Diploma Thesis

Preparation of injectable hydrogel microparticles based on silated-hydroxypropyl methylcellulose

Supervisor: Assoc. Prof. Zdeňka Šklubalová, Ph.D.
Consultant: Ing. Corine Tourné-Péteilh

Hradec Králové, 2015

Petra Husárová
Statement of originality

I declare that this diploma thesis is my own personal work and that I worked on it on my own. All literature and other resources that I used are listed in the reference list and are properly cited.

Date: Signature:
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1. **ABSTRACT**

Charles University in Prague, Faculty of Pharmacy in Hradec Králové  
Department of: Pharmaceutical Technology  
Consultant: Assoc. Prof. PharmDr. Zdeňka Šklubalová, Ph.D.  
Ing. Cori Tourné-Péteilh  
Student: Petra Husárová  
Title of thesis: Preparation of injectable hydrogel microparticles based on silated-hydroxypropyl methylcellulose

The aim of this work was to prepare hydrogel microparticles based on silated-hydroxypropyl methylcellulose (HPMC-Si). The microparticles are expected to be obtained by the self-hardening of HPMC-Si from the microdroplets formed by the emulsification in the continuous phase. Dispersion at high speed and microfluidics method were used to reach this goal.

A 3% w/w solution of HPMC-Si in the sodium hydroxide solution 0.2M (final pH of HPMC-Si solution 12.8), the HEPES buffer (pH 3.5) and a fluorescent dye FITC-Si were utilized to form microparticles. Vegetal oil was used as a continuous phase.

The formation of microparticles at high speed was based on high-performance dispensing to produce a well-dispersed emulsion. Weaker dispersing was applied to finish reticulation of microparticles. Problems like aggregation, heterogeneity of microparticles and their instability were observed with this method.

In the microfluidics method, microparticles were prepared by phase separation of a droplet in a non-miscible continuous phase by using microchannels. In order to form microparticles of controlled size and to improve their stability, various experimental conditions were tested. Parameters like temperature, speed rate of the continuous and dispersed phase, the length of the microchannel and the use of the surfactant Plurol were tested.

Although the results are preliminary, this research proved that it is possible to prepare microparticles and encapsulate FITC-Si by these two methods.
2. ABSTRAKT

Univerzita Karlova v Praze, Farmaceutická fakulta v Hradci Králové
Katedra: Farmaceutická technologie
Školitel: Doc. PharmDr. Zdeňka Šklubalová, Ph.D.
Ing. Corine Tourné-Péteilh
Posluchač: Petra Husárová
Název diplomové práce: Příprava injektovatelných mikročástic na bázi silanizované hydroxypropylmethylceluloby

Cílem této práce bylo připravit hydrogelové mikročástice na bázi silanizované hydroxypropylmethylceluloby (HPMC-Si). Příprava mikročástic byla založena na tvorbě mikrokapének vytvořených emulgací ve vnější olejové fázi a jejich následné retikulaci. K dosažení tohoto cíle byla použita metoda vysokorychlostní dispergace a mikrofluidizace.

Jako dispergovaná fáze k tvorbě mikročástic byl použit 3% w/w roztok HPMC-Si v 0,2M roztoku hydroxidu sodného s finálním pH 12,8; pufr HEPES o pH 3,5 a fluorescenční barvivo FITC-Si. Jako vnější fáze byl používán rostlinný olej.

Metoda vysokorychlostní dispergace byla založena na dispergaci vnitřní fáze při vysokých otáčkách a vytvoření homogenní emulze. K dokončení retikulace mikročástic byla následně aplikována pomalejší rychlost otáček. Při použití této metody přetrvává řada problémů, například agregace, tvorba heterogenních mikročástic a jejich nestabilita.

Principem mikrofluidizace bylo mísení dvou nemísitelných kapalin v mikrokanálcích. Jejich smíšením v T-spojení došlo k fázové separaci a tvorbě emulze. S cílem vytvořit mikročástice uniformní velikosti i struktury a zlepšit jejich stabilitu byla experimentálně zkoušena řada podmínek. Byly testovány parametry jako teplota, rychlost injektování vodné a olejové fáze, délka odvodného mikrokanálku či použití tenzidu Plurol.

I přestože se jedná o předběžné výsledky, tento výzkum prokázal možnost připravení mikročástic a enkapsulování FITC-Si za použití těchto dvou metod.
3. THE AIM OF THE STUDY

The aim of this study is to prepare spherical, non-aggregated and uniform microparticles based on silated-hydroxypropyl methylcellulose (HMPC-Si). A fluorescent dye FITC-Si is used to mark microparticles and free HPMC-Si in order to characterise the size, structure and uniformity of the prepared microparticles.

Two methods, dispersion at high speed and microfluidics, will be used. Both methods are based on inducing reticulation of HPMC-Si and effort to control the reticulation. Influencing parameters like temperature, length of microchannel, and the flux rate of oil (vegetal oil) and aqueous (solution of HPMC-Si in HEPES buffer) phases in microchannels are tested in microfluidics method.

Fluorescent and optical microscopy is used to characterise the size and structure of the microparticles.

Physical properties of phases such as viscosity, the surface and interfacial tension will be estimated.
4. INTRODUCTION

The degeneration of the intervertebral disc is one of the major causes of low back pain. The degeneration of the disc may be caused by many factors like genetics, ageing or overloading of the spine. Pain killers, anti-inflammatory drugs or suitable exercises are currently available treatment options. If these are not sufficient, surgical options are advised. Bony fusion between adjacent vertebrae or total disc replacement are usually performed. Unfortunately, these surgical solutions lead to reduced mobility. Therefore, load-bearing injectable biomaterials to replace the degenerated intervertebral disc are studied. Two main types of biomaterials are being developed. Non-degradable biomaterials are the first ones. It is assumed that these materials will be load-bearing throughout the lifetime of the patient. Mechanical properties which ensure mobility and resistance to torsion could be achieved. The degradable material which induces a regrowth of the intervertebral disc represents the second type.¹

In this thesis, the preparation of hydrogel microparticles based on HPMC-Si was the aim. This biomaterial has several convenient properties such as biocompatibility and bioadhesivity. HPMC-Si forms a three-dimensional porous network. It is possible to encapsulate active substances, cells or growth factors inside this porous network.¹,²

However, the aim of my thesis was to prepare load-bearing microparticles without active substances.

It is assumed to prepare microparticles suitable for parenteral administration. Hence, injectability, sterility and biocompatibility are indispensable.¹
5. THEORETICAL SECTION

5.1 Parenteral preparations

Parenteral preparations are sterile preparations which can be administrated by injection, infusion or implantation into the human or animal body. They have to fulfil some requirements. Excipients are used to improve the properties of parenteral preparations; examples include excipients used to make the preparation isotonic with respect to blood, to increase solubility, to adjust the pH, to prevent deterioration of the active substances or to provide adequate antimicrobial properties, while not adversely influencing the intended treatment or causing toxicity or irritations. They have to be prepared by using materials and methods which are developed to ensure sterility and to avoid the introduction of contaminants. They are stored in a sterile, airtight, tamper-proof container or a prefilled syringe. Transparent materials are convenient due to the possibility of visual inspection of the contents. Parenteral preparations comply with the test of sterility and particulate contamination: sub-visible particles of actual European Pharmacopoeia.

