Aalborg University

Department of Materials and Production

Master's Thesis

Swelling behaviour and drug release studies in alginate hydrogels

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December 13th 2019

AALBORG UNIVERSITY
STUDENT REPORT

Fourth semester of master studies of Nanobiotechnology at School of Engineering and Science

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Title:
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Project period:
February 2019 - December 2019

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Copies:
Total pages: 32
Finalized 13-12-2019

Abstract:

Hydrogels are insoluble three-dimensional polymer networks that swell in aqueous solutions. They are sensitive to environmental factors such as pH, temperature, ionic strength and can respond to the presence of specific antigens, drugs, peptides, proteins, enzymes and small molecules. They can be therefore used to design a drug delivery system where a slow or triggered release of a hydrophilic drug is achieved. Since many drugs are hydrophobic, an additional ingredient can be required – a nanocarrier, e.g. a polymeric micelle – to produce a universal drug release/delivery system. Alginate hydrogels are of particular interest for this task due to their biocompatibility and biodegradability. Furthermore, the alginate molecules are cross-linked ionically (non-covalently) by divalent cations (e.g. Ca2+) which allows for dynamic re-arrangement of their structures and also makes the crosslinking process non-toxic and biocompatible as well. In this project, a composite drug delivery system (micelle/hydrogel) will be fabricated through microfluidic channels by a single emulsion technique. The final microparticles will be characterized for their morphology, size and be studied for their drug release.

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Contents

1. Introduction

Drug delivery systems (DDS) are carrier systems that can transport and deliver drug cargos to sites in the body of clinical relevance. DDSs apply to drug carriers, composed primarily of lipids and polymers, processes, preparations, techniques and systems for distributing the drugs in the body as necessary to ensure the therapeutic effects needed are controlled [1, 2, 3]. It has been calculated that approximately 40% of licensed drugs on the market and approximately 90% still in production are poorly water-soluble [4]. Since the human body consists of 65% water, this often leads to poor biodistribution and rapid GI removal of the drug [5, 2]. Most APIs directly swallowed or injected intravenously are destroyed by the body, either by the harsh pH of the stomach or by endocrine action before reaching the target site. Some drugs may also persist in specific organs and destroy healthy tissue, leading to severe harmful side effects in patients [6]. The main objective in the field of drug delivery is to develop targeted delivery and formulations for sustainable release. In particular, the drug would only be active in the target region of the body (e.g. tumours) through targeted DDS, and the drug would be released over time under regulation, through the sustained DDS [7]. Nanotechnology has made a major contribution to the production of DDSs in the last decade. Since then, it is understood that size and shape of nanoparticles (NPs) could help navigate biological carriers, the application of nanofabrication technologies has motivated to develop more effective particulate DDS, both top-down and bottom-up. The NPs size and shape of regulates their bio-distribution. Particles smaller than 20 nm will be removed from circulation via the reticuloendothelial system (RES) within a few hours of administration intravenously, whilst larger ones will be lodged within minutes in organs such as liver and spleen [8, 9]. Researchers found that polymeric micelles($<$30nm) could effectively infiltrate poorly permeable tumour cells [10]. Manufacturing techniques such as microfluidic self-assembly, nano-precipitation, emulsion-based phase inversion, layer-by-layer synthesis and nano-imprinting were used to produce specialized DDS to carry a wide range of drugs. With a complete understanding of the prospects of particulate DDS in the necessary configurations and forms, nano-manufacturing and nanofabrication will play an ever more important role in the future [11]. Hydrogels contain 3D-network structures with unique properties such as porosity, mechanical strength and reversible swelling in aqueous environments, that can be tailored to a broad range of parameters, making them ideal for DDS applications [12]. Nonetheless, for biological and drug delivery uses, the scope of natural and synthetic hydrophilic polymers is constrained depending on their biocompatibility and biodegradability. Alginate is a natural polymer with several desirable properties including excellent biocompatibility, low costs, fast gelation, inert

nature, chemical compatibles, easy availability and viable synthesis methods that makes it an excellent polymer for engineering drug delivery and tissue engineering systems [13]. Polymeric micelles are self-assembling colloidal particles. Polymeric micelle-based DDS is clearly stable insoluble states and has well established hydrophobic and hydrophilic areas that significantly improve the dissolution of hydrophobic drugs and also allow for a high drug load capability. Limitations include overall stability of the micelles, dosage regulation, long-term release and site-specific delivery of drugs [14, 15].

Microfluidic systems show great versatility in preparing shape-controlled microdroplets with specialized chemical compositions due to excellent handling efficiency, good laminar flow and microfluidic interfacial characteristics such as interfacial polymerization, interfacial separation and droplet/ multi-emulsion fusion [16]. Droplet microfluidics is a new technology introduced in recent years for the production, processing and applicability of droplets, with sizes usually spanning from several micrometres to hundreds of micrometres in diameter [17, 18, 19]. Based on the droplet generation, alginate microspheres can be generated and gelled inside or outside the microchannels. The average size of microsphères so formed may range from 50 to 200 m [20].

New advances in engineering complex DDS, suggest the potential for developing a system with programmable release and with a better load capacity in the water medium. Consequently, this project aims to design a system capable of having hydrogel integrity while also providing better manageability over drug release through drug entrapment in micellar nano-carriers. The amphiphilic Polyvinylpyrrolidone-based micelle – hydrogel18 composite described here integrates these two schemes (hydrogels and micelle) into one entity towards a smart drug delivery system.

1.1 Hydrogels

Hydrogels are insoluble three-dimensional polymer chain networks that swell in aqueous solutions. They are sensitive to environmental factors such as pH, temperature, ions, antigens, drugs, peptides, proteins, and enzymes. Hence, hydrogels are an excellent candidate for engineering an intelligent system that can improve therapeutic efficacy. Hydrogels can swell and de-swell reversibly and, therefore, can retain large quantities of water. This hydrophilic character arises from the presence of functional groups such as hydroxyl (-OH), carboxyl (-COOH), amine ($-NH_2$), and sulfate (SO3H) which make it possible to absorb water by the hydrogel. The crosslinks between the network chains make the hydrogel water-insoluble and give it proper geometrical dimensions. Hydrogel's swelling properties are mainly dependent on external environments such as temperature, pH, ionic concentration, which in turn, can help the hydrogel to transition its volume (collapse or phase transition) [21, 22, 23]. Hydrogels can be produced using different natural and synthetic polymeric materials. Natural polymers feature a high porosity biological degradability, biocompatibility, and non-toxicity [24]. Therefore, for the delivery of drugs,

hydrogels made of natural polymers have become a research focus [25]. The commonly employed natural polymers include collagen, gelatin, hyaluronate, fibrin, chitosan, agarose, and alginate [26, 27]. Out of these polymers, alginate is unique because they will ionically crosslink in the presence of divalent cations such as Calcium (Ca^{2+}), resulting in a straightforward synthesis process. Hence, alginate is one of the most common biomaterials used for drug delivery and tissue engineering.

