



CHALMERS
UNIVERSITY OF TECHNOLOGY

Controlled Drug-Release from Mesoporous Hydrogels

Master of Science Thesis in the Master Degree Program, Materials Chemistry
and Nanotechnology

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CHALMERS UNIVERSITY OF TECHNOLOGY
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Abstract

Hydrogels are an interesting group of materials used in biomedical applications. The cross-linked polymeric network enables large water absorption and physical properties similar to soft tissue giving it excellent biocompatibility. Hydrogels with a controlled nanostructure are especially interesting for biomedical applications such as controlled drug-delivery systems since the meso-ordered structure enables loading of drugs followed by a local sustained release. This may minimize systemic side effect and give a safer and more effective delivery of drugs.

The aim of this thesis was to form meso-ordered hydrogels and hydrogel particles by the use of Lyotropic Liquid Crystals (LLC), and to evaluate them as controlled drug-release systems using the drugs Ibuprofen and ^{14}C radiolabelled Alendronate. LLC phases are formed when amphiphilic molecules mixed with water at certain concentration starts to form ordered mesostructures. A polymerizable amphiphile, a triblock copolymer with trade name Pluronic® F127 was used to form meso-ordered bulk hydrogels by photopolymerization of LLCs with cubic and hexagonal geometries. Small-Angle X-ray Scattering measurements revealed structure retention after crosslinking of the LLC gel. Meso-ordered Poly(ethylene glycol) diacrylate (PEG-DA) hydrogel particles were formed by photopolymerization in presence of surfactants in a water-in-oil emulsion. Particles with a size of 209-242 nm were formed, measured with Dynamic Light Scattering and presence of long-range order was revealed with Polarized Light Microscopy.

Ibuprofen and ^{14}C Alendronate were successfully loaded into F127 hydrogels and PEG-DA hydrogel particles. Ibuprofen incorporated into F127 hydrogels existed in an amorphous form confirmed with X-Ray Diffraction. Both hydrogels showed a controlled release of drugs, with an initial burst followed by a sustained release. There was a clear difference in release rate between Ibuprofen and Alendronate, with a more rapid release of the more hydrophilic Alendronate. Addition of the surfactant SDS in the release media resulted in an increased release rate and solubility of Ibuprofen from F127 hydrogels. Meso-ordered hydrogel particles based on PEG-DA presented significantly slower release behavior compared to F127 hydrogels. Mathematic modelling of the drug-release kinetics for the hydrogels corresponded best to the first-order model and showed good correlation to data.

Keywords: Mesoporous hydrogel, drug delivery, Lyotropic Liquid Crystal (LLC), release kinetics

List of Abbreviations

LLC	Lyotropic Liquid Crystal
PPO	Poly(propylene) oxide
PEO	Poly(ethylene) oxide
DA	Diacrylate
PEG	Poly(ethylene) glycol
MEC	Minimum Effective Concentration
MTC	Minimum Toxic Concentration
SDS	Sodium Dodecyl Sulfate
Span-80	Sorbitan monooleate
SAXS	Small Angle X-ray Scattering
XRD	X-Ray Diffraction
PLM	Polarized Light Microscopy
DLS	Dynamic Light Scattering
PdI	Polydispersity Index
UV-VIS	Ultraviolet-Visible
LSC	Liquid Scintillation Counting

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1. Introduction

Hydrogels, consisting of a cross-linked hydrophilic polymer network represent an important class of materials used in biomedical and pharmaceutical applications [1, 2]. The cross-linked polymeric structure enables large water absorption giving physical properties similar to soft tissue, and the hydrogel also have high permeability and diffusivity for water and other nutrients[1, 2]. These characteristics together with a high biocompatibility have made hydrogels interesting for biomedical applications including contact lenses, tissue engineering, biosensors, and drug-delivery [2].

In recent years there has been a growing interest in the formation of hydrogels with a controlled nanostructure. Advantages of these ordered hydrogels compared to conventional hydrogels are increased compressive strength, swelling, permeability and biological compatibility, which has sparked the interest for use in biomedical applications[3]. Meso-ordered hydrogels are interesting to use as controlled release systems since their high porosity enables loading of drug molecules into the gel matrix and subsequent sustained release of drugs, thus maintaining a high local concentration of drugs in the surrounding tissue over an extended time period[4]. This might minimize systemic effects and permit lower drug dosage and safer delivery of therapeutics[5].

Introduction of nanostructures into hydrogels can be achieved by using surfactant templates, where monomers can adsorb and crosslink. However, issues associated to the thermodynamically driven phase separation when monomers are converted to polymers, which leads to formation of hydrogels with poorly defined nanostructures, is often an problem[3]. A solution to this phase separation problem has been proposed by Guymon et al. where rapid crosslinking of monomers utilizing photo-polymerization results in a highly ordered hydrogel polymer[6]. In this present study, meso-ordered PEG-based hydrogel particles have been synthesized by photo-polymerization in the presence of liquid crystalline phases formed by surfactants. Nanostructures can also be incorporated in hydrogels by the use of polymerizable amphiphiles, and by rapid crosslinking of ordered liquid crystalline phases creating meso-ordered hydrogels. In the present study the amphiphilic triblock copolymer Pluronic® F127 has been utilized for the formation of meso-ordered hydrogels.

1.1 Objective of this study

This study aimed at forming new types of meso-ordered hydrogels and hydrogel particles by the use of Lyotropic Liquid Crystals (LLCs) either with the use of templates or with polymerizable amphiphiles and to evaluate them as controlled drug-delivery systems. The objectives can be divided into three main parts:

- Form meso-ordered hydrogels using diacrylate modified triblock copolymers (Pluronic® F127)
- Form meso-ordered hydrogel particles using diacrylate modified Poly (ethylene) glycol.
- Evaluate loading and release of the drugs Ibuprofen and ^{14}C radiolabeled Alendronate from the hydrogels

2. Theory and background

2.1 Lyotropic liquid crystals (LLCs)

Lyotropic liquid crystals (LLC) are intermediate phases between liquids and periodic crystals, so called mesophases (2-50 nm) that have a liquid like fluidity and lack short-range order but exhibit a certain degree of order over long distances. LLCs are formed by the self-assembly of amphiphilic molecules, like surfactants and triblock copolymers, in water. Different geometries, such as cubic and hexagonal, can be formed with respect to the type of amphiphile, chemistry and concentration [7] [8]. LLCs are thought to be useful for drug-delivery applications because of their ability to incorporate large amount of drugs with different physiochemical properties [9]. Different LLC phases formed by an amphiphilic triblock copolymer are shown in Figure 1.