Injections are sterile solutions, suspensions or emulsions. They can be prepared by dissolving, suspending or emulsifying the active substance and excipients in water or in a suitable non-aqueous liquid. They have to comply with the test of uniformity of dosage units, uniformity of content and test of bacterial endotoxins – pyrogens. Syringeability and injectability are important properties of parenteral preparations. Syringeability is the ability of the suspensions to pass easily through hypodermic needle on transfer from the vial prior to injection. It should be easy to handle by the surgeon and comprises the properties such as ease of withdraw, clogging and foaming tendencies and accuracy of dose measurements. Increased viscosity, density, concentration of solid particles in suspension or a greater particle size makes worse the syringeability. Needle sizes 10-13 G (internal diameter 3.5 and 2.4 mm respectively) are commonly used in vertebroplasty. It is assumed that the same size of needles will be used for the disc. Injectability allows the performance of
injection. It comprises parameters such as the pressure or force required for injection and the evenness of flow.\textsuperscript{5}

5.2 The structure of the intervertebral disc

The intervertebral disc (IVD) separates the bony vertebral bodies and acts as a shock absorber. The IVD allows a large degree of spine flexibility. It is formed by a gel-like core (the nucleus pulposus) and fibre bands surrounding the core (the annulus fibrosus). The annulus fibrosus is made of collagen fibres and acts as protective covering. The nucleus pulposus is an elastic structure with high water content. A degenerated disc has lower water content because of a starvation of the disc caused by reduced blood diffusion. Several studies were realised in order to promote a regeneration of the disc by injection of small amounts of bioactive molecules such as growth factors. Research effort is focused on the development of cartilage tissue engineering which produces gel-like matrices.\textsuperscript{1,6}

Collagen, fibrin, agarose, alginate, cellulose and its derivatives or polyglycolic acid were tested and a formation of available hydrogel was attempted.\textsuperscript{7}

Cartilage tissue engineering aims at restoring, maintaining or improving tissue function and involves seeding a biocompatible scaffold with appropriate cells (Figure 1). The scaffolds can be classified according to their nature (polysaccharide, protein), their shape (massive, porous massive, foams, viscous liquids and hydrogels) or their chemical composition. They should be biocompatible to prevent immunological responses. Their ability to constitute a three-dimensional system and to be permeable to allow the diffusion of nutrients and molecules is an important property, too. They should also be adhesive so as to enable fixation and avoid implant migration. Last, they should be bioactive in order to enable homogeneous and controlled release of bioactive molecules and they should be injectable so as to allow mini-invasive surgery.\textsuperscript{7}
5.3 Hydrogels

Many scaffolds have been investigated for cartilage tissue engineering. Hydrogels are probably the most promising candidates because of their structure and properties.\(^2\)\(^,\)\(^7\) Hydrogels are three-dimensional, cross-linked networks of water-soluble polymer. They can be virtually created by any water-soluble polymers. The chemical composition and physical properties can be very diverse. They can adopt different forms such as microparticles, nanoparticles, coatings, slabs and films.\(^2\) Hydrogels are composed of chains of synthetic or natural absorbent macromolecules. Cross-linking agents such as pH, temperature, irradiation or chemical substances induce chemical modifications and this causes the formation of a reticulated hydrogel.\(^7\)

They are highly porous and the porosity and structure of the macromolecular network can be influenced by the affinity of the hydrogels for the aqueous environment and density of the cross-links in the gel matrix. The active substances can be loaded into the porous structure. Drug release can be influenced by the diffusion coefficient through the gel network.\(^2\)\(^,\)\(^9\)
In general, hydrogels are highly biocompatible. Biocompatibility of hydrogels is enabled by high dimension of water and by their physicochemical similarity to the native extracellular matrix, both mechanically and compositionally. The advantageous property is their relative deformability which allows them to assume a shape like the surface to which they are applied. The muco- or bioadhesive properties of some hydrogels can be important for medical applications. Another important property is the time of biodegradation which influences the duration of drug release.\textsuperscript{1,2}

However, certain problems such as the low mechanical stability of hydrogels or their low tensile strength persist in hydrogel-based therapies. These problems can cause premature dissolution or a flow away from the targeted local site. The high dimension of water and large pore size of most hydrogels lead to relatively rapid drug release, over a few hours to a few days. The quantity of drugs loaded in hydrogels is limited. It can be a problem to load hydrophobic active substances into hydrogels because of high water content inside hydrogels. For surgical implantation, hydrogels have to be injectable.\textsuperscript{2}

### 5.4 Hydroxypropyl methylcellulose (HPMC) and silated-hydroxypropyl methylcellulose (HPMC-Si)

HMPC is a water-soluble polymer derived from cellulose, the most abundant polymer in nature. It is a methylcellulose modified with a small amount of propylene glycol ether groups attached to the anhydroglucose of the cellulose (Figure 2).\textsuperscript{10} HPMC is stable over a pH range of 2.0 to 13.0. Surface tension of HPMC is 43-55 mN·m\textsuperscript{-1}. It acts as a surfactant and it is able to reduce surface or interfacial tension. HPMC also thickens the aqueous phase. These properties are used in the pharmaceutical industry to stabilise emulsions. HPMC is certainly a non-toxic material.\textsuperscript{10,11,12}

The combination of HPMC solution and biphasic calcium phosphate granules was developed for biomedical applications to be injected into the intervertebral disc like a biomaterial of the first generation. This viscous suspension had a good
biocompatibility and advantageous rheological properties for injection administration; however, a tendency to flow after implantation was observed in vivo. Biomaterials of the second generation were developed. HPMC was silated and HPMC-Si with improved properties was prepared.\textsuperscript{11}

![Figure 2: Chemical structure of HPMC.\textsuperscript{10}](image)

HPMC-Si has got better mechanical properties, less flow tendency and convenient self-hardening properties. HPMC-Si cross-links under easily controllable conditions. Cross-linking is induced by a decrease of the pH and can be accelerated by increasing the temperature.\textsuperscript{11, 12}

The powder of HPMC-Si was dissolved in a strong basic medium of the sodium hydroxide solution (0.2M, pH 13.2) and the final pH of 12.8 was obtained after the dissolution. This basic pH leads to the silane’s ionization into sodium silanolate (\(-\text{SiO}^-\text{Na}^+\)). For sodium silanolate stabilisation, the minimal values of pH have to be 12.1-12.2. Below these values, sodium silanolate transforms into silanols (-SiOH). It allows condensation reactions between silanols and the formation of a three-dimensional network (Figure 3).\textsuperscript{11}
Figure 3: Silane behaviour linked in the structure of HMPC. (a) Dissolution in basic medium leads to silanolate function formation. (b) Decreasing pH transforms silanolate into silanol. (c) Silanol condensation leads to the formation of a three-dimensional network.\textsuperscript{12}

A mixture of HPMC-Si and buffer shrinks gelation transition at low pH and forms elastic hydrogel. A buffer HEPES at pH 3.2 and different ratios between HPMC-Si and buffer were tested in the laboratory INSERM in Nantes, France.\textsuperscript{11} The HPMC-Si concentration was set at 3\% (w/w). Self-hardening study was made with ratios of HPMC-Si and HEPES 1:0.5 and 1:1. HPMC-Si with a ratio of one volume of the HPMC-Si and 0.5 volume of buffer reached the final polymer concentration of 2 \% (w/v) and the final pH near 7.4. A mixture of HPMC-Si and HEPES with ratio of 1:1 reached the final polymer concentration of 1.5 \% (w/v). Hydrogel formed by HPMC-Si and buffer with a ratio 1:0.5 showed faster kinetics of cross-linking in comparison with the ratio of 1:1. The ability of gelation of the HPMC-Si in these conditions was proved.\textsuperscript{11}

A decrease in gelation time with an increase in temperature was verified. Higher temperature has a catalytic effect and accelerates silanol condensation. Additional mechanism such as the association of the hydrophobic zones in the polymer chain may probably occur and it can also accelerate silanol condensation and the formation of hydrogel.\textsuperscript{13, 14}
A dye fluorescent isothiocyanate conjugated silane (FITC-Si) (Figure 4) can be used to bind on HPMC-Si. The covalent bind with HPMC-Si can be created and bonds -Si-O-Si- can be formed.  