1.1.1 Alginate

Alginate is a natural anionic polymer harvested from brown algae. The crude polymer from the algae is treated with sodium hydroxide and purified to yield sodium alginate [28]. Alginate is a linear copolymer family comprising blocks of residues of (1,4)-linked β-d-mannuronate (M) and α-l-guluronate (G). The blocks contain consecutive residues of G, M, and alternating G-M residues. Depending on the source of the alginates, the ratio of the M and G content varies [29]. (Figure 3.5)

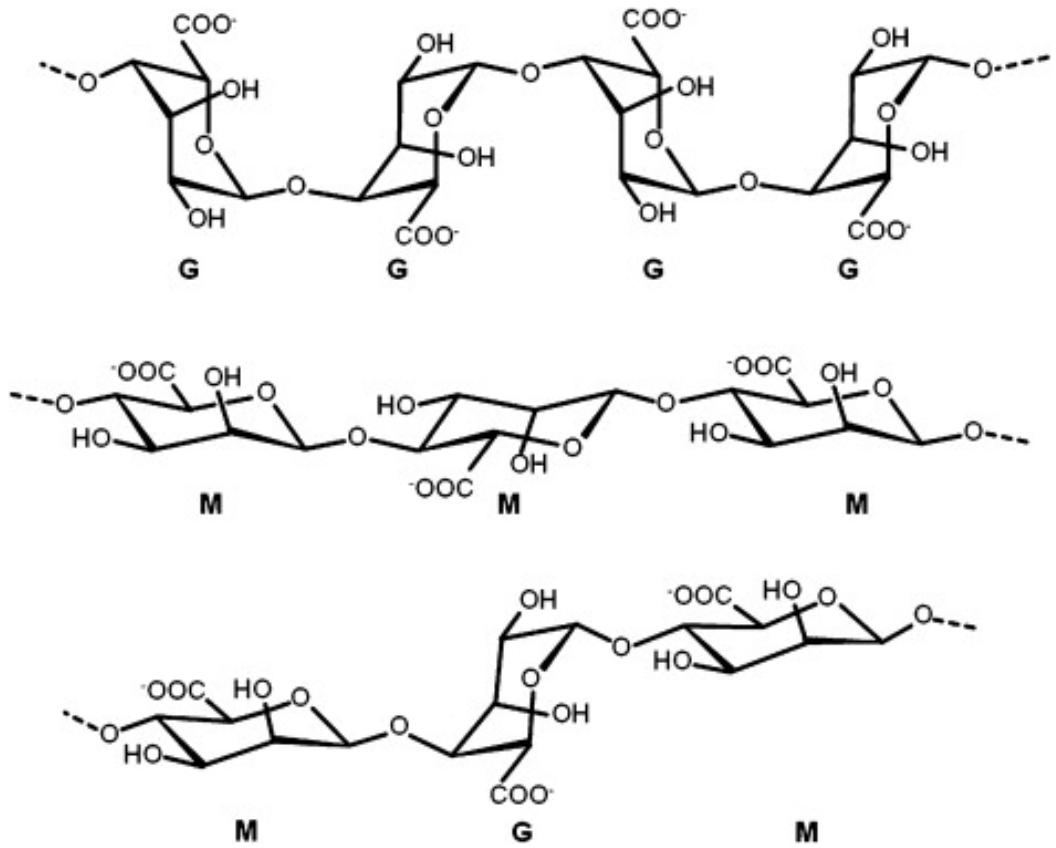

Figure 1.1: G block, M block and alternating block structures in alginate [30]

Commercially available sodium alginates weigh between 32,000 and 400,000 g/mol. Manipulation of molecular weight and its distribution in alginate polymers will significantly increase gels ' elastic moduli [31]. Alginate solutions ' viscosity increases as pH decreases and reaches a peak of around pH 3–3.5, as alginate backbone carboxylate groups protonate

and form hydrogen bonds. Alginate polymers have several superior characteristics over other polymers. This includes biocompatibility, ease of gelation, inert nature, natural origins, ease of availability, low cost and feasible method of synthesis, making it an ideal choice of a polymer of applications in drug delivery and tissue engineering [13]. Various drug molecules, from small chemical drugs to macromolecular proteins, can be trapped and released from alginate matrices in a programmed approach, using different cross-linking methods and cross-linking agents. Furthermore, alginate gels can be orally administrated or injected into the body in a minimally invasive manner, which allows extensive applications in the pharmaceutical sector.

1.1.2 Fabrication of alginate hydrogels

Alginate hydrogels are prepared through different synthesis routes. Chemical and physical cross-linking of the polymers are typical approaches to form hydrogels. In addition to the molecular weight and chemical composition of polymers, physicochemical properties are highly dependent on the cross-linking type and cross-linking density [32, 15].

Ionic crosslinking

The most common method of preparing hydrogels from an aqueous alginate solution is cross-linking with ionic cross-linkers such as divalent cations. The affinities of alginate towards the divalent ion vary in the descending order of Pb > Cu > Cd> Ba > Sr > Ca > Co, Ni, Zn > Mn [33]. Due to its non-toxicity in contrast to the other cations, Ca^{2+} is the most widely used in alginate gelation. M monomer blocks form weak junctions with divalent cations in an alginate solution. The interactions between blocks of G monomers and divalent cations, however, form tightly held junctions. The mechanism is through the formation of an egg-box structure through the coordination of divalent ions with four-carboxy groups as depicted in figure 1.2 [34].

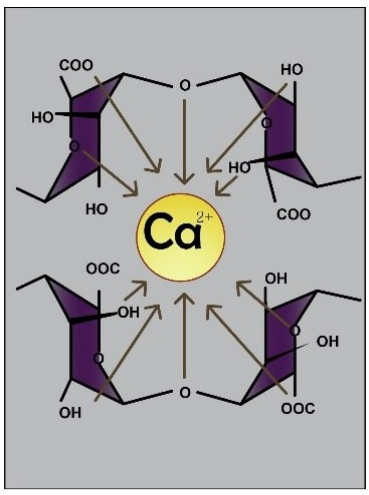

Figure 1.2: The egg-box model [35]

Calcium chloride ($CaCl_2$) is one of the most frequently used agents to crosslink alginate ionically. Though, due to its high aqueous solubility, it causes rapid gelation and results in a gel with undesirable properties. In combination with $CaCl_2$, a retardant agent containing phosphate groups can be used to slow the gelation cycle. The phosphate groups will interact with the alginate's carboxylate groups during reaction with Ca^{2+} ions. The gelation temperature affects the gelation rate and the final mechanical properties of the gels. At lower temperatures, ionic cross-linker reactivity (e.g., Ca^{2+}) is decreased, and the crosslinking becomes slow. So, the resulting crosslinked network structure is more structured and improves mechanical properties [36] The poor physiological stability is a critical drawback of an ionically crosslinked hydrogel. When the surrounding media is rich in monovalent cations anions, these hydrogels gels get dissolved by exchange divalent ions [37, 38]. Yet, this process may be positive or negative, depending on the target application. Calcium alginate hydrogels can be made to remain stable by inducing sufficiently higher concentrations of calcium in the medium [39]. Ionic crosslinking of alginate can be carried out by two routes, namely external or internal gelation.