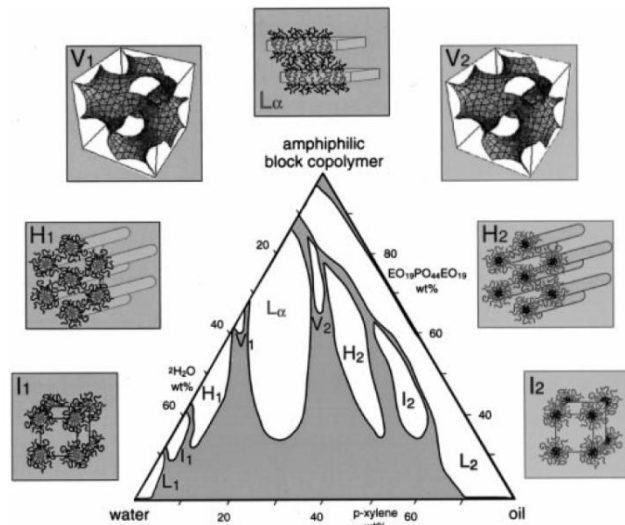


Figure 1. Ternary phase diagram of an amphiphilic triblock copolymer forming different LLC phases when mixed with water and oil[10].

2.2 Meso-ordered hydrogels from LLCs

In this study, triblock copolymers with the trade name Pluronic® have been used to form LLC phases with micellar cubic and hexagonal geometries, to create meso-ordered hydrogels. These block copolymers have excellent biocompatibility and are interesting to use in biomedical applications[11]. Built up of two blocks of hydrophilic poly (ethylene) oxide (PEO) attached to one block of hydrophobic poly (propylene) oxide (PPO) they exist in a variety of chain length of different blocks. In this study the triblock copolymer Pluronic F127, (PEO)₁₀₀(PPO)₇₀(PEO)₁₀₀ has been used to form LLCs (Figure 2).



Figure 2. Chemical structure of the amphiphilic triblock copolymer F127 (trade name Pluronic®) used in this study showing hydrophilic Polyethylene oxide (PEO) blocks in blue and hydrophobic Polypropylene oxide (PPO) blocks in red.

By modifying the hydrophilic endgroups of the triblock copolymer with cross-linkable groups a polymerizable amphiphile can be formed. Mixing the modified polymer with solvent at a certain concentration followed by rapid crosslinking then creates meso-ordered hydrogels. [12, 13].

2.3 Meso-ordered hydrogel particles from LLCs

An alternative method for introducing nanostructure into hydrogels is the use of templates, directing the formation of polymer networks into structures with different architecture. LLCs have extensively been used as templates in the formation of meso-ordered silica and titania [14, 15]. The use of LLCs as soft templates provides control over both pore size and morphology, which is dictated by the choice of amphiphile and its concentration. This enables formation of hydrogel networks with mesostructures ranging from lamellar, hexagonal and bicontinuous structures [16]. In the present study, diacrylate-modified (DA) Polyethylene glycol (PEG) based hydrogel particles have been formed in a water-in-oil emulsion to form meso-ordered particles. Figure 3 shows a templating route using surfactants for the formation of meso-ordered hydrogels based on PEG-DA.

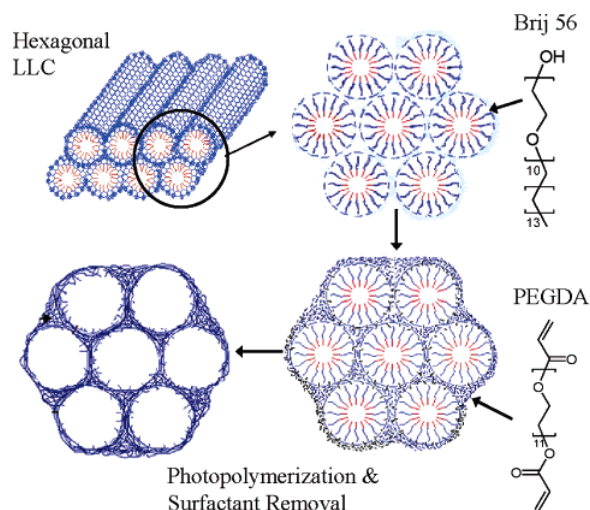


Figure 3. Schematic illustration of the LLC templating process. Clockwise rotation: Hexagonal LLCs mesophases formed by surfactants, 2D illustration of the hexagonal phase, 2D illustration of how PEG-DA monomers adsorb onto LLCs, photopolymerization and surfactant removal results in a meso-ordered PEG-DA hydrogel[6].

2.4 Controlled drug-release

Controlled drug release represents a rapidly advancing area in pharmaceutical science and aims to improve the effectiveness of drug therapy by controlling drug exposure over time, overcome physiological barriers and prevent premature degradation of the drug. A controlled release of drugs minimizes the patients' compliance by reducing the frequency of administration. Conventional administration routes where drugs enter through the systemic circulation often suffer from drug toxicity and side effects related to absorption of non-target tissue. Local drug-delivery will permit direct release to the target tissue and thus provide lower drug dosage to reach the desired effect and less exposure to other tissue [17, 18].

Controlled drug release over an extended duration is often beneficial, especially for drugs that are rapidly released and eliminated. The release should result in a concentration between the minimum effective concentration (MEC) and the minimum toxic concentration (MTC), as shown in Figure 4. A controlled release system may maintain the drug concentration within the therapeutic window for a longer time and thus avoid toxic side effect or underexposure and enable fewer administrations [18, 19].

