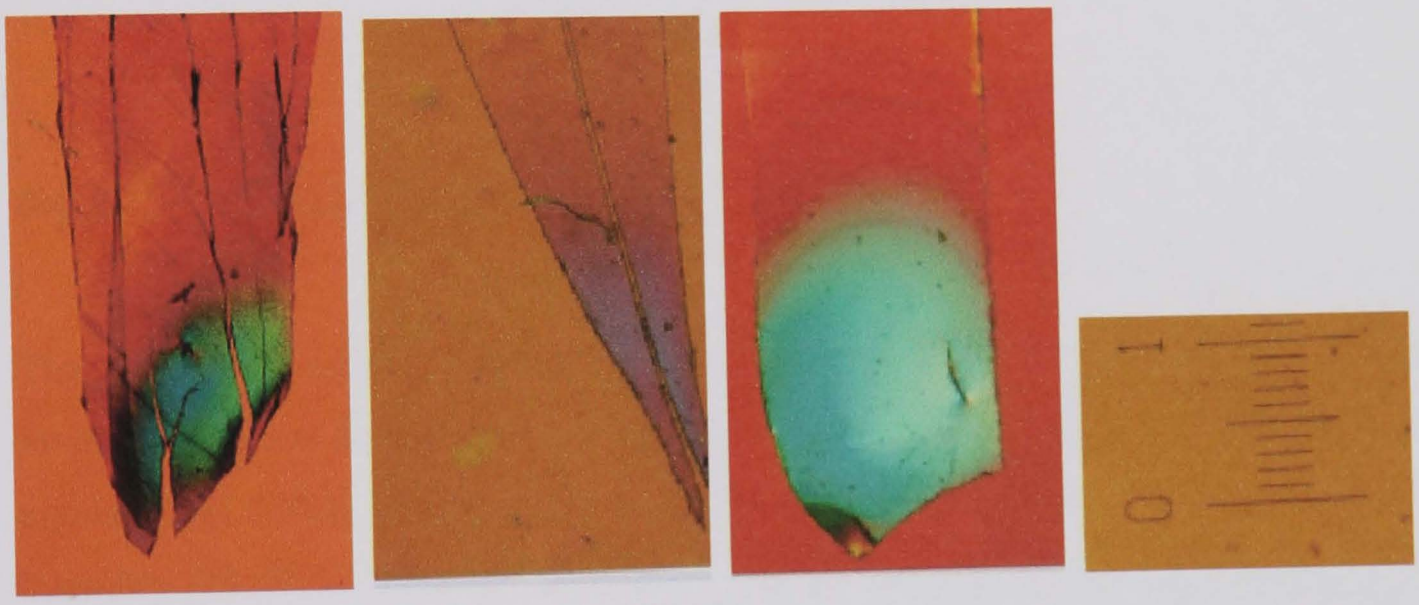
Figure 8-1: Piercing time of gelatin and caseinate films in water, gastric fluid and artificial gastric juice (gastric fluid and pepsin).



8.3. MICROSCOPY OBSERVATION

Caseinate films did not produce the same regular fracture pattern as the ones observed previously for gelatin, HPMC or alginate. Many of the fractured samples shattered and the number of lines could not be counted. Therefore, the radial orientation was only observable on some films. The colour intensity hence the orientational order obtained during deformation was between that of gelatin and HPMC (Figure 8-2).

Figure 8-2: Samples around the puncture centre observed under polarised light. From left to right: caseinate, gelatin, HPMC and scale bar (mm).

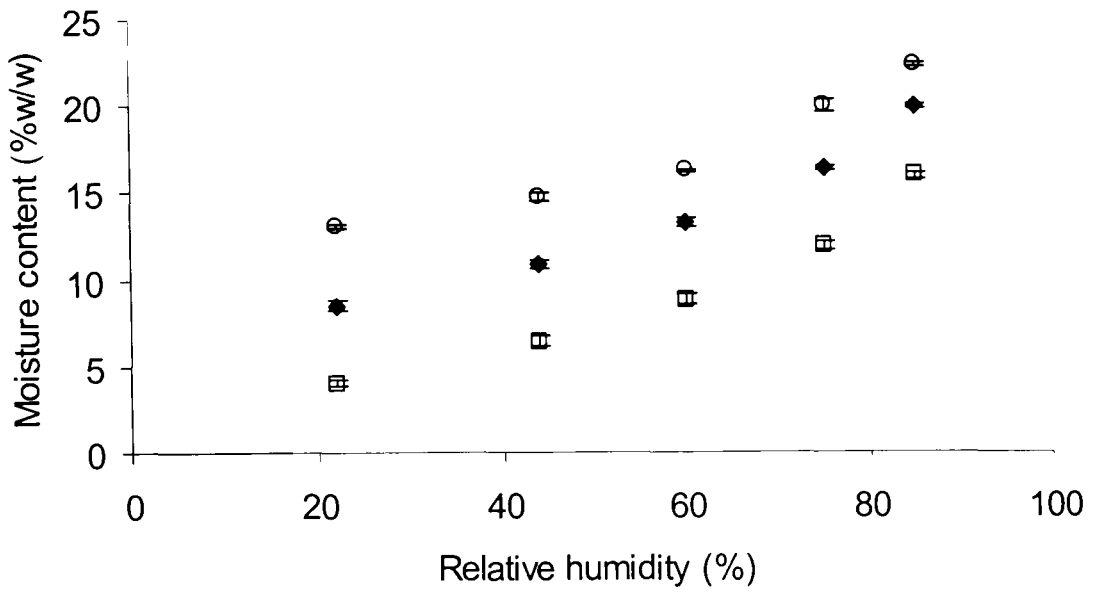


8.4. EFFECT OF RELATIVE HUMIDITY AND MOISTURE CONTENT ON MECHANICAL PROPERTIES

8.4.1. Sorption isotherm

The sorption isotherm of caseinate films is given in Figure 8-3. The moisture content of caseinate lies between the moisture content of gelatin and HPMC. This indicates the more hydrophobic character of caseinates compared to gelatin.

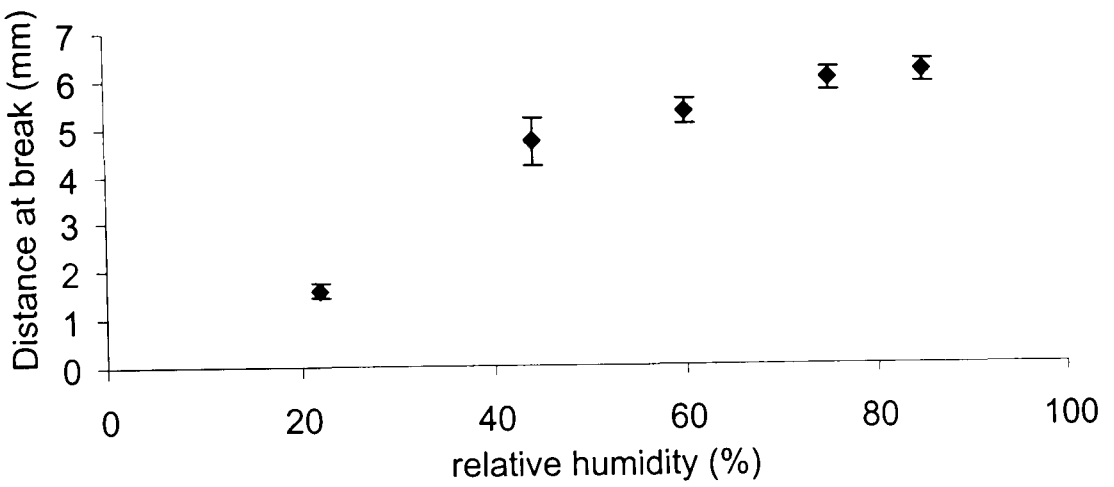
Figure 8-3: Sorption isotherm of caseinate films (filled diamonds) compared to gelatin (circle) and HPMC (square) films.

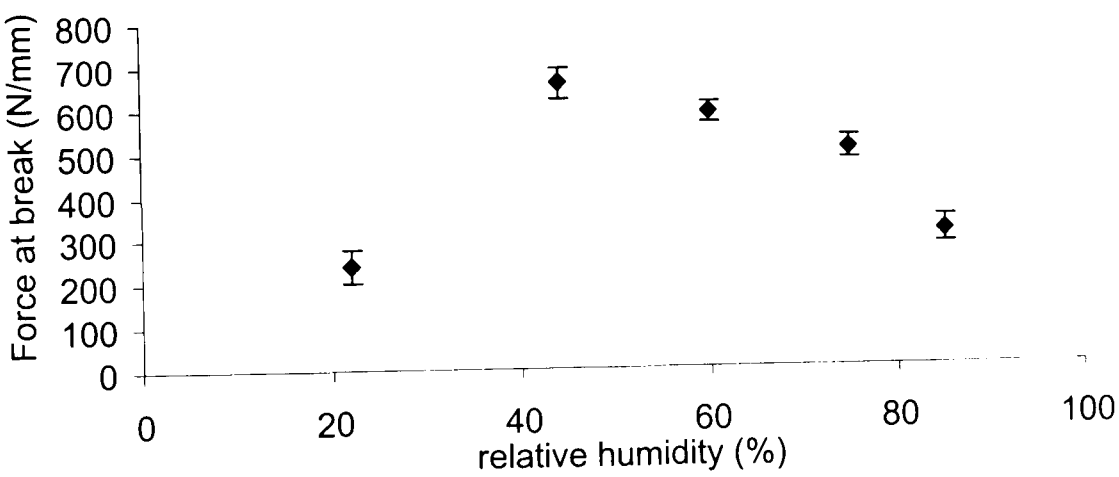


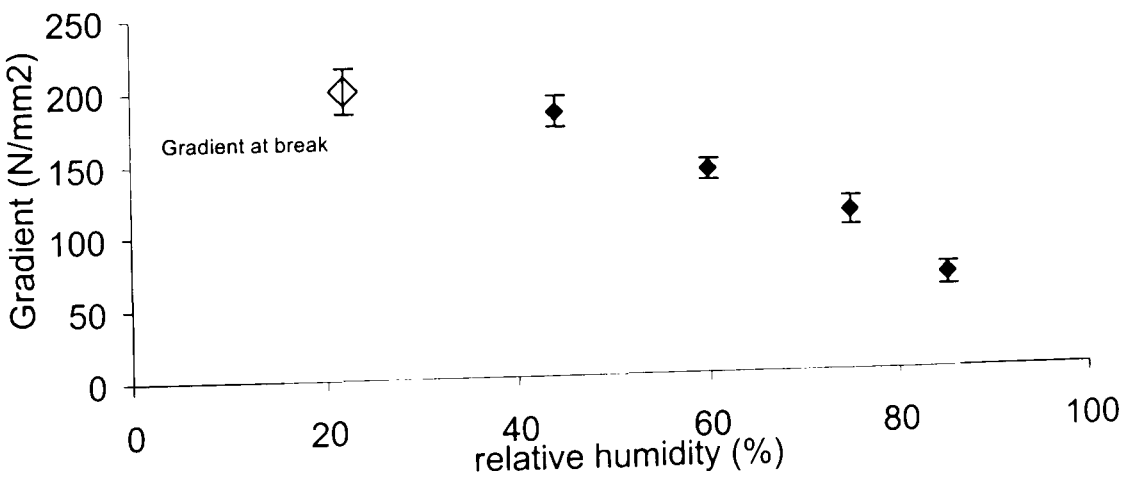
8.4.2. Effect of relative humidity

The distance at break of caseinate films is continuously increasing with relative humidity (Figure 8-4). This is different from the behaviour observed for gelatin, alginate and HPMC films where a plateau was observed at intermediate relative humidity (30-50%). Very low distance at break is obtained at low relative humidity indicating the very brittle behaviour of caseinate films at low moisture content. The gradient decreases with increasing relative humidity.

Figure 8-4: Effect of relative humidity on mechanical properties of caseinate films (gradient at break ◇is used when fracture occurred before 2.5mm) (average and 95% confidence interval as defined in Figure 4-6).







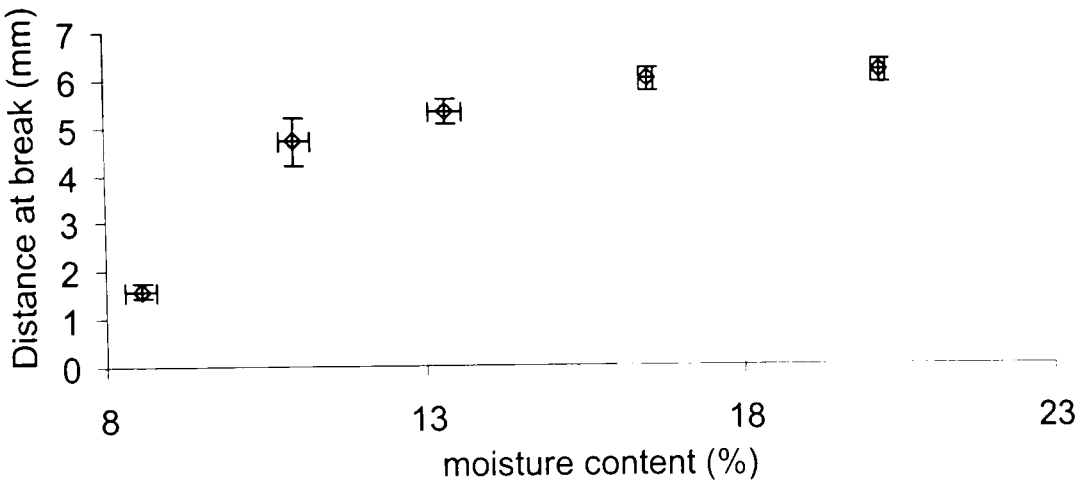
8.4.3. Effect of moisture content

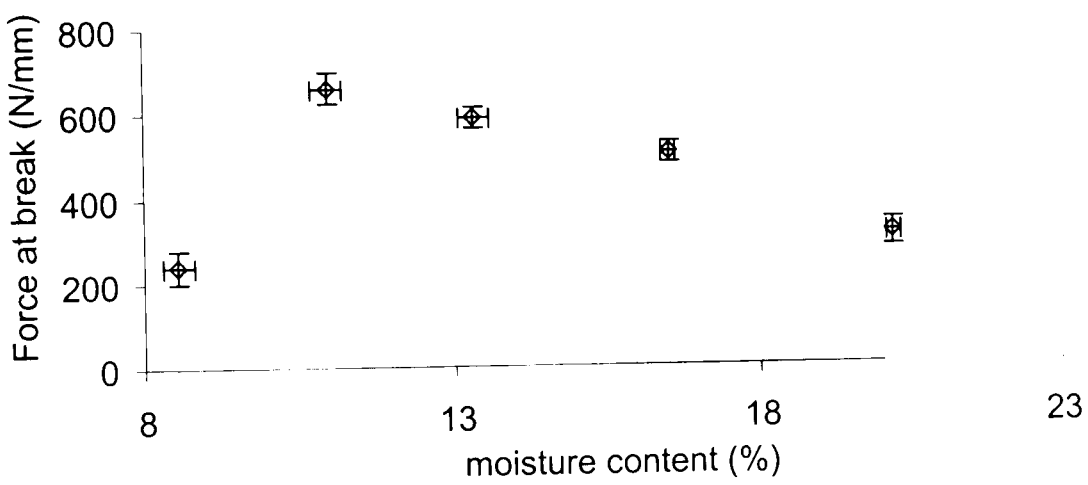
The distance at break of caseinate films with moisture content lower than 10% are very low (Figure 8-5). Moisture content and gradient are linearly correlated. Zero moisture content gradient G_0 is 304N/mm^2 and G_1 is 3.88%.