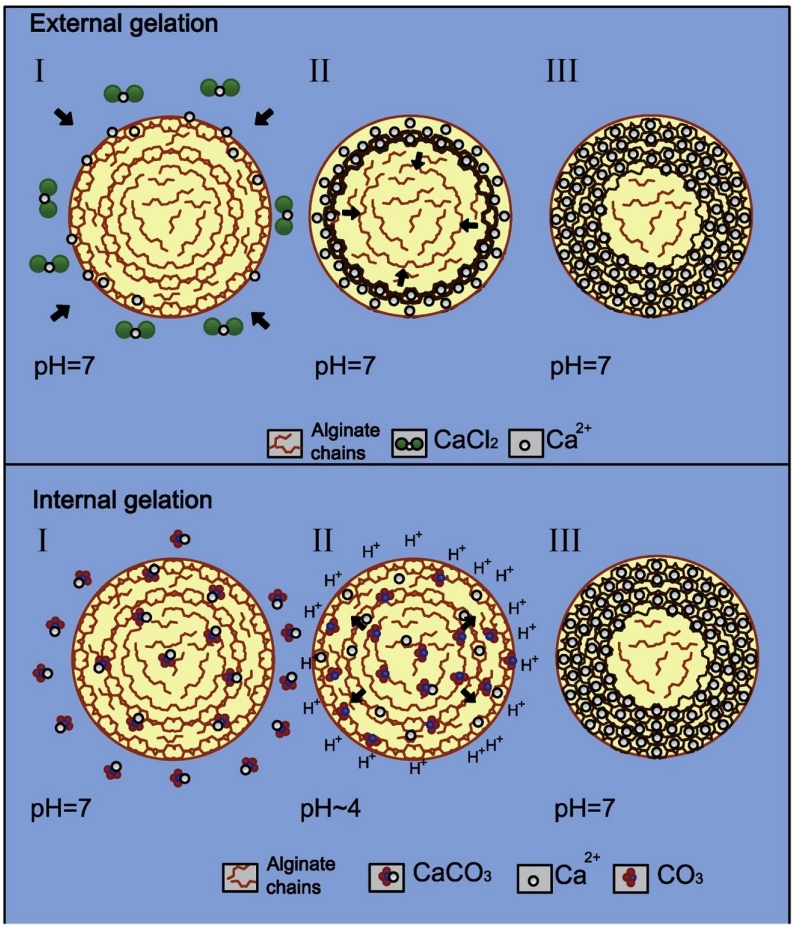

Figure 1.3: Gelling mechanisms in ionic gelation of alginate particles. [35]

Alginate gelled using external gelation is a straightforward method. As illustrated in

figure 1.3, Ca^{2+} ions are introduced into alginate droplets by addition to the surrounding medium. This allows the diffusion of Ca^{2+} ions into the spaces produced between the alginate polymer chains, leading to the cross-linkage formation. Once the alginate polymer chains come into contact with Ca^{2+} ions, cross-links start to develop at the alginate droplet peripheries, forming semi-solid membranes containing liquid cores [10]. Prolonging the incubation time of droplets in a Ca^{2+} ion bath allows the diffusion of more Ca^{2+} ions across the membranes due to changing concentration gradient and in effect allowing the alginate gel cores to gelate [40].

When the crosslinking and gelation step initiated from within the alginate droplets, it is known as internal gelation. Using a divalent cationic salt with low solubility allows for controlled gelation at neutral pH; following this, an acidification step will allow for the release of cations for better control of gelation kinetics and forming homogeneous gels. Insoluble salts mainly rely on pH for better solubility [41]. Low-soluble calcium salts such as Calcium sulfate ($CaSO_4$), Calcium carbonate ($CaCO_3$), Calcium lactate, and calcium gluconate have been used for controlled gelation approaches. Gelation rate is a critical parameter in regulating gel uniformity and strength when divalent cations are used, and slower gelation results in more uniform structures and greater mechanical integrity [42]. Figure 1.3 shows an example of this internal gelation, where an alginate solution containing insoluble calcium carbonate particles mixed into an oil phase. Lowering the pH using acetic acid led to the dissolution of calcium carbonate into Ca^{2+}, carbon dioxide, and water [43]. This method has allowed for the production of alginate gel microbeads with different sizes ranging from 20-1000 m [41, 44, 45].

Covalent crosslinking

Other approaches base upon covalently cross-linking the hydrogels, to create strong and irreversible chemical bonds. Covalent cross-linking has been found to improve physical properties. When stress is applied to ionically cross-linked alginate, eventually losing water from the gel, causing plastic deformation. Though water movement occurs in covalently cross-linked gels, leading to stress relaxation, the inability to dissociate and reform bonds leads to significant elastic deformation [46]. The issue in this method is that it employs toxic reagents, and the unreacted reactant residues may need to be thoroughly eliminated from gels. Photo cross-linking is an exciting approach to in situ gelations that utilizes covalent cross-linking. Photo cross-linking can be achieved by incorporating reactive groups like methacrylates, even in direct contact with drugs and cells. A high energy light source such as laser excites the photoinitiators and triggers production of free radicals. Then these reactive species spread in solution across the alginate prepolymer medium, thereby generating new free radicals and parallelly making cross-links between the polymer chains. As the reaction progresses, the number of cross-links in the process grows, and a network structure is achieved through a chain-growth mechanism [47]. Photo cross-linking reactions characteristically involve the use of a light sensitizer or acid release,

which could be dangerous to live cells and the body [48, 49].

1.1.3 Swelling and deswelling mechanism

Hydrogels display a sponge-like behavior by absorbing large quantities of water and biological fluids through hydrophilic groups present in their structure. Control of kinetics and degree of hydrogel swelling is possible by tuning of parameters such as charge, concentration, pK_a of ionizable groups, hydrophilic to hydrophobic balance, cross-link density, degree of ionization, interaction with the counter ion in the medium and pH [49]. The flexibility of monomers in the structure enables hydrogels to react in a short space of time to changes in pH, temperature, salt, light, biomolecules, ion strength, and magnetic fields [50, 51]. The following equation describes the swelling ratio [52, 53, 54]:

$$\text{Swelling ratio} = \frac{m_\text{s} - m_\text{d}}{m_\text{d}} * 100 \tag{1.1}$$

in where, m_s is the mass of swollen hydrogel in equilibrium at a given temperature and m_d is the dry mass of sample.