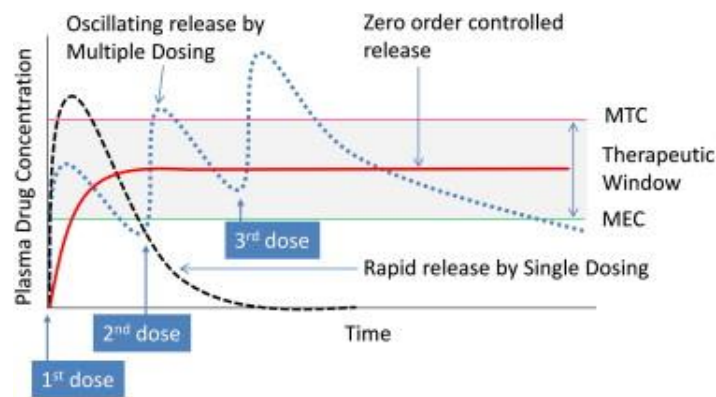


Figure 4. Plasma drug concentration obtained by different dosage forms, single dosing (black line), multiple dosing (dotted line), zero-order controlled release (solid line). The range between MTC and MEC represents the therapeutic window [20].

2.5 Drug-release kinetics

In-vitro drug delivery studies often involve mathematical modelling of the release behavior for the prediction of the release kinetics of the drug delivery system. Several kinetic models have been developed for different dosage forms, such as tablets, polymers etc.[21, 22]. In this study, the zero-order model and the first-order model were used to interpret the data obtained from the release studies.

2.5.1 Zero-order model

Pharmaceutical dosage forms that do not disaggregate and release the drug slowly can be represented by the zero-order model. Here, the drug release is only dependent on time

$$Q_0 - Q_t = Kt \quad (1)$$

Where Q_0 is the initial amount of drug in the dosage form, Q_t is the amount of drug in the dosage form at time t and K is the proportionality constant. Dividing the equation by Q_0 and simplifying leads to:

$$F_t = K_0 t \quad (2)$$

Where $F_t = 100(1 - (\frac{Q_t}{Q_0}))$ and F_t represent the percentage of drug released at time t . K_0 is the zero-order release constant.

2.5.2 First-order model

This model is typically used to describe absorption and/ or elimination of drugs. The first-order model, derived from first-order kinetics states that the concentration change with time is only dependent on concentration.

$$\frac{dC}{dt} = -KC \quad (3)$$

Where K is a first-order proportionality constant and C is the concentration of drug. Equation 3 can be expressed as:

$$Q_t = Q_0 e^{-Kt} \quad (4)$$

Where Q_t is the concentration of drug in the dosage form at time t and Q_0 is the initial concentration of drug. Equation 4 can be rewritten to:

$$F_t = 100(1 - e^{-K_1 t}) \quad (5)$$

Where $F_t = 100(1 - (\frac{Q_t}{Q_0}))$ and represents the percentage of drug released at time t , and K_1 is the first-order release constant expressed in t^{-1} .

3. Materials and methods

^{14}C radiolabeled Alendronate was purchased from Moravek Biochemicals. All other chemicals were purchased from Sigma-Aldrich and used as received. Experiments were performed in room temperature ($23 \pm 2^\circ\text{C}$).

3.1 Surfactants and drugs used in this project

3.1.1 Ibuprofen

Ibuprofen is a hydrophobic, non-steroidal anti-inflammatory drug derived from propionic acid, commonly used to treat inflammation, relieve pain and reduce fever. It exists in two isomers, R- and S, where the S-isomer is the most biologically active [23]. The structure of Ibuprofen is shown in Figure 5.

3.1.2 Alendronate

Alendronate is a bisphosphonate and an osteoporosis drug that has shown to improve bone mass density and reduce the risk of bone fractures[24]. For this study, Alendronate (Figure 5) with a ^{14}C -isotope was evaluated in drug release studies from the micellar cubic (I_{1B}) F127-hydrogel.

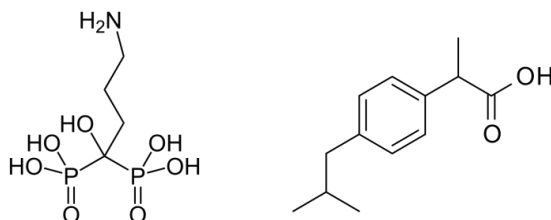


Figure 5. Molecular structures of the two drugs used in this work, Alendronate (left) and Ibuprofen (right).

3.1.3 Sodium dodecyl sulfate

Sodium Dodecyl Sulfate (SDS) (Figure 6) is an anionic surfactant used in various cleaning and hygiene products. In this project, it was used to enhance the solubility of the drug Ibuprofen in the release studies from the F127-hydrogels.

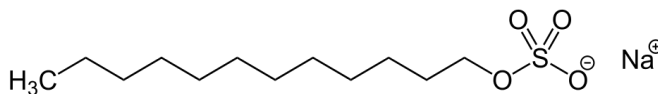


Figure 6. Molecular structure of the surfactant sodium dodecyl sulfate.

3.1.4 Sorbitan monooleate

Sorbitan monooleate (Span-80) (Figure 7) is a nonionic surfactant often used as an emulsifier. In the present study it was used as a template and emulsifier in the formation of PEG-DA hydrogel particles.

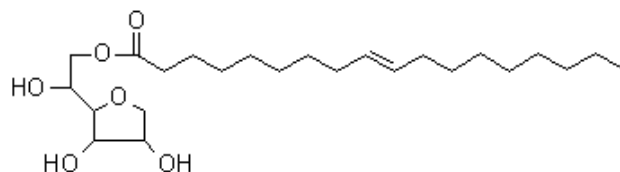


Figure 7. Molecular structure of the emulsifier sorbitan monooleate (Span-80).

3.2 Synthesis of diacrylate modified triblock copolymer

The acrylate derivate of Pluronic® F127 was synthesized by reacting the triblock copolymer with acryloyl chloride (Figure 8). To a solution of F127 in chloroform and with twice the molar amount of triethylamine, acryloyl chloride dissolved in chloroform was added drop-wise under N_2 atmosphere and magnetic stirring. After 24h reaction the product was washed three times with Na_2CO_3 (5 %), dried over anhydrous magnesium sulfate ($MgSO_4$), vacuum filtrated followed by solvent removal at reduced pressure. The diacrylate derivative of F127 was synthesized with an end product yield of 85-90%.