8.4.4. Fracture behaviour

The fracture pattern for caseinate films is not as simple as the ones observed for gelatin or HPMC. Some samples presented a large number of lines that split into many further lines during the crack propagation (Figure 8-6) while others shattered into few pieces. It was therefore impossible to measure the number of lines appearing on the samples. We can only conclude that the number of lines observed is very large (higher than for gelatin) and that the crack propagation pattern is different that in the films previously studied.

Figure 8-5: Effect of moisture content on mechanical properties of caseinate films (gradient at break ◇is used when fracture occurred before 2.5mm) (average and 95% confidence interval as defined in Figure 4-6).





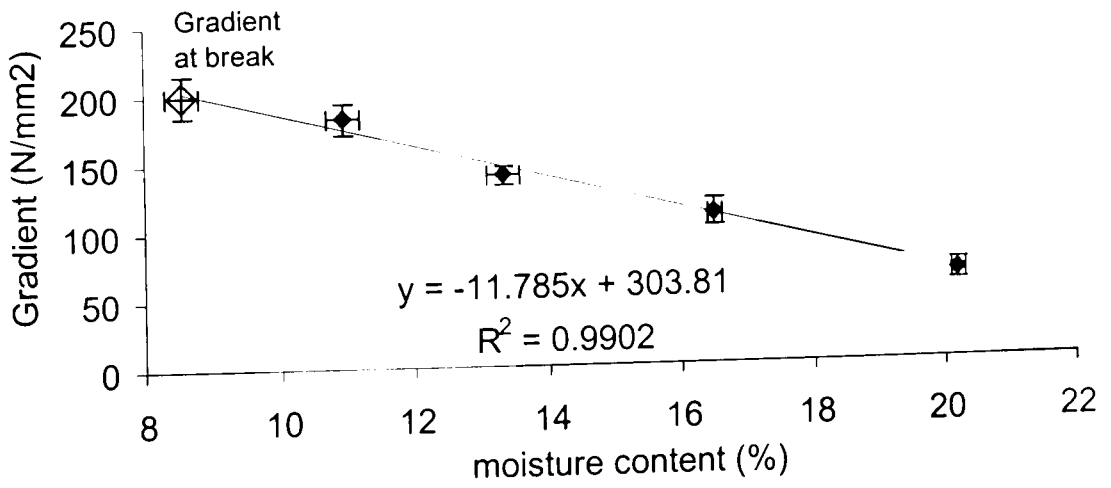
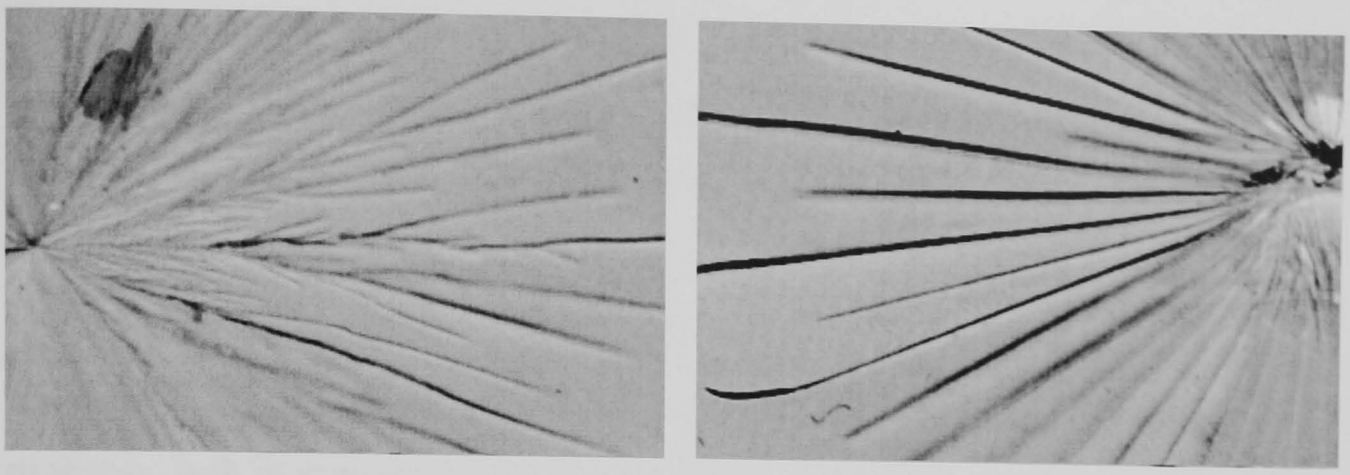


Figure 8-6: Fracture lines for caseinate (left) and gelatin (right) films used in puncture test at a relative humidity of 22%. Each picture represents a 10mm wide sample.



8.4.5. Discussion

Caseinate films are very sensitive to relative humidity changes. The distance at break is highly affected and the use of caseinate for capsule production would require very strict relative humidity control. The distance at break at a relative humidity of 44% is close to that of alginate films (Table 8-1). However, the gradient is the lowest of the four films studied. G_0 for caseinate is much smaller than gelatin and alginate and the values of G_1 lies between gelatin and HPMC values.

Table 8-1: Summarised results for caseinate, HPMC, gelatin and alginate films.

		Caseinate	Gelatin	HPMC	Alginate
Data for a relative humidity of 44%	Distance at break (mm)	4.45	5.78	5.22	4.46
	Gradient (N/mm^2)	181	291	188	205
	Force at break (N/mm)	635	1656	976	817
	Number of lines	high	36	7	15
Relative humidity independent data	G_0 (N/mm^2)	304	543	251	600
	G_1 (%)	3.88	3.10	4.27	3.20

The sorption isotherm showed that caseinates presented a hydrophobicity level between gelatin and HPMC. The molecular structure also presents intermediate characteristics. The chemical structure is closer to gelatin (both protein) but the molecular weight is much smaller (about 20000 Da). This implies that unlike gelatin,

molecular entanglements must be limited. Furthermore, caseinate do not form junction zones like gelatin or crosslink like alginate.

The large number of lines formed after fracture is a clear indication that energy is stored during deformation. However, some slippage occurs during deformation as observed by microscopy. The value of G_0 and G_1 are very different from alginates and gelatin.

Caseinates are therefore very different from all previously studied films. They probably lack in entanglements but can not slip as much as HPMC explaining their relatively poorer mechanical properties. Furthermore, differences in the fracture pattern suggest than the crack propagation is very different. We might argue that slippage is limited which implies that ultimately, crazing occurs. However the crazes will not be stabilised by entanglements and the crack propagation might spread more easily than for alginate and gelatin films. The major source of craze stabilisation would be the hydrogen bonding. In fact, the hydrogen bonding might prevent efficient slippage without providing a good craze stability.

Although these explanations are highly speculative, they explain well the differences observed and are consistent with molecular structure and fracture behaviour.

8.5. EFFECT OF COUNTERION

Caseinates can be produced with various counterions. The results obtained with sodium caseinate were compared with the data for other salts. The gradient is almost unaffected by the changes in counterions (Figure 8-7). The distance at break is decreasing in the order sodium, potassium, calcium and magnesium. The differences in moisture content can not explain these differences (Figure 8-8).

Figure 8-7: Effect of counterion type on mechanical properties of caseinate films obtained by puncture at a relative humidity of 44% (average and 95% confidence interval as defined in Figure 4-6).

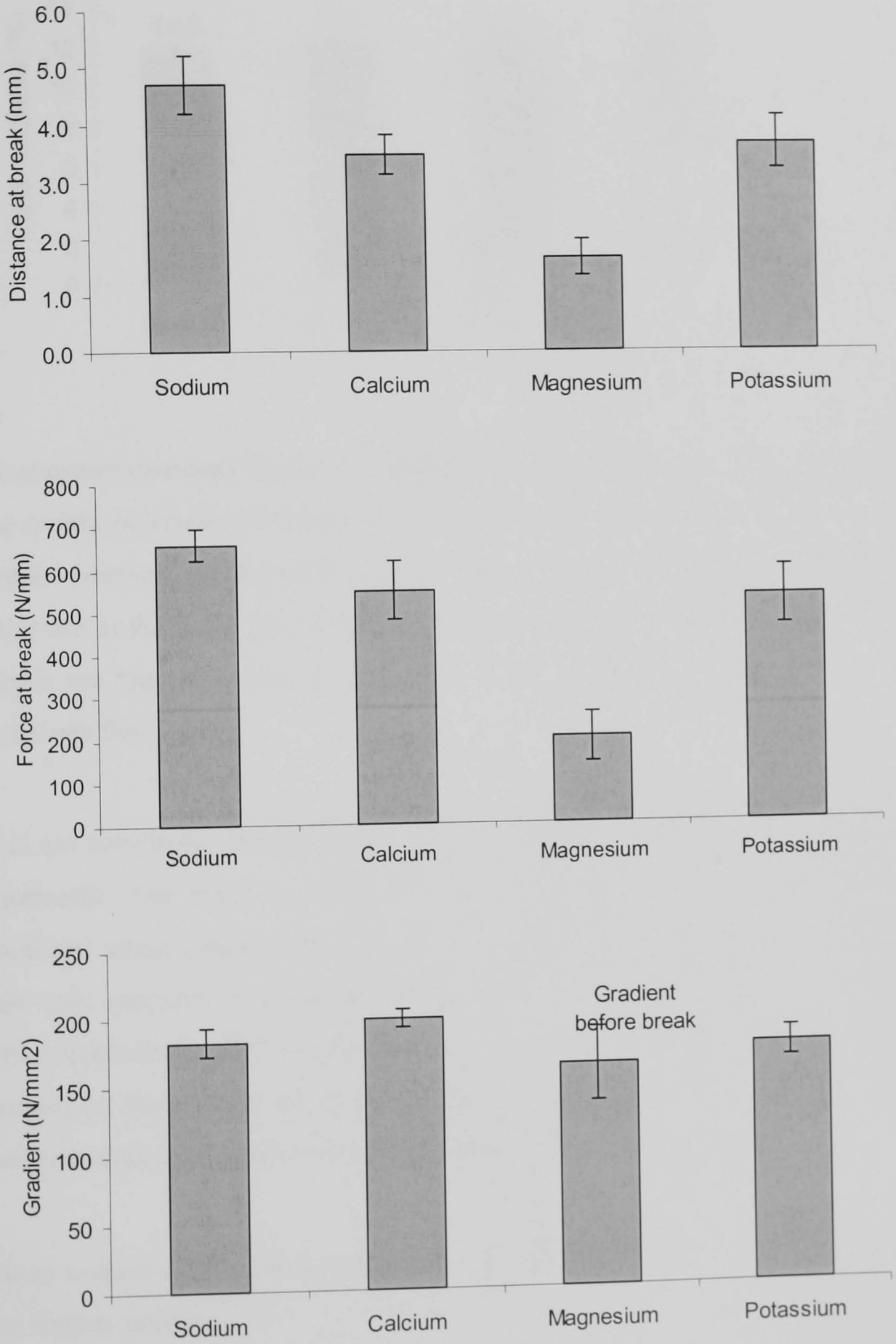
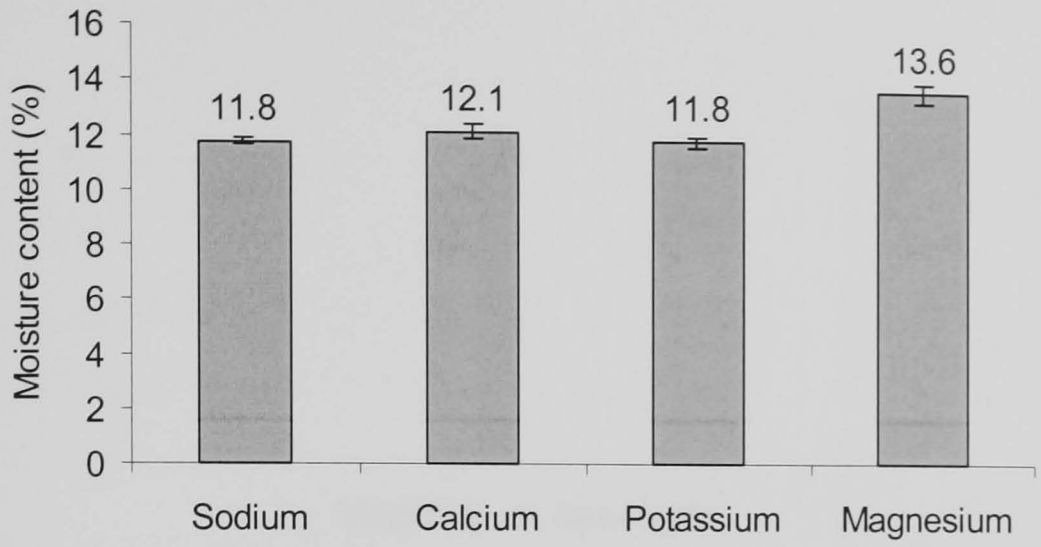


Figure 8-8: Moisture content of caseinate with different counterions at a relative humidity of 44%.

