Microfluidic-Assisted Nanoprecipitation to Produce Personalised Dosage Forms

Sarvenaz Hosseini, MPharm

In collaboration with the

Advanced Materials and Healthcare Technologies Research Division

School of Pharmacy,

University of Nottingham

Submitted toward the qualification award of

MRes

Submitted: October 2021

Abstract

The rise of polypharmacy and knowledge of pharmacogenetics has the potential to revolutionise dosing regimens for patients. Already, many single nucleotide polymorphisms have been identified as cause for alternate dosing in particular patient populations. Current manufacturing techniques in the pharmaceutical industry fall short on producing on demand, personalised, and patient tailored dosage forms. During this study, we propose that personalised multiparticulate drug delivery systems encompassing both nanoparticles and microparticles, produced through on-demand microfluidic-assisted production methods could help integrate current and future therapeutics for geriatric patients with high pill burdens. Microfluidic assisted particle production enables the user to have greater control on particle size through flow rate ratios and chip design along with traditional parameters already used in the field of polymeric nanoprecipitation such as solvent choice, polymer concentration, polymer characteristics and surfactant use. Through the application of this technology to drugs of different biopharmaceutical class types, this project aims to introduce the microfluidic platform as a strategy to produce 'on demand' personalised dosage forms. Currently, the project is using the FDA approved Poly(lactic-*co*-glycolic acid) (PLGA), a biocompatible and biodegradable polyester. Particle sizes of 80 nm to 400 nm have been achieved by using a 190 µm droplet junction microfluidic device. At polymer concentration of 2.5 mg/mL particle size was increased from 74 nm ± 1.6 nm to 128nm ± 1.3 nm through reducing continuous phase flow rate while keeping the polymer phase flow rate constant. Similar size control was observed at all concentrations studied except where agglomeration and consequent chip blockages resulted in unreliable results. Altering the polymer phase to aqueous phase flow ratio within the microfluidic system as well as reducing polymer concentration helped prevent

blockages, however resulted in an overall reduced particle size with all the samples collected having a PDI < 0.15. A ‘levelling off’ of particle size is observed at which point NP size does not alter significantly even with changing flow rates and concentrations when mixing conditions are optimal. The model drug chosen is the dihydropyridine calcium channel blocker Nifedipine for encapsulation with PLGA. Future works include release studies and surface analysis techniques to characterise Nanoparticles.

Table of Contents

Abbreviations

AI	Artificial Intelligence
API	Active Pharmaceutical Ingredient
BCS	Biopharmaceutics Classification System
DCM	Dichloromethane
DI	Deionised
DLS	Dynamic Light Scattering
DMSO	Dimethyl Sulfoxide
DoE	Design of Experiments
EMA	European Medicines Agency
FDA	Food and Drug Administration
HPLC	High Performance Liquid chromatography
NP	Nanoparticle
OrbiSIMS	3D OrbiTrap™ secondary ion mass spectrometry
PDI	Polydispersity Index
PLGA	Poly(lactide-*co*-glycolic acid)
PVA	Poly(vinyl alcohol)
Qcon	Continuous phase flow rate
Qdis	Dispersed phase flow rate
RPM	Rotations Per Minute
SEM	Scanning Electron Microscopy
SHM	Staggered Herringbone Mixer
TEM	Transmission Electron Microscopy

1. Background

1.1. Polypharmacy

In 2013, the King's Fund report on polypharmacy and medicines optimisation concluded that 'There is a need to develop systems that optimise medicines use where there is polypharmacy so that people gain maximum benefit from their medication with the least harm and waste' (1). As a result of this report, the Royal Pharmaceutical Society and the National Health Service have issued guidance to clinicians to tackle polypharmacy. Polypharmacy is defined as the concurrent use of multiple medicines by an individual, often defined as 5 or more medicines (2,3). The rise of multimorbidity in the geriatric population is also correlated with the rise in polypharmacy. Renal and hepatic function changes in this patient population, altered body mass, problems with cognition and dexterity can each be a reason for personalised dosing which is both convenient and more effective than current clinical strategies. One fifth of the people in the UK are aged 65 or over and the proportion is rising (1). In a study on 1.7 million patients residing in Scotland, 70% had at least one chronic condition by the age of 60 and almost half of those with a chronic condition had multi-morbidity (1). 'Pill burden' is known to be one of the primary reasons for non-adherence and is currently being tackled from different clinical angles of deprescribing, depot injections, fixed dosage polypills, less frequent dosing regimens and the error prone monitored dosage systems prepared in the pharmacy (4,5).

With everchanging clinical guidelines and increased understanding of pharmacogenetic (6) and organic disease influences on medicines absorption, distribution, metabolism and elimination in individual patients, there is a lack of 'on demand' production methods for individualised and personalised medicines.

The development of such technologies has the potential to revolutionise the industry and enable clinicians to formulate personalised dosage forms to meet the needs of their patients on an individual basis.

1.2. The direction of Pharma

After the decline in approval of new molecular entities and biologics between 2000-2010, there has been a steady increase in new approvals since. Thanks to an increased knowledge of the molecular biology of disease manifestation, the majority of new approvals encompass oligonucleotides and biologics, with a record high of new approvals by the FDA taking place in 2018 and the second highest in 2020 (7). As a result, there has been an increase in biotechnology stock prices, increasing by 20% in 2020 (8). Both oligonucleotides and biologics can require delivery systems in order to bypass *in vivo* delivery challenges, as a result of this, drug modifications, microenvironment modifications and drug delivery systems have been implemented in the area to overcome biological barriers to the delivery of this growing class of therapeutics (9). Precision medicine, a layer deeper into personalisation of treatments, aims to understand disease extensively to develop more targeted therapy (10). This growth in the development of precision medicines shows the shift in the pharma-sphere from ‘one-size fits all’ approach to a more targeted and personalised philosophy. This begs the question, should a revolution in dosage form production techniques follow suit?

1.3. Personalisation

‘Personalisation’ within healthcare remains a verb which can have differing meanings to the reader. While formulation personalisation can imply having control over drug release rates in a co-formulated medicine (11), in precision medicine, personalisation bases treatment choice on the growing fields of proteomics, transcriptomics and metabolomics alongside pharmacogenetics to reduce adverse effects or improve

patient outcomes (6,12). Greater understanding of disease progression at the molecular level in specific patient populations has increased the potential of successful treatments for patients, especially in the field of oncology where specific genetic markers can define drug treatment, particularly when combined with companion diagnostics (13). Although, unspecified, personalisation in healthcare should always have one unifying core at its centre, the patient.

1.3.1. The Genetic Angle

Genetic differences between patients can be used in predicting therapeutic effectiveness and adverse drug reactions. Pharmacogenomics, known as the relationship between genetics and the response to medicines, adds another layer of complexity to treatment plans. The previous barriers in this field, namely cost, have dramatically reduced with advancing technology. The first human genome sequencing project has been estimated to cost between 0.5-1 billion dollars (14), three million times more costly than a commercial genome sequencing service provided by a private company in the US in 2021 (15). The UK government has recently published a policy paper on ‘Genome UK’ announcing its recent strategy on the future of UK healthcare with pillar 1 involving diagnosis and personalised medicine. By 2024, the National Health Service (NHS) is committing to sequencing 500’000 whole genomes (16). In the US, the Food and Drug Administration (FDA) is also pushing for scientists and pharmaceutical companies to submit information on gene-drug risks on drug monographs (17). Some examples include; Clopidogrel sensitivity (CYP2C19), Simvastatin (SLCO1B1), Warfarin (CYP2C9 and VKORC1) (18). It is evident that with the field of pharmacogenetics advancing through reduced cost, policy implementation, increased funding, and regulation, current production methods will not be able to meet the needs of the pharmacogenetic treatment plans of the future.

1.3.2. The Formulation Angle

Dosage form personalisation is a practice already undertaken by the pharmacy profession. This can involve personalising formulation by choosing formulation flavours which are more favourable by the patient to more complex formulation designs such as particulate systems to enable successful delivery through a nasogastric tube. Dosage form personalisation through combining multiple different drug entities into one formulation to reduce pill burden is a relatively modern invention, with the first poly-pill invented in 1999 for cardiovascular disease (19). Poly-pills however remain at the mercy of mass production systems unable to account for personalised drug strengths or release systems required by individual patients with individualised and pharmacogenetic needs. Minitabs™ a commercialised mini-tablet which can be comprised of multiple mini tablets ≤ 3 mm (20) with different release patterns or active compounds formulated within a capsule is also a solution to polypharmacy and dosage form personalisation.

3D printing has been paving the way for personalised dosage forms in recent years. From 3D printed tablets with braille patterns for the visually impaired (21) to producing five-in-one polypills with defined release rates (11). Methods used in 3D printing include fused deposition modelling(22), selective laser sintering (23) and Inkjet printing (24). Although 3D printing technology has been applied to produce drug loaded microparticles (25), Nanoparticle (NP) production itself through 3D printing remains a less explored area (25) perhaps due to physical restrains imposed by current production technologies. Furthermore, the optimisation of ‘inks’ as well as the use of high temperatures during production creates a formulation challenge that needs to be addressed (26).

1.3.3. Comprehensive personalisation

Both genetic and formulation personalisation can help create pharmaceutical regimens with the potential of reduced side effects, improved patient outcomes and reduced long term healthcare costs. However, to truly produce personalised dosage forms of the future, the 'five rights' of medication administration (27), commonly used in clinical practice can be applied to formulation design with the patient at the centre. a summary of which can be found in **Table *1***.

Table 1 - Applying the 'five rights' of medication administration principles to the personalisation of drug formulation in personalised dosage forms

Five rights	**Application of 'personalisation'**
The right patient	Genetic testing to eliminate drugs unsuitable for patients
The right drug	Genetic testing to choose drugs suitable for patients e.g., Trastuzumab for HER2+ cancers
The right time	*Clinical, social, and drug physiochemical factors* Clinical – Patient specific pharmacokinetic-pharmacodynamic complexities Social – Patient occupation, preferences, and lifestyle Drug physiochemical factors – Stability, Solubility, Mode of action, among others
The right dose	
The right route	

1.3.4 Clinical & Legal Challenges

Practically, the future of a personalised and tailor-made dosage formulation production method will require a clinical and legal framework within which to operate.

In the UK, The National Institute for Health and Care Excellence (NICE) is responsible for the appraisal of health-care technologies. Evidential backing is required to demonstrate the economical and health benefits of new technologies if they are to be employed for use in the NHS. As a result, clinical and cost analysis studies will need to take place if such a technology is to revolutionise the pharmaceutical care sector in the UK.

Legal framework for preparing one-off unlicensed medication for an individual patient supplied from a pharmacy in accordance with a prescription already exists under the Human Medicines Act 1968 and Regulation 4 of the Human Medicines Regulations 2012 (28). However, currently, manufacturing medicines in a community pharmacy setting is very rare. ‘Off licence’ manufacturing of special medicines most often occurs at a central production facility on a ‘made to order’ basis. For a more seamless transition, this centralised model could be utilised to produce personalised dosage forms, allowing for more robust and expensive quality assurance processes to be utilised.

As the area of personalised medicines expands, logistical and legal development alongside technology realisation will be required to integrate new personalised manufacturing methods into the healthcare setting.

The technology proposed has the potential to disrupt and shake up the current pharmaceutical business model. If an instrument was to be produced with the ability to create personalised dosage forms as per need of the patient, quality assurance and current legal frameworks need to be put in place to ensure safety and quality. Central or community-based production facilities will be required to meet patient need. Each of which will have various implications such as patient access or potential costs and benefits to the healthcare economy. Additionally, clinical equivalence or even enhancement of therapeutics will need to be established for such a technology to be accepted by the healthcare community and the wider public. In addition to this, the healthcare workforce will need to be involved in determining the production model to ensure integration within current healthcare systems. With pharmacists at the centre of pharmaceutical care of patients, such a technology will involve redefining the role of pharmacists, pharmacist technicians and dispensers in adjunct to training

requirements needed to ensure safe working practices. As a result, consultation with the workforce would be necessary to envisage this change in geriatric care towards personalised dosage form production. Although there is much to be done and studied, this space remains an exciting new avenue for the future of healthcare.

1.4. Multi-unit drug delivery systems

As mentioned previously in 1.3.2., multi-unit drug delivery systems have previously been developed using 3D-printing technologies. However, with the expansion of oligonucleotide and biological therapeutics, delivery systems able to bypass biological barriers are of interest, namely nanoparticulate vehicles for drug delivery (29).

As a result of the physical constraints imposed by 3D printing, a versatile production platform able to produce varying particle sizes from nano to micro, using a multitude of materials based on formulation need, is needed to meet formulation personalisation for geriatric patients of the future.

Presently, to aid geriatric and vulnerable patients with their medication adherence, four times a day monitored dosage systems (MDS) are prepared in community pharmacies. Although helpful for patients, MDS' are error prone, highly time consuming to prepare and can be rigid to alter in the case of medication changes (30). Based on this knowledge, on demand personalised production of integrated but defined release systems for each drug within a multi-unit dosage form has the potential to reduce tablet burden, reduce medication errors and improve health outcomes. A strategy to address this issue can be by encapsulating different drugs in unique particles with defined release kinetics in one delivery medium such as a capsule.

Several theories for drug release from particles have been proposed, including but not limited to desorption of drug bound to particle surface, diffusion of drug through the polymer matrix, diffusion through the polymer matrix, diffusion through the polymer

wall of nano capsules, NP matrix erosion and combined erosion-diffusion process (31). The main consensus with drug release from NPs being that an increase in size of the particle decreases the release rate (32). Therefore, a simple way to control drug release on an individualised basis could be through controlling drug-particle size.

1.5. Nanoprecipitation

Nanoprecipitation or interfacial deposition was first described by Fessi *et al.* in 1989 (33) as a method of NP production. Polymer precursor dissolved in a water-miscible solvent such as acetone is emulsified in an aqueous solution in which the polymer is insoluble. The mixing of the two phases results in the diffusion of the water-miscible solvent into the aqueous phase. Consequently, local areas of unimer supersaturation within the aqueous phase result in the nucleation of NPs as unimers aggregate together. To achieve homogenous supersaturation and as a result, a homogenous NP size distribution, extremely rapid mixing between the solvent and aqueous phase is required with the nanoprecipitation method (34).