![Figure 4](image)

**Figure 4:** Fluorescent isothiocyanate (FITC) conjugated with AminoPropyl-Triethoxysilane.  

### 5.5 Microparticles

Microparticles are particles with dimensions between 1µm and 1 mm. Below this dimension, one talks about nanoparticles. They can have different forms. Microparticles can contain active substances, it is possible to entrap from 5 to 90 % mass of active substance. They can be formed from natural or synthetic polymers or lipids. Among polymers of natural origin, gelatine, chitosan, agarose, alginate or casein can be listed. Derivatives of cellulose like ethylcellulose, hydroxypropylmethylcellulose (hypromellose) or carboxymethylcellulose can be mentioned among semi-synthetic polymers. Among synthetic polymers, copolymers of acrylic and methacrylic esters and copolymers of lactic and glycolic acid are included. Among lipids, fatty acids (stearic acid, palmitic acid), fatty alcohols, cholesterol derivatives or waxes can be listed.

Two main kinds of microparticles are distinguished – microcapsules and microspheres. Microcapsules are composed of a solid shell and a core-forming space which can enclose a solid or liquid active substance. Microspheres are formed by macromolecular or lipid matrices without any distinct outer layer. The active substance is dispersed in the whole microparticle.
Microparticles are prepared to ensure the protection, stability and compatibility of the active substance. They can regulate release properties and provide a sustained or retarded drug release. They are produced to cover up the taste or smell. Microparticles are used in the pharmacy, cosmetics, perfumery and chemical and food-processing industries.\textsuperscript{16}

The procedures for the preparation of microparticles can be classified as physical-chemical processes, mechanical processes, chemical processes and microfluidics processes.\textsuperscript{16} Physical-chemical processes are based on influencing the solubility and inducing precipitation or changing the state (from liquid to solid). Thermal gelification, precipitation by a non compatible separating agent or by changing temperature or pH can be listed among these methods. Mechanical processes are based on forming a dispersion or emulsion by mechanical forces. Spray-drying (pulverisation), prilling (formation of drops or droplets) and extrusion can be mentioned. Chemical processes are based on \textit{in situ} formation of matrices by polycondensation and radical or anionic polymerization. Microfluidics approaches are based on phase separation of a droplet in a non-miscible continuous phase by using microchannels and by manipulating a small volume of fluids inside them.\textsuperscript{16,18,19}

It is necessary to choose a convenient chemical composition, accurate ratios between chemicals and a suitable process for the formation of microparticles and encapsulation of active substances. A convenient composition influences the stability of microparticles, the kinetics of drug release and the conditions for the liberation of active substances. Preparation process influences the size of microparticles, the distribution in size, the extent of encapsulation, the final form and the conditions and kinetics of drug release.\textsuperscript{16}

### 5.6 Microfluidics

Microfluidics is the manipulation of fluids in channels with dimension of tens to hundreds of micrometres. Microchannels, T-junction and syringe-pumps are essential microfluidic devices. The main advantages of this method include very small quantities of samples and reagents high resolution and sensitivity of the separations and detections carried out. Reactions can be performed in significantly less time.
Hours of reaction time using standard laboratory technologies could be decreased to seconds.\textsuperscript{19, 20}

The basic principle is based on the phase separation of a droplet by diffusion of the separating agent. In general, two immiscible fluids are used to generate emulsions. In the first stage, droplets are formed in a flow-focusing geometry when two immiscible fluids are introduced (Figure 5).\textsuperscript{19, 21}

![Figure 5: T-junction configuration. The dispersed and continuous phase meets in a junction perpendicularly.\textsuperscript{22}](image)

The droplets are separated and form a single phase. Multiple emulsions, including double, triple and quadruple emulsions can be produced by altering the rate of phase separation. The reticulation of droplets can be induced by increasing the temperature, irradiation or other factors.\textsuperscript{23}

Microfluidic approaches are developed due to design emulsions with a high degree of flexibility and control. Double emulsion can be prepared in the one-step process and the emulsion is applied to simultaneously encapsulate bioactive molecules. Complex emulsions with various architectures can be formed by using mutually insoluble compounds in the disperse phase.\textsuperscript{23}

Different geometry of junctions is used to produce particles with a different structure or compartments inside the particles (Figure 6). For example anisotropic Janus particles (see Figure 6) with advanced complexity and refinement can be created in the one-step process by combining phase separation and dewetting phenomena. Phase separation can be induced by the diffusion of the separating agent. Dewetting transition at the interface of the inner droplet can be caused by the surfactant which diffuse from the continuous fluid into the droplets, decrease the surface free energy
at the interface of the double or triple emulsion drop and initiate a dewetting transition and acorn-like shapes are formed.\textsuperscript{23, 24}

The formation of droplets depends on the viscosity of the disperse phase and characteristic time for complete phase separation. The higher viscosity of the droplets obstructs the diffusion of the separating agent, results in in the slow rate of the phase separation and permits several separation steps of the droplet. It takes approximately 1.5 sec for double emulsion, 2 sec for triple emulsion and 3 sec for quadruple emulsion (Figure 7). It means that morphology of the emulsions and microparticles can be controlled the by influencing the viscosity of the dispersed phase and the rate of phase separation. The velocities of the continuous and the dispersed phases are essential parameters for the size of droplets.\textsuperscript{23, 25}

The materials and dimensions of microfluidic devices also strongly influence the size of droplets. Many microfluidic devices are fabricated using poly(dimethyl)siloxane.\textsuperscript{20} The advantages of this material are a relative low price and elasticity; however, strong organic solvents cause his deformation. Hence, other materials with higher solvent resistance such as silicon, thiolene or glass are used. The size of the orifice of the T-junction also influences the size of the particles. Smaller particles can be formed by using a T-junction with smaller dimensions.\textsuperscript{20}

Hydrophilicity or hydrophobicity of the channel surface is another important factor. To prevent the dispersed phase from adhering to the surface of microchannel walls, hydrophobic channels are used for the production of W/O droplets and O/W emulsions are formed in hydrophilic microchannels. The surface wettability can be altered with hydrophobic treatments such as silanization or siliconization to make a hydrophilic surface hydrophobic. Hydrophilic treatments such as oxygen plasma or polyvinyl acetate coating are used to create a hydrophilic surface.\textsuperscript{20}

Figure 6: Different geometries of junctions can be used to produce different types of particles. Janus particles are on the left picture.\textsuperscript{21}
Figure 7: Double, triple or quadruple emulsion can be formed by microfluidics.\textsuperscript{23}

5.6.1 Physical principles in microfluidics

Microfluidics profits from certain important differences between the physical properties of fluids moving in large channels in comparison to micrometre-scale channels. At the micrometres scale, the huge increase in surface area relative to volume is appeared. Mass and heat transfer is more efficient. The creation and the homogenization of emulsion or temperature gradients are faster at the micrometre scale.\textsuperscript{26}

The important difference is flow. On large scales, fluids mix convectively. In systems reduced in size, flow is laminar without eddies or turbulences and the only mixing is caused by the diffusion of molecules across the interface between the fluids.\textsuperscript{26}

Another important difference is that diffusion, viscosity and surface tension become more important than gravity and inertia. Capillary forces are also predominant.\textsuperscript{26}
The capillary number $Ca$ is the ratio between viscous and capillary forces. Capillarity is the rise or depression of a liquid in a small passage, for example in a thin microchannel. It is a dimensionless number defined as:

$$Ca = \frac{U \cdot \eta}{\gamma}$$  

Equation 1

Where $U$ is the average velocity scale of the fluid in the microchannel [m·s$^{-1}$], $\eta$ is the dynamic viscosity [Pa·s] and $\gamma$ is the interfacial tension between the continuous and dispersed phase [N·m$^{-1}$]. Viscous forces are negligible compared with interfacial forces for capillary numbers smaller than one. Viscous forces dominate for capillary number greater than one. Capillary number is usually large for high-speed flows and low for low-speed flows.$^{26,27}$