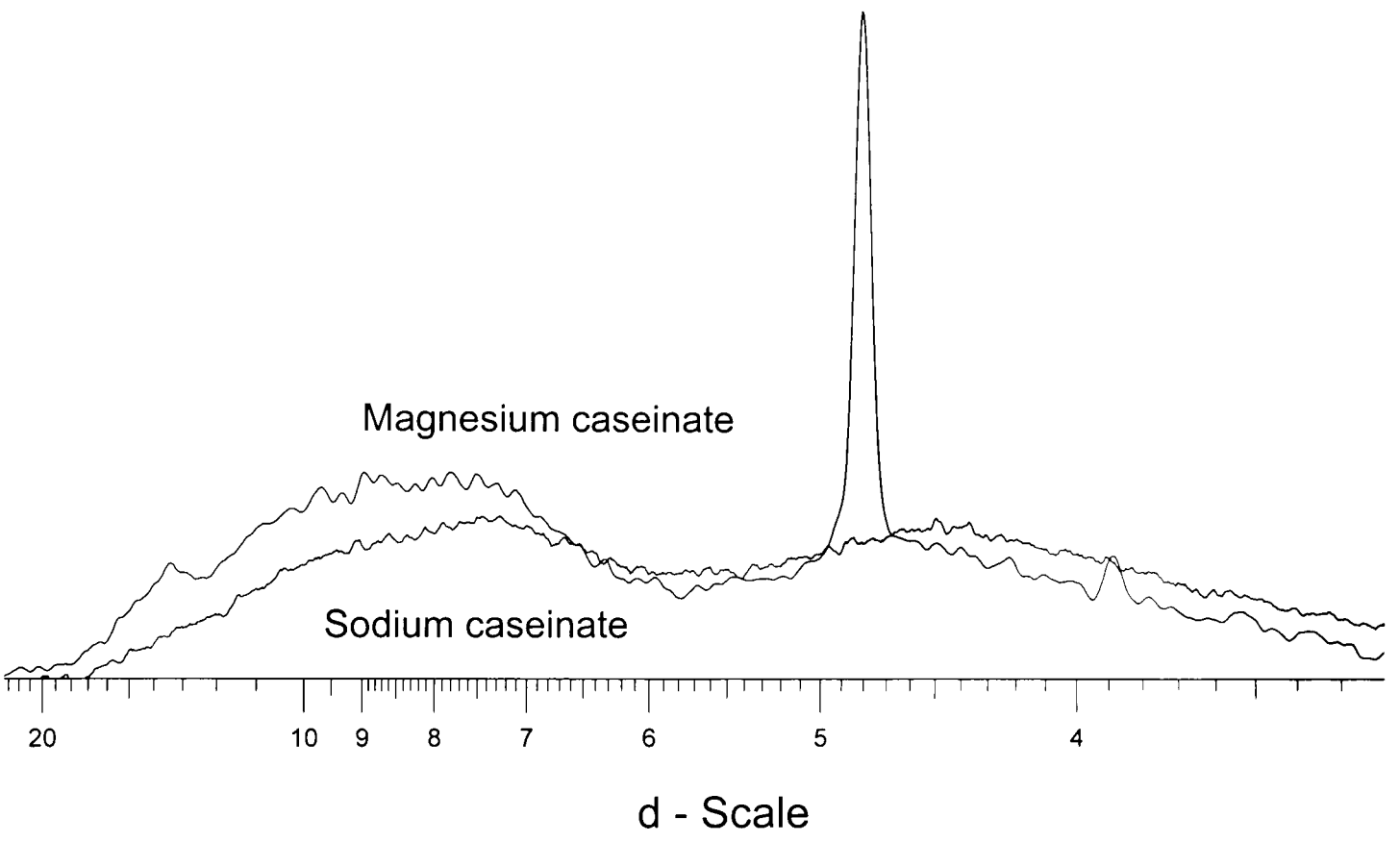


Magnesium caseinate films were slightly white indicating the presence of particles in the matrix or some phase separation. It was confirmed that the very low distance at break observed for magnesium caseinate was due to the presence of crystalline structure in the dried film. Magnesium caseinate films presented sharp X-ray peaks, which are typical of crystal (Figure 8-9). No such peak was present in any other caseinate film.

It is not easy to explain the effect of the counterions on the mechanical properties of caseinates. The presence of crystal in magnesium caseinate films could generate localised stress concentration leading to early break. For the other counterions, we can only speculate that the molecular packing is modified due to size differences. Films made from calcium caseinate do not present significantly different mechanical properties from those of film cast from potassium caseinate. The difference in valence of the ions seems not to affect the mechanical properties.

Since sodium caseinates are the best of all caseinates studied here, they will be used for further studies.

Figure 8-9: X-ray diffraction of sodium and magnesium caseinate films.



8.6. PLASTICISER

It was shown that caseinate (sodium) films had similar distance at break to alginates for a relative humidity of 44% (Table 8-1). However, the mechanical properties at low moisture content were very poor and caseinates could only be used if they were improved. The use of various plasticisers was studied in order to increase the distance at break.

8.6.1. Results

The effect of glycerol on the mechanical properties is shown in Figure 8-10. The distance at break shows an initial decrease at low glycerol content. On increasing the glycerol content further, the distance at break increases linearly. The changes in force at break with increasing plasticiser content are not significant. However, an initial drop is observed and is reported in Table 8-2. The gradient decreases linearly with glycerol content. However, changing the plasticiser content also changes the

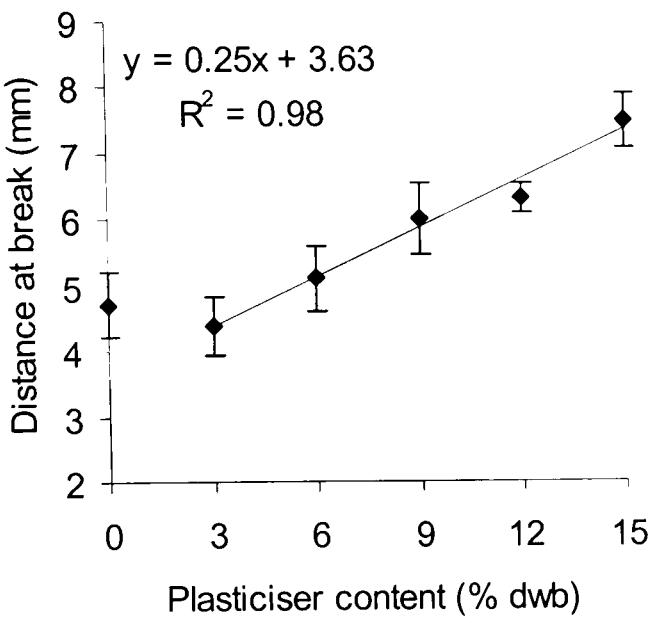
moisture content of the film at a relative humidity of 44% (Figure 8-10). This is also true when the moisture content is given using a polymer weight basis. This implies that all the changes observed are due to the combined effect of increasing plasticiser and decreasing moisture content.

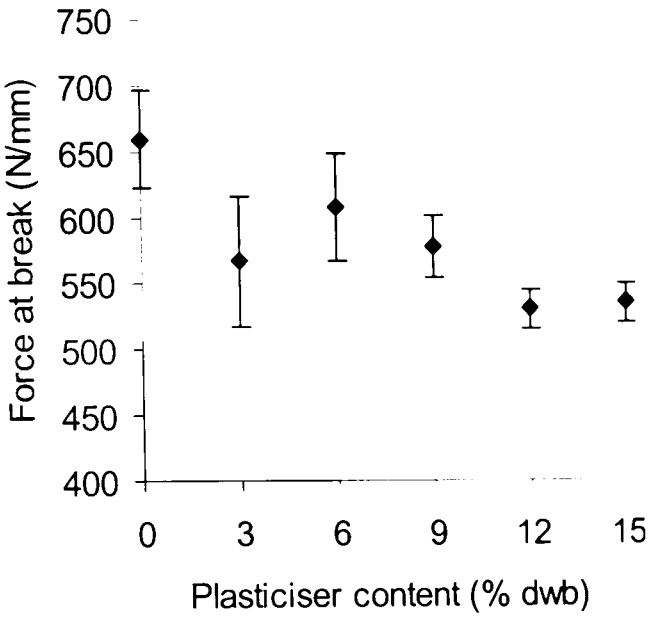
In order to understand the effect of the plasticiser on the mechanical properties, regardless of moisture content changes, the zero moisture content gradient was calculated for each plasticiser content and plotted versus the plasticiser content. We used the previously described values of G_1 and G_0 and made the assumption that the measured gradient resulted from a simple addition of the effects of water and plasticiser in the form $G(mc, pc) = G_0\left(1 - G_1.mc - G_{plasticiser}.pc\right)$ where pc and mc are respectively the plasticiser content and the moisture content. The effect of the plasticiser on the zero moisture content gradient, $G_{glycerol}$ can be calculated in a similar way as for G_1 ($G_{glycerol}$=7.95/321=2.48%).

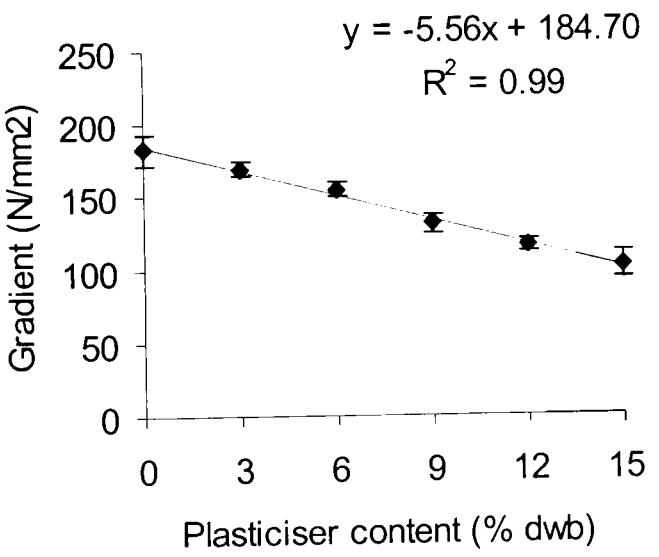
The behaviour of caseinates films with increasing sorbitol, PEG 400 or PG content is qualitatively identical to glycerol plasticised films (Figure 8-11 to Figure 8-13). However, the distance at break showed a much larger initial decrease at low plasticiser content for PEG. Furthermore, the force at break is lower with plasticiser than without plasticiser. This effect is clearly observed for PEG 400. The amount of plasticiser does not affect the force at break.

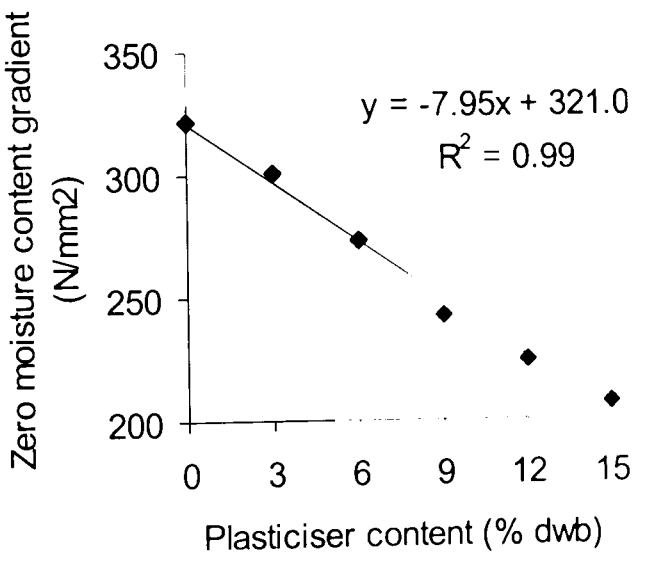
The moisture content changes are observed for all films. However, when the dry polymer basis is used, the moisture content is not affected for PEG 400 and sorbitol.

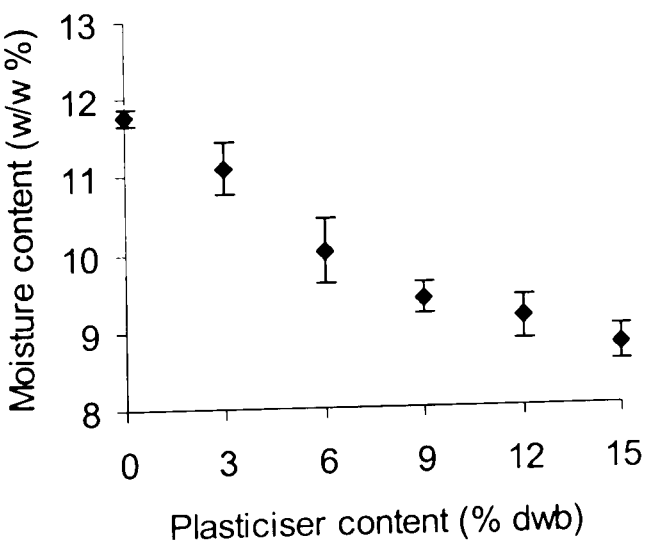
Figure 8-10: Effect of glycerol content on mechanical properties of caseinate films (average and 95% confidence interval as defined in Figure 4-6).