The nucleation-growth mechanism theory can be summarised in a 3-step process:

1- Nucleation of NPs in local areas of supersaturation.

2- Growth of NPs through aggregation of unimers.

3- Kinetically locked NPs as concentration of unimers drop and water-miscible solvent mixes into the aqueous phase.

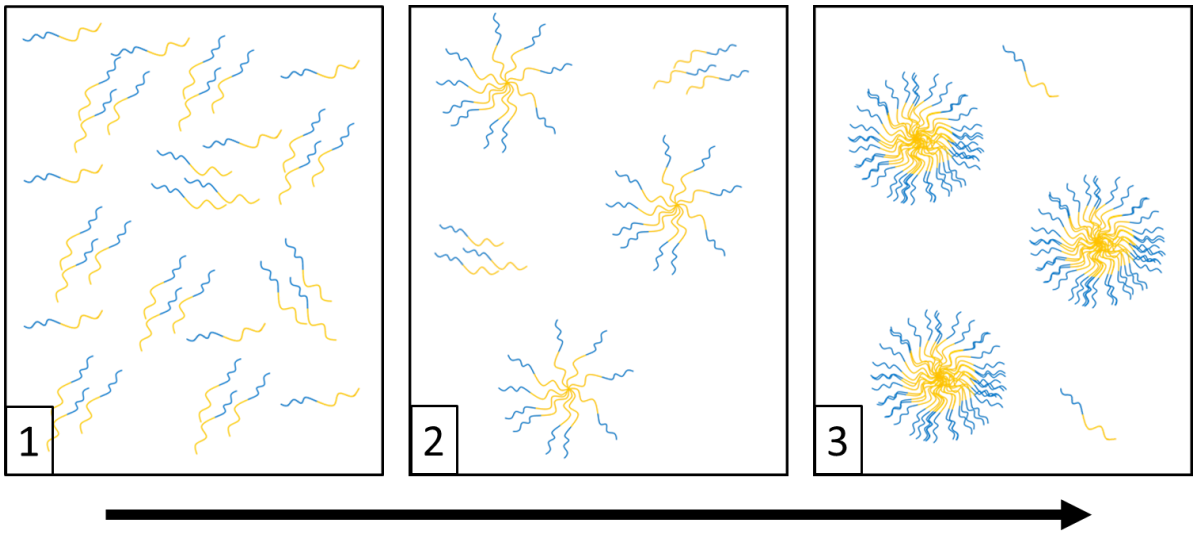

Figure 1. Schematic diagram of the nucleation-growth mechanism theory of NP formation.

Mixing time between the aqueous and solvent phase needs to be ultra-rapid (in the older of milliseconds) as it determines particle size and size distribution (35,36). To ensure low polydispersity of the particles, mixing time needs to remain shorter than the time taken for the aggregation of unimers to complete into the kinetically locked NPs described in (32). The two-dimensional model developed by Karnik *et al*. (32) can be used to estimate mixing time of the solvent and aqueous phase.

$$1.\quad \tau_{mix} \approx \frac{w^2}{9D\left(1+\frac{1}{R^2}\right)}$$

Where τ_{mix} is the mixing time expressed in seconds, w is the microfluidic channel width expressed in μm, D represents the diffusivity of water (approximately 10^{-9} m^2/s) and R is the ratio of flow rate of the polymeric phase to the total flow rate of water (Q_{dis}/Q_{con}).

However, once NPs are formed, particle size can change over time. Their growth will also depend on diffusion limited cluster–cluster aggregation (34). NPs may also aggregate further through the process of Otswald ripening if solvent removal is not complete (34).

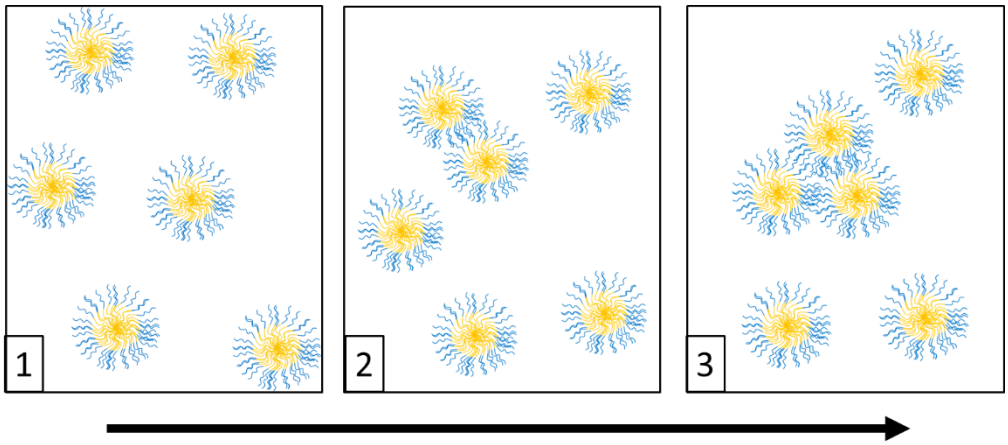

Figure 2. 1) Newly precipitated NPs 2) Cluster-cluster aggregation; occuring when there is a high concentration of NPs, increasing the chance of collision and growth of aggregates 3) Ostwald ripenning; a thermodynamically favourable process by which smaller particles deposit onto the larger aggregates.

Historically, nanoprecipitation has been achieved using batch production methods through sonication (37), magnetic stirring and homogenisation (38). As mixing of the two phases determines the particle size and particle size distribution, batch particle production can cause non-homogenous mixing conditions. Eddies produced upon mixing of the solvent and anti-solvent vary in size depending on relative distance to the mixing vortex. Smaller eddies closer to the centre of the vortex result in smaller particles while larger eddies further away result in larger particles. Consequently, this can result in a greater polydispersity index or particle size distribution as evidenced by Recent work by Donno *et al.* (39). This is unfavourable in drug delivery as particle behaviour would be non-uniform as a result.

1.6. Milli-fluidic and Microfluidic particle production

NP production in flow was introduced using the milli-fluidic confined impinging jet mixers used by Johnson and Prud'homme in 2003 (40). Other milli-fluidic mixers such as multi-inlet vortex and coaxial mixers have also been used to achieve nanoprecipitation (41).

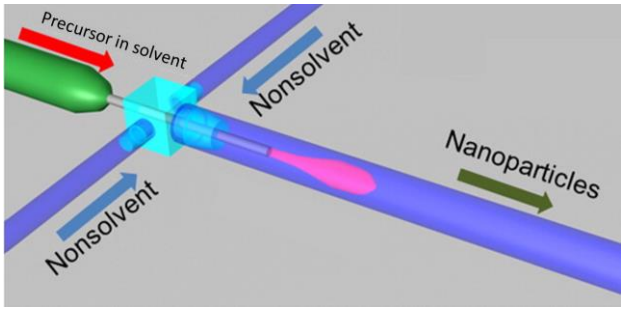

Figure 3. Milli-fluidic NP production in flow. The precursor solution containing dissolved polymer in solvent is shown in green. The non-solvent of the polymer is introduced into the jet mixer from the side, shown in blue. The nanoparticles are then collected from the outlet (41).

By introducing the polymer and aqueous phase through separate inlets, rapid mixing between the two phases occurs within the device, resulting in nanoprecipitation.

Altering solvent (Q_{dis}) and aqueous (Q_{con}) phase flow rates through the device enables for increased production parameter control, confers further influence over particle size and size distribution compared to batch nanoprecipitation methods. In comparison to batch production methods, in-flow production results in superior reproducibility of particles due to the establishment of homogenous mixing conditions within the in-flow reactors (41). Within the last two decades, microfluidic-assisted nanoprecipitation has grown to be a continuation of the 'In-flow' NP production story. With reduced reagent utilization, and an ability to be a platform for microparticle and NP production, microfluidic devices are ideal for economical high-throughput screening of novel formulation prototypes (35,42,43). As a result, microfluidic devices can tackle problems associated with prevailing particle production technologies (44,45).

There are two types of fluid flow, turbulent and laminar. The turbulent flow regimen is characterised by vortices, eddies, and fluctuations in fluid flow in space and time (46). Conversely, laminar flow regimens are characterised by fluid flowing in parallel with mixing among fluids dominated by molecular diffusion (46,47). Therefore, to obtain optimal mixing of the two phases, a turbulent flow or a controlled mixing regimen is needed within the microfluidic device (see **Figure *4***). This can be acquired through

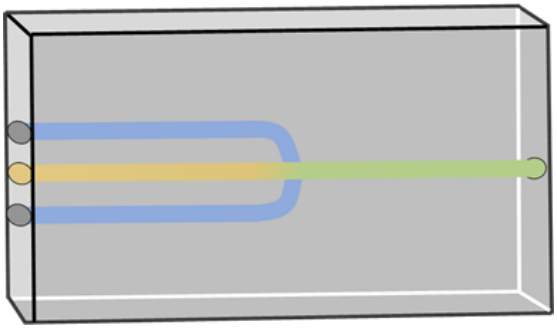

Figure 4. Microfluidic assisted nanoprecipitation device schematic. Aqueous solution (blue) and precursor solution (yellow) enter through the left channels and meet at the junction where the nucleation-growth mechanism gives rise to the nanosuspension (green).

high flow rates in larger microfluidic chips or through the alteration of the chip geometric design.

Whitesides *et al.* (48) have developed the staggered herringbone mixer (SHM) topology which reduces mixing time and as a result particle size and polydispersity. The SHM design results in the crisscrossing of the fluid routes within the chip (See **Figure 5**) resulting in a three dimensional twisting of the fluids (35), consequently inducing chaotic stirring, efficient mixing, and swift mass transfer (49).

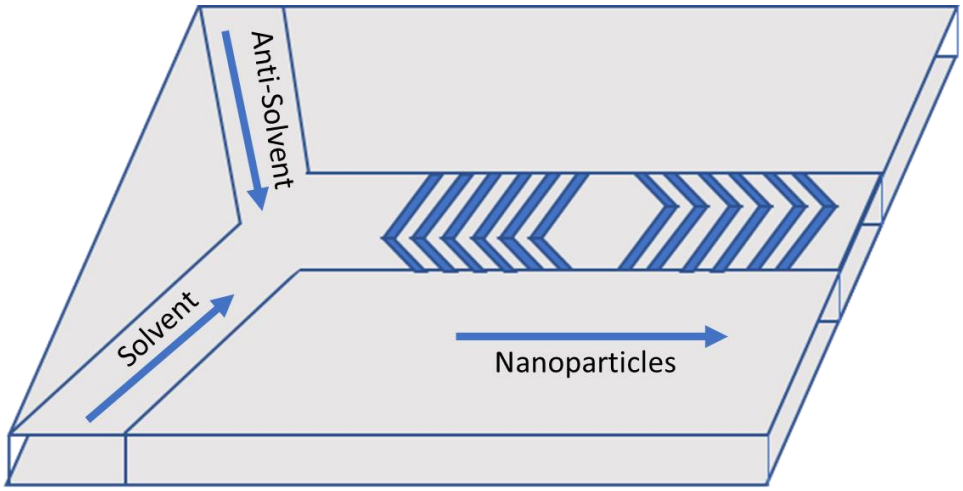

Figure 5 – Adapted SHM diagram (35).

Indeed, this microfluidic design is currently commercialised by Precision NanoSystems Inc. ® and has been employed by a variety of research groups for drug delivery purposes (49,50). However, the effect of the geometric shape of the microfluidic chip remains a less studied within the field of NP production (36).

To inform particle formulation design, a classification system is required from which examples of APIs can be used as proof of concept. An example of a pharmaceutical classification system for oral delivery of drugs is the Biopharmaceutics Classification System.

1.7. Biopharmaceutics Classification system

The biopharmaceutics classification system (BCS) was developed in 1995 (51). Using this system, active pharmaceutical ingredients (APIs) are classified by two characteristics; permeability and solubility when orally administered. Accordingly, based on their classification, different regulatory procedures apply to become licensed medicines. The European Medicines Agency (EMA) has defined high solubility as when the dose administered to the patient can be dissolved in 250 mL of aqueous buffers in the pH range between 1.2 and 6.8 (52). To determine permeability across the gastrointestinal tract, the EMA approved published human data with the gold standard reference being the absolute bioavailability; comparing API blood levels gained from an intravenous dose to the orally administered dose. However, the FDA allows for use of Caco-2 monolayer cell lines or animal studies for passively absorbed APIs (51).

Multimorbidity and polypharmacy in the geriatric population results in patients having to consume a combination of BCS drugs, taken daily at multiple times a day. Polypharmacy has been defined as when a patient takes five or more medicines by the Royal Pharmaceutical Society (3). Using drug delivery systems such as polymeric NPs, it is possible to manipulate the bioavailability of drug molecules and create multidose systems with distinct release profiles for each drug which is not possible with classical tabletting. Utilising a capsule with drug loaded nano and microparticles, would create unique micro and nano ‘environments’ for each specific drug, preventing direct physical and chemical interaction between the drugs. Ultimately, if successful, patients would be able to benefit from reduced daily intake of medicines and additionally have a personalised dosage formulation specific to their own bodies’ pharmacokinetics.

In this thesis, the encapsulation of two different drug molecules; Propranolol hydrochloride and Nifedipine were investigated using the microfluidic assisted nanoprecipitation method (see **Table 2.**) as they have different BCS classifications and are commonly used in pharmaceutical management of patients in the UK.

Table 2. BCS table with example drugs used within this thesis in bold (53).

	High solubility	**Low solubility**
High Permeability	**Class I** **Propranolol hydrochloride** Diltiazem Bisoprolol	**Class II** **Nifedipine** Piroxicam Phenytoin
Low permeability	**Class III** Ranitidine Gabapentin Atenolol	**Class IV** Furosemide Chlorothiazide Ritonavir

2. Parameters of Consideration for Microfluidic-Assisted Nanoprecipitation of Drug Loaded NPs

2.1. Polymer Choice

The distinct characteristics of the polymer used in NP production impacts the characteristics of the particles produced, namely; shape, size and charge which all have a crucial influence on bioactivity (54).