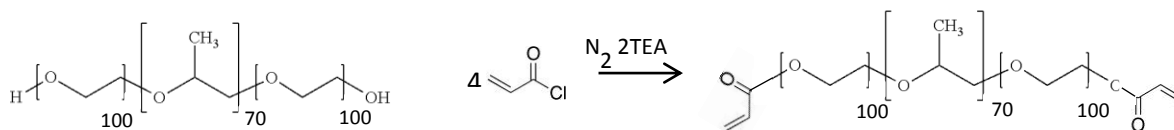


Figure 8. Reaction scheme of the synthesis route of the diacrylate modified triblock copolymer Pluronic F127.

3.3 The LLC system

The phase behavior of F127 has extensively been studied by Alexandridis et. al (25). In Figure 9 the ternary phase diagram for F127/water/butanol is shown. In this study, only the F127/water system has been studied and the phases of interest have been marked in the figure: the micellar cubic (I_1) and hexagonal (H_1) phase. Table 1 shows the composition by weight of the components forming the LLCs.

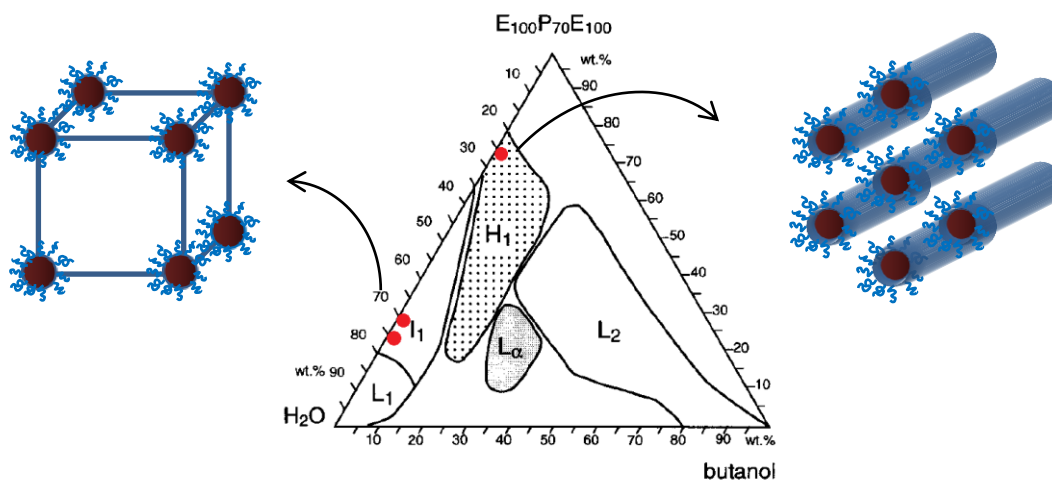


Figure 9. Ternary phase diagram for F127/water/butanol system with the phases of interest marked with red[25].

Table 1. LLC phases studied for the DA-modified F127 copolymer and water with the relative weight composition of each component.

Triblock copolymer	Phase	Designation	% copolymer	% water
Pluronic F127	Micellar cubic I_1	I_{1A}	25	75
Pluronic F127	Micellar cubic I_1	I_{1B}	30	70
Pluronic F127	Hexagonal H_1	H_1	75	25

3.4 Polymerized LLCs: formation of meso-ordered hydrogel

Hexagonal and micellar cubic liquid crystal gels were prepared by mixing of acrylate modified F127, water and photoinitiator, 2-Hydroxy-2-methylpropiophenone, at different ratios presented in Table 1. The initiator had a concentration of 1 wt. % of the amphiphile. The components were manually mixed with a spatula in a vial forming a thick homogenous gel. The gel was then applied onto glass slides (gel thickness ~ 2 mm) and cross-linked under UV-light (90 W lamp, $\lambda = 252$ nm) for 10 min creating a rubbery polymerized liquid crystal (hydrogel).

3.5 Formation of meso-ordered PEG-DA particles

The formation procedure of meso-ordered PEG-DA particles was directly adopted from Wallin et. al and performed as described before[26]. Diacrylate modified poly(ethylene) glycol (PEG-DA) (Figure 10) (1500 g/mol) had previously been synthesized and was used as received.

Formation of PEG-DA hydrogel particles was performed in a water-in-oil emulsion. A nonpolar solution was prepared by mixing 1,12 g emulsifier (Span-80) with 20 ml of hexane. The solution was rapidly stirred using a homogenizer (Silent Crusher M, Heidolph, Schwabach, Germany) followed by slow addition (~ 1 min) of PEG-DA mixture containing 5 wt. % PEG-DA (1500 g/mol), Milli-Q water and photoinitiator (2-Hydroxy-2-methylpropiophenone) with a concentration of 1 wt. % in respect to PEG. The formed water-in-oil emulsion was under constant stirring put under UV-light for 1h to ensure complete crosslinking of PEG-DA. The surfactant template was removed by liquid-liquid extraction (Milli-Q water: hexane, 1:3) four times, followed by water removal by Freeze-drying 24h.

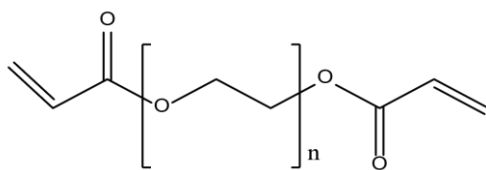


Figure 9. Chemical structure of diacrylate modified poly(ethylene) glycol (PEG-DA).

3.6 Loading and release of drugs

3.6.1 Determination of Ibuprofen concentration in F127 hydrogel

The total amount of drug absorbed in the hydrogel was determined by dissolution, using triplicate of samples. After polymerization of LLCs the hydrogel was freeze-dried for 24h and cut into appropriate sizes (0.015 g). The dried hydrogel was soaked in a 20 mg/ml Ibuprofen: ethanol solution for 24h, then taken out of solution to let the ethanol evaporate. The loaded hydrogel was put in 1.5 ml sodium hydroxide (NaOH) solution (1M) for 24h until complete dissolution was observed. Samples were then taken out, properly diluted and analyzed with UV/VIS Spectroscopy to determine the total drug uptake in the hydrogel.