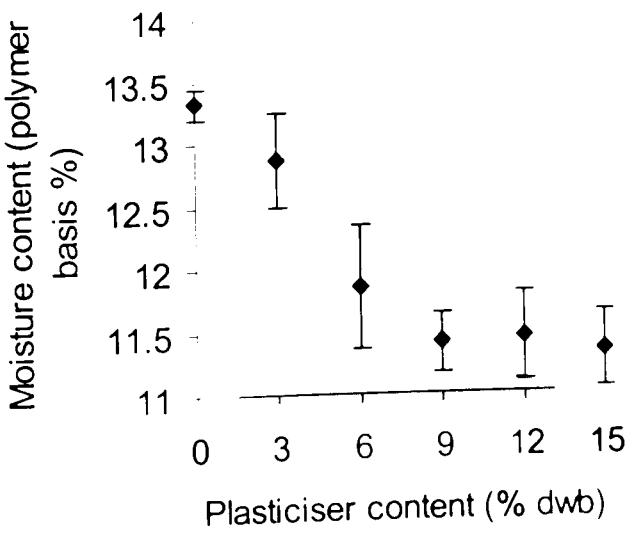
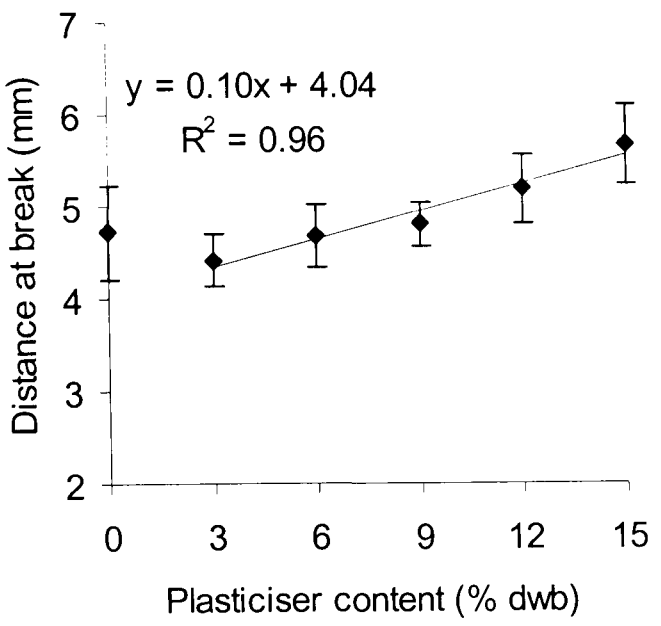
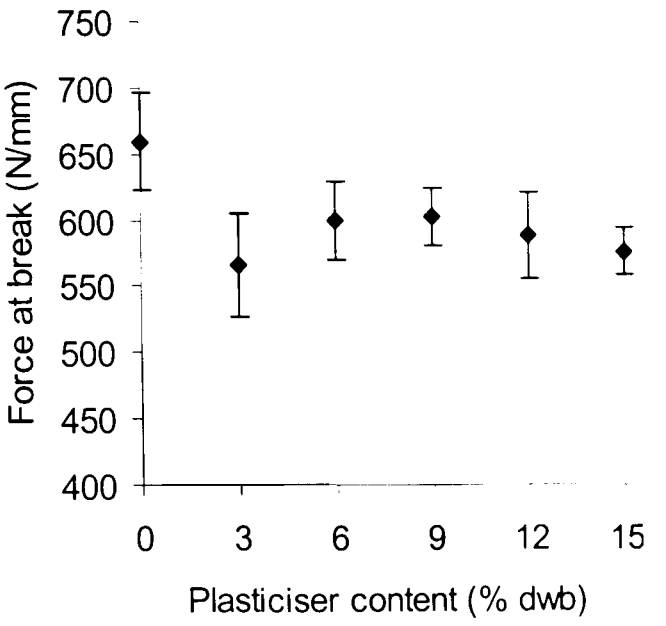
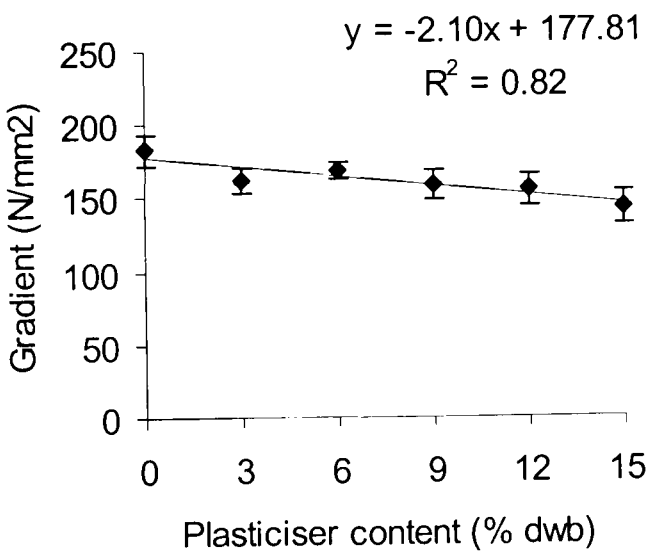
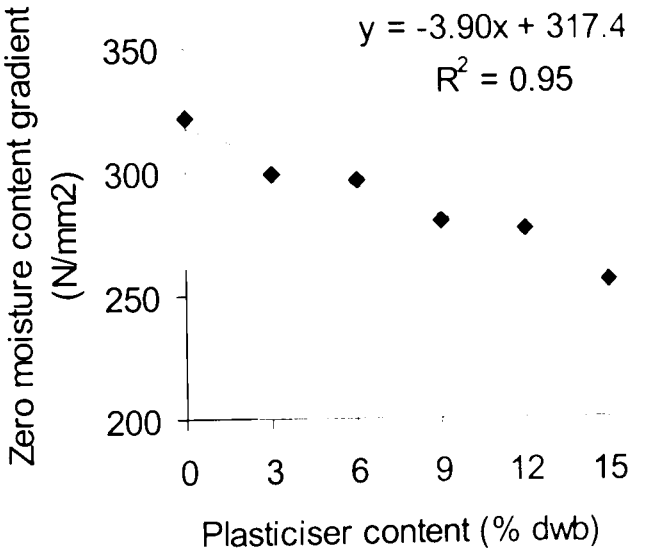


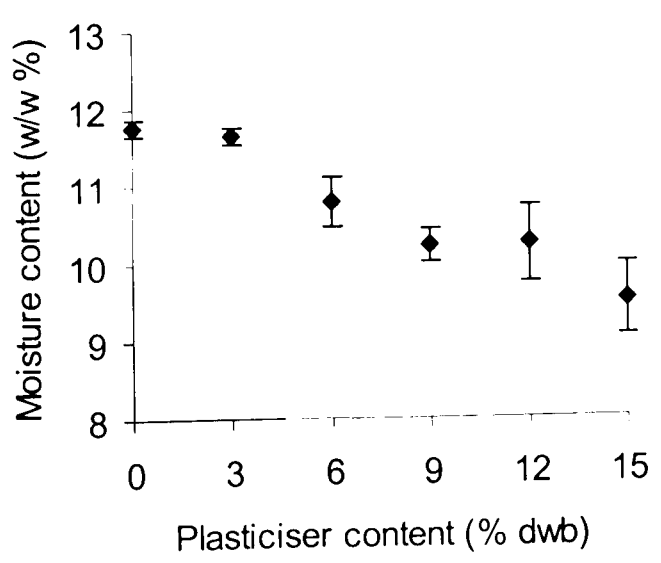
Figure 8-11: Effect of sorbitol content on mechanical properties of caseinate films (average and 95% confidence interval as defined in Figure 4-6).











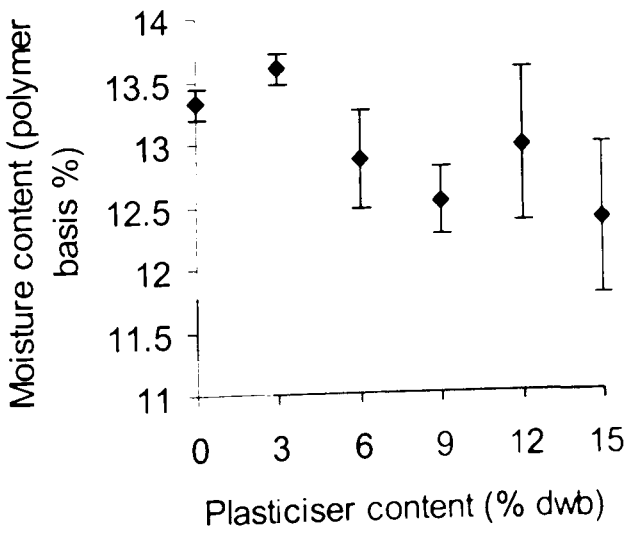
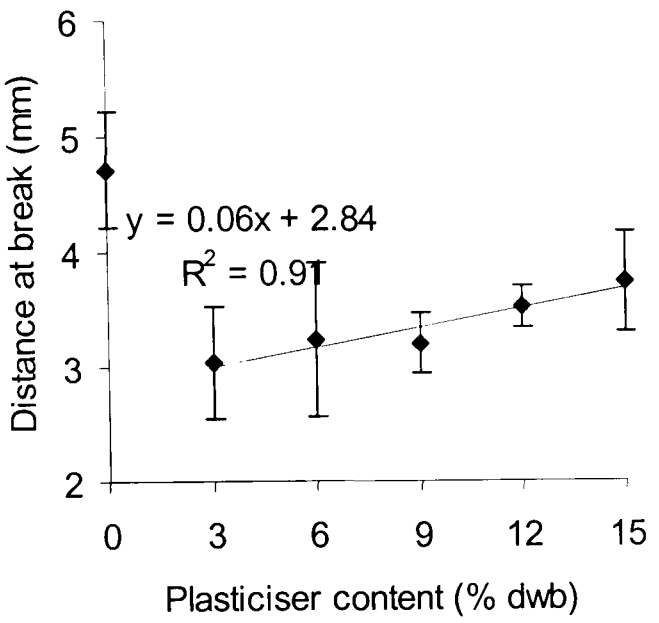
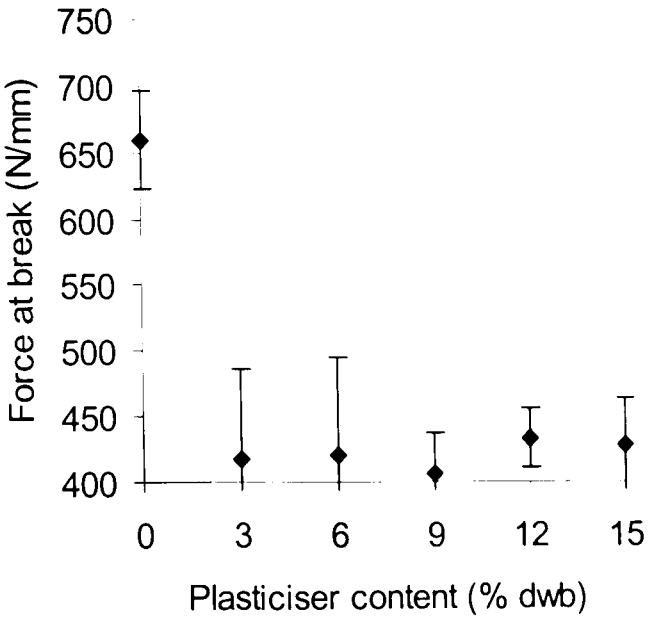
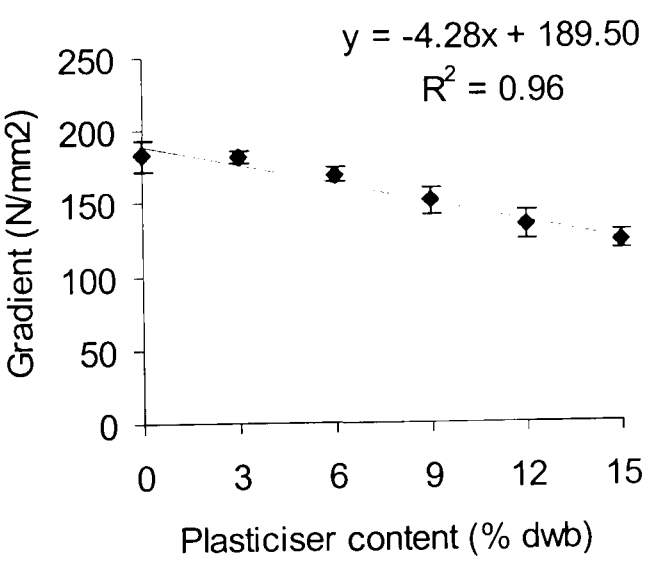
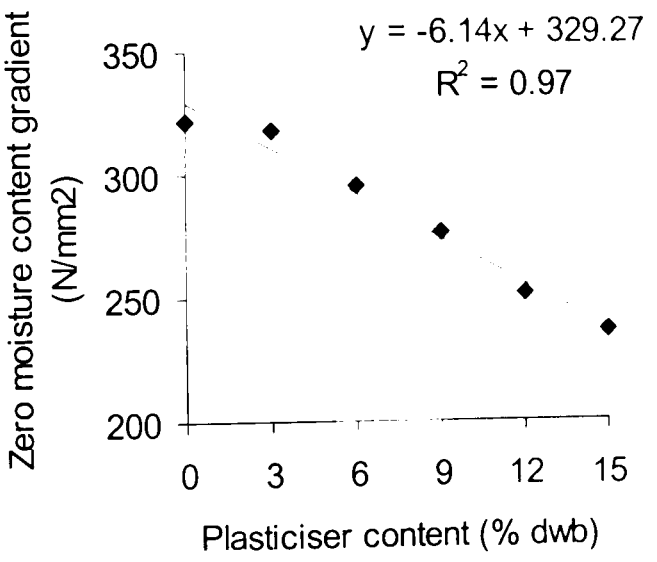


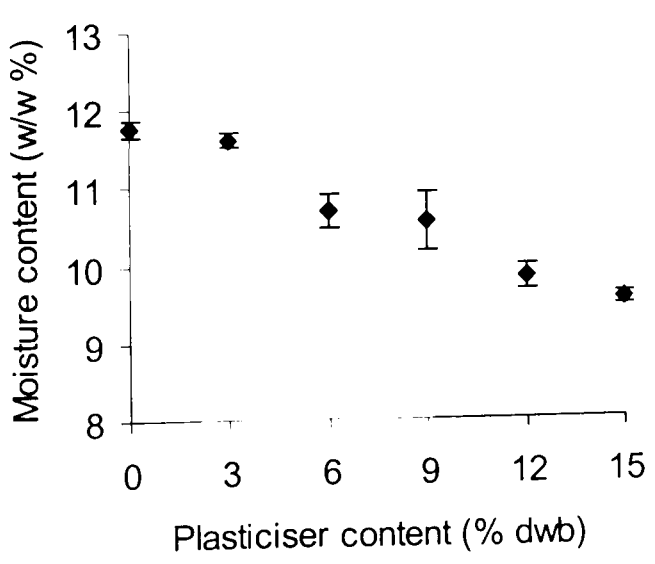
Figure 8-12: Effect of polyethyleneglycol 400 content on mechanical properties of caseinate films (average and 95% confidence interval as defined in Figure 4-6).