The Reynolds number $Re$ measures the ratio of inertial and viscous forces and subsequently quantifies the relative importance of these two forces in the system. $Re$ characterises the flow. It decides if the flow is laminar or turbulent.$^{26}$

Viscosity is the internal friction of a fluid. It induces a resistance to shear and a fluid has a tendency to move in parallel layers known as laminar flow. Inertia is the tendency of a fluid in motion to retain its initial motion and in the end can lead to turbulent flow. Viscous forces are more important than inertia and the flow is usually laminar at the micrometre scale.$^{26}$

It is a dimensionless number defined as:

$$Re = \frac{a \cdot U}{\nu}$$  

Equation 2

Where $U$ are the same physical unit as mentioned above, $a$ is a microchannel diameter [m] and $\nu$ means the kinematic viscosity of the fluid [m$^2$·s$^{-1}$].$^{26}$

It can be expressed also as:

$$Re = \frac{\rho \cdot a \cdot U}{\eta}$$  

Equation 3

Where $a$, $U$ and $\eta$ are the same physical units as mentioned above, $\rho$ is density of continuous phase [kg·m$^{-3}$].$^{28,29}$ The Reynolds number is less than 1 for the laminar flow.$^{26,27}$
The Péclet number Pe relates the effectiveness of mass transport by convection to the effectiveness of mass transport by diffusion or dispersion. It is a dimensionless number calculated as:

\[ Pe = \frac{a \cdot U}{D} \tag{Equation 4} \]

Where \( a \) and \( U \) are the same physical units as mentioned above and \( D \) is the diffusion coefficient of the particle or molecule of interest [m\(^2\)·s\(^{-1}\)]. The diffusion is the most important transport mechanism for Péclet numbers smaller than one.\(^{20,26}\)

Due to the importance of viscosity and interfacial tension at the micrometre scale, these parameters were evaluated.

### 5.7 The importance of physical properties in microfluidics

Viscosity is essential data for calculating Reynolds number. The viscosity is a parameter dependent on temperature and viscosity decreases with increasing temperature. The viscosity of tested continuous phase (vegetal oil) and other potential oils was measured at different temperatures by rotating viscosimeter method.

Interfacial tension is necessary information for calculating capillary number. HPMC acts as a surfactant and her surface tension is 43-55 mN·m\(^{-1}\). It can be expected HPMC-Si will also influence surface and interfacial tension. To verify this hypothesis and for a better understanding of the microfluidic system, surface and interfacial tension was measured by Wilhelmy plate method.

#### 5.7.1 The measurement of viscosity by the rotating viscometer method

The principle of the method is to measure the force acting on a rotor when it rotates at a constant angular velocity (rotational speed) in a liquid. Commonly used types of rotating viscometers and rheometers measure shearing forces in a liquid medium placed between two coaxial cylinders. One cylinder is driven by a motor and the other is made to revolve by the rotation of the first. The apparatus measures the angle...
of deflection of the cylinder made to revolve, which corresponds to a moment of force expressed in Newton meters. The viscosity can be determined from these data. The viscosity of Newtonian (shear-independent viscosity) and non-Newtonian liquids (shear-independent viscosity) can be measured.\textsuperscript{30}

Rotating viscometers can be divided in two groups, namely absolute and relative viscometers. The flow in the measuring geometry is well defined for absolute viscometers. The values are absolute and they can be compared with any other absolute values. The flow in the measuring geometry is not defined for relative viscometers. The obtained results are in relative viscosity values and they cannot be compared with other absolute or relative values. They can be compared only with values obtained by the same relative viscosimeter method.\textsuperscript{30}

The used viscometer arrangement should be chosen according to the viscosity of the fluid. Two concentric cylinders (cup and bob), cone-plate or two parallel plates can be used.\textsuperscript{30}

Figure 8: Concentric cylinder viscometer (absolute viscometer).\textsuperscript{30}
5.7.2 The measurement of surface and interfacial tension by the Wilhelmy plate method

A thin plate measures equilibrium surface or interfacial tension at air-liquid or liquid-liquid interfaces. The plate is usually made of platinum or glass and it is oriented perpendicularly to the interface. The apparatus measures the force exerted on the plate (Figure 9).\textsuperscript{31}

The plate is moved towards the surface. The tensiometer carefully connects the plate with the meniscus of the liquid and measures the force acting on the plate due to its wetting.\textsuperscript{31}

It is a very sensitive system and any contamination of the plate can affect the measurement. It is necessary to clean the plate thoroughly with a suitable solvent or the flame.\textsuperscript{31}

Figure 9: The principle of Wilhelmy plate method.\textsuperscript{31}
6. EXPERIMENTAL SECTION

6.1 Chemicals

Sterile solution of HPMC-Si 3% in sodium hydroxide 0.2M, INSERM and Laboratoire Ostéo-Articulaire et Dentaire, Nantes, France
FITC-Si, M= 527 g/mol, provided by Pr. SUBRA, G., Institut des Biomolécules Max Mousseron, Montpellier, France
4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), M = 238.30 g/mol, Sigma-Aldrich, USA
Liquid paraffin, Eur Pharm grade, Interchimie, Compans, France
Light liquid paraffin, Eur Pharm grade, Interchimie, Compans, France
Refined sesame oil, Cooper, Melun, France
Olive oil, Eur Pharm grade, Cooper, Melun, France
Miglyol 812 (Caprylic/Capric Triglyceride), Interchimie, Compans, France
Oleic acid, Eur Pharm grade, Fluka, Sigma-Aldrich, USA
Novec 7500 (3-Ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-(trifluoromethyl)hexane), 3M, Cergy-Pontoise Cedex, France
Poloxamer 188 (Lutrol F 68), BASF, Germany
Polyglyceryl-3-diisostearate (Plurol diisostearate CG), Gattefossé, Saint-Priest, France
Deionised water made in the laboratory

6.2 Equipment

Syringe Pumps PHD 2000 Programmable, Harvard Apparatus, Massachusetts, USA
Centrifuge Sigma 2K15, Sigma Laborzentrifugen GmbH, Osterode am Harz, Germany
Microscope EVOS ® FL, Life Technologies, Saint Aubin, France
Vortex-Genie 2, Scientific Industries, New York, USA
Ultrasonic cleaning units Elmasonic S 30H, Elma Schmidbauer GmbH, Singen, Germany
Stirring hotplate IKA® RH basic KT/C, IKA®-Werke GmbH & Co. KG, Staufen, Germany
Disperser T25 digital ULTRA-TURRAX, IKA®-Werke GmbH & Co. KG, Staufen, Germany
Laboratory stirrer Eurostar Power Control-Visc Stirrer, IKA®-Werke GmbH & Co. KG, Staufen, Germany
Rotating shear-rate imposed rheometer RM 200, LAMY RHEOLOGY, Champagne au Mont d’Or, France
Tensiometer K100, KRÜSS, Hamburg, Germany
Electronic contact thermometer ETS-D5, IKA®-Werke GmbH & Co. KG, Staufen, Germany
Hand-held thermometer EcoScan JKT meter, Eutech Instruments, Nijkerk, Netherlands
Analytical balances (precision 0.01 mg), Discovery DV-215CD, OHAUS, Nänikon, Switzerland
Precision balances Secura (precision 0.1 mg), Sartorius AG, Goettingen, Germany
Integral Water Purification System Milli-Q, Merck Millipore, Darmstadt, Germany
Autoclave VAPOUR-Line Eco, VWR, Vienna, Austria
Syringe filters Minisart hydrophilic 0.20 µm, Sartorius AG, Goettingen, Germany
Microsyringe – 10 µl, Hamilton Company, Bonaduz, Switzerland
Microchannels PEEK Tubing Orange 1/16” OD x .020” ID, IDEX Corporation, Illinois, USA
Connection Luer female PP, MMSI, Kouba, Algerie
Vessel to measure surface and interfacial tension SV10 Glass Vessel, volume 43.5 ml, width 51 mm, height 29 mm
6.3 Methods