3.6.2 Release of Ibuprofen from F127 hydrogel

Meso-ordered hydrogels of micellar cubic (I_{1A}) and hexagonal phase (H_1) were investigated for Ibuprofen drug-release. Freeze-dried hydrogels were loaded with Ibuprofen as described in 3.6.1. The release was performed with 6 replicas in Milli-Q water or SDS (1 wt. %) as release media, with constant stirring using a shaker -plate (Yellow Line OS2 basic). Samples were taken out at specific time intervals and the amount of drug released was measured with UV/VIS spectroscopy.

3.6.3 Determination of Ibuprofen concentration in PEG-DA particles

Freeze-dried PEG-DA particles were soaked in a 20 mg/ml Ibuprofen/ethanol solution for 24h. The solution was centrifuged at 3500 rpm for 30 min, the supernatant removed with a plastic pipette and soaked particles were put on glass slides to dry for 24h. The loaded particles (0.01 5g) were put in 1.5 ml sodium hydroxide solution (1M) for 24h to allow complete dissolution. After 24h, particles had agglomerated and the solution was therefore filtered through 0.8 μ m filters prior to UV-VIS Spectroscopy analysis to determine total amount of drug uptake in the particles.

3.6.4 Release of Ibuprofen from PEG-DA particles

Freeze-dried PEG-DA particles were loaded with Ibuprofen as described in 3.6.3. Ibuprofen loaded particles were transferred to a dialysis membrane (Spectra/Por) with 1ml Milli-Q water. The membrane was put in Milli-Q water for release measurements under magnetic stirring.

3.6.5 Determination of ^{14}C -Alendronate concentration in F127 hydrogel

The polymerized F127 hydrogel was oven-dried at 40°C for 24h and was then cut into pieces (0.045 g) and put in ^{14}C Alendronate water solution to soak for 24h. Dissolution of the hydrogel was performed as described in 3.6.1. Samples were collected and analyzed with Liquid Scintillation Counting.

3.6.6 Release of ^{14}C -Alendronate from F127 hydrogel

Meso-ordered hydrogels of micellar cubic (I_{1B}) phase were studied as drug-delivery systems for Alendronate. The soaked ^{14}C Alendronate loaded hydrogel was then placed in Milli-Q water for release measurements. Samples were taken out at specific time periods and the β -radiation released from the hydrogel was measured using Liquid Scintillation Counting.

3.7 Analytical methods

3.7.1 Small Angle X-ray Scattering

Small angle x-ray scattering (SAXS) is a technique where the inelastic scattering of X-rays (1-2 Å) at low angles (1-10°) provides structural information of a material at a length scale of 1-100 nm. Evaluation of the out-coming scattering patterns can provide morphological information like particle size, pore size distribution and more. The structure in LLC phases can be studied by SAXS by looking at relative distance between peaks to detect e.g. hexagonal and cubic phases. For instance H_1 phases can be distinguished by peak position ratios of 1, $\sqrt{3}$, 2, $\sqrt{7}$, and the micellar cubic phase (I_1) with primitive (P) cubic structure by relative distances of 1, $\sqrt{2}$, $\sqrt{3}$, $\sqrt{4}$. In this study, SAXS was used to study the meso-ordered hydrogels to confirm that the ordered LLC phases were retained after polymerization. Synchrotron SAXS measurements were performed on beamline I911 at the Max Lab synchrotron facility in Lund, Sweden.

3.7.2 X-ray diffraction

X-ray diffraction (XRD) is a tool for determining the molecular structure and crystallinity of a material by obtaining information about lattice parameters. The principle is to bombard the sample with an X-ray beam with different incoming angles generating a diffraction pattern. Constructive interference is observed when Bragg's law (eq.6) is fulfilled resulting in peaks in the diffraction pattern.

$$2d \sin \theta = n\lambda \quad (6)$$

Where n is any integer, θ is the scattering angle, λ is the wavelength of the X-rays. The obtained data can be compared with the Joint Committee on Powder Diffraction Standards (JCPDS) registry to determine the crystal structure of the material. In the present study, a Bruker D8 Advance X-ray diffractometer (Cu-K 1 radiation and $\lambda = 1.54056 \text{ \AA}$) with a 2θ range of 20-60°, step size 0.050° and data acquisition time of 30 min was utilized. XRD was used to determine the crystallinity of Ibuprofen absorbed in the F127 hydrogel.

3.7.3 Polarized Light Microscopy

Polarized Light Microscopy (PLM) is a technique that uses a transmission light microscope equipped with two polarizing filters placed perpendicular to each other and is normally used to detect birefringence in samples, like LLC phases. Placing a LLC sample having an isotropic structure like the cubic phase between the filters will result in a dark image since the two polarizers will block the light. For an anisotropic sample, the polarized light will interact with the sample and change direction resulting in structure induced birefringence patterns characteristic for different phases; the hexagonal phase shows rod-like streaks while the lamellar phase shows fan-like patterns. PLM measurements were performed on fully swollen PEG-DA particles with a Zeiss microscope (Axio Scope A.1, Carl Zeiss Microscopy, Germany) equipped with an AxioCam ICc5 camera (40x objective) and polarized filters.

3.7.4 Dynamic Light Scattering

Dynamic Light Scattering (DLS) is a technique that can be used to measure the size distribution of small particles in suspensions, typically emulsions, micelles, polymers and nanoparticles. The principle of the technique is the illumination of the sample by a laser beam and the fluctuations of the scattering are detected at a known scattering angle and collected by the detector. Scattering of light occurs if the particles are smaller compared to the wavelength of the light (<250 nm). The scattering fluctuations due to Brownian motion of the particles yields information of hydrodynamic radius or diameter of the particles calculated via the Stokes-Einstein relation. In the present study, PEG-DA particles (1 mg/ml) were filtered through 0.8 µm filters and the size distribution was measured with DLS using a Zetasizer Nano-ZS instrument (Malvern Instruments, Worcestershire, UK).