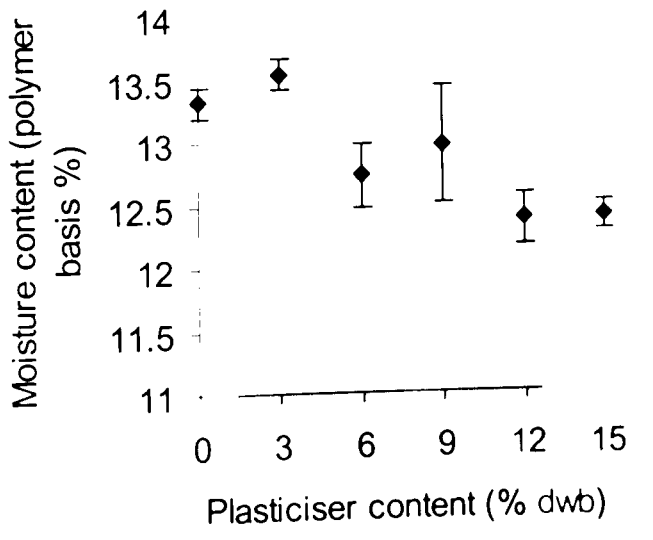
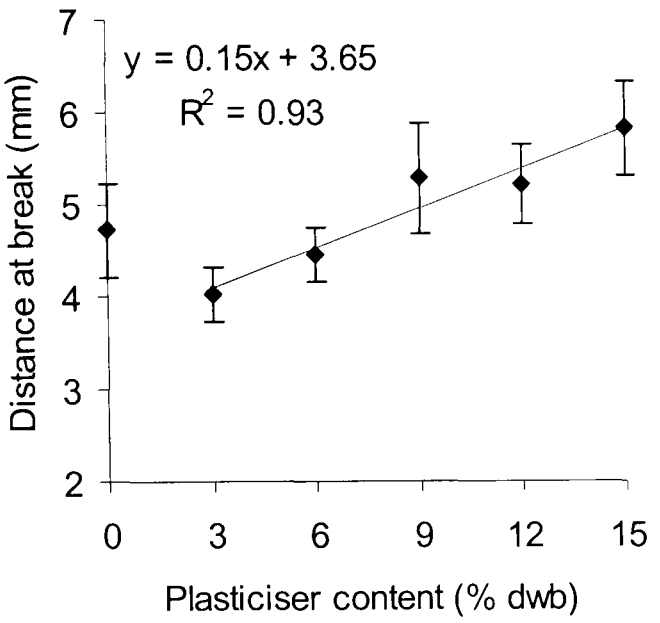
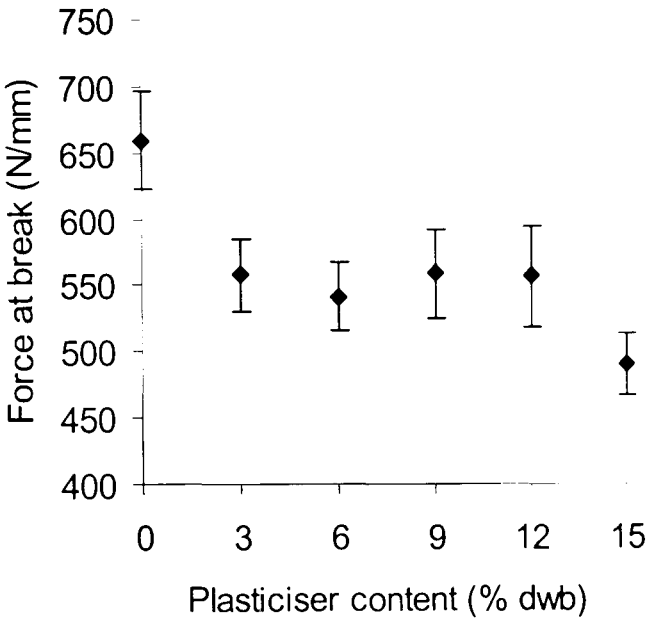
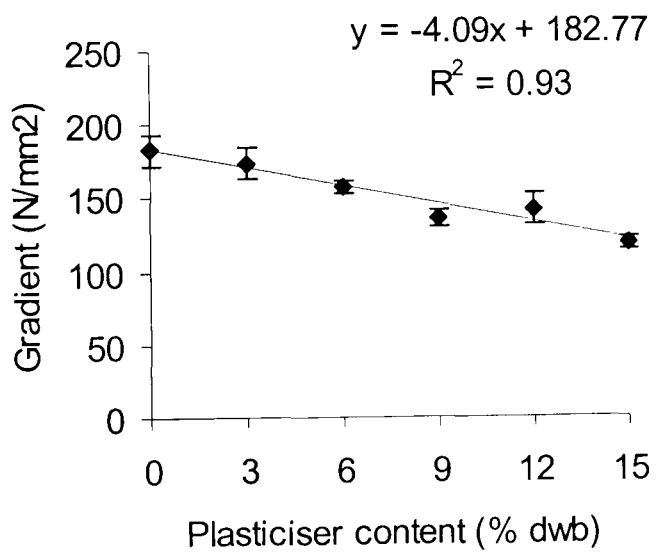
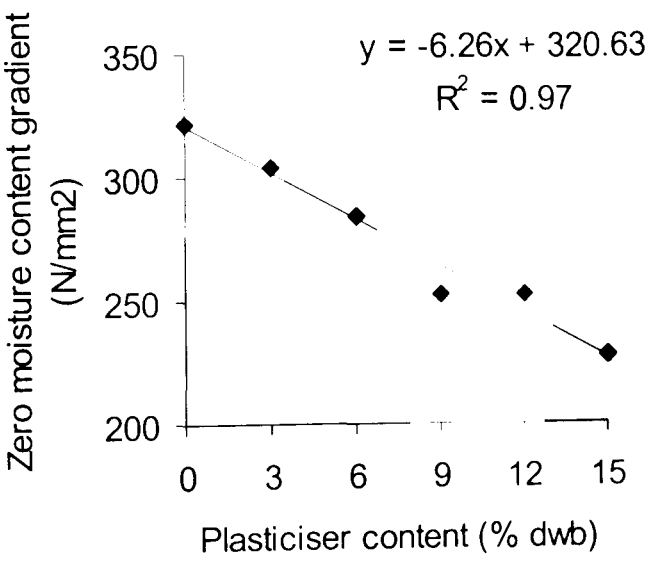


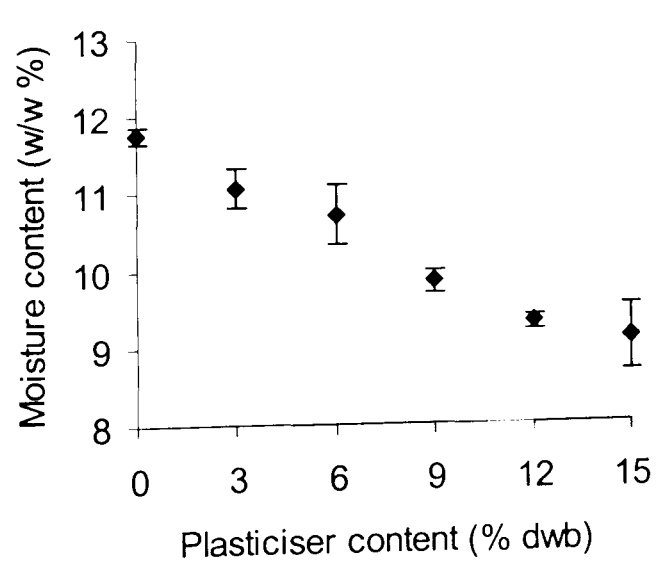
Figure 8-13: Effect of propylene glycol content on mechanical properties of caseinate films (average and 95% confidence interval as defined in Figure 4-6).

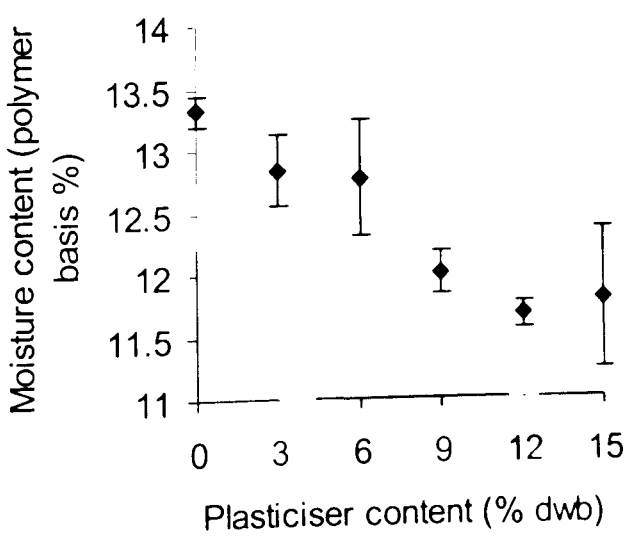












8.6.2. Quantitative analysis

Results indicate that all plasticisers systematically generated similar behaviour changes. The distance at break increases after an initial decrease. For water, such effects were not observed in our study but very low water contents were not investigated. The moisture content and the gradient both decrease with increasing plasticiser content. The force at break decreases initially and stays unchanged with increasing plasticiser content.

Caseinate-starch blends (50/50) have been studied in the past with various amounts glycerol, sorbitol and xylose present. The elongation at break measured in tensile mode did not show any initial decrease for glycerol or sorbitol (Arvanitoyannis and Biliaderis 1998). However, with xylose the elongation at break was reduced significantly at low level of plasticiser. For starch plasticiser systems, the elongation at break increased with glycerol, sorbitol or xylose whereas it decreased for sucrose (Arvanitoyannis et al. 1996). Such behaviour was related to antiplasticisation at low plasticiser content (Gaudin et al. 1999; Lourdin et al. 1997). Lowering of the distance at break in puncture test of crosslinked calcium caseinate films in presence of PG or triethylene glycol was not observed at a relative humidity of 56% (Mezgheni et al. 1998).

In this study, the influence of the plasticiser content and its associated moisture content changes were derived from the changes in distance at break and gradient. The slopes obtained in both graphs (S_{db} and S_g) and the values of $G_{plasticiser}$ are given in Table 8-2. We also considered the initial drop in the distance at break (I_{ddb}). This was measured as the difference between the experimental value of the distance at break of the pure caseinate film and the zero plasticiser content distance at break obtained by fitting (3-15% plasticiser content at a relative humidity of 44%). The first four parameters described in Table 8-2 represent the real effect of using a plasticiser whereas $G_{plasticiser}$ gives an estimate of the plasticising effect of the plasticiser alone, regardless of moisture content changes. The absence of drop in the distance at break for water will not be discussed here since the lower water content studied are much higher than for the other plasticisers.

It can be seen that glycerol is the strongest plasticiser (after water). The initial drop is limited (I_{ddb} is 1.09mm) and the increase in distance at break is the largest (S_{db} is 0.25mm). The effect on the gradient is also the largest. The value of $G_{glycerol}$ shows that this is due to real plasticisation of the glycerol rather than limited water loss compared to the other plasticisers.

PEG 400 has a slightly stronger effect on the gradient than propylene glycol. However, the comparison of G_{PEG} and G_{PG} values shows that this is due to a lower moisture content decrease in the case of PEG 400. Although both plasticisers are affecting similarly the gradient, PG increases more the distance at break. Furthermore, the initial decrease of the distance at break and force at break for PEG 400 are very high, which makes PEG plasticised films more brittle that unplasticised caseinate films.

Table 8-2: Quantitative effect of plasticiser on mechanical properties of caseinate films.

	Glycerol	Sorbitol	PEG 400	PG	Water
Slope for the distance at break (mm): S_{db}	0.25	0.10	0.06	0.15	Not linear
Slope of gradient (N/mm2): S_g	5.56	2.10	4.28	4.09	11.8
Initial drop in the distance at break (mm): I_{ddb}	1.09	0.67	1.88	1.06	Nd
Initial drop in the force at break (N/mm)	92	93	243	101	Nd
$G_{plasticiser}$ (%)	2.48	1.23	1.87	1.95	3.88

Nd: Not determined

Sorbitol is the worst plasticiser used in our study when the gradient is considered. However, the distance at break is more increased than when PEG 400 is used. Furthermore, the initial drop in the distance at break for sorbitol is the smallest (I_{ddb} of 0.67mm).

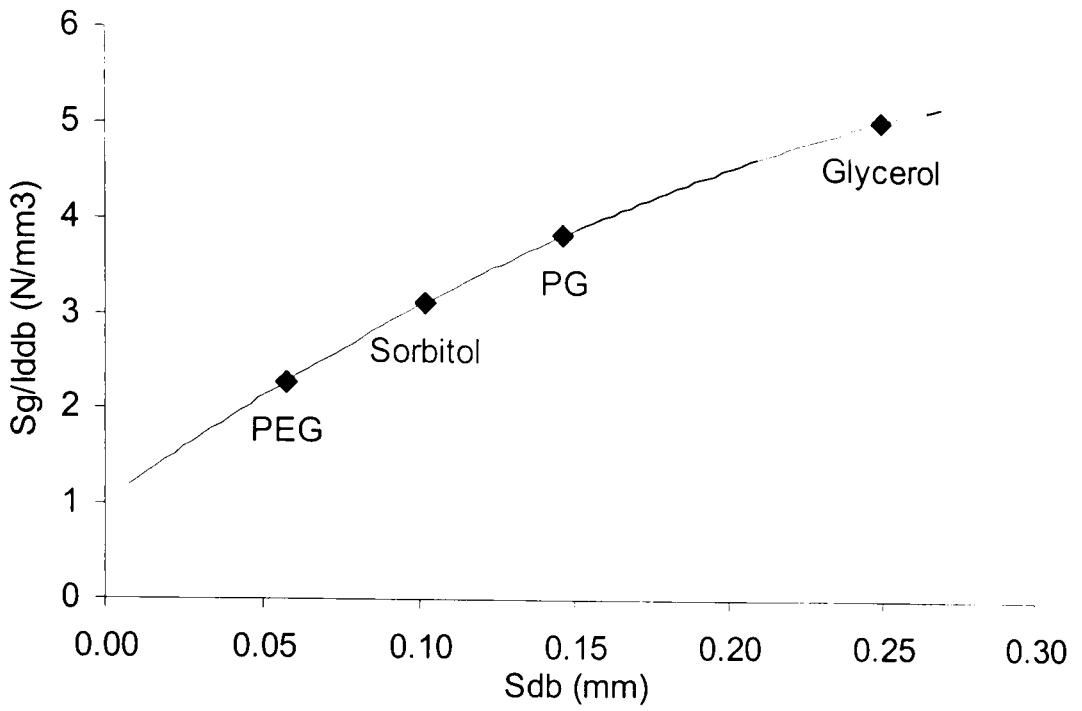
8.6.3. Discussion

All plasticisers affect the mechanical properties of caseinate films in a similar way. The gradient is decreased and, after an initial drop, the distance at break increases. The force at break initially decreases.

It was observed that none of the parameters described in Table 8-2 were directly correlated. This is surprising since the effects of the plasticiser on the various mechanical parameters measured by puncture should be related. For the gradient, the plasticising effect could be estimated by the value of $G_{plasticiser}$ or S_g but the plasticising effect on the distance at break is more complex. Two parameters are needed to account for the behaviour of the distance at break with increasing plasticiser content: I_{ddb} and S_{db}.