Some technical details were used in accordance to the previously published article of Fatimi et al.\textsuperscript{11}

6.3.1 Preparation of buffer HEPES

Aqueous solution of buffer HEPES was prepared by dissolution of HEPES in deionised water. The pH value was measured and adjusted to pH 3.5 using HCl 1M. Deionised water was added to obtain 250 ml of buffer. Buffer was sterilized in an autoclave and kept in the freezer at temperature -22°C.\textsuperscript{11}

6.3.2 Preparation of microparticles by dispersion at high speed

Small volume of vegetable oil was placed into the tube. 0.200 ml of 3.0% solution of HPMC-Si and FITC-Si solution was stirred slowly with two syringes to form a homogeneous mixture with as few air bubbles as possible (Figure 10). 0.100 ml of HEPES buffer and the mixture of HPMC-Si and FITC-Si were mixed slowly in the same way. The mixing had to be performed quickly because of the gelification of HPMC-Si which occurs after addition of HEPES within few minutes. The mixture of HPMC-Si was immediately added to the tube with vegetal oil and dispersed by ultraturax at approximately 10 000 rpm for 5 minutes. After 5 minutes, the tube was placed into a water bath at 45°C and agitated again for 10 minutes. The temperature inside the tube was controlled. Then, the mixture was diluted with 5 ml of fresh vegetal oil. The tube was kept at room temperature over night under agitation. It was protected from light irradiation by an aluminium film attached outside the tube. The microparticles were observed by optical and fluorescence microscope. Prepared microparticles are shown in the Figure 12 and 13. Then, purification of the microparticles started.
6.3.3 Calculating the Reynolds number

The laminar conditions are estimated by calculating the Reynolds number, Re, which has to be $0 < \text{Re} < 1$.\textsuperscript{22,27}

The Reynolds number was calculated from the known values according to Equation 3. Actual values of variables see below:

- $\rho_{\text{sesame oil}} = 915 \text{ kg m}^{-3}$ (from supplier data sheet)
- $\eta_{\text{sesame oil}} = 69.3 \cdot 10^{-3} \text{ Pa.s}$ (measured values at room temperature 21 °C)
- $U = 0.4 \text{ ml min}^{-1} = 0.034 \text{ m s}^{-1}$
- $a = 500 \mu m = 500 \cdot 10^{-6} \text{ m}$

$\text{Re} = 0.22$

So that, laminar conditions were respected.

6.3.4 Preparation of microparticles by microfluidic device

The aqueous phase consisted of 3% solution of HPMC-Si, solution of FITC-Si and HEPES 6.2%. The mixture of HPMC-Si, FITC-Si and HEPES was prepared in the same way as preparation of microparticles by dispersion at high speed. Vegetable oil was the continuous phase. Figure 11 shows microfluidic devices and direction of the flow. The fluids were injected into the inlets of microfluidic channels from syringes (BD Luer-Lok 1 ml, BD Plastipak 50 ml). The fluid injection was controlled by syringe pumps. When a formation of quite regular particles started, the particles were collected.
The experiments were repeated with different temperatures in outlet microchannel and/or in the T-junction, speed of injection (rate of the aqueous phase from 0.001 ml/min to 0.020 ml/min, rate of the oil phase from 0.400 ml/min to 1.000 ml/min) or with different solutions to conserve the microparticles (oil at the room temperature, preheated oil from 37°C to 50 °C, solution of HEPES 6.2%, surfactant Plurol 0.5-3%) in order to stabilize them.

![Microfluidic devices and direction of the flow](image)

Figure 11: Photos shows microfluidic devices and direction of the flow.

### 6.3.4.1 Evaluation of microparticles

Microscopy was performed and pictures of microparticles were taken with the microscope. The pictures were observed with the enhancement 400 (scale 400 μm in the pictures) or 1000 (scale 100 μm in the pictures). Then, the evaluation of the microparticles size and structure were done with the ImageJ programme. The size was measured for at least 100 microparticles and by using at least two different photos. The distribution of size was graphically expressed in percentages for each 10 μm. The results of the produced microparticles are presented as optical microscopy.
and fluorescence microscopy pictures. The structure of the particles is presented in percentage.

6.3.4.2 Purification of microparticles

Centrifugation at 3600 rpm for different time was performed in order to separate microparticles and dissolve the free HPMC-Si into the supernatant oil. The supernatant was observed in the microscope after the centrifugation and the amount of the free HPMC-Si was qualitatively evaluated by optical and fluorescence microscopy. The supernatant was eliminated from the tube by a pipette and if it was necessary to continue with the elimination of the free HPMC-Si, fresh oil was added and centrifugation was again performed. After the elimination of the free HPMC-Si, oil was replaced by the purified water or a solution of buffer and centrifugation in order to eliminate the oil phase and the process of obtaining purified microparticles started.

Filtration as a means of eliminating oil and free cellulose and obtaining purified microparticles was also tested.

If large aggregates of microparticles were observed, ultrasonification was performed.

6.3.5 Measurement of viscosity

The viscosity of the continuous phase (oil phase) was measured by shear-rate imposed rheometer at 7.7, 21.0, 38.0 and 48.0 °C. The temperature was controlled by cryothermostat. 18 ml of oil was filled within the annulus of one cylinder inside another. It was necessary to wait for the temperature to stabilize the temperature and verify this with a thermometer. The velocity was continuously increased (from 64.6 to 1290.0 s⁻¹). The force exerted on the cylinder (diameter 30 mm) was measured and converted to a shear stress. The measurement of viscosity was repeated three times.

The viscosity of sesame oil, olive oil, Novec 7500, liquid paraffin, Miglyol 812, light liquid paraffin, isopropylmyristat and oleic acid was measured.
The average values for each temperature are presented in the Table 11. In the last column of the Table 11, there is a difference $\Delta \eta$ between viscosities of oils measured at 7.7 and 48 °C ($\Delta \eta = \eta_{7.7^\circ C} - \eta_{48^\circ C}$). The results are graphically expressed in the Figure 21.

### 6.3.6 Measurement of surface and interfacial tension

The surface and interfacial tension was measured by Force Tensiometer –K100 using the Wilhelmy plate method. The experiments were performed at room temperature (23°C). The tension was measured for the same oils as were mentioned for viscosity measurements.

The surface tension was measured at interphase oil/air and/or HPMC-Si solution/air. The concentrations of the aqueous solution of HPMC-Si between 0.03%- 3.75·10⁻⁶ % were used. In the first step, the vessel with 18 ml of fluid was prepared. The plate was oriented perpendicularly and moved towards the surface. A tensiometer connected the plate with the surface of the fluid and measured the force acting on the plate. The values were measured for 300 seconds and 50 values were obtained. The measurement of surface tension was repeated three times. The average value and standard deviation (SD) was counted. The results for oil/air measurements are presented in the Table 12; in Figure 22 the results for HPMC-Si are shown.

The interfacial tension between all oils mentioned above and water and/or between sesame oil and a solution of HPMC-Si was also measured. First, a vessel with 23 ml of the lower density fluid was prepared, the plate was plunged completely and tensiometer measured a tare. The plate was cleaned thoroughly. The vessel with 18 ml of the higher density liquid was introduced and the plate was plunged to a depth of 2 mm. The lower density liquid was slowly added to plunge all of the plate and it was necessary to wait 15 minutes for the fluids to stabilize. Then, the interfacial tension was measured for 300 seconds and 50 values were taken. The plate was cleaned by surfactant, deionised water and fire. Then, the experiment was repeated with another liquid.