1.1.4 Drug entrapment and release

Hydrogels can be loaded with drug cargos and programmed to intelligently deliver the drug in a sustained manner for clinical applications. Such abilities emerge from adjustable hydrogel porosity or by using physical and chemical approaches [55]. Modifying the degree of cross-linking in the hydrogel structure allows for an appropriate increase or decrease in the release of the drug. Higher the degree of cross-linking between polymer chains reduces the molecular space through which the drug could travel, thus increasing the time to diffuse out of the gel matrix. This diffusion is also dependent on the nature and size of the drug used [56]. Apart from the pore size of the gel, the physiological conditions like temperature, pH, ionic concentration also play a role in drug release. For example, anti-cancer drug doxorubicin had been successfully loaded in alginate microspheres subject to pH-triggered release [57].

1.1.5 Biodegradation and biocompatibility

While alginate comes from natural sources, various impurities such as heavy metals, endotoxins, proteins, and polyphenolic compounds might still present. Notably, alginate processed to a very high purity through a multi-step extraction procedure did not cause unusual foreign body reactions when administered in animals [58]. Commercially produced high purity alginate did not cause any severe inflammatory reaction after subcutaneous injection into mice [59]. The average molecular weight of alginate is higher than the kidney's renal clearance threshold, so it likely to be not completely removed from the body [60]. An effective strategy to counter this problem would be to oxidize the alginate

chains partially. Partially oxidized alginate can degrade in aqueous environments. The degradation rate is further influenced by the degree of oxidation, pH, and temperature [61]. Alginate is essentially non-degradable in mammals due to the absence of alginase enzyme that cleaves the polymer chains; however, ionically cross-linked alginate hydrogels can still be dissolved by exchanging the divalent ions present in the gel cross-links with monovalent cations such as sodium ions present in surrounding media.

1.2 The microfluidic approach

Microfluidics is a technique that involves injecting non-miscible fluids through interconnected micron-scaled channels. This immiscibility of the fluids promotes the formation of a liquid microstructure inside the dispersing phase; followed by gelation in the presence of external sources like light or through interaction between ionic polymers with multivalent ions. Thus, the yielded microstructures are known as microgels [62]. Different types of shapes can be produced based on variables such as the relative position of microchannels, injection speed, number of liquids used, and their viscosity. Furthermore, cells can be encapsulated by suspending them into the desired precursor solution that will form a microstructure, before they are injected into the microfluidic channel [63, 64]. More complex microfluidic systems can be used to obtain structures such as core-shell spheres, hollow tubes, and Janus particles (particles including two assigned and new surfaces) for drug delivery and multi-layered cell scaffolding applications [65, 66, 67, 68]. Microfluidics has gained popularity due to several advantages of this technique, including small sample volumes and minimal quantities of reagents used for testing, the ability to sort and classify specimens at high-resolution and sensitivity and rapid analysis [69].

1.2.1 Materials for fabrication of microfluidic devices

First microfluidic devices were commonly constructed of transparent polymers, glass, stainless steel, silicon, and ceramics. The first microfluidic chips were commonly fabricated using transparent polymers, glass, stainless steel, silicon, and ceramics. Silicon was the first material used to fabricate microsensors in the early 1970s. Non-silicon based materials were developed as technology progressed; however, which led to the creation of a new field called microelectromechanical systems (MEMS). The rapid development in this field had led, for example, to the introduction of new technology among researchers: microfluidics and lab-on-a-chip (LOC) [70]. The choice of silicon as the material made it possible for reactions requiring high temperatures, and strong solvents. Glass, a material with properties similar to that of silicon and robust optical properties, was the next choice for making the microchips. Nevertheless, these materials proved to be disadvantageous as fabricating the chips was laborious, the chips were brittle, and production costs were expensive. So a better candidate material was sought, and hence polymers were implemented for making the microchips [17]. Polymers like polydimethylsiloxane (PDMS), polymethyl methacrylate

(PMMA), and polycarbonate (PC) showed better characteristics than silicon and glass such gas permeability, chemical resistance, optical properties and inexpensive moulding techniques for large scale production [71]. The microfluidic chip used in this project is made from Cyclo-olefin-copolymer (COC), a plastic material having outstanding optical characteristics, very low water uptake, and extremely low permeability for water vapour. It was bought from microfluidic ChipShop.

1.2.2 Physical considerations in microfluidics

Microfluidics involves manipulation and analysis of fluids in microscale structures. To understand and work with microfluidics, its necessary to understand the physical phenomena at the microscale. Different forces rule at the microscale in comparison to those present in the macro-world [72]. For instance, the capillary forces dominate the microfluidic system over gravity. While miniaturizing existing large devices, the chips must be designed to take advantage of forces that work on the microscale.

Reynolds number

Reynolds number (R_e) describes the ratio of fluid momentum to frictional forces occurring in contact with channel walls. The Reynolds number can be calculated by [73, 74]:

$$R_e = \frac{\rho \upsilon D_h}{\mu} \tag{1.2}$$

Where ρ is the fluid density, υ is the characteristic velocity of the fluid, μ is the fluid viscosity, and D_h is the hydraulic diameter [75]. The hydraulic diameter is a measured value depending on the cross-sectional geometry of the channel. From Reynold's number, fluid flow can be classified as two central systems: Laminar flow and turbulent flow. Fluid flow is assumed to be laminar if Re is low, typically $R_e < 2300$. As the Re values approach, the fluid may show signs of turbulence, and the flow is said to be turbulent at $R_e > 2300$.

Laminar flow

In a Laminar flow, all the particles in a fluid stream follow a path in layers, with each layer moving smoothly along the other with little or no mixing. The small dimension of microchannels allows the flow to be almost always laminar [76]. Therefore two or more streams in a laminar flow will not mix on contact except through a diffusion process. Laminar flow also can be used to generate fluid packets that can withhold their integrity, with diffusion occurring in the terminals. Such packets can be transferred in a controlled way, thereby allowing for various opportunities in cellular and molecular analysis.

Diffusion

Diffusion is a phenomenon in which the particles move from a higher concentration region to lower concentration until the concentration reaches an equilibrium. Brownian motion is responsible for this particle movement. Diffusion can be defined in one dimension by the following equation:

$$d^2 = 2Dt \tag{1.3}$$

Where d is the distance, a particle moves in a time t, and D is the diffusion coefficient of the particle. As the distance is changed by square power, microchannels allow for shorter diffusion times compared to diffusion in macro-systems for the same particle [75].

Surface area to volume ratio

The surface area is an essential factor influencing processes taking place at the microscale. As we scale down from the macroscale to the microscale, the volume is reduced. At lower volumes, surface-related force becomes dominant over the volume-related force. Consequently, the surface area to volume (SAV) ratio increases by large magnitudes. The large SAV ratio allows for molecules to quickly diffuse between the fluid streams or droplets formed inside the microchannels [77].