Owing to its biocompatible, biodegradable, and non-toxic qualities, within the pharmaceutical industry, the FDA approved Poly(lactide-*co*-glycolic acid) (PLGA) (**Figure 6**) is used in extended and modified release formulations, as a gelling and microencapsulating agent (55). As a versatile polyester, PLGA can be chemically functionalised with various biochemical entities to suit the need of the application. PLGA also has a rigid chain structure with a glass transition temperature (Tg) above 37 °C (56).

Furthermore, as PLGA is extensively used as a model polymer in research, ostensibly shown with the topic search of 'PLGA and drug delivery' revealing 6,624 publications from the past 20 years on 'Web of Science' search engine alone. it was chosen as a model polymer within this report to investigate microfluidic-assisted nanoprecipitation. More homogenous dispersion of the drug in the polymer matrix is achieved using D,L-PLA over L-PLA (56). Increasing PLGA molecular weight has shown to decrease NP size due to a more rapid precipitation as a result of reduced solubility within the aqueous phase, using the nanoprecipitation method (39). End-capped PLGA using esters as opposed to free carboxylic acid end groups in uncapped PLGA results in slower degradation of PLGA. The free carboxylic acid end group is able to create an acidic condition through which hydrolysis of PLGA is favoured (37). Finally, copolymer composition in the case of PLGA can impact biodegradation and drug delivery

capability (56). By altering lactide:glycolide molar ratio, the biodegradation rate of the polymer can be altered due to an increase in the rate of hydrolysis with higher ratios of glycolide. This effect has been previously utilised with the commercialised Zoladex® depot injections with one-monthly injections having a 50:50 lactide to glycolide ratio whereas Zoladex LA® being three-monthly depot injections having a 95:5 ratio (57).

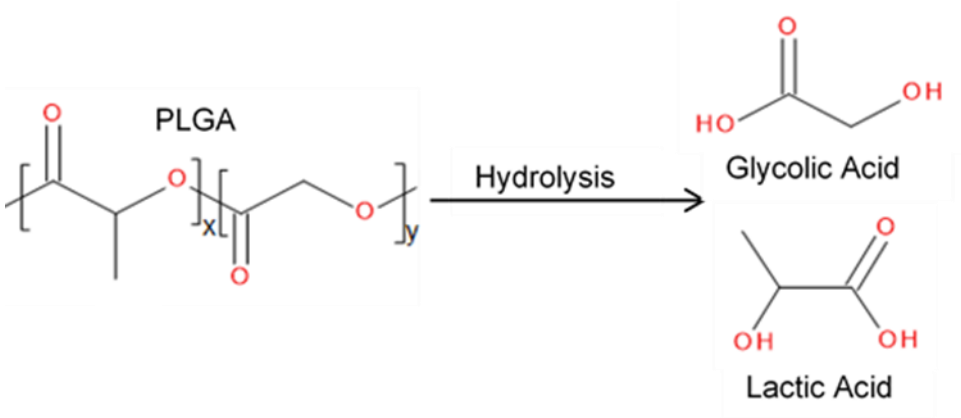

Figure 6. PLGA copolymer ratio is a rate determining factor on *in vivo* hydrolysis. The monomers glycolic and lactic acid are both metabolised in the body through the Krebs cycle (58).

Drug loading can be achieved during NP production or through adsorption of drug onto the NP through incubation of the NPs in a drug solution (59). Generally, drug loading during NP production results in higher drug encapsulation compared to incubation of NPs within a drug solution post production to achieve drug adsorption (59). With this method, the drug chemistry and conditions of NP production affect the encapsulation efficiency. The encapsulation efficiency of drug loading can be calculated using the following equation:

$$2. \quad EE = \frac{A}{B}$$

Where EE is encapsulation efficiency expressed in %, A is the amount of drug bound and B is the total amount of drug used for NP production.

It is generally accepted that with an increase in polymer concentration, an increased capacity for drug loading is achieved (60). Polymer-solvent-drug interactions are also key in successfully producing drug loaded particles.

2.2. Solvent Choice

Solvent properties and solvent-polymer interactions can have a considerable impact on NP properties. In nanoprecipitation, water-miscible solvents such as acetone are required to ensure optimal mixing conditions, furthermore, the solvent and polymer concentration chosen for the precursor solution should be optimal in order to ensure unimers being able to act as ideal chains (**Figure *7***) as this is when the best conditions for nanoprecipitation can be achieved (34).

Solvent characteristics such as volatility and viscosity determine the solvent evaporation rate which can also affect NP size. It is therefore important to maintain conditions affecting solvent evaporation rate when conducting experiments. This can include but not limited to rotations per minute of the magnetic stirrer bar used in the drying step, temperature of the medium as well as the application of vacuum.

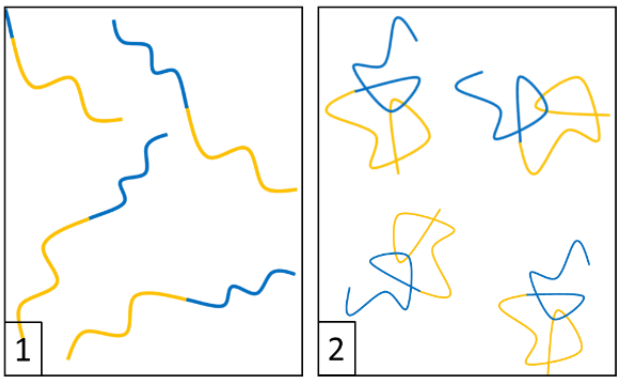

Figure 7. 1) favourable interactions between the unimers and a solvent at an appropriate concentration resulting in the unimers having an ideal chain composition. 2) Unfavourable polymer-solvent interactions resulting in the unimer chains coiling on themselves. Both these processes are thermodynamically driven.

2.3. Made for Nanoencapsulation, The Hydrophobic APIs

The encapsulation of hydrophobic drugs within PLGA such as Nifedipine (61), Indomethacin(33), Risperidone (62) and Doxorubicin (63) have been enabled using the nanoprecipitation technique (58). As the solvent diffuses into the aqueous phase, hydrophobic APIs remain attached to NP nuclei, whereas hydrophilic APIs are less likely to interact with the solvent phase and consequently the PLGA NP nuclei, making them harder to encapsulate. Consequently, the highest reported encapsulated APIs are hydrophobic and belonging to the class II BCS.

2.4. Overcoming The Hydrophilic API Problem

Although the encapsulation of hydrophilic drugs within PLGA such as Propranolol hydrochloride (64), Acetylcysteine (49) and proteins (58) have been reported, often times the method of production involves non-microfluidic assisted methods of production such as w/o/w double emulsion technique (58).

However, manipulations of microfluidic production parameters have shown to be successful in encapsulating hydrophilic APIs within PLGA and these can be subcategorised into chemical or physical approaches.

i) *The chemical approach.*

a. Using surfactants such as Poly(vinyl alcohol) (PVA) (65)
b. PEGylation of PLGA; giving PLGA an amphiphilic nature (66)
c. Using mixed solvents Dimethyl sulfoxide (DMSO) and Dichloromethane (DCM) (63)

ii) *The physical approach.*

a. Employing a SHM; by inducing chaotic mixing and increasing rapid mass transfer, N-Acetylcysteine loaded PLGA NPs have been produced (49).

3. Parameters of consideration with analysis

3.1. Lyophilisation

By removing water, lyophilisation enables the long-term storage of PLGA NPs. As explained previously, PLGA is prone to hydrolysis and if kept in an aqueous solution over time, PLGA NPs degrade. In addition to this, to be able to undertake dissolution and release studies, it is important to set a t=0, where drug release is not already in equilibrium with the surrounding environment, therefore, lyophilisation is a key step by which drug-NPs are ‘locked’ so that the release behaviour of the NP can be investigated.

Lyophilisation itself can cause the aggregation of NPs, preventing successful reconstitution. As a result, cryoprotection with pharmaceutically accepted excipients such as Trehalose, Sucrose or Mannitol can be necessary to recover NPs post lyophilisation (67).

3.2. Drug Release

To establish drug release behaviour from delivery systems, dissolution studies are required. Most described release behaviour from PLGA NPs is the ‘burst-release’ phenomena within literature. The ‘burst’ portion of release is describing the rapid release of the loaded drug from the particles, hypothesised to be adsorbed drug on the surface of the particles (31,68). While the ‘release’ portion refers to a steady release overtime mainly governed by the degradation of the NP (37).

Investigating the physico-chemical interaction between the encapsulated drug and the NP has been analytically challenging historically. This not only highlights the importance of post-production strategies to separate unbound drug from drug bound NPs but also sheds light on the importance of the latest developments in surface characterisation techniques such as secondary ion mass spectrometry (3D-OrbiSIMS).

3.3. The future is Automation

Scalability is often cited as a concern for microfluidic particle production, however, parallelising and in-tandem production (**Figure *8***) is a solution to this issue for applications within industry. Due to the nature of microfluidic devices, once particle production has been optimised, it is possible to automate the production process with minimal manipulation required by the operator (66). To achieve this, it is important to understand the design space in which the system operates. A solution to this is using Design of Experiments (DoE) to create a robust model for particle production can optimise parameters to attain desired size distribution, particle size or drug loading efficiencies. Machine learning has been predicted to replace DoE in the future (69).

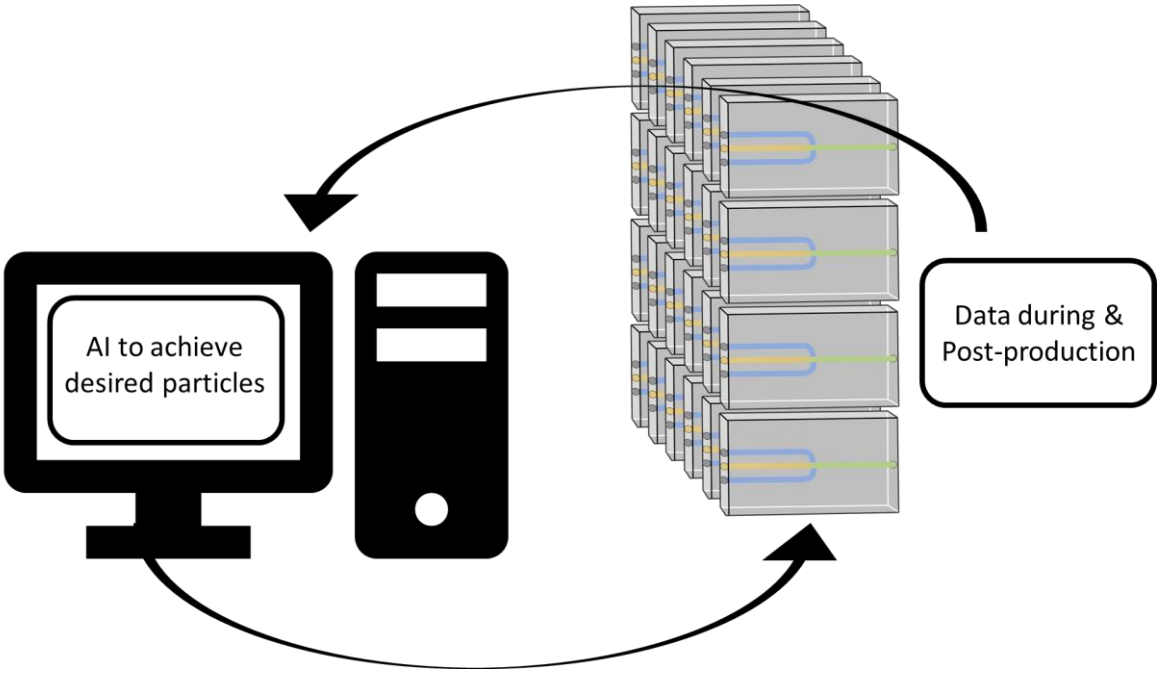

Figure 8. Automation of production using artificial intelligence (AI) and parallel particle production by using multiple chips at any one time can scale up production while maintaining control over production parameters.

Already, a computationally guided random forest model has been designed to understand drug-NP interactions (70) and AI has been applied to microfluidic-assisted PLGA microparticle production (44). Furthermore, with increased development in the

field of ‘big data’, the combining of NP-Biological interaction datasets, patient specific information and production parameters will help to produce bespoke, personalised dosage forms for the need of the future patient (71). Additionally, ‘in-line’ analytical elements such as HPLC, Spectrophotometers or bench-top NMR can be connected to the production outlet for quality control and monitoring (72).

Semi-automated, commercialised microfluidic production systems are now available such as the Asia ® series by Syrris ® have shown to have a wide range of applications in research including; chemical synthesis (73), biocatalysis (72) and hybrid liposome-metal NP production to name a few (74). The latest clinical translation of microfluidic technologies has occurred with the microfluidic manufacture of mRNA lipidic NPs for vaccine development (75).

Aims

The aims of this study were to investigate the production of PLGA NPs using the microfluidic-assisted nanoprecipitation method. The encapsulation of two different APIs of BCS I and II, Propranolol hydrochloride and Nifedipine, respectively, were explored and the presence of a surfactant during nanoprecipitation, Poly (vinyl alcohol) was investigated.

Propranolol hydrochloride (**Figure 9**) is a BCS class I drug (Fig. 11) (76). A hydrophilic non-selective beta-adrenoreceptor blocker, propranolol is clinically indicated for cardiac arrhythmias, anxiety and migraine prophylaxis (77). As a weakly basic drug, propranolol is formulated as a salt (Hydrochloride) to improve solubility *in vivo*. As a result, propranolol is highly soluble in water. To formulate propranolol-NPs, propranolol was introduced to the production system through the aqueous phase.