The initial drop in the distance at break (I_{ddb}) and the slope in the distance at break (S_{db}) result from the fitting of the distance at break data for increasing plasticiser content. S_g and $G_{plasticiser}$ result from the fitting of gradient data for various plasticiser contents. They do not depend on the fracture point of the film and therefore, I_{ddb} and S_{db} are mathematically independent of S_g and $G_{plasticiser}$. It is interesting to observe that a relation is obtained between S_{db} and the ratio S_g/I_{ddb} (Figure 8-14). This implies that the effect of the plasticiser on the distance at break and the gradient are indeed related.

Figure 8-14: Plot of puncture parameters obtained for four plasticisers.



Plasticisation phenomena and their influences on mechanical properties have been widely studied. In most cases, the system is considered homogeneous. Indeed, if phase separation occurred whitening of the films should be obtained. This was observed in methylcellulose films plasticised with high molecular weight PEG (Donhowe and Fennema 1993). No whitening was observed within the timescale of our study. Nevertheless, the low molecular weight compounds can be unevenly distributed within the polymer matrix without resulting into large-scale phase separation and some areas within the material could therefore be preferentially plasticised (amorphous versus crystalline, hydrophobic versus hydrophilic).

Plasticisation results in changes in glass transition temperature T_g which is related to the molecular mobility within the materials. Models predicting the T_g of plasticised systems using the T_g of both plasticiser and polymer have been developed (Roos 1998; Nielsen and Landel 1994). These models predict that the T_gs of the mixture polymer-plasticiser are lower for the plasticisers with the lowest T_g. The T_gs of the plasticisers used are given in Table 8-3. Comparing these data with the results in Table 8-2, it is clear that the T_gs of the plasticisers are not directly related to the plasticising effect on the mechanical properties observed by puncture. Knowing the

T_g of plasticisers is therefore not sufficient in predicting the plasticising ability of the plasticisers used here.

Table 8-3: T_g of plasticisers used in the study

Compound	T_g (K)	Source
Water	134	(Lourdin et al. 1997)
PG	166	(Takeda, Murata and Yamashita 1998)
Glycerol	187	(Lourdin et al. 1997; Takeda et al. 1998)
Sorbitol	271	(Lourdin et al. 1997)
PEG 400	198	(Verhoeven et al. 1989)

It is also noticed that decreases in the distance at break occurred for all the plasticisers used. At low plasticiser content antiplasticisation can occur. This would arise from the formation of a closely packed system in the presence of little plasticiser. Decrease of free volume, limited mobility, changes in β-relaxation (changes in temperature, decrease of intensity or absence of relaxation) and early fracture can all occur at low plasticiser content (Lourdin et al. 1997; Nielsen and Landel 1994). It is possible that the initial drop in the distance at break is related to antiplasticisation. This effect was especially strong for PEG 400. PEG differs chemically from the other plasticisers by the lack of hydroxyl groups and the presence of ether groups. This should lead to a different hydrogen bonding ability. The presence of CH_2 along the PEG chain would also allow hydrophobic interaction with caseinate. It could then be possible that PEG is preferentially placed within the hydrophobic region of caseinates. Therefore the interaction between the caseinates and the PEG may differ in intensity and location. The effect observed in our study resembles the antiplasticisation effect. However, this should also lead to an increase in the gradient, which was not observed here.

The main process involved in plasticisation is loss of polymer-polymer interactions and the creation of new polymer-plasticiser interactions. When these new interactions allow the polymer molecules to move away from each other during application of stresses, active plasticisation occurs.

Interactions between hydrophobic groups are relatively weak compared to hydrogen bonding that can occur between hydrophilic groups. Hydrogen bonding between

macromolecular structures reinforces the resistance to deformation hence the modulus. When hydrophilic plasticisers are introduced, they will preferentially surround hydrophilic structures allowing relative mobility of the hydrophilic part of the polymer. The applied stresses are relieved in plasticised films due to the quick relaxation obtained compared to unplasticised systems. This results in lower gradient and increased distance at break.

This would explain the ranking observed for the plasticisation of the distance at break: glycerol, PG and PEG. The behaviour observed with sorbitol could be explained by its higher molecular weight. The presence of a plasticiser of higher molecular weight might lead to bridging between polymer molecules which in turn decreases the plasticising efficiency. This could result in low S_{db}, S_g and $G_{plasticiser}$.

In our study, the loss of water does not compensate for the addition of plasticiser at constant relative humidity. The low molecular weight compound content (water and plasticiser) therefore increases from 11% (w/w) (no plasticiser) up to 24% (w/w) (15% plasticiser). On adding a plasticiser to the polymer-water mixture, the system becomes therefore very complex with the creation of new interactions (plasticiser-polymer, plasticiser-plasticiser and plasticiser-water) and the possible decrease of some interactions (polymer-polymer, water-polymer and water-water).

The combined effects of these interactions led to a difficult pattern. As mentioned earlier, the possibility of local concentration of plasticiser due to the natural disparity of hydrophobicity along the caseinate chain could result in more possible differences. Overall, it appears that water, glycerol and PG are the best plasticisers. Low molecular weight and high hydrogen bonding ability might be the reasons for these strong plasticising effects.

Plasticisation using glycerol could be an interesting way of improving the mechanical properties of caseinate films. However, the gradient decreases significantly and is much lower than for HPMC or gelatin. It would also be

interesting to consider the effect of relative humidity on the mechanical properties of plasticised films.

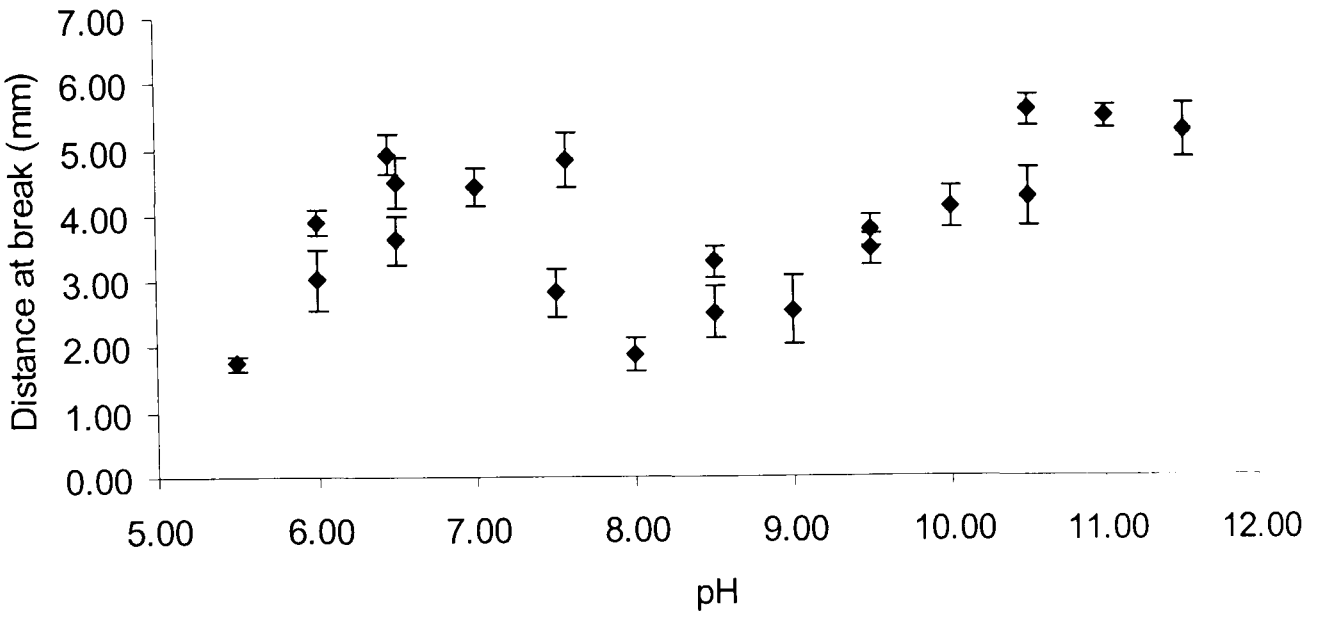
8.7. EFFECT OF pH

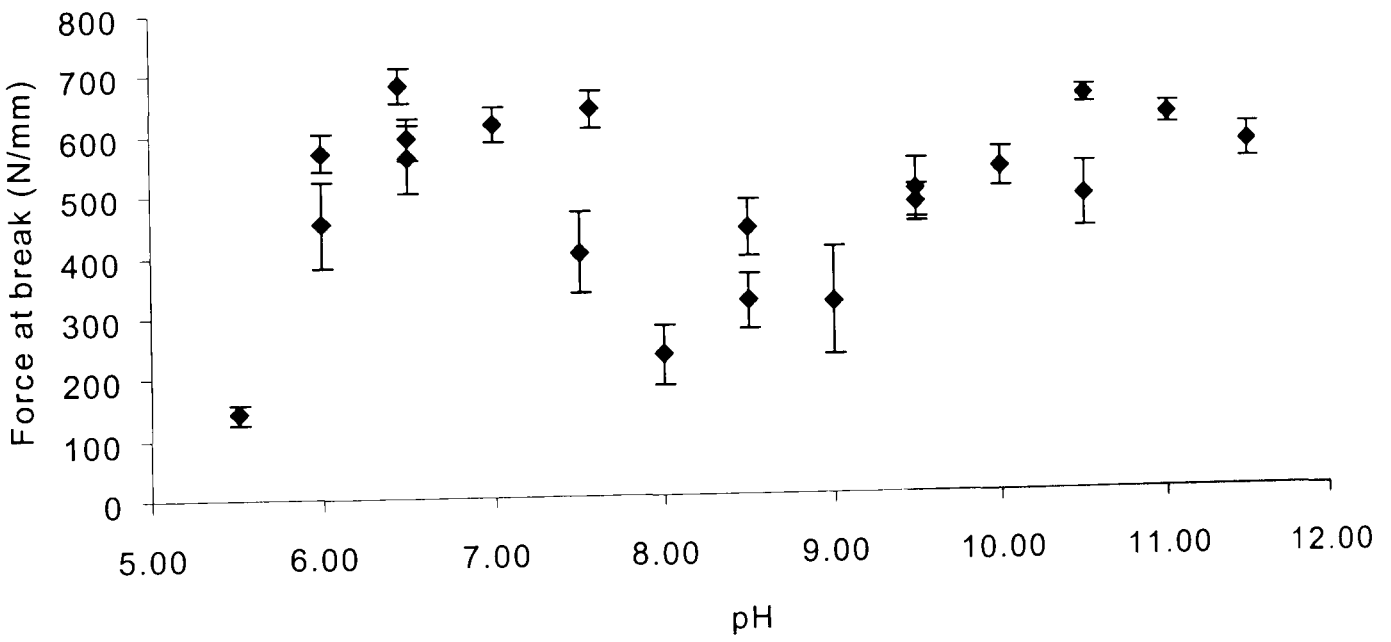
For all the experiments carried out so far, the pH of the caseinates solutions was not controlled. Sodium caseinates at a concentration of 10% (w/w) had typically a pH of about 6.5.

8.7.1. Results

The experiments were repeated three times. The data obtained with various pH's are much more scattered than usually observed. For pH's below 5.5 a gel formed and no films were cast. At pH 5.5, the solution became white indicating some precipitation. However, no gel was formed and a film was produced. All the data from the puncture tests are gathered in Figure 8-15. The distance at break shows a local maximum at pH 6.5 and increases beyond pH 8. The gradient is relatively constant with a slight decrease at high pH. The moisture content of the samples was affected by the pH as shown on Figure 8-16.

Figure 8-15: Effect of the pH (10% solution) on puncture data of sodium caseinate films (average and 95% confidence interval as defined in Figure 4-6).





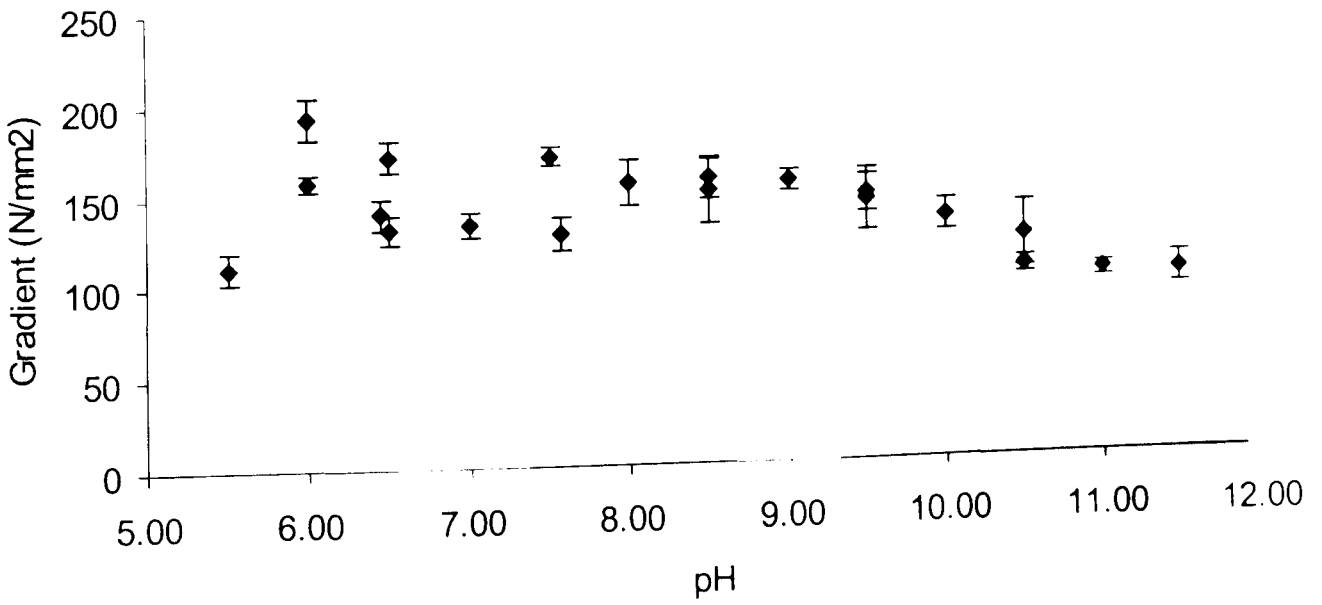
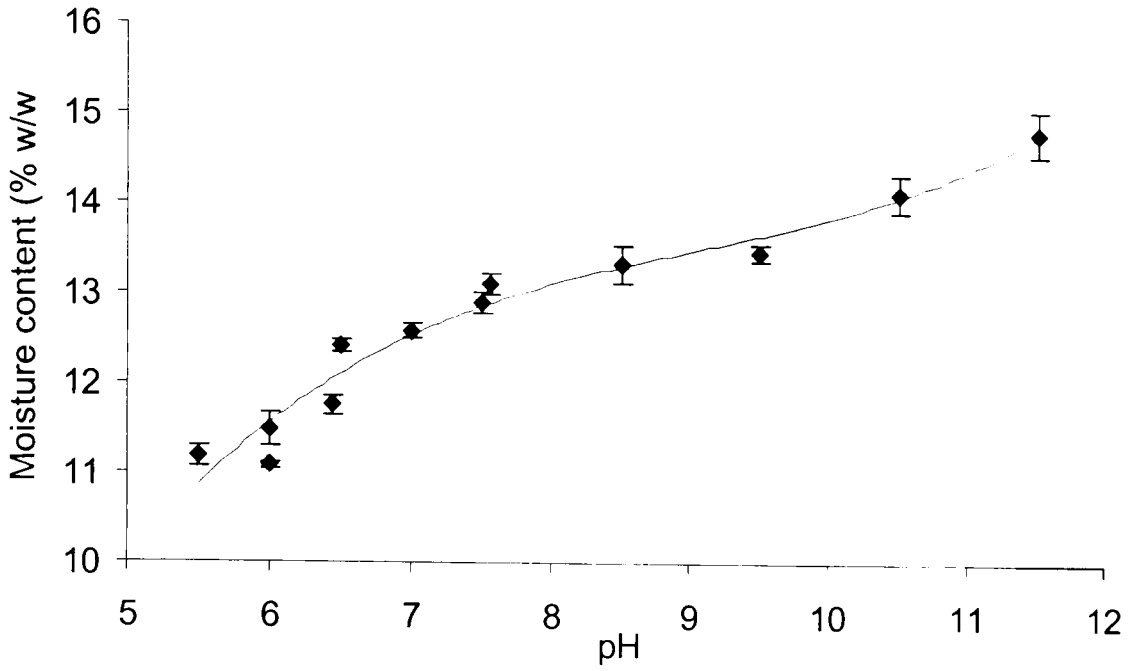