1.3 Polymeric Drug Delivery System

Controlled drug delivery is among the most challenging and rapidly-growing scientific areas. Drug delivery systems (DDS) based on nanoparticles were known to help improve bioavailability and extend the circulation of clinically relevant drugs. DDS using polymer-based nanostructures such as dendrimers, micelles, nanoparticles, or nanogels have been investigated, with sizes ranging from 1 to 1000 nm [78, 79, 80, 81, 82, 83]. It can deliver many advantages compared to traditional dosage forms such as improved absorption rates and diminished immunogenicity, targeted delivery, drug protection from cellular enzymes, enhanced biodistribution when controlling drug release [84].

1.3.1 Polymeric micelles

Polymeric micelles feature unique properties such as high molecular weight, significantly low CMC, higher stability, slower dissociation rate, more excellent retention of encapsulated drugs and higher site-specific deposition of drugs at the target site [8]. Amphiphilic blocks or copolymers are identical to the behaviour of traditional amphiphiles. A significant difference between the micelles of conventional surfactant monomers and polymeric surfactants is the presence of covalent linkage in individual polymeric surfactant molecules within the hydrophobic core. Hence monomers are not dynamically exchanged between free solution and the micellar pseudo-phase [85]. Due to this, polymeric micelles possess conformational rigidity and stability [86]. Polymeric micelles can range 10 to

200 nm in diameter. The factors that control the size of the polymeric micelles include the molecular weight of the block copolymer, aggregation number, the relative fraction of the hydrophilic and hydrophobic chain, the amount of solvent trapped in the micellar core and the preparation method [87, 88]. Amphiphilic block copolymers may mainly assemble themselves into spherical micelles, worm-like or cylindrical micelles, and polymer vesicles or polymers. Main factor regulating micellar morphology is the hydrophilic-hydrophobic copolymer block equilibrium determined by the hydrophilic volume fraction, f. For amphiphilic block copolymers with a value of f = 35%, polymer vesicles form although, at a value of f > 45%, spherical micelles are observed. Other experimental variables in micelle formation are concentration, temperature, pH, ionic strength, degree of swelling of the corona and sample preparation [89, 90, 91]. Many hydrophilic polymers have been researched which include biodegradable and non-biodegradable copolymers, namely poly(amino acid)s, polyglycerol, poly(2-oxazoline)s, polyacrylamide, poly[N-(2-hydroxypropyl) methacrylamide and polyvinylpyrrolidone (PVP) [92]. PVP is a biocompatible, non-ionic, water-soluble polymer that can interact with a wide range of hydrophilic and hydrophobic drugs [93]. PVP is attractive with its cryoprotective and lyoprotective properties while freeze-drying, thereby preventing nanoparticle aggregation [94, 95, 96].

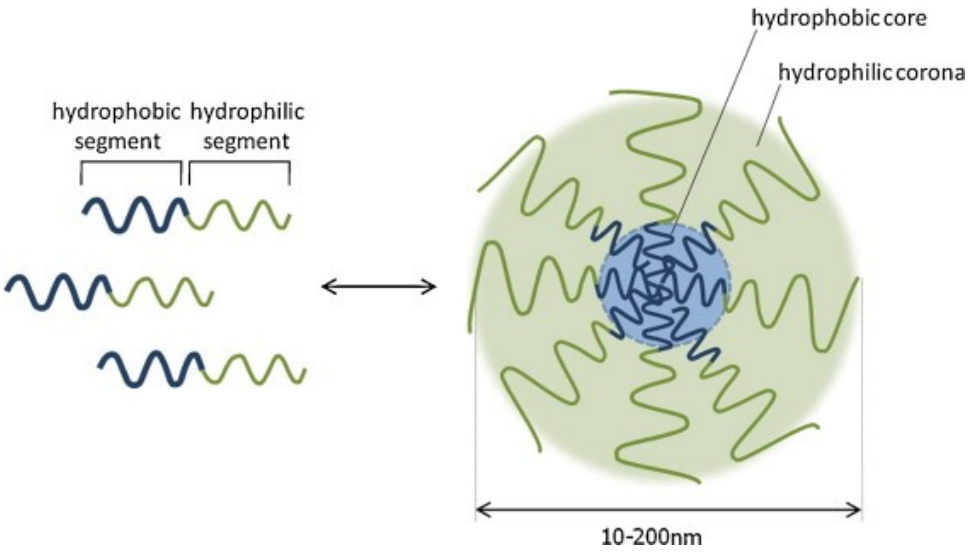

Figure 1.4: Schematic illustration of amphiphilic polymers self-assembling into micelles [97]

1.3.2 Micellization

Micelle formation in aqueous solution is a result of active interaction between surfactants ' hydrophobic regions. The main factor behind this self-interaction is the system's reduction in free energy. The free energy reduces as an outcome of removing hydrophobic moieties from the aqueous environment and forming a micelle core reinforced with hydrophilic

moieties exposed to water. Change in free energy for micellization is described as:

$$\Delta G^{o}{}_{\text{mic}} = RTln(CMC) \tag{1.4}$$

where R is the gas constant, T is the temperature of the system, and CMC is the critical micelle concentration [98]. The amphiphilic blocks, assemble only when a specific minimum concentration is reached, known as the critical micelle concentration. At low, medium concentrations, amphiphilic molecules exist separately and tend to be sub-colloidal. Below CMC, the amount of amphiphile adsorption at the air-water interfaces increases as the amphiphile concentration rises. When the concentration is equal to CMC, Monomers saturate the interface and bulk phase. Any additional amphiphilics added above CMC trigger in the aggregation of monomers throughout the bulk phase, reducing the system's free energy [99, 100].

1.3.3 Drug loading of polymeric micelles

Polymer micelles can be used for solubilizing hydrophobic molecules in its core region by hydrophobic interactions and ionic interactions. Badly water-soluble drugs could easily be incorporated into polymeric micelles to solve issues of drug solubility in aqueous conditions [84, 101]. The addition of the drug into the micellar core contributes to the in vitro stabilization of the drug as it is secured from disrupting agents in the surrounding system. The hydrophilic exterior and nanoscopic dimensions prohibit the physical elimination of micelles by filtration or ion the spleen during application [102]. This property is helpful to increase the circulation of the drug in the body. Nanoscopic size of micelles reduce the risk of capillary embolism, as opposed to larger drug carriers [102]. This section summarizes, some of the techniques for passive drug loading in micelles.

Solvent evaporation/thin-film formation

It is the commonly used micelle preparation process. In this method, the drug and polymer are dissolved in an organic solvent, followed by the entrapment drug on thin polymer film after the organic solvent is removed by evaporation(usually 12–24 h). The thin film is then rehydrated in water to produce drug-loaded polymeric micelles. The non-trapped drug is removed by dialysis [85, 103, 102]. Nonetheless, this approach is unsuitable when for long-chain and higher hydrophobicity core-forming blocks [104].

Direct dissolution

It is the simplest method used when polymers have small chains and low molecular weight blocks that are insoluble in water. In brief, amphiphilic polymers and drugs are solubilized directly in water at or above CMC with stirring, thermal, and ultrasound treatments [85, 105]. The main limitation here is reduced drug loading [102, 104].