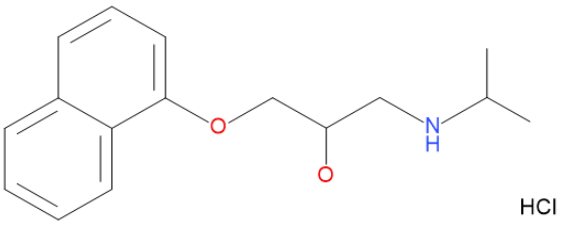

Figure 9. Propranolol Hydrochloride chemical structure

Nifedipine is a BCS class II drug (78) belonging to dihydropyridine voltage gated calcium channel blockers (**Figure 10**). Through influencing the displacement of calcium ions on myocardial cells, Nifedipine reduces myocardial contractility. Extended-release nifedipine formulations are therapeutically indicated for hypertension and angina prophylaxis (77). Due to high solubility in acetone, nifedipine was introduced to the production system through the solvent phase.

Poly(vinyl alcohol) (PVA) (**Figure 11**) is a water-soluble polymer with varying grades conferring its viscosity levels. Commercially, PVA is used as an emulsion stabilising agent. In the field of NPs, it is commonly used as an emulsifier (65).

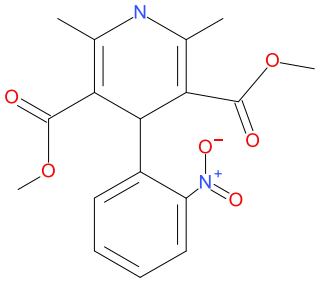

Figure 10. Nifedipine chemical structure

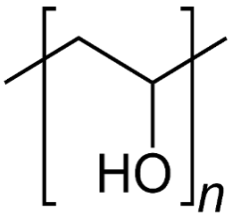

Figure 11. Chemical structure of PVA

4. Experimental

4.1. Materials

Poly(D,L-lactic-*co*-glycolic acid) (PLGA) (acid terminated) (50:50, Mw 30,000-60,000 g/mol, Poly(vinyl alcohol) (PVA), (Mw 9,000-10,000, 80% hydrolysed) and (+/-)-Propranolol hydrochloride were purchased from Sigma-Aldrich, USA, Japan and Belgium respectively. Acetone, dimethyl sulfoxide (DMSO) and methanol were purchased from Thermo-fisher Scientific, UK, USA, and Belgium, respectively. Unless specified, all solvents used were HPLC grade. Nifedipine (98%) was purchased from ARCOS organics, China. Deionised (DI) water with >18.2 MΩ electrical resistance was used as the aqueous phase.

Large droplet junction chip with a 190 µm channel size (part no. 3200130) with the connection PTFE tubing 1/16" OD x 0.5 mm ID (part no. 3200067) were acquired from The Dolomite Centre Ltd, Royston, UK. 10 mL borosilicate glass syringe with a gas tight PTFE plunger and Conventional 20 mL polycarbonate syringes with polypropylene plunger rods were acquired from Trajan SGE®, Australia and Beckton, Dickinson and Company, USA, respectively. Syringe pumps PHD ULTRA™ were obtained from Harvard Apparatus, Massachusetts, USA.

4.2. Preparation of Solutions

PLGA was dissolved in acetone at different concentrations. The FB15051 ultrasonic bath (Fisher brand, Germany) and Vortex-Genie 2 (Scientific Industries, USA) were used to dissolve PLGA in acetone. 1 mg/mL PVA in DI water was used as the aqueous phase when specified. For drug loading experiments, propranolol hydrochloride was dissolved in DI water at a concentration of 1 mg/mL. A stock solution of Nifedipine in acetone at 0.05 mg/mL was prepared to then produce PLGA in acetone solutions of 0.5 mg/mL and 1.0 mg/mL.

4.3. General NP Production

PLGA concentration, solvent flow rate (Q_{dis}) and aqueous flow rate (Q_{con}) were altered to achieve particle size control, see **Figure 12**. Q_{dis} was set at 100 µL/min, unless specified. A glass syringe and Becton, Dickinson and Company plastic syringes were used to contain the solvent and aqueous phase, respectively. The solvent phase was introduced into the chip through the central channel. NP suspensions were collected after 2 minutes run time to ensure equilibration of the system in a 20 mL glass scintillation vial from the outlet for 5 minutes. One sample was collected each time unless specified for analysis. To remove acetone, suspensions were left under magnetic stirring at 80 rotations per minute (RPM) using the multi-channel stirrer (Jeto Tech, MS52M, Korea) overnight with the vial caps loosely on.

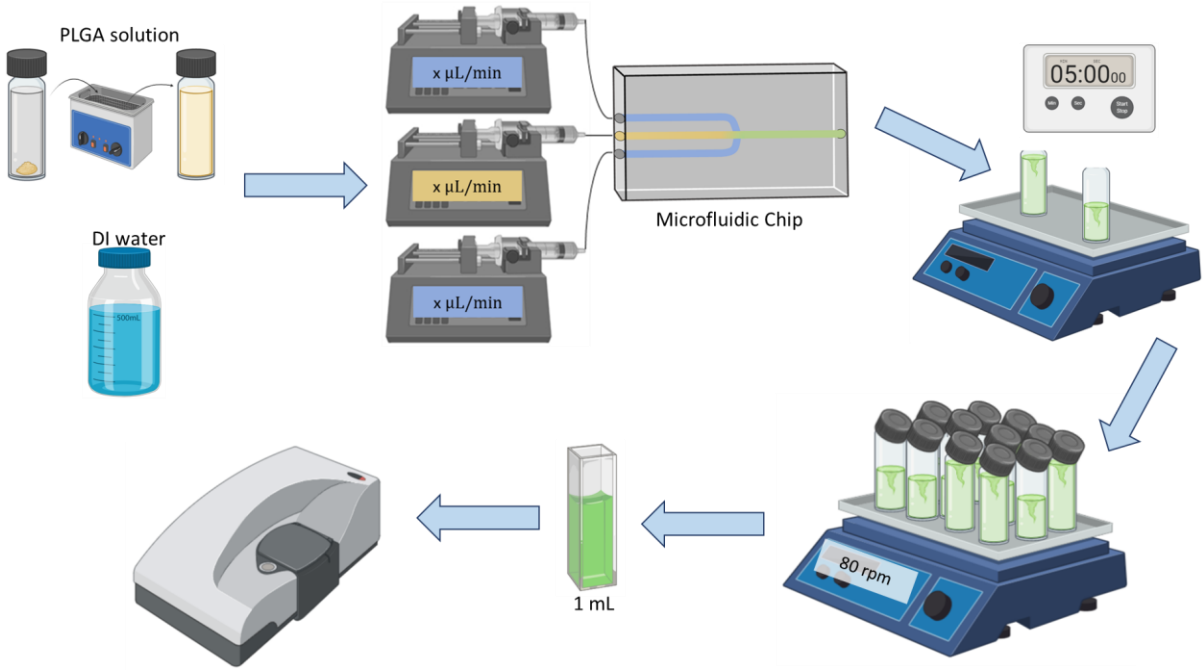

Figure 12 Microfluidic-assisted nanoprecipitation method. Yellow, blue, and green represent PLGA solution, aqueous phase, and NP suspension, respectively.

4.3.1. Investigating NP production

Samples were produced (See **Figure 12**), collected in triplicate and analysed. PLGA concentration was varied at 2.5, 5.0, 7.5, 10.0 mg/mL. Q_{dis} was kept at 100 µL/min while Q_{con} was altered at 400, 800, 1200, 1600 µL/min.

4.3.2. Nifedipine-NP production

The experimental set-up was as described**.** PLGA concentration of 0.5 mg/mL and 1.0 mg/mL were used. Q_{dis} was kept at 300 µL/min while Q_{con} was altered at 300, 900 and 1500 µL/min. One sample was collected at each point and analysed.

4.3.3. Propranolol hydrochloride-NP production

Both blank and propranolol hydrochloride containing particles were produced using a 2.5 mg/mL PLGA in acetone solution (Refer to **4.3.**) at a Q_{con} of 400 µL/min with a collection time of 6 minutes per sample. One sample was collected at each point for analysis.

4.3.4. NP production with PVA

PLGA in acetone at 2.5 mg/mL was used in microfluidic production (Refer to **4.3.**) Q_{con} was set at 400, 800, 1200 and 1600 µL/min and samples were collected for 6 minutes. One sample was collected at each point for analysis.

4.4. Statistical analysis

Where noted, a two-way ANOVA with post-hoc Tukey test was performed. A non-parametric one-way ANOVA followed by a multiple-comparisons test was carried out when analysing the change of one variable. Differences were considered significant for values of $p < 0.05$. GraphPad Prism version 9.1.2 for Windows (GraphPad Software, San Diego, California, USA) was used to perform statistical tests.

4.5. Nanoparticle Characterisation

4.5.1. Dynamic Light Scattering

The PLGA NPs were characterised by dynamic light scattering (DLS). A Zetasizer Nano ZS (Malvern Instruments, Ltd., Malvern, UK) particle size analyser was utilised for this purpose at a backscattering angle of 173° to the incident beam. All measurements were made in triplicate (n=3) and mean values ± SD were reported at 25 °C. Each measurement was undertaken with 6 sub runs (10 seconds each). The hydrodynamic radius was calculated using the Stokes-Einstein relation. Polydispersity index (PDI) was calculated using a two-parameter fit to the correlation data.

4.5.2. Zeta Potential Determination

Zeta potential was determined by electrophoretic mobility using a Zetasizer Nano ZS (Malvern Instruments Ltd., Malvern, UK). NP suspensions were measured in triplicate at 25 °C ± 1 °C using a DTS1061 disposable capillary cell (Malvern Instruments Ltd., Malvern, UK).

4.6. Encapsulation Efficiency

Nifedipine encapsulation efficiency

A 2 mg/mL stock solution of Nifedipine in methanol was produced. This was diluted to 200 µg/mL and a serial dilution was carried out to produce the final lowest concentration of 0.2 µg/mL. The spectra were then collected in triplicate at each concentration using an Agilent Cary 3500 UV-VIS spectrophotometer in the range of 190 nm and 700 nm in 1 nm steps (Agilent Technologies, Australia) at 15 °C (**Figure 13**).

Simple linear regression at the chosen absorption peak (236nm) was performed using GraphPad Prism version 9.1.2 for Windows (GraphPad Software, San Diego, California, USA). Through suspension of nifedipine-NPs in methanol, the nifedipine-

NPs can be suspended in methanol to solubilise nifedipine while PLGA remains insoluble, the solution obtained can then be used to calculate encapsulation efficiency using the calibration curve (**Figure 14**) produced.

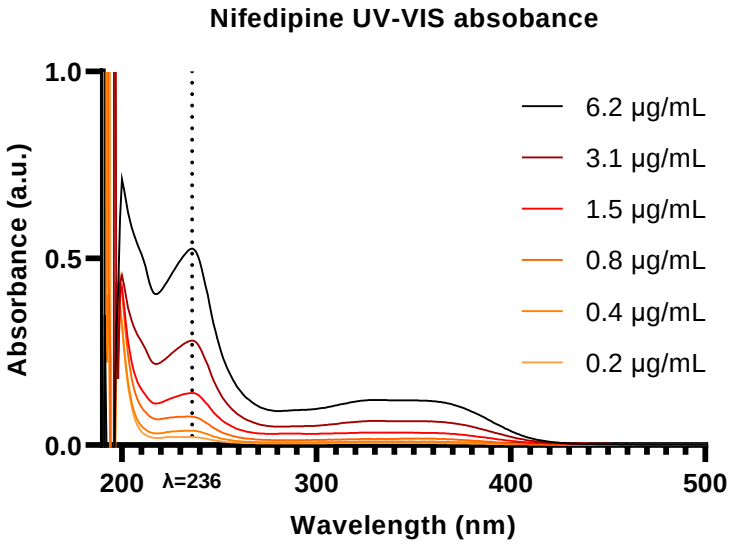

Figure 13 Nifedipine UV-VIS absorbance. The wavelength of 236 nm was chosen to produce a concentration calibration curve seen in **Figure 14**.

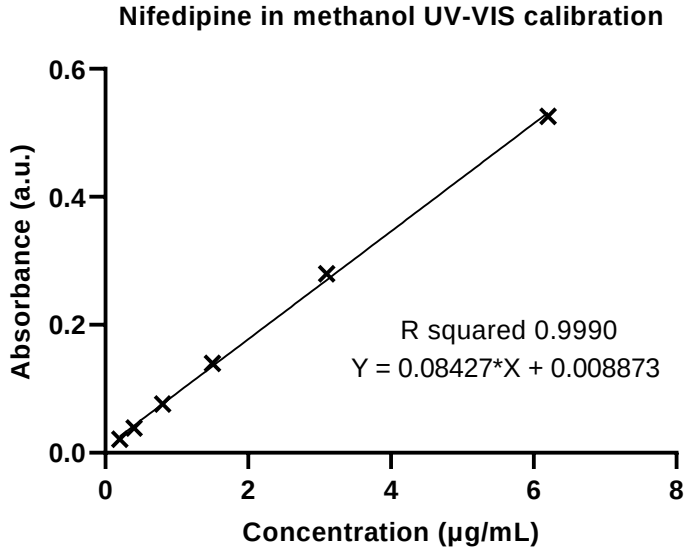

Figure 14 Nifedipine in methanol UV-VIS calibration curve

5. Results & Discussion

5.1. Microfluidic-assisted nanoprecipitation

When first investigating the microfluidic-assisted nanoprecipitation of PLGA, the lowest Q_{con} investigated (400 µL/min) gave rise to inconsistent particle size with larger PDI observed at higher PLGA concentrations of 7.5 mg/mL and 10 mg/mL as shown in **Figure 15**. At lower flow rates and higher PLGA concentrations, the microfluidic chip was prone to blockage. This can be explained by a reduced shearing force acting on the precursor solution flowing out of the inlet channel. This resulted in backflow into the central inlet channel within the microfluidic chip and consequently resulting in PLGA agglomerate formation. This effect within the microfluidic chip was later determined to be diagnostic of large PDI values post analysis as precipitation within the channel was inconsistent. Larger PDI values have been observed with increasing PLGA concentrations which in return has an impact on mixing conditions, therefore, agglomerate formation being a sign of increased PDI values during the analysis stage of production is not surprising and in line with literature (34).