3.7.5 Ultraviolet-Visible spectroscopy

Ultraviolet–Visible (UV-VIS) Spectroscopy involves absorption of radiation in the Ultraviolet (180–400 nm) and the visible (400–800 nm) region in the electromagnetic spectrum, where the absorption induces excitation of electrons from ground state to a higher energy state. This technique has applications mainly in quantitative measurements of transition metals, conjugated organic compounds and biological macromolecules. Lambert-Beer's law (eq. 7) expresses the proportionality between absorption, concentration in solution and path length.

$$A = \log_{10} \left(\frac{I_0}{I} \right) = \epsilon cl \quad (7)$$

Where A is the Absorbance, I_0 is the intensity of incident radiation, I is the intensity of the transmitted radiation, ϵ the molar absorptivity or molar extinction coefficient, c concentration of solution (mol/l), and l the path length of the sample. For an organic compound the molar absorptivity is constant at a certain wavelength, thus a calibration curve with different concentrations can be constructed, and the concentration of a sample can be determined. In the present study, UV-VIS Spectroscopy was used to measure the release of Ibuprofen from the F127 hydrogels and the amount of drug absorbed in F127 hydrogels. Measurements were performed with 1 ml quartz cuvettes, using an Agilent 8453 UV-VIS Spectrophotometer.

3.7.6 Liquid Scintillation counting

Liquid scintillation counting (LSC) is a technique measuring low energy emitting radiation such as β -emitting isotopes. The principle of LSC is that energy is emitted from radioactive decay to a scintillation cocktail consisting of an organic solvent and scintillators (fluors). The energy is absorbed by the solvent and transferred to the fluor molecules, which upon de-excitation emit photons of visible light. These flashes of light are then detected by a photomultiplier tube and converted into a flow of electrons and measured as an electric pulse (Figure 10). [27]. In this study, LSC was used to measure drug-release of ^{14}C labeled Alendronate by detecting emitting β -radiation. Samples were taken out (0.1 or 0.2 ml) and added to scintillation vials filled with 12 ml Emulsifier-Safe liquid scintillation cocktail (PerkinElmer) and shaken to obtain a homogenous solution. The measurements were performed using a Wallac Guardian 1414 Liquid Scintillation Counter (PerkinElmer).

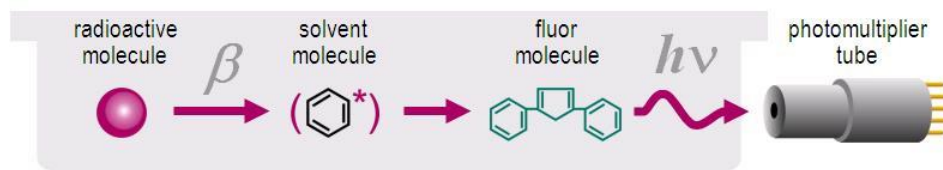


Figure 10. Basic sketch of the scintillation process[28].

4. Results and discussion

The aim of this study can be divided into two parts, where the first part aimed to synthesize two types of meso-ordered hydrogels; based on crosslinking of LLC phases formed by a triblock copolymer, and formation of PEG-based hydrogel particles using LLC templates. The second part of the project involved drug-release studies from the hydrogels, examining the release behavior of the drugs Ibuprofen and ^{14}C radiolabeled Alendronate.

4.1 Material characterization of MF127

4.1.1 LLC gel and hydrogel formation

The formed hexagonal and cubic LLC gels were transparent and highly viscous (Figure 11, left). After UV-polymerization the gels turned into rubbery hydrogel polymers showing that the covalently crosslinking was successful (Figure 11, right). However, further structure determination was necessary to confirm the structure retention of the hydrogel.

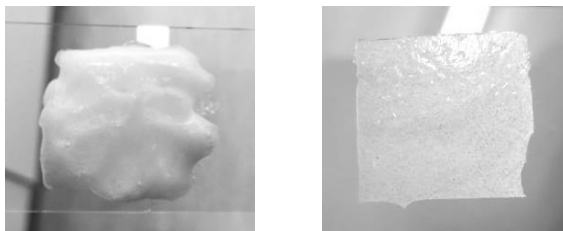


Figure 11. Image of the I_1 LLC gel (left) and hydrogel polymer (right).

4.1.2 Phase behavior of the F127 hydrogel

In order to confirm that the LLCs retain their ordered structure after polymerization, SAXS measurements were performed on the LLC gel and the polymer (hydrogel). Data from SAXS for the hexagonal LLC gel and polymer (hydrogel) (Figure 12, left) shows reflections with relative peak distance of adjacent peaks of the scattering vector (q) to be $1:3^{1/2}:2:7^{1/2}$, which confirms the hexagonal structure for both the gel and hydrogel polymer [29]. For the micellar cubic structure, which is built up of spherical micelles arranged in corners of a cube, the relative peak distance of adjacent peaks is $1:2^{1/2}:3^{1/2}:2$. An important note is that for both phases the peaks for the polymerized gel are slightly shifted to the left and to the right for the hexagonal and cubic phase, respectively. It is difficult to give a definitive explanation of this phenomenon, but it could be due to insufficient crosslinking, or other structure inhomogeneity.

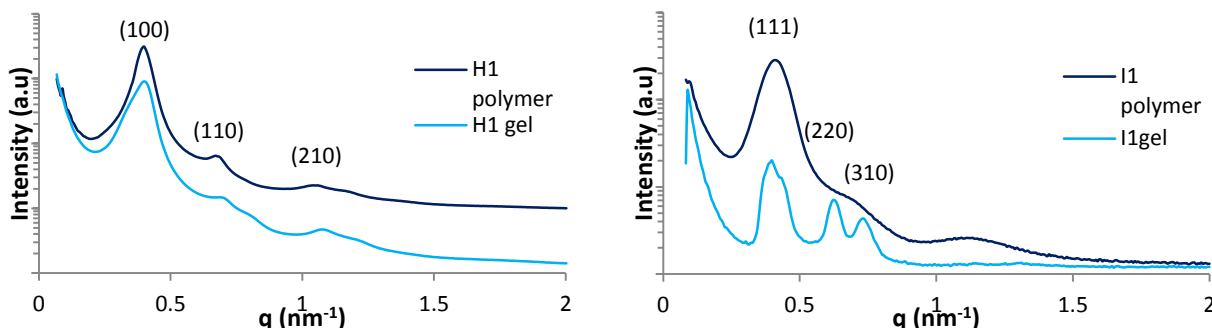


Figure 12. SAXS scattering patterns for the hexagonal (H_1) gel and polymer (left figure) and the cubic (I_1) gel and polymer (right figure).