O/W emulsion

This process involves the dissolution of the copolymer in organic or the aqueous solvent and the drug in a volatile organic phase miscible in water. The solvents used are mostly acetone, tetrahydrofuran, ethyl acetate, chloroform and methylene chloride. Drug-loaded polymer micelles are obtained by constantly stirring the aqueous phase as the organic phase is steadily introduced. However, this method involves the usage of toxic solvents which require complex purification steps [87, 106]

Direct dialysis

This approach is typically used if the core blocks are strongly hydrophobic and have long sequences [104]. Here both the drug and the polymer are dissolved in a water-miscible organic phase. The dialysis bag containing the drug solution is then submerged several hours into the water and causes micelle formation by solvent exchange [105, 107]. While dialysis is commonly used, it is a time-consuming process and can lead to changing morphology because of the incremental modification of solvent properties [85, 108].

Freeze drying/lyophilization method

Freeze drying is a simple, one-step and economical technique, where the drug is dissolved together with the polymer in an organic phase and water mixture, and then lyophilised. Reconstituting the freeze-dried product in water will yield micelles, and further dialysis would remove the non-encapsulated drug [103, 107].

Cosolvent evaporation

Via cosolvent evaporation, the drug and the amphibolic copolymer blocks are dissolved first in a water-miscible organic solvent. After this, water is gradually added until a point where drug and polymer are still soluble. The mixture is allowed to evaporate until the organic solvent is wholly eliminated. The slow removal of the organic solvent forces the drug to go inside the micellar core as the water concentration increases [6].

2. Materials and Methods

2.1 Materials and Chemicals

Material	Manufacturer	Description
1-Undecanol	TCI	>98%
Calcium iodide	Sigma-Aldrich	99 %
Dye aye (DiI)	Invitrogen	λ_{max} - 549 nm
EDTA	Sigma-Aldrich	>98.5 %
Ethanol	Kemetyl	Absolute
	VWR	96 %
Fluorescin free acid	Fluka	λ_{max} - 490 nm
Phostphate buffer saline	Lonza	
PVP		
Rhodamine B	Sigma-Aldrich	λ_{max} - 543 nm
Sodium alginate	Sigma-Aldrich	
Span – 80 (sorbitan oleate)	Sigma-Aldrich	

Table 2.1: Materials used in this project

Equipment	Manufacturer	Description
Axioskop 2 plus	Zeiss	Compound microscope
Centrifuge	Eppendorf	5804R
DP controller	Olympus	Software
Fluidic 537	Microfluidic-ChipShop	Droplet generator chip; Topas material
NanoSight LM10	Malvern	Sample chamber, microscope and camera
NanoSight NTA 3.0	Malvern	Software
NE-300	New Era Pump Systems Inc.	Syringe Pump
Olympus IX71	Olympus	Inverted Microscope System; DP70 CCD camera
Vibra Cell	Sonics	Sonication Probe

Table 2.2: Equipments and software employed in this project

2.2 Methods

2.2.1 Production of micellar nanocarriers

The current section describes the synthesis of polymeric micelles through different strategies: the co-solvent method and the sonication method. The micelles were loaded with DiI as a hydrophobic model molecule.

DiI loaded PVP micelles using sonication method

100 mg of 12 kDa PVP polymer was weighed and dissolved in 8 mL of Milli-Q water, heated up to 50 °C in a beaker. The polymer was mixed in the water rapidly, with the assistance of a magnetic stirrer. Next, 5 mg of DiI was measured and dissolved in 1 mL of acetone. The DiI solution was then added dropwise to the polymer. This mixture was allowed to mix for 30 mins, after which it was transferred to Greiner tubes and sonicated using sonication probe for 10 minutes with fixed parameters: pulse: 1 s on, 2 s off at an amplitude of 40%. During the sonication procedure, the polymer solution remained immersed in ice to prevent overheating. Then, the tubes were carefully immersed into liquid nitrogen for 15 minutes approximately and lyophilised in vacuum freeze-drier for two days. After lyophilisation ended, the tubes were carefully covered with paraffin film and aluminium foil to protect the sample against moisture and light. The tubes remained then stored at room temperature.

DiI loaded PVP micelles using Co-solvent method

30mg of 12 kDa PVP polymer was weighed and dissolved in 10 mL of Milli-Q water and sonicated using sonication probe for 10 minutes with fixed parameters: pulse: 1 s on, 2 s off at an amplitude of 40%. Also, 3mg of DiI was dissolved in 100 mL of 96% ethanol by rapidly mixing using a magnetic stirrer for 2 hours. After this, the polymer solution was slowly introduced into the ethanol solution along with rapid stirring. The solution was then evaporated utilising a rotary evaporator for solvent removal. The rotary evaporator was operated at 40 textdegree C and stopped when the solution was reduced to approximately 3 mL in volume. This solution was transferred to Greiner tubes and then carefully immersed into liquid nitrogen for 15 minutes approximately, and lyophilised in vacuum freeze-drier for two days. After lyophilisation ended, the tubes were carefully covered with paraffin film and aluminium foil to protect the sample against moisture and light. The tubes remained stored at room temperature.

2.2.2 Characterization of micellar nanocarriers

Nanoparticle tracking analysis of micelles

For the DiI loaded polymeric micelles, the size distribution analysis was performed using the technique of Nanoparticle Tracking Analysis (NTA). The sample chamber used for the

experiment was bought from NanoSight (Malvern). The principle here is based on the light scattering properties of molecules and their Brownian motion when suspended in a liquid [109]. The micelle sample for analysis was prepared in many steps. The lyophilised sample was dissolved in PBS to get a final concentration of 1mg/mL. The solution was then sonicated using sonication probe for 5 minutes with fixed parameters: pulse: 1 s on, 2 s off at an amplitude of 40%. The sample solution was then filtered through a 0.45 μm and 0.25 μm syringe connected in series to remove any debris and large agglomerates. The sample was loaded in the sample chamber, and an averaged size distribution was obtained from three measurements.

2.2.3 Production of alginate microgels

This section describes the steps in the fabrication of alginate microgels using a microfluidic chip, its recovery and characterization.

2.2.4 Setup of microfluidic droplet generation system

Microfluidic droplet generation kit was purchased from microfluidic-ChipShop, Germany. The chip used in this study was Fluidic-537; it is made from topas (cyclo-olefin copolymer) material and consists of four parallel emulsion units on the same chip. The chip's dimensions are as described in the schematic drawing. (Figure-) The standard accessories provided by the manufacturer were equipped on to the chips, which includes the Luer Fluid Connectors, Luer Plugs, Silicone and PTFE Tubing. The tubings were then connected to two syringes, each containing the oil phase and water phase. The syringes were then mounted into their syringe pumps. After setting up, the chip was attached on to the stage of an Olympus IX71 inverted microscope. The microscope is coupled to an Olympus D70 CCD camera for viewing and capturing the droplet generation process. After mounting the loaded syringes, the flow rates are set in the syringe pumps. Starting off the pump initiates fluid flow inside the chip. The flow inside the channel is observed through the microscope's camera software, DPcontroller. At a specially designed cross-junction channel, the aqueous phase is hydrodynamically focussed by the two oil streams through a narrow orifice of the outlet microchannel. This flow-focussing will form emulsions in the form of spherical microdroplets inside the microchannel. The effect of flow rate and concentration dependency of surfactant was also studied to determine the optimal setting for microdroplet formation.