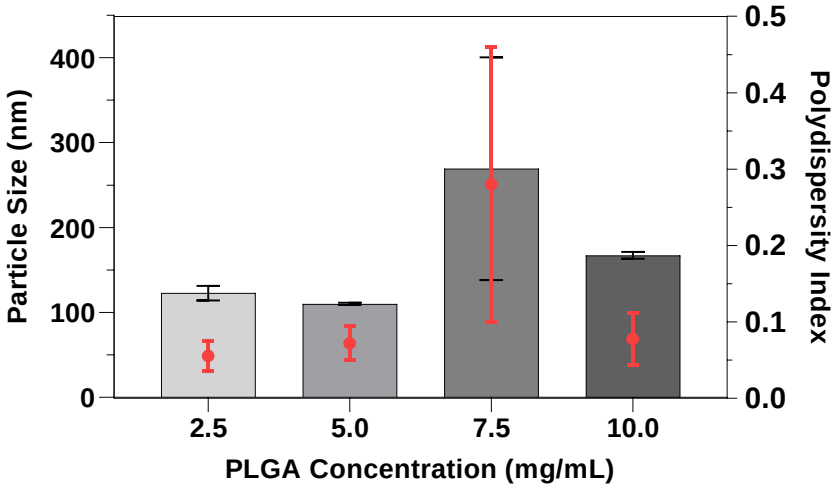

Figure 15. Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced at Q_{con} = 400 µL/min.

With mixing conditions within the microfluidic chip being vital in determining particle size and size distribution as previously described in **Section 1.5. Nanoprecipitation**, optimisation of the experimental setup was necessary to ensure control over particle size. To systemically investigate the effect of production variables on particle size and PDI, PLGA concentration and Q_{con} were varied while Q_{dis} was kept constant at 100 µL/min to elucidate how significantly a particular variation in the experimental setup influenced the outcome.

At certain concentrations such as 2.5 mg/mL PLGA, control over particle size was attainable through altering Q_{con}, whereas at higher concentrations of PLGA (10 mg/mL) this was not possible as can be seen by **Figure 16** and **Figure 17**.

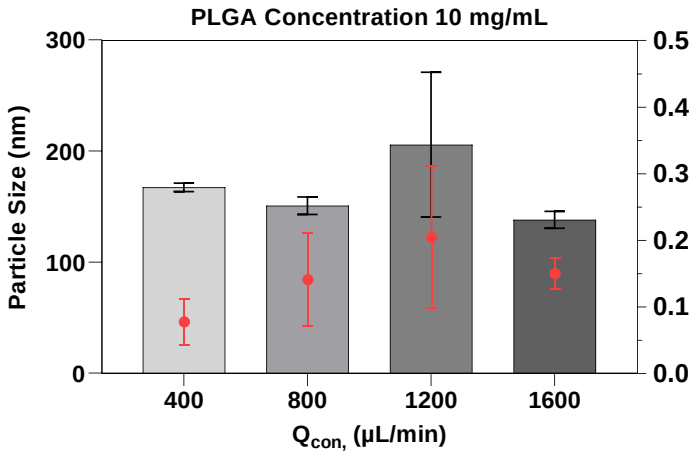

Figure 16. Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced with 10 mg/mL PLGA.

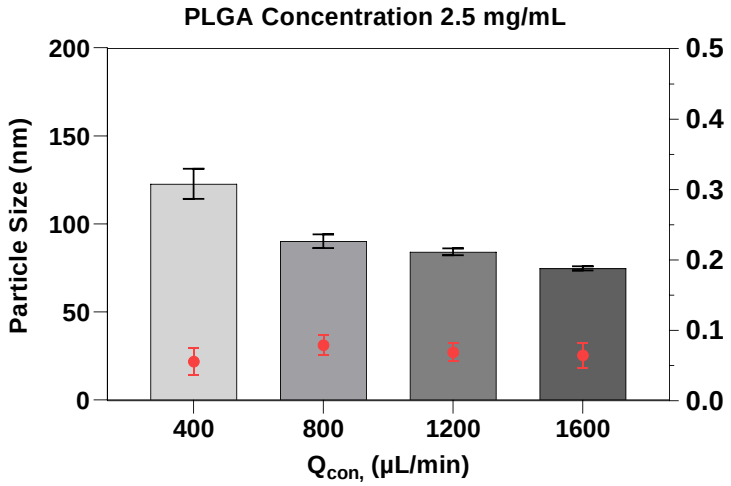

Figure 17. Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced with 2.5 mg/mL PLGA.

At a 2.5 mg/mL polymer concentration, although between smaller variations of Q_{con} (e.g., between 1200 and 1600 µL/min) a statistically significant difference in particle size was not observed, at larger variations (between 400 and 1600 µL/min) the results were statistically significant. Similar to the work of Donno *et al.* (39), increasing Q_{con} causes the reduction in particle size which is observable with an overall trend at all flow rates examined at this concentration. However, as flow rate changes did not result in a trend in particle size at higher concentrations of PLGA (**Figure 18**), this indicated that to achieve a purely microfluidic control over NP size, a set limit exists on the concentration of PLGA.

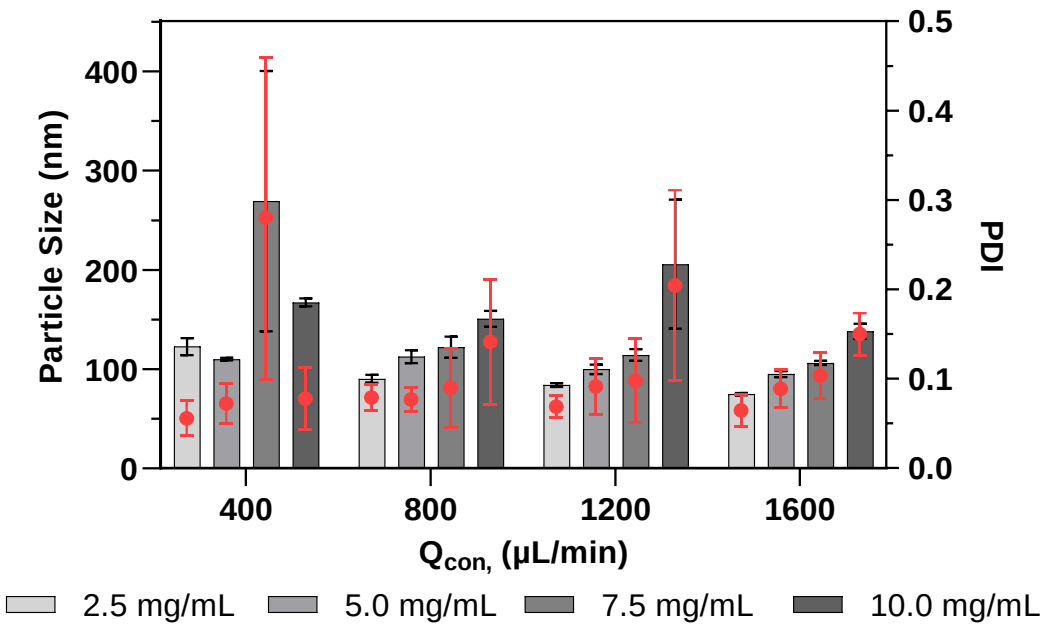

Figure 18. Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced at varying conditions to investigate microfluidic-assisted nanoprecipitation of PLGA NPs.

Further investigations with increasing PLGA concentration did however reveal a positive correlation with particle sizes produced at 800, 1200 and 1600 Q_{con} µL/min (**Figure 18**) this was not surprising as this effect is widely accepted within literature regarding nanoprecipitation (79). Increasing PLGA concentration causes an increase in the number of available unimers able to nanoprecipitate and aggregate during and after nanoprecipitation. In addition to this, the viscosity of the polymer phase also increases with increasing concentration. Viscosity is a parameter which impacts T_{mix} and can consequently result in larger particle formations as mixing time is increased (34).

During the production of particles with PLGA concentration higher than 2.5 mg/mL, turbulent and unpredictable flow regimens due to agglomeration within the chip may

have resulted in non-homogenous mixing conditions, resulting in large particle size distributions. This is reflected by the large range in PDI value of 0.1-0.5 for samples collected from 7.5 mg/mL at 400 Q_{con}. Generally, higher PDI ranges were observed with increasing polymer concentration. At lower polymer concentrations, smaller NPs are produced and as such a smaller PDI range is attained due to a more homogenous and reproducible NP system (34).

5.2. Optimising Parameters

To optimise and prevent agglomeration within the microfluidic chip at 2.5 mg/mL, Qdis was increased to 300 µL/min and the Qcon was set at 300, 900 and 1500 µL/min while PLGA concentration was also reduced to 1.0 mg/mL and 0.5 mg/mL.

Comparable to previous results, increasing polymer concentration correlated positively with NP size (**Figure 19**). Through altering production parameters as described, the PDI of all samples collected were below 0.15 with no significant difference between the samples. For clinical applications of polymeric nanomaterials, a PDI of 0.2 and lower is regarded satisfactory (80). Therefore, we have shown the ability of the optimised system to be potentially clinically applicable. For further confirmation of the data, additional repeats of this experiment may be required.

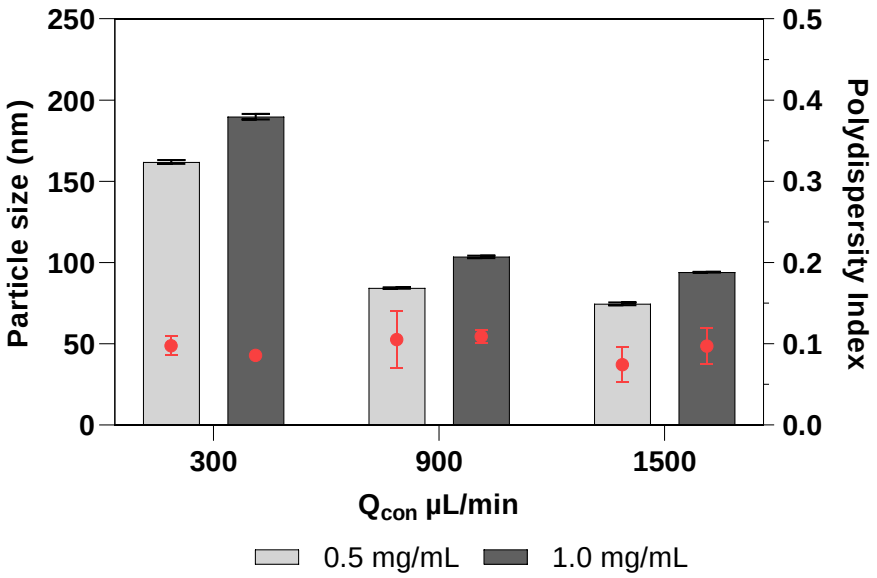

Figure 19. Particle size and polydispersity index (PDI) of PLGA NPs at a PLGA concentration of 0.5 and 1 mg/mL. Key: Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced at varying conditions.

There was a greater difference in particle size between Q_{con} of 300 and 900 µL/min, compared to 900 and 1500 across both concentrations of PLGA. This could show that with increasing aqueous flow rate, particle size decreases in a sigmoidal manner. In work done by Johnson and Prud'homme using a coaxial confined impinging jet mixer, increasing flow rates were shown to reduce particle size until a levelling point at which particle size remained unchanged (40). In this experiment, similar particle size levelling off was observed between 900 and 1500 µL/min where change was minimal at an average of ~10nm at both concentrations. Estimated mixing time within this experimental setup would have been 2, 0.39 and 0.15 seconds for Q_{con} of 300, 900 and 1500 µL/min, respectively. The reduced mixing time at higher Q_{con} increases the rate at which NPs become kinetically locked. This is because unimers are no longer dissolved in a solvent in which they can exist as ideal chains and NPs are only able to grow until the energy barrier for insertion of unimers becomes too high and the particles become ‘kinetically locked’ (34).

5.3. Nifedipine

With the greater microfluidic control over the particle production at the optimised settings described above, the encapsulation of Nifedipine was investigated.

Within literature, particle size increase between blank systems and drug loaded systems is often used to insinuate successful drug loading (81). However, as can be compared between **Figure 19** and **Figure 20**, the average particle size obtained at Q_{con} of 300 µL/min, the presence of nifedipine had an opposite effect.

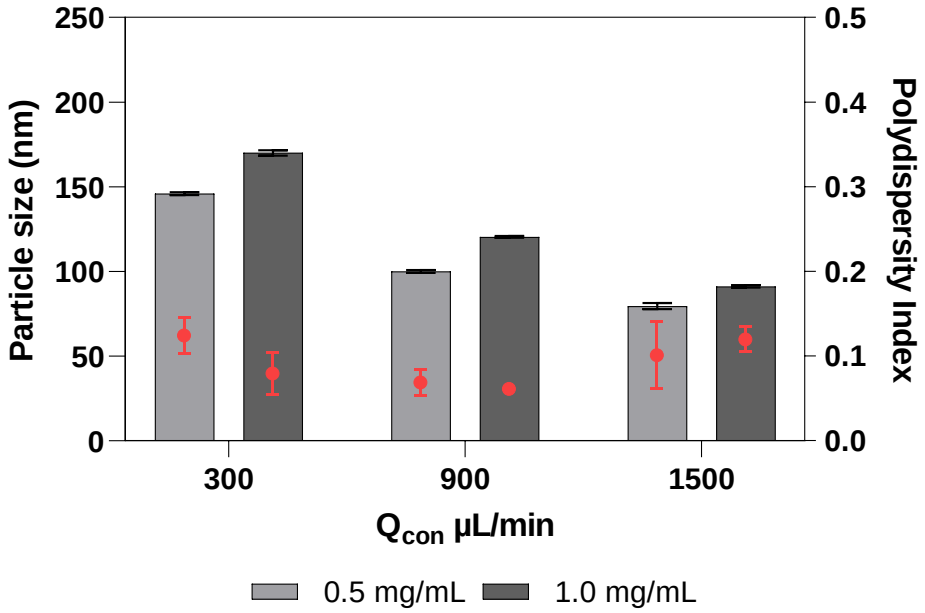

Figure 20. Particle size and PDI of *nifedipine*-PLGA NPs at a PLGA concentration of 0.5 and 1 mg/mL. Key: Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced at varying conditions.