4.1.3 Determination of drug crystallinity

XRD was used to determine the crystallinity of the drug Ibuprofen, both as dry powder and when incorporated into F127 hydrogels with cubic and hexagonal phase. Most drugs form crystals at room temperature, existing in multiple crystal forms (polymorphs) or as crystal hydrates. The type of polymorph affects drug dissolution which plays an important role in determining the release behavior[18]. For hydrophobic drugs, like Ibuprofen poor water solubility is an issue in pharmaceutical applications since it lowers the bioavailability. Converting the drug to a more soluble sodium salt or specific polymorphic form can increase solubility and thus improve the bioavailability[30].

In Figure 13 diffraction patterns for the cubic and hexagonal phase for the soft and freeze-dried hydrogel with and without Ibuprofen, and Ibuprofen powder are shown. The Ibuprofen powder shows diffraction peaks corresponding to high crystallinity. The dry hydrogel shows two distinct peaks due to crystallization after water removal, also seen in the hexagonal soft hydrogel. However, when incorporating Ibuprofen in the freeze-dried hydrogel matrixes no diffraction peaks from Ibuprofen could be identified, which imply that that the hydrogel has disrupted the crystalline structure of the drug.

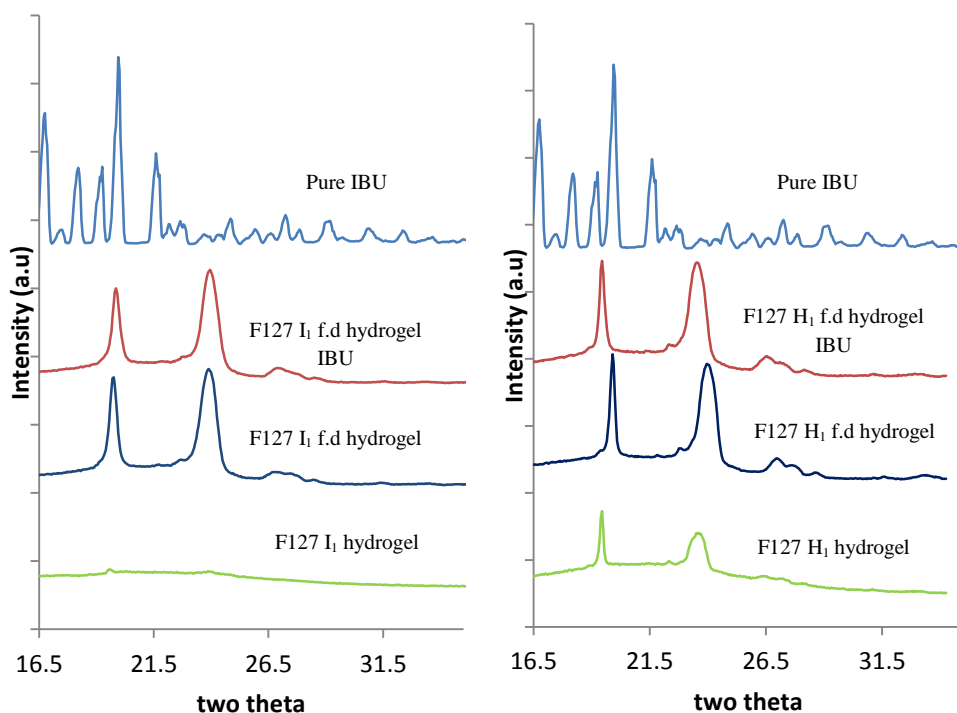


Figure 13. XRD patterns for the F127 hydrogel for soft polymer, freeze-dried polymer and freeze-dried polymer with Ibuprofen, and pure Ibuprofen. Cubic phase (I₁) (left) and hexagonal phase (H₁) (right).

4.2 Material characterization of PEG-DA hydrogel particles

4.2.1 Formation of meso-ordered PEG-DA particles

The formed PEG-DA particles appeared as a white fluffy powder, and when re-dispersed in water the particles swelled, confirmed that the crosslinking was successful. However, further structure determination was necessary to confirm the order and phase of the material.

4.2.2 Phase behavior of PEG-DA hydrogel particles

In order to detect presence of anisotropy in synthesized PEG-DA, PLM measurements were performed. Figure 14 shows a micrograph of the hydrogel particles showing clear birefringence patterns, and thereby confirming long-range order in the sample. It is however difficult to tell whether the patterns correspond to hexagonal or lamellar phases and to further determine the exact phase SAXS scattering measurements have to be performed.

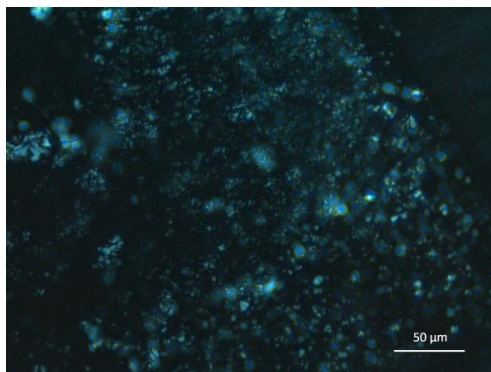


Figure 14. PLM micrograph of the hydrogel particles showing birefringence confirming that the particles have an anisotropic long-range order.

4.2.3 Size determination of PEG-DA hydrogel particles

The size distribution of the PEG-DA particles was measured with DLS, presented in Figure 15. The intensity distribution of particle size corresponds to the scattering intensity of each particle fraction, and hence larger particles give a larger contribution. Therefore, also the number average distribution is presented, where smaller particles contribute more. The polydispersity index (PdI) is a dimensionless value between 0 and 1 estimating the size distribution. As can be seen in Figure 15, the size distribution for the PEG-particles is quite narrow, with a PdI of 0.35 and particle size estimated to 242 and 209 nm for intensity and number average, respectively.