2.2.5 Preparation of emulsion fluids

For generating microdroplets, a two-phase oil/water system was utilized. Sodium alginate was dissolved in PBS at different concentrations (1% - 2% w/v). Two dyes, namely Fluorescin free acid and Rhodamine B, was added to the aqueous phase for real-time monitoring of microdroplet formation. The alginate solution served as the aqueous phase.

For the oil phase, 1-Undecanol was chosen as it is immiscible in water and negligibly soluble. For the cross-linking experiments, Calcium iodide was dissolved in 1-Undecanol and used as the cross-linker at a concentration range of 0.15 - 0.3% w/v. Span-80 was added to the oil phase and used as the surfactant (1% v/v).Surfactants are used to reduce the surface tension at the oil/water interface. Finally, the liquids were loaded into their respective syringes and mounted to the syringe pumps.

2.2.6 Crosslinking of microdroplets

For the cross-linking reaction, 1 mL of 2% w/v alginate solution, 1 mL 1-Undecanol containing 0.3 % w/v CaI2 and 1 % v/v surfactant was loaded into pump syringes. The syringes were connected to appropriate channels on the microfluidic chip. When the flow was started, microdroplets of alginate solution were formed at the cross-junction channel. The droplets were collected in oil containing Ca^{2+}. The droplets were allowed to gelate in the oil phase for 1 hour and were recovered by washing three times in PBS.

2.2.7 Microdroplets size measurement

For the cross-linking reaction, 1 mL of 2% w/v alginate solution, 1 mL 1-Undecanol containing 0.3 % w/v CaI2 and 1 % v/v surfactant was loaded into pump syringes. The syringes were connected to appropriate channels on the microfluidic chip. When the flow was started, microdroplets of alginate solution were formed at the cross-junction channel. The droplets were collected in oil containing Ca^{2+}. The droplets were allowed to gelate in the oil phase for 1 hour and were recovered by washing three times in PBS.

3. Results and discussion

3.1 Production of micelles

Ploymerics micelles were successfully synthesized using 12 kDa of PVP using co-solvent evaporation and soication methods. The lyophilized micelles were reconstituted in PBS and filtered using 0.2 μm filter syringe for further analysis.

3.2 Size distribuition of micelles

The size distribution of the micelles produced in the previous section was analyzed to study any possible implication of the polymer, loading drug, and pretreatment used on their size. For this assay, micelles in dehydrated powder were prepared following the method described in Section 3.2 3.1: Dehydrated powder was dissolved in milli-Q water up to a concentration of 1 mg / ml, later pre-treated with sonication. The solution was analyzed in a NanoSight sample chamber and the recorded information was processed by the software Origin Pro 8 to produce the different figures presented in this section.

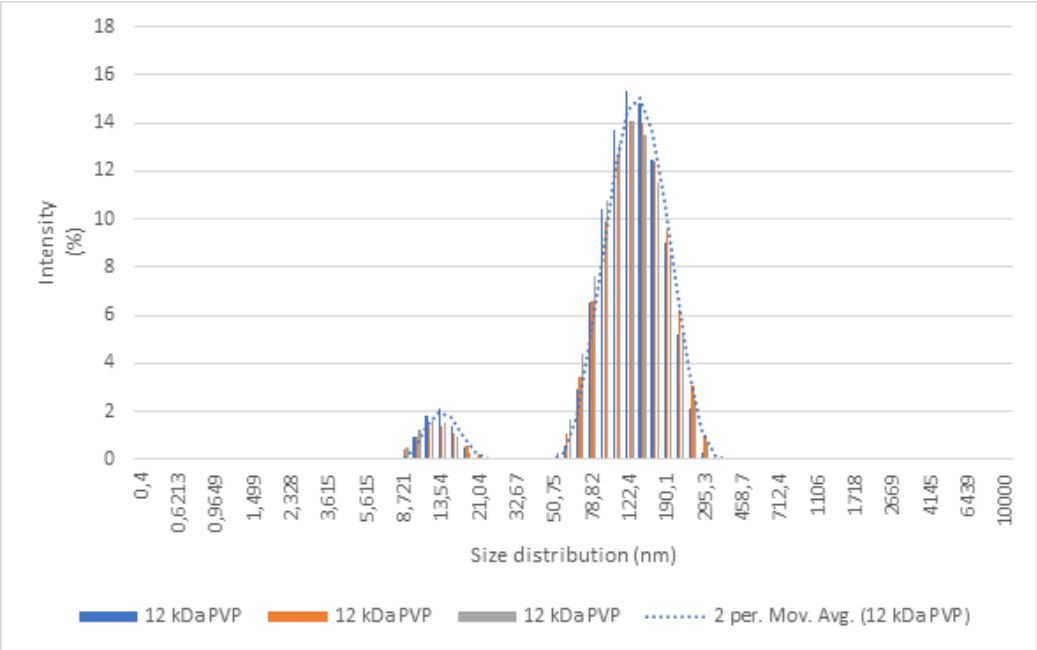

Figure 3.1: DLS size distribution for 12 kDa PVP micelles loaded with DiI

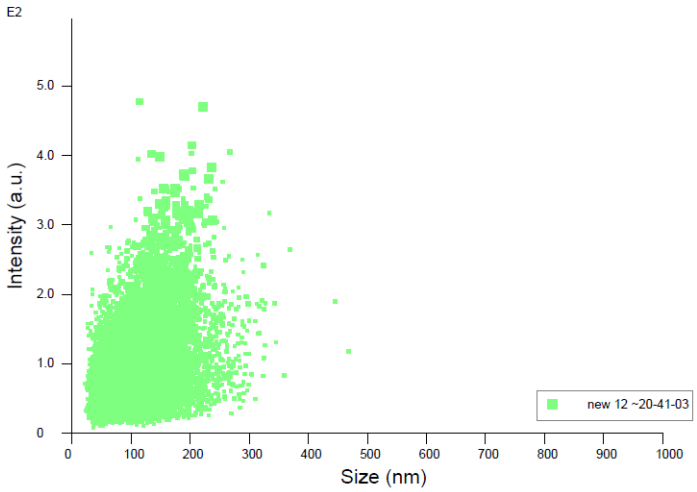

Figure 3.2: NTA distribution for 12 kDa PVP micelles loaded with DiI

3.3 Production of alginate microgels

3.4 Effect of surfactant concentration

Surfactant reduces the surface tension at the water/oil interface. Therefore, the effect of surfactant concentration during flow-focusing and the consequent droplet formation was studied. For this, Span 80 was solubilized at different concentrations in 1-Undecanol with cross-linker absent. The concentration range was from 0 - 1% v/v.