Figure 8-16: Moisture content of sodium caseinate films prepared from 10% solutions at various pH's.



8.7.2. Discussion

The effect of pH on ionisable groups is very important. The thermodynamic equilibrium between the two forms (charged-uncharged) can be displaced substantially by changing the pH. For polyelectrolytes, each ionisable group is affected and the overall behaviour of the polymer can be modified. These changes explain the coagulation of the caseinates at low pH, due to neutral net charge (Mulvihill and Fox 1989; Mulvihill 1989). At high pH, it is expected that the caseinate became highly negatively charged. These changes should induce conformational changes such as unfolding or alignment due to electrostatic repulsive interactions.

Very little effect of the pH on the gradient was observed in our study. The increase of the moisture content at high pH could be the reason for these effects. As mentioned earlier, the data for the distance at break are difficult to interpret. It would seem however that the best results are obtained either at pH 6.5 or at very high pH.

It was surprising that so little effect was obtained on the polyelectrolyte on varying pH. However, it is important to remember that the effects of the pH are only important when water is abundant enough. As the water evaporates, the ionisable groups become less hydrated and ultimately, in the 'dried' film, pH has less relevance. Therefore the possible effects described above are valid until a certain stage in the drying process. If the molecular mobility of the polymer is high enough at this stage, these effects could be cancelled. Because caseinates are relatively small molecules, it is indeed likely that once most of the charges have disappeared, the polymer can adopt the most stable conformation, and cancel out the original effect of pH.

8.8. MAILLARD CROSSLINKING

8.8.1. Introduction

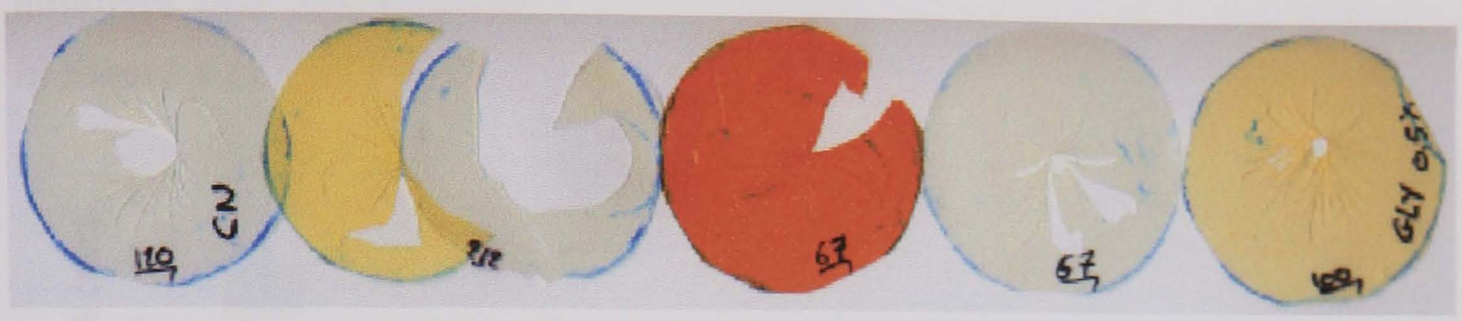
It was shown that plasticisation could improve the mechanical properties of caseinate films in term of their distance at break. In order to increase the gradient, crosslinking via Maillard type reaction was studied.

Maillard reactions are a group of chemical reactions that occur on heating between protein and sugar and lead to browning of the product. Such reactions have been used to crosslink proteins and polysaccharides (Kato et al. 1992).

8.8.2. Results

Films containing glyoxal (0.1 and 0.5%), xylose (10%), sucrose (10%) and glucose (10%) were prepared. Before heat treatment, only the samples containing glyoxal and xylose were coloured. Browning during the heating process showed that Maillard reaction occurred to different extents in the various samples (Figure 8-17). The colour is an indication of Maillard reaction but is not directly related to the crosslinking level. It is not known if additional linkage actually occurred in these studies.

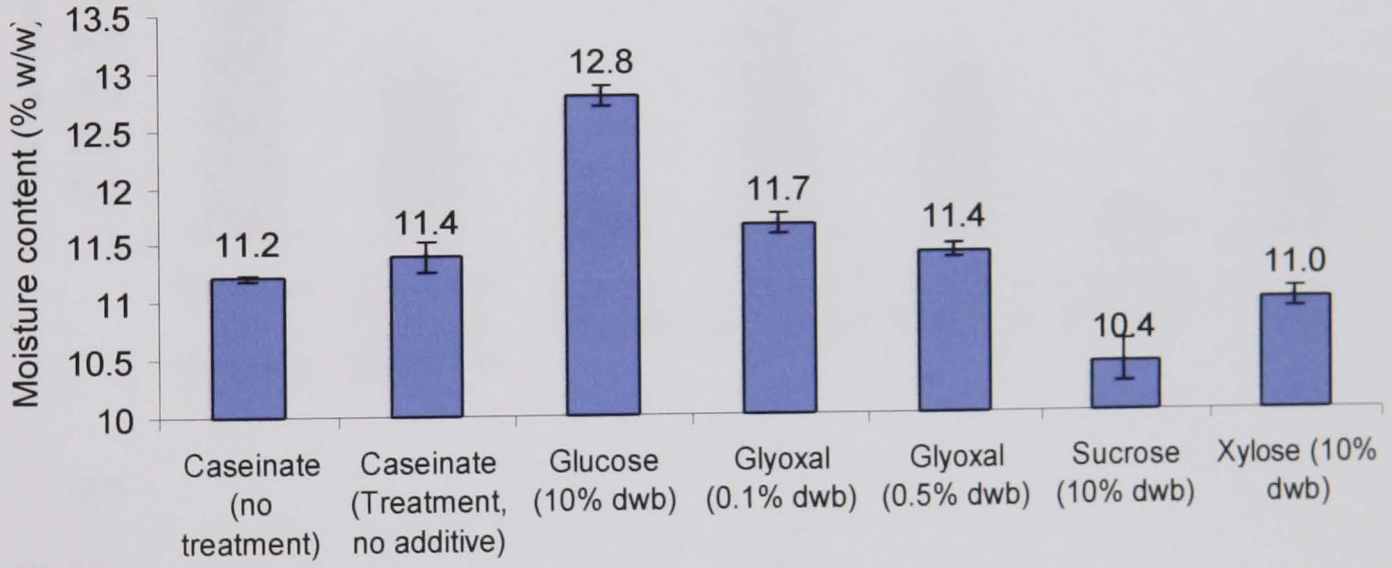
Figure 8-17: Films obtained using various additives after heat treatment (observed after puncture test).



None | Glucose (10%) | Sucrose (10%) | Xylose (10%) | Glyoxal (0.1%) | Glyoxal (0.5%)

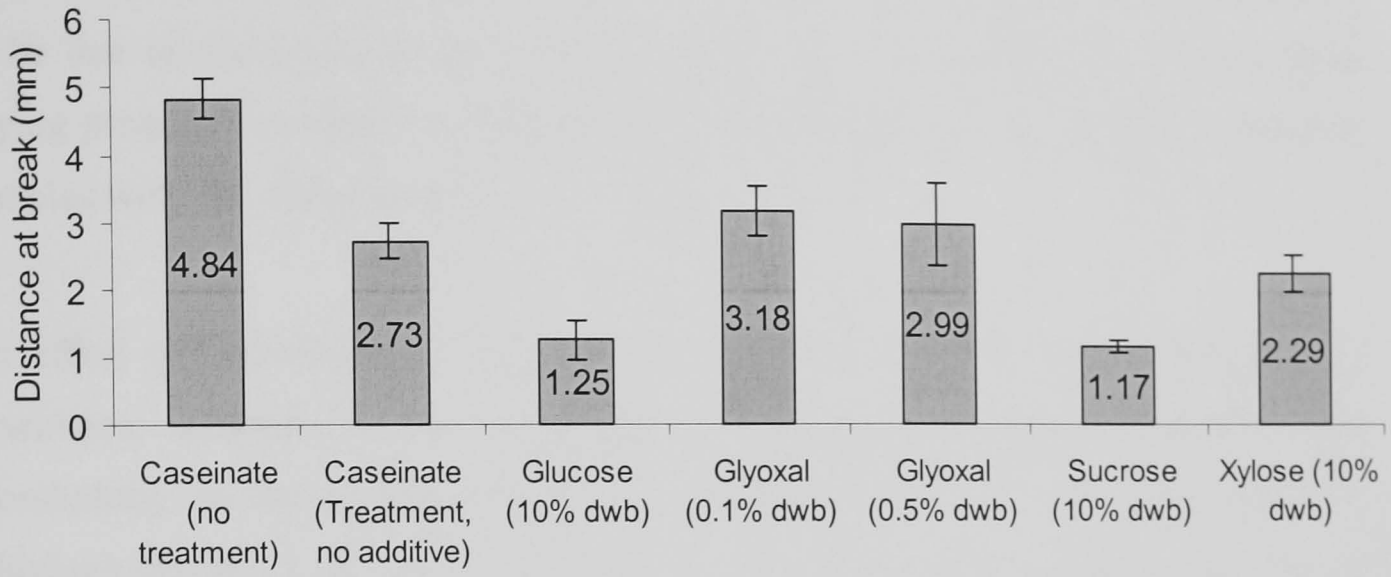
The moisture content of each film was measured and is given in Figure 8-18. The moisture contents for caseinate films before and after heat treatment are not significantly different ($p<0.05$). However, some differences are observed for samples with glucose and sucrose.

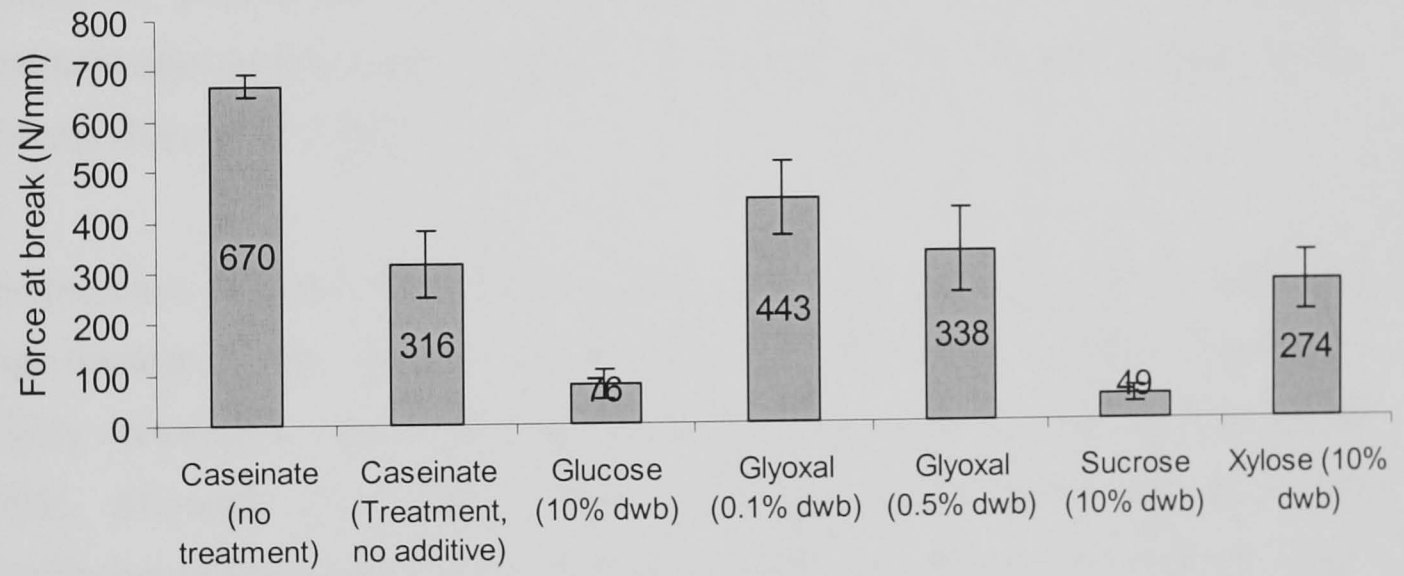
Figure 8-18: Moisture content of caseinate films containing 'Maillard additives' at a relative humidity of 44%.