The values of interfacial tension for oil/water measurements are presented in Table 13.
7. RESULTS

7.1 Preparation of microparticles by dispersion at high speed

Figure 12: Microparticles in oil phase after single centrifugation. (a) Optical microscopy. (b) Fluorescence image which shows the encapsulation of a dye FITC-Si into the microparticles formed by HPMC-Si

Figure 13: Microparticles in oil phase after four centrifugations.
7.2 Preparation of particles by microfluidics

7.2.1 Optimisation of the microfluidics device and operating conditions

Figure 14: The photos show microfluidic devices and parameters which were optimised. a) T-junction and microchannels and the direction of the flows. Microfluidic channels had an internal diameter of 500 µm. b) Microfluidics system device: 1) syringe pumps, 2) temperature before and in T-junction, 3) microchannel length of the outlet microchannel, 4) temperature in the outlet microchannel.
7.2.2 Influence of flux ratio $Q_{w/o}$ on the size distribution and morphology of microparticles
Figure 15: Influence of the ratio $Q_{w/o}$ on the size and structure of microparticles. Microparticles observed in the vegetal oil.
7.2.3 The structure of the microparticles

Table 1: The influence of flux ratio $Q_{w/o} = 0.1250$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>243</td>
<td>79.15</td>
</tr>
<tr>
<td>Pair</td>
<td>2</td>
<td>0.65</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple</td>
<td>62</td>
<td>20.20</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>307</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 2: The influence of flux ratio $Q_{w/o} = 0.0625$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>163</td>
<td>66.00</td>
</tr>
<tr>
<td>Pair</td>
<td>2</td>
<td>0.81</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>5</td>
<td>2.02</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple</td>
<td>77</td>
<td>31.17</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>247</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 3: The influence of flux ratio $Q_{w/o} = 0.0500$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>130</td>
<td>69.15</td>
</tr>
<tr>
<td>Pair</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>4</td>
<td>2.13</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple</td>
<td>54</td>
<td>28.72</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>188</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Table 4: The influence of flux ratio $Q_{w/o} = 0.0200$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>202</td>
<td>74.26</td>
</tr>
<tr>
<td>Pair</td>
<td>16</td>
<td>5.88</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>10</td>
<td>3.67</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>13</td>
<td>4.80</td>
</tr>
<tr>
<td>Multiple</td>
<td>31</td>
<td>11.39</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>272</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 5: The influence of flux ratio $Q_{w/o} = 0.0125$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>77</td>
<td>74.76</td>
</tr>
<tr>
<td>Pair</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple</td>
<td>26</td>
<td>25.24</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>103</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 6: The influence of flux ratio $Q_{w/o} = 0.0062$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>76</td>
<td>72.38</td>
</tr>
<tr>
<td>Paires</td>
<td>16</td>
<td>15.24</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>6</td>
<td>5.71</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>6</td>
<td>5.71</td>
</tr>
<tr>
<td>Multiples</td>
<td>1</td>
<td>0.96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>105</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
Table 7: The influence of flux ratio $Q_{w/o} = 0.0050$ on microparticle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>56</td>
<td>52.83</td>
</tr>
<tr>
<td>Pair</td>
<td>28</td>
<td>26.42</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>22</td>
<td>20.75</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiple</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>106</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

7.2.4 The stability of microparticles

![Image of microparticles]

Figure 16: Test of the stability of microparticles at $Q_{w/o} = 0.0625$, and temperature $T_1$. (a) Microparticles after the preparation. (b) Microparticles after 5 weeks. (c) Microparticles after 3 months.
Figure 17: Test of the stability of microparticles at $Q_{w/o} = 0.0062$ and temperature $T_2$. (a) Microparticles after the preparation. (b) Microparticles after 3 weeks. (c) Microparticles after 3 months.

7.2.5 The effect of Plurol on the structure, size and stability

Plurol 3%
Figure 18: The influence of surfactant concentration on the structure of the microparticles after the preparation. Microparticles conserved in oil with Plurol.
Figure 19: The influence of Plurol concentration on the stability of the microparticles after 2 weeks of storage at room temperature.

Figure 20: The influence of Plurol concentration on particle size distribution.
Table 8: The influence of Plurol concentration 3% on particle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>130</td>
<td>94.20</td>
</tr>
<tr>
<td>Paires</td>
<td>2</td>
<td>1.45</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>6</td>
<td>4.35</td>
</tr>
<tr>
<td>Multiples</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>138</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 9: The influence of Plurol concentration 1% on particle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>145</td>
<td>98.64</td>
</tr>
<tr>
<td>Paires</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiples</td>
<td>2</td>
<td>1.36</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>147</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 10: The influence of Plurol concentration 0.5% on particle structure.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Amount</th>
<th>Distribution (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Simple</td>
<td>91</td>
<td>77.12</td>
</tr>
<tr>
<td>Paires</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (3-5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Aggregates (&gt;5)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Multiples</td>
<td>27</td>
<td>22.88</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>118</td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
7.3 Study of the physical parameters which are important in microfluidics method for future approaches

7.3.1 Viscosity

Table 11: The influence of temperature on viscosity $\eta$ (mPa·s) of the oils at constant shear rate $D = 358$ s$^{-1}$.

<table>
<thead>
<tr>
<th>Oils</th>
<th>Temperature (°C)</th>
<th>$\Delta\eta$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>7.7</td>
<td>21.0</td>
</tr>
<tr>
<td>Novec 7500</td>
<td>4.4</td>
<td>4.1</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>137.0</td>
<td>69.3</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>425.0</td>
<td>171.0</td>
</tr>
<tr>
<td>Olive oil</td>
<td>150.0</td>
<td>72.6</td>
</tr>
<tr>
<td>Miglyol 812</td>
<td>57.9</td>
<td>31.7</td>
</tr>
<tr>
<td>Light liquid paraffin</td>
<td>61.2</td>
<td>29.9</td>
</tr>
<tr>
<td>Isopropylmyristat</td>
<td>10</td>
<td>6.6</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>60.5</td>
<td>32.4</td>
</tr>
</tbody>
</table>
Figure 21: Dependence of viscosity on temperature.

7.3.2 Surface and interfacial tension

Figure 22: Dependence of surface tension on the concentration of HPMC-Si solutions.
Table 12: Measured and literature values of the surface tension between oil and air.

<table>
<thead>
<tr>
<th>Oil</th>
<th>Measured surface tension</th>
<th>Literature surface tension (20-25 °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Novec 7500</td>
<td>15.96</td>
<td>16.20</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>33.23</td>
<td>26.00</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>31.69</td>
<td>35.00</td>
</tr>
<tr>
<td>Olive oil</td>
<td>32.90</td>
<td>23.20</td>
</tr>
<tr>
<td>Miglyol 812</td>
<td>29.84</td>
<td>31.10</td>
</tr>
<tr>
<td>Light liquid paraffin</td>
<td>30.47</td>
<td>Below 35.00</td>
</tr>
<tr>
<td>Isopropylmyristat</td>
<td>28.66</td>
<td>29.60</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>32.56</td>
<td>32.79</td>
</tr>
</tbody>
</table>