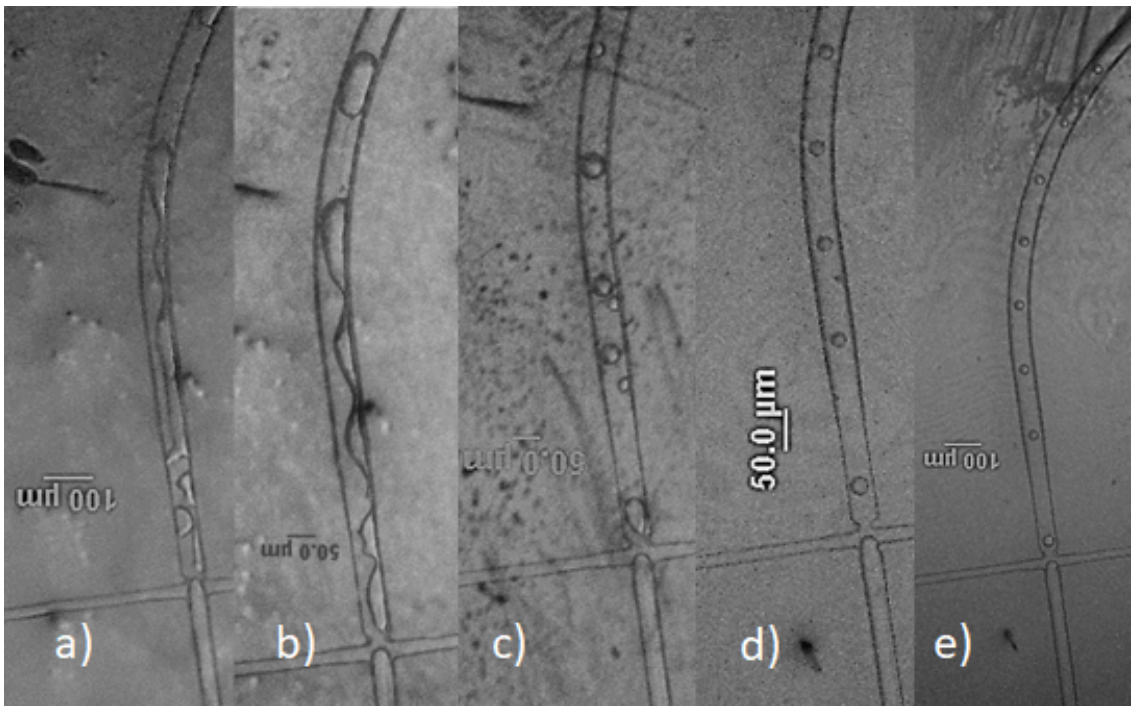

Figure 3.3: Droplet generation relative to increasing concentration of surfactant a) to e)

3.5 Effect of flow rate

To further optimise microdroplet formation, the relationship between flow rate and droplet size was studied. The size of droplets is defined by the flow-rate ratio between continuous and disperse phases (Qw/Qo).At a constant flow rate of the oil phase(Qo), the flow rate of the aqueous phase(Qw) was varied.

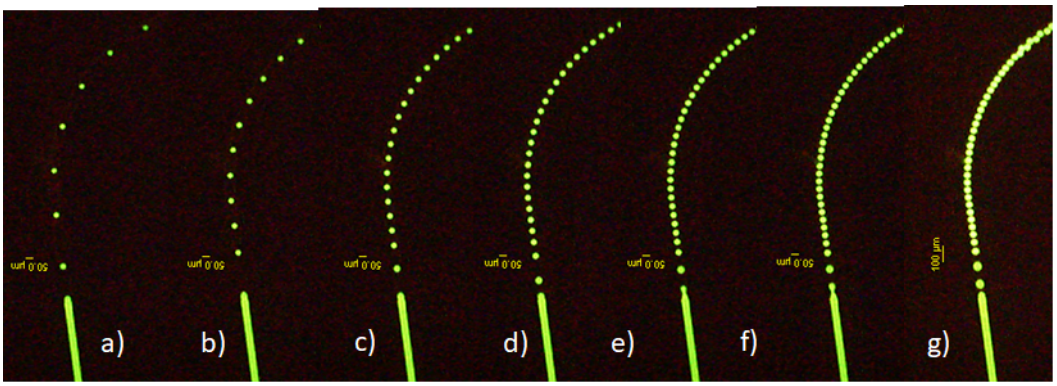

Figure 3.4: Microdroplet generation relative to ratio of flow rate Qw/Qo

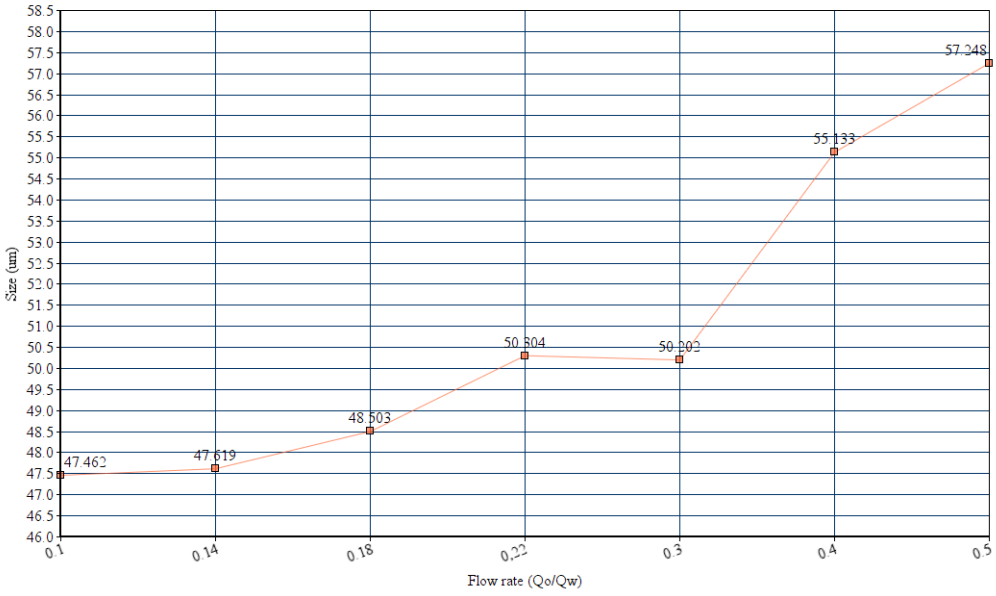

Figure 3.5: Plot of flow rate ratio versus diameter of microdroplet

It has been possible to create emulsions of microdroplets using alginate solution and 1-Undecanol. Although the gelation was not successful, the srudies indicate the possibilities in manipulating and controlling droplet formation. This would allow for a future possibilty in precise fabrication of monodisperse alginate microspheres.

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