Still, the overall trend of increasing Q_{con} and reducing polymer concentration on reducing particle size was maintained. Similarly, to blank NPs produced, there was a ~10% greater difference in particle size between Q_{con} of 300 and 900 µL/min, compared to 900 and 1500 across both concentrations of PLGA which again supports the theory of 'levelling off' previously described (34).

The PDI of Nifedipine-PLGA-NPs remained at below 0.2 at all samples with the average PDI of all samples being 0.092 ± 0.031. Jog, Unachukwu and Burgess (82) produced pH sensitive crystalline nifedipine Eudragit® L100-55 and PVA NPs through a batch emulsion solvent diffusion method. They were able to produce optimised NPs with an average PDI of 0.135 ± 0.008. Thus, a more homogenous particle size distribution was achieved using the reported microfluidic setup compared to that found in literature, albeit using a different manufacturing method.

During production of Nifedipine-PLGA-NPs, there were no visible agglomerate formation within the microfluidic chip as can be seen in **Figure 21**. Although further investigations such as encapsulation efficiency and dissolution studies are required to understand the delivery system produced. The optimised parameters in microfluidic production for continuous production of PLGA-NPs with minimal operator manipulation enabled the agglomerate-free production of nifedipine loaded PLGA NPs.

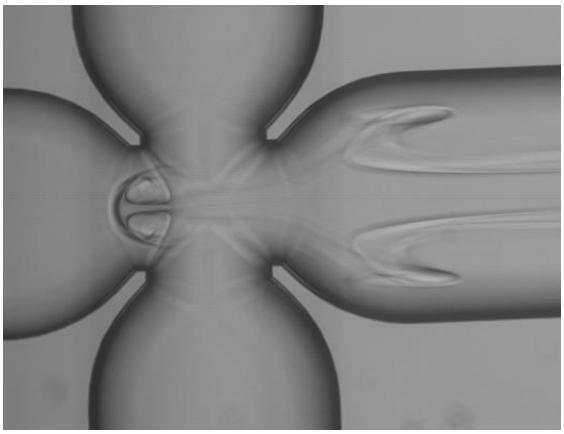

Figure 21. Optical microscope image of the microfluidic chip while producing Nifedipine-PLGA-NPs.

5.4. Propranolol Hydrochloride

As the encapsulation of the hydrophobic drug Nifedipine in PLGA NPs was successfully achieved, the next aim of this study was to compare the application of the microfluidic-assisted nanoprecipitation method to a hydrophilic drug, Propranolol hydrochloride.

PropranololHCl-PLGA-NPs have previously been produced using a water-in-oil-in-water (W/O/W) multiple emulsion technique utilising a homogeniser for the emulsification (64). However, to the authors knowledge, microfluidic assisted nanoprecipitation has not been applied to encapsulating propranolol hydrochloride. Therefore, a preliminary study was carried out to compare the production of Propranolol-PLGA-NPs to Nifedipine-PLGA-NPs.

In contrast to the production of the blank particles, during the manufacture of propranolol particles, the presence of propranolol hydrochloride resulted in extensive agglomeration (**Figure 22**) within the chip and the connection tubes disrupted production.

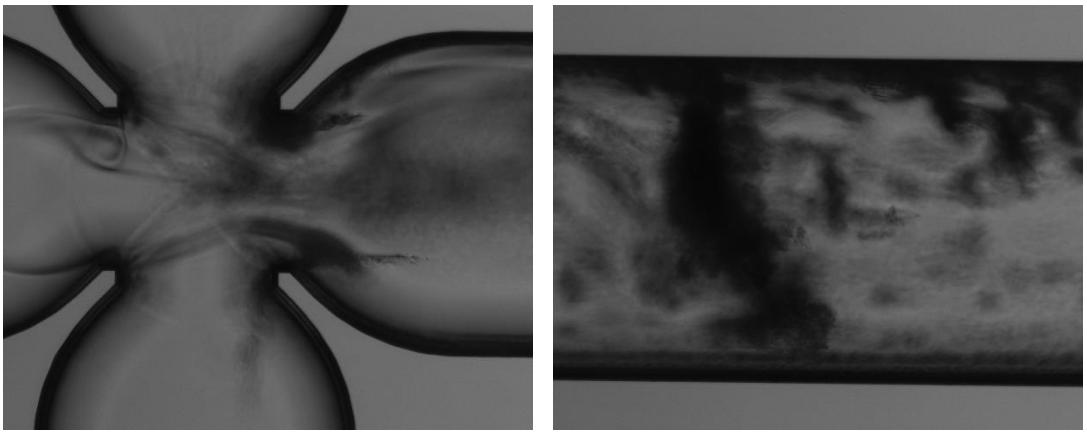

Figure 22. Chip blockage during Propranolol-PLGA-NP production

The propranolol particles had an average size and zeta potential of 2.3 µm ± 0.75 and 8.7 mV± 2.3, respectively. In comparison, the blank particles had an average size and

zeta potential of 130 nm ± 2.5 and -45 mV ± 1. Reduced colloidal stability within the nanosuspension due to the presence of both cations and anions in the form of cationic propranolol and anionic chloride ions may have resulted in the large particle size increase in the drug loaded system in comparison to the blank particles. This is supported by the fact that propranolol as a weakly basic drug with a pKa of 9.6 is a ~100% ionised at a pH of 7. Although a pH reading of the nano-emulsion was not obtained, it is likely for the emulsion system to have a pH lower than 7.

Deprotonation of drugs is a method utilised to improve encapsulation within NP systems (63,83). Through removing the salt present in commercially available medicines such as hydrochloric acid in propranolol hydrochloride, the drug is less likely to ionise in solution and is rendered more hydrophobic with favourable loading capability within the drug delivery system. However, in doing so, the product is no longer in its licensed or approved format. Additional chemical changes to drug entities are also unfavourable with the aims of this project.

As propranolol hydrochloride is a highly water-soluble drug, the potential for the drug escaping PLGA NPs into the aqueous phase while the particles dry is great. However, through increasing the pH of the aqueous system, an increase in propranolol hydrochloride loading into particles has been observed previously by Ubrich *et al.* (64). Although hydrolysis of the PLGA polymer is a concern at the extremes of the pH scale, an optimisation study also involving a wider range of microfluidic parameters such as an increased mixing time could be investigated.

Ultimately, PLGA remains a polymer with higher affinity toward the encapsulation of hydrophobic APIs. Furthermore, the nanoprecipitation method requires for both the drug and polymer to be soluble within the same solvent system for favourable

encapsulation results. Therefore, the use of other polymers with affinity toward hydrophilic APIs may be necessary in achieving successful encapsulation. For example, modification of PLGA with polyethylene glycol (84) or alginate (85) have been shown to be effective strategies to encapsulate hydrophilic APIs. The nanoprecipitation method has also been shown to be applicable to polysaccharides such as starch (86), modified cellulose (87) and chitosan as well as other polyesters such as polycaprolactone (88). Exploring other polymeric material during production could enable the progress of the project toward the goal of encapsulating a wide range of APIs from all four BCS categories to facilitate the production of personalised dosage forms.

Surfactants used during production can improve the interaction between the NP and hydrophilic API through ionic interactions or the formation of hydrogen bonds between the hydrophilic moieties of the surfactant and the hydrophilic API. In work done by Meikle *et al.* (50), the use of PVA resulted in the doubling of rifampicin encapsulation efficiency. To investigate the production of PLGA NPs in the presence of a surfactant, the use of PVA in the aqueous phase was investigated.

5.5. Poly(vinyl alcohol) as a surfactant.

To understand the effect of surfactant presence on the PLGA-NPs produced through the microfluidic-assisted nanoprecipitation method, Poly(vinyl alcohol) (PVA) was used. Although precipitation within the chip was reduced by decreasing PLGA concentration to 0.5 mg/mL and 1 mg/mL, it may be necessary to increase PLGA concentration in future formulations. Therefore, the water-soluble polymer PVA was utilised as not only a future strategy to increase hydrophilic drug loading but also to study precipitate formation within the microfluidic chip.

It has been reported that with increasing PVA concentration, the increased viscosity of the aqueous phase, results in an increase in mixing time between the solvent and aqueous phase can cause particle growth during nanoprecipitation (49). However as only a 1 mg/mL concentration was used, increased viscosity was an unlikely factor in increased particle size.

The association of PVA and PLGA during the nanoprecipitation process can occur when the hydrocarbon backbone of PVA enters the organic phase and remains attached onto the polymer matrix (49) this leaves the hydroxyl groups facing the aqueous phase by forming hydrogen bonds. However, PVA is insoluble in acetone and this theory may therefore not explain the increase in the size of NPs when PVA was present during nanoprecipitation (**Figure 23**).

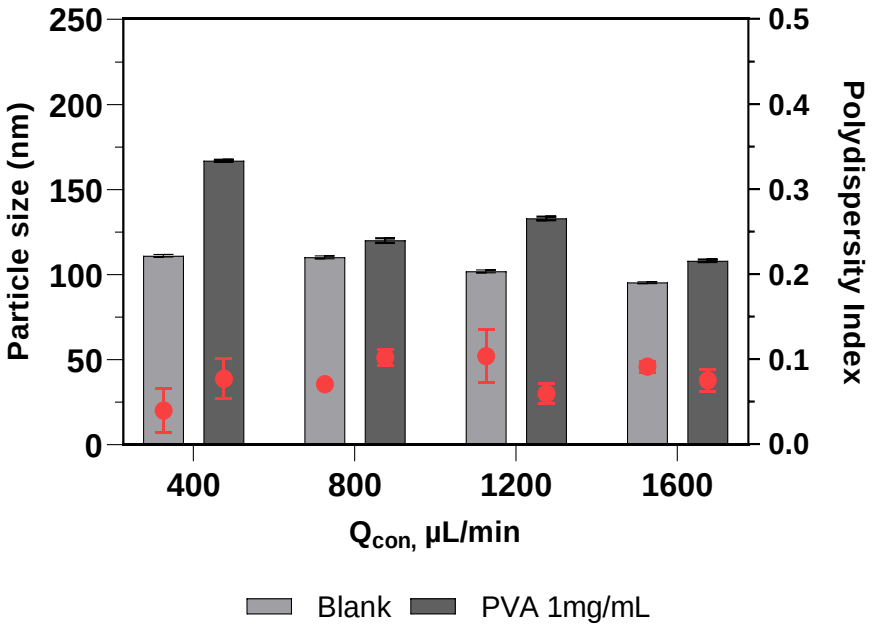

Figure 23. Particle size and PDI of NPs at a PLGA concentration of 2.5 mg/mL. Key: Average particle size (nm) ± SD and average PDI ± SD are shown as greyscale and orange correspondingly for particles produced at varying conditions.

Instead, PVA hydroxyl groups could be forming hydrogen bonds with the carboxyl groups on PLGA. Alas, the presence of PVA increased the Zeta potential of PLGA NPs to -18.5 ± 6.7 mV from -44.7 ± 1.5 mV (**Figure 24**). The reduction in the strength of electrophoretic mobility suggests a reduction in repulsion between particles. Supporting the theory that PVA may be blocking the anionic carboxyl groups from interacting with the aqueous media as PVA itself does not have ionisable functional groups.

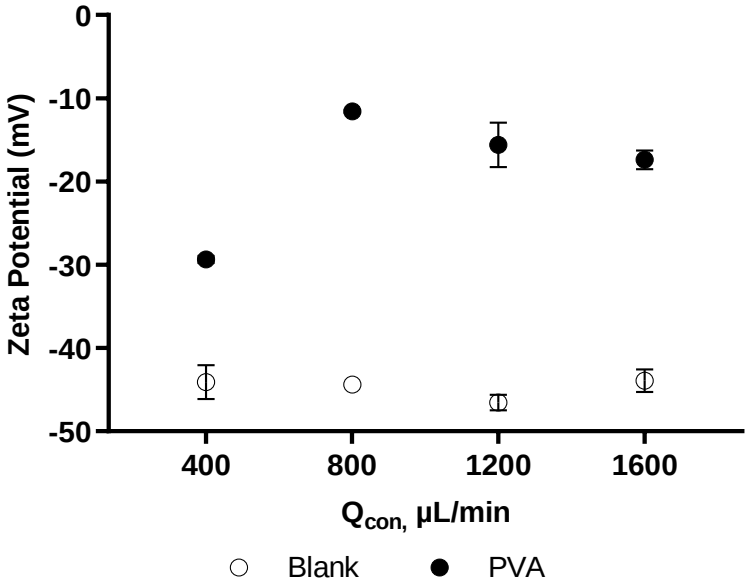

Figure 24. Zeta Potential of blank PLGA NPs compared to PLGA NPs produced in the presence of 1 mg/mL PVA.

The magnitude of Zeta potential is important in ensuring colloidal stability (89). A neutral Zeta potential can compromise colloidal stability as NPs experience reduced 'inter-particle' repulsion (90). Ironically, using PVA to reduce agglomeration within the microfluidic chip during production, could itself result in agglomeration of particles within the emulsion after production over time, however, through lyophilisation this process can be prevented as the aqueous phase is removed.

6. Critical assessment of results achieved

During the undertaking of this research, an understanding of the optimisation parameters in the field of microfluidic polymeric NP production was gained. Once an understanding of parameters affecting production are increased, Design of Experiments studies can help to further optimise parameters and prevent blockages, consequently defining the unique range of production constraints in which a specific drug, solvent, polymer, surfactant, and chip combination can operate in to result in the required product. Other polymers as well as surfactants will be used to optimise parameters for different BCS category APIs to show the ability to continuously manufacture personalised dosage forms with the microfluidic platform.

The shape and size of the produce NPs will need to be investigated using technologies such as TEM and SEM. Additionally drug loading determination of the particles will be important in further characterising the particles, this will involve dissolving the drug-loaded particles in a suitable solvent and measuring drug loading using High Performance Liquid Chromatography with a UV-VIS detection.