Table 13: Values of the interfacial tension between oil and water

<table>
<thead>
<tr>
<th>Oil</th>
<th>Interfacial tension oil/water (mN·m⁻¹)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\gamma_1$</td>
<td>$\gamma_2$</td>
</tr>
<tr>
<td>Novec 7500</td>
<td>34.30</td>
<td>53.99</td>
</tr>
<tr>
<td>Sesame oil</td>
<td>34.63</td>
<td>35.12</td>
</tr>
<tr>
<td>Liquid paraffin</td>
<td>25.73</td>
<td>28.57</td>
</tr>
<tr>
<td>Olive oil</td>
<td>33.02</td>
<td>32.31</td>
</tr>
<tr>
<td>Miglyol 812</td>
<td>38.29</td>
<td>41.15</td>
</tr>
<tr>
<td>Light liquid paraffin</td>
<td>53.01</td>
<td>63.44</td>
</tr>
<tr>
<td>Isopropylmyristat</td>
<td>17.12</td>
<td>17.15</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>31.98</td>
<td>36.15</td>
</tr>
</tbody>
</table>
8. Discussion

The goal of this thesis was to prepare hydrogel microparticles based on siliconated-hydroxypropyl methylcellulose. These microparticles are finally intended for the parenteral application. Therefore, the injectability of microparticles is crucial demand. Microparticles had to be uniform and their size is an important parameter. It is expected that microparticles will be injected into the intervertebral disc and flow away is unwanted. Hence, the risk of thrombosis or embolism is not increased. It is important that needle will not be blocked by too big microparticles. Because needle sizes of 10-13 G are expected, microparticle size should be 50-90 µm (less than 100 µm) to respect these requirements.

8.1 Preparation of microparticles by dispersion at high speed

First, preparation of microparticles by dispersion at high speed which combines mechanic and chemical process was studied.

Three experiments were performed and the results are shown in Figure 12 and in Figure 13. Microparticles appeared in green fluorescence due to encapsulation of FITC-Si.

The microparticle sizes were roughly evaluated due to the poor yield of production. The observed internal structures showed different organisations that were defined on the fluorescence intensity (qualitatively):

- simple particles, fluorescence were distributed in the entire volume of the microparticles;
- multiple particles, different grade of fluorescence separated in different spherical compartments.

Problems like aggregation, heterogeneity of microparticles and their instability were observed by this method as well as a lot of air bubbles entrapped. Nevertheless, it was possible to estimate a distribution encompassed between 5 and 30 µm (Figure 12). On the other hand, many undefined structures, probably composed of aqueous phase and air bubbles were observed (Figure 13).
Certain processes such as centrifugation, ultrasonification, use of surfactant Lutrol 1 % and membrane filtration were tried in order to isolate microparticles. Unfortunately, these processes were generally unsuccessful or microparticles were even destroyed.
So, other approaches were searched and microfluidics was started.

8.2 Preparation of microparticles by microfluidics device

Microfluidics process allows to produce droplets in a controlled and reproducible manner and to create highly regular monodisperse droplet stream. The main condition is to achieve laminar flow of liquids in microchannels. Therefore, Reynolds number was calculated using equation 3 with the actual values of variables; the value of 0.22 was found as mentioned at page 28.
However, microfluidics device is a complex system. It is necessary to optimise many conditions including the rate of the continuous and dispersed phases, temperature and length of microfluidics channels.

Figure 14 illustrates the microfluidics device that was optimised in order to control the temperature along the microfluidics channels and the residence time of the continuous and dispersed phases.
Preparation of microparticles was successful for 17 trials. Pictures were taken and it was made counting of microparticles, evaluation of their size, distribution in size and structure.

8.2.1 Influence of flux ratio $Q_{w/o}$ on the size distribution and morphology of microparticles

Tice et al studied the influence of flux rate on the formation of microparticles. They observed that at higher flow velocities, drops are not separated immediately at the junction, the laminar segment is formed before the separation of drops, the length of drops fluctuates and the size of drops decreases noticeably. This theory was verified in several experiments.
Different rates of dispersed aqueous phase ($Q_w$) and continuous oil phase ($Q_o$) were tested. To compare the effect of velocity on the microparticles production, the results were expressed in form of $Q_{w/o}$ as the ratio $Q_w/Q_o$.

Flow rate of oil phase $Q_o$ was fixed to 0.400 ml/min after some trials to form non-aggregated microparticles. At lower speed rates, microparticles joined in the microchannel and fusion of microparticles or obstruction of the microchannel was observed. At higher velocities, microparticles moved too quickly, reticulation was not sufficient and microparticles were deformed.

The flow rate of water phase $Q_w$ was changed experimentally in this thesis. At higher flow velocities, many small microparticles and some bigger or aggregated microparticles were observed. In opposite, bigger and more uniform microparticles were observed at lower flow velocities (see Figure 15). At these low flow velocities, drops are immediately separated from the fluid of continuous phase and the size of drops was reproducible.

The influence of $Q_{w/o}$ ratio on the particle structure is illustrated in Tables 1-7. The fluorescence inside the microparticles was heterogeneous in intensity that could ascribe to heterogeneities in the HPMC-Si/HEPES mixture preparation. Indeed, different structures were observed such as simple particles, pair of particles, aggregates as well as multiple ones. The best results were noted at $0.0050 \leq Q_{w/o} \leq 0.0062$. In this range, it was possible to obtain individual microparticles with no visible intra-particular structures (homogeneous fluorescence signal). In these cases, the size distributions were larger (50-90 µm).

Table 14: The dependence of microparticle size on ratios $Q_{w/o}$.

<table>
<thead>
<tr>
<th>$Q_{w/o}$</th>
<th>The size of microparticles (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Less than 0.0050</td>
<td>No microparticles</td>
</tr>
<tr>
<td>0.0050-0.0062</td>
<td>50-90 µm</td>
</tr>
<tr>
<td>0.0125-0.1250</td>
<td>10-40 µm</td>
</tr>
</tbody>
</table>

In summary, it was proved that the rate of the continuous and dispersed phase is a principal parameter.
8.2.2 Influence of temperature in the microchannels and length and temperature in the outlet microchannel

The intention was to form the homogeneous microdroplets in the T-junction and to reticulate droplets in the outlet microchannel. It can be influenced by the temperature and the length of the microchannel.

The temperature had to be optimised to different temperatures in different parts of equipment. After several unsuccessful trials, temperature in front of and in T-junction where microdroplets are formed was decreased below room temperature in order to avoid reticulation in the microchannels and create homogeneous emulsion in T-junction. In opposite, increase in temperature in the outlet microchannel was necessary in order to evocate reticulation and to form microparticles from microdroplets. Higher temperature accelerates the reticulation and helps to avoid the fusion of microparticles after the preparation.14

The residence time in the microchannel is the second important influencing parameter. This parameter depends on the length of the microchannel and rate of flow. It was observed that the reticulation of microparticles was improved and the fusion to one big drop was avoided if the length of the microchannel was elongated. However, reticulation was not completely finished in the outlet microchannel and some microparticles fusioned. The oil in the tube for conserving microparticles, therefore, was also preheated to approximately 50 °C in order to finish reticulation and avoid the fusion of microparticles.

To obtain microparticles of regular size, the optimal conditions in the microfluidic system were concluded as folows: low temperature in the T-junction, temperature approximately 50 °C in the outlet microchannel, the longer outlet microchannel to prolong the residence time in the microchannel, and the flux ratio between 0.0050-0.0062.
8.2.3 Evaluation of microparticles stability

To study stability, microparticles were stored in oil at normal laboratory conditions (temperature of 23±2°C).

After several weeks, the microparticles prepared at lower temperature conditions (T1) settled. Figure 16 shows that the size and the absence of aggregation were maintained up to 3 months. This is a good indication of the stability. The microparticles were well reticulated, the fusion did not occur. It was easy to resuspendate the microparticles.

Microparticles prepared at higher temperature conditions (T2) were well formed after preparation with the homogeneous structure as illustrated in Figure 17a. After 3 weeks, the fluorescent particles were still visible (Figure 17 b) but the number of microparticles generally seemed to be decreased. After 3 months, the tube with microparticles was centrifugated for 45 min at 4500 rpm. Some fluorescent microparticles were observed as can be seen in Figure 17 c.