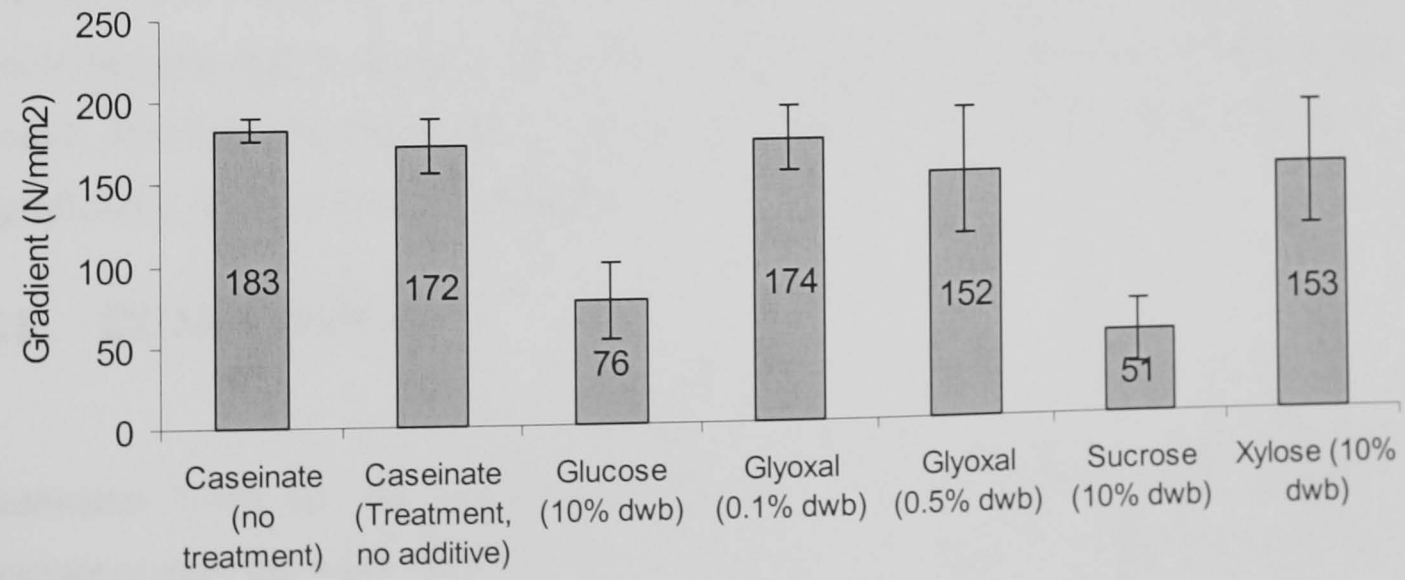


The results of the puncture test are given in Figure 8-19. The distance at break of pure caseinate is decreased by the heat treatment. This distance at break of the samples with sugar (xylose, glucose and sucrose) was lower than the control. The gradient of these samples must be analysed cautiously since very short distances were reached allowing only the gradient at break to be measured. The samples with glyoxal or xylose gave results close to the control.

Figure 8-19: Puncture results on caseinate films with various amounts of additives after heat treatment (average and 95% confidence interval as defined in Figure 4-6).







8.8.3. Discussion

The heat treatment did not affect either the moisture content or the gradient of pure caseinates. However, the distance at break was lowered significantly. This is likely to be due to the strong bending that occurred after heat treatment. A very quick drying process occurred when the samples were removed from the oven leading to samples with non flat geometry.

An effect of crosslinking is to make sample insoluble. However, in the case of caseinates, solubility in the presence of enzymes should be maintained. The crosslinking processes that occur during Maillard reactions are very complex. Although the pathways are still unclear, glyoxal seems to be a key compound in linking the protein via the lysine groups (Lederer and Klaiber 1999). The heat treatment used here is known to allow the formation of crosslinking in protein sugar systems (Kato et al. 1992).

The presence of crosslinking did not increase the distance at break of the films. The large decrease in the case of glucose and sucrose could be due to phase separation. Highly crosslinked elastomers present higher moduli and T_g (Nielsen and Landel 1994). Although crosslinking could improve the mechanical properties, the distribution of the distance between crosslink may play a major role. According to Nielsen (1994), random crosslinking can lead to the presence of short chains that would become highly stressed and make the material brittle. Controlled crosslinking would therefore be necessary in order to promote long chains and improve significantly the mechanical properties.

8.9. CONCLUSION

Caseinates films can be produced easily from 10% solution or more. Sodium caseinates gave the most promising films. The use of different salt could however be interesting for mixed systems or in the case where very low viscosities are needed.

The fracture behaviour and ultimate deformation processes involved are likely to be intermediate between gelatin and HPMC films. This was supported by the effect of moisture content on the mechanical properties.

The use of plasticisers allowed increased distance at break. Glycerol and propylene glycol were the most interesting plasticisers. It was argued that molecular weight and hydrogen bonding ability of the plasticisers might be the key properties determining the quality of the plasticiser. pH changes and Maillard type reactions did not improve the mechanical properties of the films.

CHAPTER 9. DISCUSSION AND FUTURE WORKS

Medical drugs are mostly delivered as tablets and capsules. The possibility of producing capsule with a non gelatin based system is a very appealing idea. This would lead to a much a wider acceptance by the consumer and also a possible reduction of the price. However, the task is very challenging since the material must fulfil severe requirements, especially if similar production processes are to be kept.

Gelatin provides the gel and the quantity of material to form an even coating for each capsule end. To match this, a mixed systems was proposed (gelling agent - filler), however the gelling behaviour might be affected by the presence of the filler. This is especially important because the filler concentration must be very high for capsule production. In this work, phase separation has been considered and how it could affect the gelling behaviour in a mixed system.

On a theoretical basis, it was shown that the limit for gelation could not be defined by the binodal curve. The requirements to prevent gelation are that the mixed system is above the tie line that meets c^*_b and above the inversion line. Nevertheless, a shift in the position of the binodal would result in a shift of both these lines. The consideration of the effects of the charge of each polymer (gelling agent and filler) on the gelling ability of the gelling agent has been undertaken. The results were not symmetrical and this was explained by the difference in binodal shifts that are required for preventing gelation. The effect of charge density differences is explained by the effect of the entropy of the counterion on the phase diagram. On a practical aspect, the system will gel when only one of the polymers is charged or when the gelling agent is more charged than the filler.

In order to choose a set of materials of interest, a sensory approach has been used. Although this approach is very arguable in term of its ability in measuring fine

differences, it proved useful in discriminating among a large set of films. This showed that cellulose and alginates derivatives were very promising in term of their mechanical properties. It also provided a definition for some terms and an easy way of assessing new films via the flick-test.

A low speed puncture test using a homemade device was used throughout the project for comparing the mechanical properties of the films. The results for HPMC and gelatin showed large differences. Both film showed strong correlation between the gradient (slope of the force-distance plot) and the moisture content. However, the relative slopes were very different. Differences in fracture patterns and orientational order were also demonstrated. The study of the alginate films showed similarities between alginate and gelatin. A theoretical model was envisaged for ultimate deformation behaviour of alginate, gelatin and cellulose derivatives films. It is proposed that the deformation of alginate films proceeds through unfolding and crazing which would explain both fracture pattern behaviour and orientational observation. Such system would be dependent on entanglement and crosslinkage. A different model for the cellulose derivative was suggested where intermolecular slippage occurred, leading to energy loss and the creation of orientational order on deformation. This latter model would be consistent with the very low molecular weight of the HPMC used and with the hydrophobicity of the polymer.

For the caseinate systems, the mechanical properties are relatively poor. Ultimate deformation is thought to occur mostly via crazing. Sodium caseinate formed the best film. It is possible to enhance the mechanical properties using a plasticiser. Low molecular weight and hydrogen bonding ability of the plasticiser seems to be the key to effective plasticisation. The deformation model proposed is intermediate between the two previous limit models.

The two limit models of ultimate deformation proposed here are raised from the known deformation processes, the observation of the fracture pattern and orientation, the influence of the molecular weight and the knowledge of the structural differences of the films. However, although these models match the experimental evidence, they

are hypothetical. Further experimental data are required in order to confirm these hypotheses (DMTA, stress relaxation...).

At this stage, it was not possible to propose a serious candidate for replacing gelatin for the production of hard capsules. Nevertheless, some of the products, which had a good flick score by sensory analysis, have not yet been studied further. However, it seems unlikely to find a product that would fulfil the mechanical properties requirements. There is indeed a general awareness for the replacement of gelatin for hard capsule production. The use of plasticiser to enhance existing product's quality is arguable. Increasing plasticiser content usually results in mechanical properties with increased sensitivity to changes in relative humidity.

In order to find new solutions, novel systems would have to be devised. The use of mixed filler systems could be of importance. However, if phase separation occurred, the resulting mechanical properties are likely to be reduced, due to bad adhesion between the phases. Polymer compatibility is very unlikely at the very low moisture content. Nevertheless, thermodynamic equilibrium can be avoided if the system is either very viscous or gelled. Another option would be to enhance the adhesion and make use of the blending laws to tailor the mechanical properties. It would also be possible to make use of controlled crosslinking in the concentrated state (Maillard, PGA-Protein).

If different production processes were considered (moulding, hot pressing, extrusion), then the constraints would be lowered and the use of high viscosity non gelling systems could also be considered. There would be some possibility of using PGA for instance but also a wide range of high viscosity products in the starch area. Furthermore, phase separation of mixed systems should be less problematic due to the high viscosities and temperatures.

Understanding the processes of deformation, fracture and their relations to film structure and chemical structure would be essential in predicting the potential new candidates. A low speed puncture test for comparing the films has been used in this

study. Any mechanical testing device could have been used. It would be interesting to model the stress states of a real capsule in 'normal' stress conditions. This might result in a complex situation where different parts of the capsule are subjected to very different stress states: tension, compression, shear, and torsion. It would then be possible either to use testing conditions of films that mimic these, or to set up new sample geometry that allow such complex stresses to be established. This would be a step forward in relating the mechanical measurements results to film quality for capsule production. The fracture mechanisms can also provide some insight in the source and nature of fracture involved.

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Orientational order in surface layers of gelatin films. **European Polymer Journal 2000** (in Press)

APPENDIX 1: SUPPLIER INFORMATION

ADM (Archer Daniels Midland)
1251 Beaver channel parkway
Clinton, IA 52732
USA

AE Staley
Decatur, IL 62525
USA

Allchem
Broadway house
21 Broadway
Maidenhead
Berkshire
SL6 1NJ
England

Amylum
Thames Bank House, Tunnel Avenue
Greenwich
London
SE10 OPA
UK

Armor Proteine SAS
35460 Saint Brice en Cogles
France

Avebe
M&O-weg 11
9563 TM Ter Apelkanaal
The Netherlands

Capsugel
Avenue de Timken
Colmar
France

Cerestar France
7 Rue du Marechal Joffre BP 109
59482 Haubourdin Cedex
France

Citrus colloids
Pomona Place
Hereford
HR4 0DA
UK

Dow Food Stabiliser
Midland, MI 48674
USA

GPC (Grain Processing Corporation)
Muscatine, Iowa 52761-1494
USA

Hercules
Aqualon
1313 North Market Street
PO Box 8740
Wilmington, Delaware 19899-8740
USA

Kelco
Waterfield, Tadworth
Surrey, KT20 5HQ
UK

Meyhall chemical AG
CH 8280 Kreuzlingen
Switzerland

Midwest Grain Products, Inc.
1300 Main
PO Box 130
Atchinson, Kansas
66002-0130
USA

National Starch
Prestbury Court, Greencourts Business Park
333 Styal Road, Manchester
M22 5LW
UK

New Zealand Milk Products (Europe) GmbH
Postfach 11 65 . 25452 Rellingen
Germany

Roquette (UK) limited
The Pantiles house
2 Nevill Street
Tunbridge wells
Kent TN2 5TT
UK

Shin-Etsu
SEH Europe Ltd.
Wilson Road
Toll Roundabout
Eliburn
Livingston
West Lothian
EH54 7DA
UK

APPENDIX 2: PRODUCT BATCH NUMBER

Product	Company	Batch number
Gelatin	Capsugel	
Kelcoloid LVF	Kelco	230321
Kelcoloid O	Kelco	65669A
Kelcoloid S	Kelco	60014
Manucol ester ERK	Kelco	57334A
Manucol LB	Kelco	683740
Manucol DH	Kelco	500374
Manugel DMB	Kelco	500771
Blanose 7	Hercules	73383
HPMC 606	Shin-Etsu	
Methocel E15	Dow	
Methocel E50	Dow	
Methocel E4M	Dow	
Klucel EFF	Hercules	FP10 13070
Klucel LF	Hercules	FP10 13868
Klucel MF	Hercules	FP10 13725
Alacid 710	New Zealand Milk Products	P3062
Alacid 741	New Zealand Milk Products	N4080
Alaren 799	New Zealand Milk Products	
Alanate 380	New Zealand Milk Products	P3066
Alaplex 1180	New Zealand Milk Products	R2002
Alanate 180	New Zealand Milk Products	
Potassium caseinate	Armor Proteine	
Magnesium caseinate	Armor Proteine	
Amylogum CLS	Avebe	
C* Avatex	Cerestar	75700
C* Cream Polartex 06716	Cerestar	
C* Cream Polartex 06718	Cerestar	
C* Set	Cerestar	6598
Clinco 460	ADM (Archer Daniels Midland)	
Clineo 718	ADM	
Colflo 67	National Starch (NS)	111768
Crisp film	NS	CG4784
Crystal Gum S	NS	
Dextran	Sigma	
FirmTex	NS	AHK3084
Flojel 45	NS	121458
Flojel 60	NS	121457
Glucidex 2	Roquette	652244
Hylon VII	NS	115818
Instant Clearjel Coarse	NS	97MGD316
K4484	NS	BHX14625
LVAWS	Midwest	
LVHPWS	Midwest	
LVOSWS	Midwest	959/98
Midsol 35	Midwest	H790609
Miracap	AE Staley	121456
Nadex 771	NS	

Appendix 2: Product batch number

Nadex 8781	NS	121455
National 1900	NS	MFI 7684
N-Lite L	NS	JGX23168
N-Lite LP	NS	HFI7882
N-Tack	NS	95JE7531
Pure Cote 760	GPC (Grain Processing Corporation)	S9523511
Pure Cote 790	GPC	S9704201
Stadex90	AE Staley	SD8C3495A
Textra	NS	97KGB048
Meyproguat 7	Meyhall	
Sunfiber R	Allchem	
X98001	Citrus colloids	
ExPro	Amylum	
Solpro 500	Amylum	
SWP 050	Amylum	
SWP 100	Amylum	
Film forming wheat protein isolate	Midwest	