To study the release kinetics of the drug loaded NPs based on oral release, dissolution rigs will need to be set up according to US Pharmacopoeia standards. Additional release studies will be needed to understand the effect of the encapsulation material itself on the release of APIs. Ensuring the pH of the release media used is equivalent to the pKa of the API in question, will eliminate the solubility of each drug affecting release rates.

7. Conclusions and further work

In this work we were able to showcase the ability of the microfluidic platform to produce NPs ranging from 80 nm - 400 nm through altering concentration and flow rate. We were able to optimise the microfluidic-assisted nanoprecipitation method to maintain continuous and controllable production of NPs (ranging from 90 – 170 nm) even in the presence of the hydrophobic API, nifedipine. Additionally, we have presented the microfluidic platform as a NP production method which has the potential of being scaled up while maintaining low PDI at < 0.2. Finally, we investigated the possibility of including a surfactant during production to improve hydrophilic drug loading to enable the encapsulation of a variety of APIs in the future belonging to different BCS categories. Progressing towards the production of personalised and tailored dosage forms as the control over particle size will control drug release.

For this research to succeed in reaching the aim of producing personalised and tailored dosage forms, a cycle of production exists (**Figure 25**) in which every parameter may affect the next stage. Particle size will alter drug release, polymer choice may alter particle size and so on. Through the analysis of the data, an optimised design of parameters can be achieved, however, to systematically achieve this, a Design of Experiments (DoE) should be employed. This will enable the statistical prediction of a desired particle for further study. ‘The design space’ obtained from carrying out a DoE study will enable production of particles with optimised or desired settings such as high encapsulation or reduced PDI. This in turn will help to guide future works listed below.

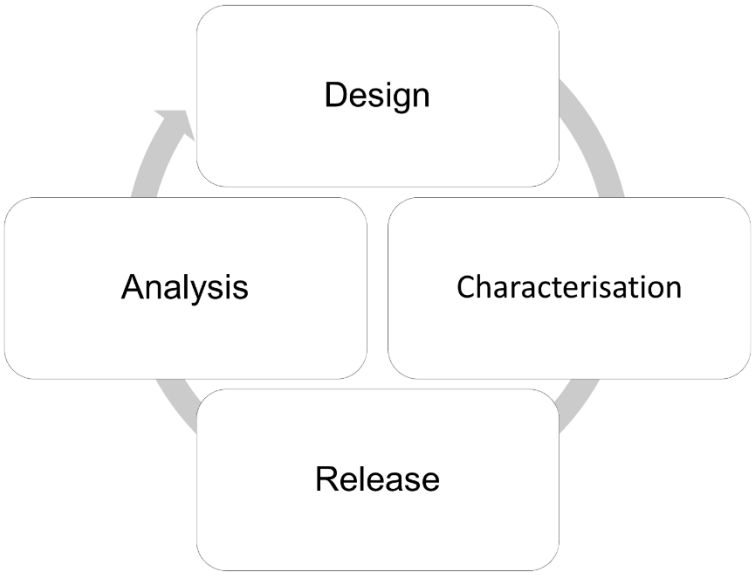

Figure 25 The cycle of particle production within this project, changing design parameters can alter encapsulation, drug release, particle size and morphology

High performance liquid chromatography (HPLC) will be used to undertake encapsulation efficiency studies of different APIs. In undertaking and developing dissolution studies to investigate release of chosen drug from the NP system, a deeper understanding of the effect of microfluidic parameters on drug dissolution can be gained. Additionally, the utilisation of Transmission Electron Microscopy (TEM) and Scanning Electron Microscopy (SEM) in conjunction with advanced particle characterisation techniques such as the 3D OrbiSIMS will aid understanding of the NP system morphology and the drug-NP interaction. Through depth profiling of the drug loaded NP, a novel understanding of the drug within the polymeric particle system can be sought.

9. References

1. Duerden M, Payne R. Polypharmacy and medicines optimisation. King's Fund [Internet]. 2013 [cited 2021 Apr 23];1–68. Available from: www.kingsfund.org.uk

2. Masnoon N, Shakib S, Kalisch-Ellett L, Caughey GE. What is polypharmacy? A systematic review of definitions. BMC Geriatr. 2017 Dec 10;17(1).

3. RPS. Polypharmacy: Getting our medicines right. Available from: https://www.rpharms.com/recognition/setting-professional-standards/polypharmacy-getting-our-medicines-right

4. García-Pérez LE, Álvarez M, Dilla T, Gil-Guillén V, Orozco-Beltrán D. Adherence to therapies in patients with type 2 diabetes [Internet]. Vol. 4, Diabetes Therapy. Springer Healthcare; 2013 [cited 2021 May 17]. p. 175–94. Available from: www.diabetestherapy-open.com

5. Kavanagh ON, Albadarin AB, Croker DM, Healy AM, Walker GM. Maximising success in multidrug formulation development: A review. Vol. 283, Journal of Controlled Release. Elsevier B.V.; 2018. p. 1–19.

6. Radhakrishnan A, Kuppusamy G, Ponnusankar S, Shanmukhan NK. Pharmacogenomic phase transition from personalized medicine to patient-centric customized delivery [Internet]. Vol. 20, Pharmacogenomics Journal. 2020. p. 1–18. Available from: https://doi.org/10.1038/s41397-019-0135-8

7. Mullard A. 2020 FDA drug approvals. Nat Rev Drug Discov. 2021 Feb 1;20(2):85–90.

8. DeFrancesco L. Financing breaks all records in 2020. Vol. 39, Nature biotechnology. NLM (Medline); 2021. p. 133–4.

9. Vargason AM, Anselmo AC, Mitragotri S. The evolution of commercial drug delivery technologies. Nat Biomed Eng 2021 59 [Internet]. 2021 Apr 1 [cited 2021 Oct 21];5(9):951–67. Available from: https://www.nature.com/articles/s41551-021-00698-w

10. Ashley EA. Towards precision medicine [Internet]. Vol. 17, Nature Reviews Genetics. 2016. p. 507–22. Available from: www.nature.com/nrg

11. Khaled SA, Burley JC, Alexander MR, Yang J, Roberts CJ. 3D printing of five-in-one dose combination polypill with defined immediate and sustained release profiles. J Control Release. 2015 Nov 10;217:308–14.

12. Chen R, Snyder M. Promise of personalized omics to precision medicine. Wiley Interdiscip Rev Syst Biol Med. 2013;5(1):73–82.

13. David V, Fylan B, Bryant E, Smith H, Sagoo GS, Rattray M. An Analysis of Pharmacogenomic-Guided Pathways and Their Effect on Medication Changes and Hospital Admissions: A Systematic Review and Meta-Analysis. Front Genet. 2021 Jul 30;0:1308.

14. Reuter JA, Spacek D V., Snyder MP. High-Throughput Sequencing Technologies. Mol Cell. 2015 May;58(4).

15. Nebula Genomics. Whole Genome Sequencing DNA Test [Internet]. 2021

[cited 2021 Oct 27]. Available from: https://nebula.org/whole-genome-sequencing-dna-test/

16. Genome UK: 2021 to 2022 implementation plan - GOV.UK [Internet]. [cited 2021 May 28]. Available from: https://www.gov.uk/government/publications/genome-uk-2021-to-2022-implementation-plan/genome-uk-2021-to-2022-implementation-plan

17. Feero WG, Guttmacher AE, Wang L, Mcleod HL, Weinshilboum RM. Genomic Medicine Genomics and Drug Response. Vol. 364, n engl j med. 2011.

18. Ashley EA. Towards precision medicine [Internet]. Vol. 17, Nature Reviews Genetics. Nature Publishing Group; 2016 [cited 2020 Oct 13]. p. 507–22. Available from: http://www.nature.com/articles/nrg.2016.86

19. Pike H. Power of one: what is holding polypills back? Pharm J [Internet]. 2019 [cited 2021 Oct 21]; Available from: https://pharmaceutical-journal.com/article/feature/power-of-one-what-is-holding-polypills-back

20. Aleksovski A, Dreu R, Gašperlin M, Planinšek O. Mini-tablets: A contemporary system for oral drug delivery in targeted patient groups. Expert Opin Drug Deliv. 2015;12(1):65–84.

21. Awad A, Yao A, Trenfield SJ, Goyanes A, Gaisford S, Basit AW. 3D Printed Tablets (Printlets) with Braille and Moon Patterns for Visually Impaired Patients. Pharm 2020, Vol 12, Page 172 [Internet]. 2020 Feb 19 [cited 2021 Oct 22];12(2):172. Available from: https://www.mdpi.com/1999-4923/12/2/172/htm

22. Aho J, Bøtker JP, Genina N, Edinger M, Arnfast L, Rantanen J. Roadmap to 3D-Printed Oral Pharmaceutical Dosage Forms: Feedstock Filament Properties and Characterization for Fused Deposition Modeling. Vol. 108, Journal of Pharmaceutical Sciences. Elsevier; 2019. p. 26–35.

23. Fina F, Goyanes A, Gaisford S, Basit AW. Selective laser sintering (SLS) 3D printing of medicines. Int J Pharm [Internet]. 2017 [cited 2021 Oct 25];529(1–2):285–93. Available from: https://reader.elsevier.com/reader/sd/pii/S0378517317305902?token=33CA20CC00DA8A88A7100080DEB9693E5AF15464E6BF4E54C8FD46E1ADD9C7FB518330D4BBD8E94843FD90FB044D6194&originRegion=eu-west-1&originCreation=20211025174720

24. Clark EA, Alexander MR, Irvine DJ, Roberts CJ, Wallace MJ, Sharpe S, et al. 3D printing of tablets using inkjet with UV photoinitiation. Int J Pharm. 2017 Aug 30;529(1–2):523–30.

25. Awad A, Fina F, Trenfield SJ, Patel P, Goyanes A, Gaisford S, et al. 3D printed pellets (Miniprintlets): A novel, multi-drug, controlled release platform technology. Pharmaceutics [Internet]. 2019 Apr 1 [cited 2020 Oct 12];11(4):148. Available from: https://www.mdpi.com/1999-4923/11/4/148

26. Goyanes A, Buanz ABM, Hatton GB, Gaisford S, Basit AW. 3D printing of modified-release aminosalicylate (4-ASA and 5-ASA) tablets. Eur J Pharm Biopharm [Internet]. 2015;89:157–62. Available from: http://dx.doi.org/10.1016/j.ejpb.2014.12.003

27. World Health Organization (WHO). Topic 11: Improving Medication Safety. WHO Patient Saf Curric Guid Med Sch [Internet]. 2009 [cited 2021 Oct 25];229–44. Available from: www.who.int/patientsafety/education/curriculum/who_mc_topic-11.pdf

28. The Human Medicines Regulations 2012.

29. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater [Internet]. [cited 2021 Oct 22]; Available from: www.nature.com/natrevmats

30. Barber ND, Alldred DP, Raynor DK, Dickinson R, Garfield S, Jesson B, et al. Care homes' use of medicines study: Prevalence, causes and potential harm of medication errors in care homes for older people. Qual Saf Heal Care [Internet]. 2009 Oct [cited 2021 Oct 27];18(5):341–6. Available from: /pmc/articles/PMC2762085/

31. Kumari A, Yadav SK, Yadav SC. Biodegradable polymeric nanoparticles based drug delivery systems. Vol. 75, Colloids and Surfaces B: Biointerfaces. Elsevier; 2010. p. 1–18.

32. Karnik R, Gu F, Basto P, Cannizzaro C, Dean L, Kyei-Manu W, et al. Microfluidic Platform for Controlled Synthesis of Polymeric Nanoparticles. Nano Lett [Internet]. 2008 Sep 10 [cited 2020 Oct 26];8(9):2906–12. Available from: https://pubs.acs.org/doi/10.1021/nl801736q

33. Fessi H, Puisieux F, Devissaguet JP, Ammoury N, Benita S. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm. 1989 Oct 1;55(1):R1–4.

34. Lepeltier E, Bourgaux C, Couvreur P. Nanoprecipitation and the "Ouzo effect": Application to drug delivery devices [Internet]. Vol. 71, Advanced Drug Delivery Reviews. Elsevier; 2014 [cited 2020 Dec 1]. p. 86–97. Available from: http://dx.doi.org/10.1016/j.addr.2013.12.009

35. Xu Z, Lu C, Riordon J, Sinton D, Moffitt MG. Microfluidic Manufacturing of Polymeric Nanoparticles: Comparing Flow Control of Multiscale Structure in Single-Phase Staggered Herringbone and Two-Phase Reactors. Langmuir [Internet]. 2016 Dec 6 [cited 2021 Jun 21];32(48):12781–9. Available from: https://pubs.acs.org/sharingguidelines

36. Abdelkarim M, Abd Ellah NH, Elsabahy M, Abdelgawad M, Abouelmagd SA. Microchannel geometry vs flow parameters for controlling nanoprecipitation of polymeric nanoparticles. Colloids Surfaces A Physicochem Eng Asp. 2021 Feb 20;611:125774.

37. Budhian A, Siegel SJ, Winey KI. Controlling the in vitro release profiles for a system of haloperidol-loaded PLGA nanoparticles. Int J Pharm [Internet]. 2008;346:151–9. Available from: www.sciencedirect.com

38. Ubrich N, Bouillot P, Pellerin C, Hoffman M, Maincent P. Preparation and characterization of propranolol hydrochloride nanoparticles: a comparative study. 2004; Available from: www.elsevier.com/locate/jconrel

39. Donno R, Gennari A, Lallana E, De La Rosa JMR, D'Arcy R, Treacher K, et al.

Nanomanufacturing through microfluidic-assisted nanoprecipitation: Advanced analytics and structure-activity relationships. Int J Pharm [Internet]. 2017 Dec 20 [cited 2020 Oct 28];534(1–2):97–107. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0378517317309560

40. Johnson BK, Prud'homme RK. Mechanism for rapid self-assembly of block copolymer nanoparticles. Phys Rev Lett [Internet]. 2003 Sep 11 [cited 2020 Oct 26];91(11):118302. Available from: https://link.aps.org/doi/10.1103/PhysRevLett.91.118302

41. Lim JM, Swami A, Gilson LM, Chopra S, Choi S, Wu J, et al. Ultra-high throughput synthesis of nanoparticles with homogeneous size distribution using a coaxial turbulent jet mixer. ACS Nano [Internet]. 2014 [cited 2021 May 10];8(6):6056–65. Available from: www.acsnano.org6056

42. Kim DY, Jin SH, Jeong SG, Lee B, Kang KK, Lee CS. Microfluidic preparation of monodisperse polymeric microspheres coated with silica nanoparticles. Sci Rep [Internet]. 2018 Dec 1 [cited 2020 Oct 27];8(1):8525. Available from: www.nature.com/scientificreports

43. Lim JM, Bertrand N, Valencia PM, Rhee M, Langer R, Jon S, et al. Parallel microfluidic synthesis of size-tunable polymeric nanoparticles using 3D flow focusing towards in vivo study. Nanomedicine Nanotechnology, Biol Med. 2014 Feb 1;10(2):401–9.