Use of the surfactant is classed between methods influencing the stability of microparticles. This method was tried when optimization of conditions were developing.

In order to avoid the fusion of microparticles, surfactant Plurol in three different concentrations of 3%, 1% and 0.5%, was added to the oil phase. Because the biggest problems with aggregation and fusion of microparticles were observed within 15 minutes after the preparation, Plurol was used immediately after the preparation of microparticles.

All experiments were performed with the same ratio Q_{w/o}= 0.0062. Stability after several days and weeks was observed.

Many small fluorescent particles were observed for all trials. It could be seen that microparticles were less dense and the fluorescence was weaker in comparison with experiments without surfactant. It was noted that the concentration of Plurol had significant effect on the microparticles stability. The results are presented in Figure 18-19.
At the lowest concentration, Plurol permitted formation of some denser, well formed microparticles. However, surfactant influenced the structure and the stability of microparticles in a negative way. Microparticles were not well formed and they were easily destroyed if higher concentrations of surfactant were used. It is possible that surfactant solubilized HPMC-Si instead of formation of microparticles.

The influence of Plurol concentration on particle structure is summarized in Tables 8 – 10. More than 94 % of microparticles were simple for the concentration of surfactant 3% and 1%. Multiple particles (nearly 23 %) were observed for the concentration of surfactant 0.5 %.

As could be visible from Figure 20, more than 90 % of microparticles had a size 10-20 µm for the concentration of surfactant 3% and more than 90 % of particles had a size 10-30 µm for the concentration of surfactant 1%. For the concentration of surfactant 0.5%, nearly 35 % of particles were bigger than 30 µm. The higher size with the lower concentration of surfactant was observed.

In conclusion, the increase in stability of particles by the addition of Plurol was not fully succesful. Based on the results, it seems that also the lowest concentration (0.5 % Plurol) was too high.

### 8.3 Future approaches

In this work, the significant effect of temperature in the microfluidics system was noted. Thus, different values of viscosity of the oil phase as well as the different Reynolds numbers could actually be in the different parts of the microchannels during particle production. In microfluidics, dynamic viscosity and the interfacial tension between the continuous and dispersed phase are necessary to know in calculation of the forces in the microchannel. Properties of various oils were, therefore, studied.
8.3.1 Viscosity

Viscosity of sesame oil, olive oil, Novec 7500, liquid paraffin, Miglyol 812, light liquid paraffin, isopropylmyristat and oleic acid was measured at 7.7°C, 21.0, 38.0 and 48.0 °C.

Except for Novec 7500, all oils displayed a Newtonian behaviour. On the contrary, Novec 7500 showed shear thickening behaviour but only negligible changes in values from 0 to 10 mPa·s were registered with the shear rates D from 64.6 to 1290 s⁻¹.

Viscosity of oils at D = 358 s⁻¹ (uniform flow region) at temperatures mentioned above was used as reference value. Data are presented in Table 11. The difference between viscosity η at 7.7°C and 48.0°C was expressed and could be listed in last column of the Table. Liquid paraffin exhibited the highest values of viscosity with the highest dependence on temperature. In opposite, isopropylmyristat and Novec 7500 showed the lowest viscosity values and they were the less sensible to the temperature.

The results were plotted in Figure 21. As could be seen, some of the oils showed a strong dependence of viscosity on temperature. Generally, viscosity decreases with increasing temperature. Again, isopropylmyristat and Novec 7500 showed the best results.

In conclusion, isopropylmyristat and/or Novec 7500 [3-Ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-(trifluoromethyl)-hexane] are perspective for further research. Apart from the less dependence of viscosity on temperature in a range of 7.7°C - 48 °C, these syntetic oils are better defined in composition when compared with natural oils like sesame or olive oil.
8.3.2 Surface and interfacial tension

Surface tension between oil and air and/or between HPMC-Si solution and air as well as and interfacial tension between oil and water (deionized) and/or oil and HPMC-Si solution (in deionized water) and sesame oil was measured to better understanding the microfluidics system. The HPMC-Si concentration of 0.03%- 3.75·10^{-6} % was used. The results are shown in Tables 12 and 13 and in Figure 22.

The dependence of surface tension on the concentration of HPMC-Si solutions is shown in Figure 22. It was noted that HPMC-Si decreases the surface tension really strong. Even the lowest tested concentration of HPMC-Si 3.75·10^{-6} % decreased the surface tension to 66.01 mN·m^{-1}. From the concentration 1.50·10^{-3} %, the values were constant with values about 48.15 mN/m. For comparison, surface tension of HPMC is 43-55 mN·m^{-1}.^{10}

The concentration of HPMC-Si 0.03 % from plateau region of Figure 22 was taken for determination of interfacial tension between HPMC-Si and sesame oil. The value of interfacial tension between sesame oil and aqueous solution of HPMC-Si 14.82 mN·m^{-1} was detected.

The measured values of surface tension \( \gamma \) (mN·m^{-1}) were compared with those ones obtained from literature references. It was verified that both are similar.

The measurement of interfacial tension was more complicated. Some values significantly varied.

The value of interfacial tension is important in calculation of the capillary number (equation 1). Viscous forces are negligible compared with interfacial forces for lower capillary numbers (smaller than one) and spherical droplets are usually formed. In opposite, viscous forces dominate for higher values of capillary number (greater than one) and it could cause deformation of the droplets and asymmetric shapes.\textsuperscript{22, 26}

Lower values of interfacial tension cause higher values of capillary number. Hence, viscous forces are more important.
9. CONCLUSIONS

From the results of this thesis the following conclusions were drawn:

1. FITC-Si is a good fluorescent dye to mark microparticles formed by HPMC-Si and to characterise their size and structure.

2. It was possible to prepare microparticles by dispersion at high speed. However, many problems were observed during reticulation and separation of microparticles including their destruction.

3. It was possible to prepare spherical non-aggregated microparticles of HPMC-Si by microfluidics. Microparticles were stable for 3 months. However, the fusion of microparticles after their preparation was not completely avoided.

4. Flux ratio $Q_{w/o}$ between water and oil phase is the main parameter for the size and structure of microparticles.
   a. The optimal flow rate of oil phase $Q_o$ was 0.400 ml/min.
   b. The optimal flux ratio $0.0050 \leq Q_{w/o} \leq 0.0062$ was noted.
   c. For $0.0050 \leq Q_{w/o} \leq 0.0062$, the particles size 50-90 µm were obtained.

5. Temperature is elementary parameter to finish reticulation of the microparticles.
   a. Higher temperature accelerates the reticulation and helps to avoid the fusion of microparticles after the preparation.
   b. To obtain microparticles of regular size, lower temperature in the T-junction and higher temperature in the outlet microchannel can be recommended.

6. Residence time in the microchannel is important to produce and stabilize microparticles. The longer outlet microchannel can be recommended to achieve longer residence time in the microchannel.

7. Surfactant Plurol in concentrations of 3%, 1% and 0.5% influenced the microparticles stability significantly.
   a. 0.5% Plurol permitted formation of some denser, well formed microparticles.
b. At higher concentrations of surfactant, microparticles were not well formed and were easily destroyed, probably for the solubilizing effect of surfactant.

8. Viscosity of the natural and synthetic oils showed a strong dependence on temperature. Out of seven substances studied, isopropylmyristat and Novec 7500 exhibited minimum dependence.

9. In conclusion, isopropylmyristat and/or Novec 7500 are perspective for the further research.
10. REFERENCES


15. Information provided by Prof. SUBRA, G., Institut des Biomolécules Max Mousseron, Montpellier (personnal information)  
   http://energy.concord.org/energy2d/reynolds.html.