44. Damiati SA, Rossi D, Joensson HN, Damiati S. Artificial intelligence application for rapid fabrication of size-tunable PLGA microparticles in microfluidics. Sci Rep. 2020 Dec 1;10(1).

45. Martins JP, Torrieri G, Santos HA. The importance of microfluidics for the preparation of nanoparticles as advanced drug delivery systems. Expert Opin Drug Deliv [Internet]. 2018;15(5):469–79. Available from: https://doi.org/10.1080/17425247.2018.1446936

46. Beebe DJ, Mensing GA, Walker GM. Physics and applications of microfluidics in biology [Internet]. Vol. 4, Annual Review of Biomedical Engineering. 2002 [cited 2021 May 19]. p. 261–86. Available from: www.annualreviews.org

47. Batchelor GK. An Introduction to Fluid Dynamics [Internet]. An Introduction to Fluid Dynamics. 2000 [cited 2021 May 19]. chapter 1 physical properties of fluids. Available from: https://books.google.co.uk/books?hl=en&lr=&id=Rla7OihRvUgC&oi=fnd&pg=PR17&ots=hklRwPGSvE&sig=_3Ax1vIJuz12t5hQEZSge0XagA4&redir_esc=y#v=onepage&q&f=false

48. Stroock AD, Dertinger SKW, Ajdari A, Mezić I, Stone HA, Whitesides GM. Chaotic mixer for microchannels. Science (80-) [Internet]. 2002 Jan 25 [cited 2021 Jun 21];295(5555):647–51. Available from: http://science.sciencemag.org/

49. Chiesa E, Dorati R, Modena T, Conti B, Genta I. Multivariate analysis for the optimization of microfluidics-assisted nanoprecipitation method intended for the loading of small hydrophilic drugs into PLGA nanoparticles. Int J Pharm [Internet]. 2018;536(1):165–77. Available from:

https://doi.org/10.1016/j.ijpharm.2017.11.044

50. Meikle TG, Drummond CJ, Conn CE. Microfluidic Synthesis of Rifampicin Loaded PLGA Nanoparticles and the Effect of Formulation on their Physical and Antibacterial Properties. Aust J Chem. 2020;73(3):151–7.

51. Cardot JM, Garcia Arieta A, Paixao P, Tasevska I, Davit B. Implementing the Biopharmaceutics Classification System in Drug Development: Reconciling Similarities, Differences, and Shared Challenges in the EMA and US-FDA-Recommended Approaches. AAPS J [Internet]. 2016 Jul 1 [cited 2021 May 28];18(4):1039–46. Available from: https://link.springer.com/article/10.1208/s12248-016-9915-0

52. Committee for Medicinal Products for Human Use. ICH M9 guideline on biopharmaceutics classification system-based biowaivers ICH M9 on biopharmaceutics classification system-based biowaivers [Internet]. 2020 [cited 2021 May 28]. Available from: www.ema.europa.eu/contact

53. Jain D, Pathak D, Pathak K. Pharmaceutical product development technologies based on the biopharmaceutical classification system. Vol. 64, Pharmazie. 2009. p. 483–90.

54. Banerjee A, Qi J, Gogoi R, Wong J, Mitragotri S. Role of nanoparticle size, shape and surface chemistry in oral drug delivery. J Control Release. 2016 Sep 28;238:176–85.

55. Paul J Sheskey, Bruno C Hancock, Gary P Moss DJG. Handbook of Pharmaceutical Excipients Ninth edition. 9th ed. London: Pharmaceutical Press; 2020.

56. Jain RA. The manufacturing techniques of various drug loaded biodegradable poly(lactide-co-glycolide) (PLGA) devices. Biomaterials. 2000 Dec 1;21(23):2475–90.

57. Pérez-López A, Martín-Sabroso C, Torres-Suárez AI, Aparicio-Blanco J. Timeline of translational formulation technologies for cancer therapy: Successes, failures, and lessons learned therefrom. Pharmaceutics. 2020;12(11):1–34.

58. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: An overview of biomedical applications. Vol. 161, Journal of Controlled Release. Elsevier; 2012. p. 505–22.

59. Soppimath KS, Aminabhavi TM, Kulkarni AR, Rudzinski WE. Biodegradable polymeric nanoparticles as drug delivery devices. Vol. 70, Journal of Controlled Release. Elsevier; 2001. p. 1–20.

60. Huang W, Zhang C. Tuning the Size of Poly(lactic-co-glycolic Acid) (PLGA) Nanoparticles Fabricated by Nanoprecipitation. Biotechnol J. 2018;13(1).

61. Zhang L, Long C, Pan J, Qian Y. A dissolution-diffusion model and quantitative analysis of drug controlled release from biodegradable polymer microspheres. Can J Chem Eng. 2006;84(5):558–66.

62. Muthu MS, Rawat MK, Mishra A, Singh S. PLGA nanoparticle formulations of risperidone: preparation and neuropharmacological evaluation. Nanomedicine

Nanotechnology, Biol Med. 2009 Sep;5(3):323–33.

63. Xu J, Zhang S, MacHado A, Lecommandoux S, Sandre O, Gu F, et al. Controllable Microfluidic Production of Drug-Loaded PLGA Nanoparticles Using Partially Water-Miscible Mixed Solvent Microdroplets as a Precursor. Sci Rep [Internet]. 2017 Dec 1 [cited 2020 Oct 20];7(1):1–12. Available from: www.nature.com/scientificreports

64. Ubrich N, Bouillot P, Pellerin C, Hoffman M, Maincent P. Preparation and characterization of propranolol hydrochloride nanoparticles: a comparative study. J Control Release [Internet]. 2004;97:291–300. Available from: www.elsevier.com/locate/jconrel

65. Sahoo SK, Panyam J, Prabha S, Labhasetwar V. Residual polyvinyl alcohol associated with poly (D,L-lactide-co-glycolide) nanoparticles affects their physical properties and cellular uptake. J Control Release. 2002 Jul 18;82(1):105–14.

66. Rios De La Rosa JM, Spadea A, Donno R, Lallana E, Lu Y, Puri S, et al. Microfluidic-assisted preparation of RGD-decorated nanoparticles: exploring integrin-facilitated uptake in cancer cell lines. Sci Rep [Internet]. 2020 Dec 1 [cited 2020 Oct 1];10(14505). Available from: https://doi.org/10.1038/s41598-020-71396-x

67. ABDELWAHED W, DEGOBERT G, STAINMESSE S, FESSI H. Freeze-drying of nanoparticles: Formulation, process and storage considerations☆. Adv Drug Deliv Rev [Internet]. 2006 Dec 30 [cited 2021 Jun 10];58(15):1688–713. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0169409X06001840

68. Chen Y, Mohanraj VJ, Parkin JE. Chitosan-dextran sulfate nanoparticles for delivery of an anti-angiogenesis peptide.

69. Freiesleben J, Keim J, Grutsch M. Machine learning and Design of Experiments: Alternative approaches or complementary methodologies for quality improvement? Qual Reliab Eng Int. 2020 Oct 17;36(6):1837–48.

70. Reker D, Rybakova Y, Kirtane AR, Cao R, Yang JW, Navamajiti N, et al. Computationally guided high-throughput design of self-assembling drug nanoparticles. Nat Nanotechnol [Internet]. 2021 Mar 25 [cited 2021 Apr 6]; Available from: http://www.nature.com/articles/s41565-021-00870-y

71. Poon W, Kingston BR, Ouyang B, Ngo W, Chan WCW. A framework for designing delivery systems [Internet]. Vol. 15, Nature Nanotechnology. Nature Research; 2020 [cited 2020 Oct 6]. p. 819–29. Available from: http://www.nature.com/articles/s41565-020-0759-5

72. Benítez-Mateos AI, Contente ML, Roura Padrosa D, Paradisi F. Flow biocatalysis 101: design, development and applications. React Chem Eng [Internet]. 2021;6(4):599–611. Available from: http://xlink.rsc.org/?DOI=D0RE00483A

73. Baker BJM, Kerr WJ, Lindsay DM, Patel VK, Poole DL. A sustainable and scalable multicomponent continuous flow process to access fused imidazoheterocycle pharmacophores. Green Chem. 2021;23(1):280–7.

74. Al-Ahmady ZS, Donno R, Gennari A, Prestat E, Marotta R, Mironov A, et al. Enhanced Intraliposomal Metallic Nanoparticle Payload Capacity Using Microfluidic-Assisted Self-Assembly. Langmuir. 2019 Oct 15;35(41):13318–31.

75. Buschmann MD, Carrasco MJ, Alishetty S, Paige M, Alameh MG, Weissman D. Nanomaterial delivery systems for mrna vaccines [Internet]. Vol. 9, Vaccines. MDPI AG; 2021 [cited 2021 Jun 21]. p. 1–30. Available from: https://doi.org/10.3390/vaccines

76. Wu CY, Benet LZ. Predicting drug disposition via application of BCS: Transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. Vol. 22, Pharmaceutical Research. 2005. p. 11–23.

77. Joint Formulary Committee. British National Formulary. 81st ed. London: BMJ Group and Pharmaceutical Press; 2021.

78. Nader AM, Quinney SK, Fadda HM, Foster DR. Effect of Gastric Fluid Volume on the In Vitro Dissolution and In Vivo Absorption of BCS Class II Drugs: a Case Study with Nifedipine. AAPS J [Internet]. 2016 Jul 1 [cited 2021 May 11];18(4):981–8. Available from: https://link.springer.com/article/10.1208/s12248-016-9918-x

79. Huang W, Zhang C. Tuning the Size of Poly(lactic-co-glycolic Acid) (PLGA) Nanoparticles Fabricated by Nanoprecipitation. Biotechnol J [Internet]. 2018 Jan 1 [cited 2021 Mar 1];13(1):1700203. Available from: http://doi.wiley.com/10.1002/biot.201700203

80. Danaei M, Dehghankhold M, Ataei S, Hasanzadeh Davarani F, Javanmard R, Dokhani A, et al. Impact of particle size and polydispersity index on the clinical applications of lipidic nanocarrier systems [Internet]. Vol. 10, Pharmaceutics. 2018 [cited 2021 Jun 27]. Available from: www.mdpi.com/journal/pharmaceutics

81. Mohan P, Rapoport N. Doxorubicin as a molecular nanotheranostic agent: effect of doxorubicin encapsulation in micelles or nanoemulsions on the ultrasound-mediated intracellular delivery and nuclear trafficking. Mol Pharm. 2010;7(6):1959–73.

82. Jog R, Unachukwu K, Burgess DJ. Formulation design space for stable, pH sensitive crystalline nifedipine nanoparticles. Int J Pharm [Internet]. 2016 [cited 2021 May 4];514(1):81–92. Available from: http://dx.doi.org/10.1016/j.ijpharm.2016.08.039

83. Kataoka K, Matsumoto T, Yokoyama M, Okano T, Sakurai Y, Fukushima S, et al. Doxorubicin-loaded poly(ethylene glycol)-poly(b-benzyl-L-aspartate) copolymer micelles: their pharmaceutical characteristics and biological significance [Internet]. Vol. 64, Journal of Controlled Release. 2000. Available from: www.elsevier.com/locate/jconrel

84. Park J, Fong PM, Lu J, Russell KS, Booth CJ, Saltzman WM, et al. PEGylated PLGA nanoparticles for the improved delivery of doxorubicin. Nanomedicine Nanotechnology, Biol Med. 2009 Dec 1;5(4):410–8.

85. Abdelghany S, Parumasivam T, Pang A, Roediger B, Tang P, Jahn K, et al.

Alginate modified-PLGA nanoparticles entrapping amikacin and moxifloxacin as a novel host-directed therapy for multidrug-resistant tuberculosis. 2019; Available from: https://doi.org/10.1016/j.jddst.2019.05.025

86. Chin SF, Pang SC, Tay SH. Size controlled synthesis of starch nanoparticles by a simple nanoprecipitation method. Carbohydr Polym. 2011 Oct 15;86(4):1817–9.

87. Plucinski A, Lyu Z, Schmidt BVKJ. Polysaccharide nanoparticles: from fabrication to applications. J Mater Chem B. 2021;

88. Colmenares Roldán GJ, Agudelo Gomez LM, Carlos Cornelio JA, Rodriguez LF, Pinal R, Hoyos Palacio LM. Production of polycaprolactone nanoparticles with low polydispersity index in a tubular recirculating system by using a multifactorial design of experiments. J Nanoparticle Res [Internet]. 2018;20(3). Available from: https://doi.org/10.1007/s11051-018-4168-8

89. Bhattacharjee S. DLS and zeta potential - What they are and what they are not? [Internet]. Vol. 235, Journal of Controlled Release. 2016. p. 337–51. Available from: http://dx.doi.org/10.1016/j.jconrel.2016.06.017

90. Stolnik S, Garnett MC, Davies MC, Illum L, Bousta M, Vert M, et al. The colloidal properties of surfactant-free biodegradable nanospheres from poly(β-malic acid-co-benzyl malate)s and poly(lactic acid-co-glycolide). Colloids Surfaces A Physicochem Eng Asp. 1995 Apr 28;97(3):235–45.