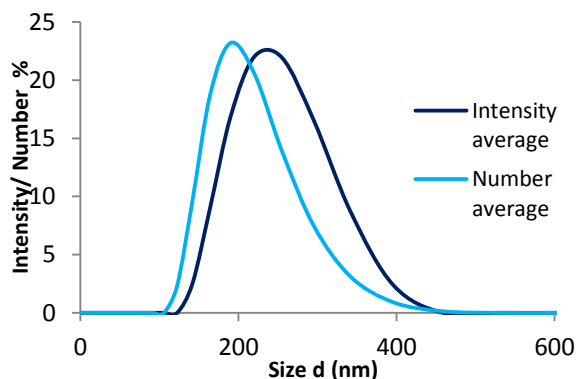


Figure 15. Intensity- and number average size distribution for formed PEG-DA hydrogel particles.

4.3 Drug-loading and release

4.3.1 Loading of Ibuprofen

Ibuprofen was loaded in F127 hydrogels and PEG-DA hydrogel particles by soaking in an ethanol-Ibuprofen solution followed by solvent evaporation. The total drug amount absorbed was determined by dissolving the hydrogels in sodium hydroxide solution, and measure the drug concentration with UV-VIS Spectroscopy. The F127 hydrogel did easily dissolve and left a homogenous clear solution, while the PEG-particles formed long thread-like aggregates which had to be filtered prior to analysis.

The total drug loadings (Table 2) were quite low for F127 hydrogels, around two percent of the polymer weight. For the PEG-DA particles a substantially higher loading was observed, which could be due to the small particles high surface area, and that there remained excess solution around the particles after removal of drug solution.

Table 2. Total amount of Ibuprofen absorbed in the F127-hydrogels and in PEG-DA hydrogel particles.

Sample	Sample weight (g)	Amount IBU* (mg)	Standard deviation (SD)	Fraction IBU in dry hydrogel (%)
F127 Hexagonal (H _I)	0.015	0.32	$4.05 \cdot 10^{-5}$	2.1
F127 Cubic (I _{IA})	0.015	0.35	$2.25 \cdot 10^{-5}$	2.3
PEG-DA particles	0.015	1.8	-	12

*mean value from 3 samples

4.3.2 Loading of ¹⁴C Alendronate

Radiolabeled Alendronate was loaded into the cubic F127 hydrogel by soaking the dried polymer in an Alendronate- water solution. The total amount absorbed was determined by dissolution in sodium hydroxide solution, which gave a clear homogeneous solution that was analyzed with LSC. Since LSC is a very sensitive technique measuring β -radioactive decay, a much lower drug-loading was used compared to the Ibuprofen loaded samples.

4.3.3 Drug-release

Drug-release measurements were performed with UV-VIS Spectroscopy and LSC to investigate the hydrogels ability to give a controlled release of the two drugs Ibuprofen and ¹⁴C Alendronate. Mesoporous hydrogels are thought to show a sustained release behavior since the pores enables absorption of drugs and a subsequent diffusion-controlled release from the pores to the surrounding media controlled by the diffusion coefficient of the small drug molecules through the gel network[4]. In Figure 16-19 release profiles for hydrogels used in the present study are presented together with kinetic models fitted to the release data.

Release of Ibuprofen from the F127 hexagonal (H_1) hydrogels is presented in Figure 16, with water and SDS-solution as release media. For both curves an initial burst release is observed (around 25-40 %) followed by a slow and sustained release of drug. Clear differences in release rates between SDS-solution and water can be observed, with a faster initial release and a higher equilibrium concentration for SDS 87.7 vs 73.3 % (inserted figure in Figure 16). In the present study, the surfactant SDS was used to increase the solubility of Ibuprofen in water, and thereby it also increased the rate of diffusion from the hydrogel.

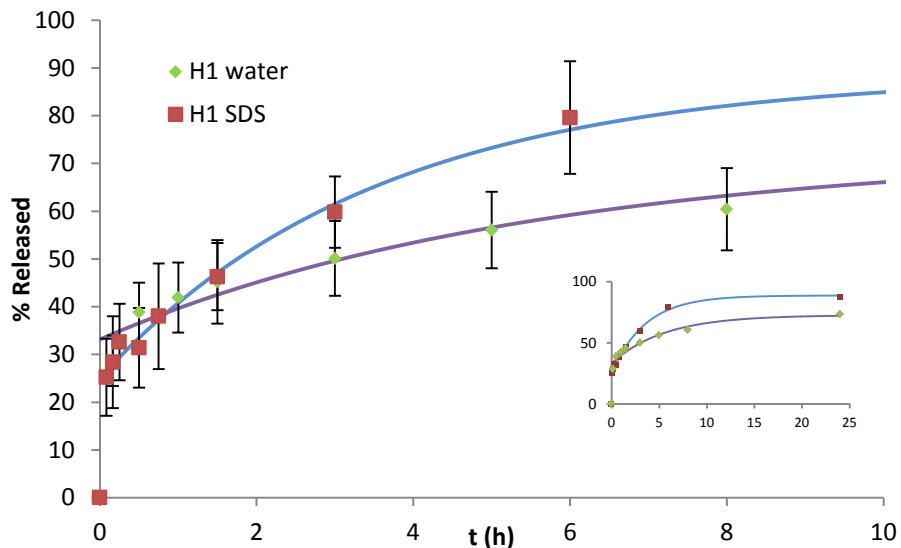


Figure 16. Release profiles for Ibuprofen from F127 hexagonal (H_1) hydrogels in water (green rhombs) and 1 % SDS solution (red squares) for the first 10 hours with first-order release models. Inserted figure shows the release profiles over 24 hours.

For the cubic (I_{1A}) hydrogels the release behavior of Ibuprofen in water and 1 % SDS (Figure 17) showed a similar arrangement as discussed previously for the hexagonal phase, with equilibrium concentrations of 76.7 and 71 % for SDS and water, respectively. Comparing the two phases reveals a more rapid initial release of drug for the hexagonal phase, and also more drug is released with time. It is difficult to assign the exact mechanism behind this phenomenon, and the variations between measurements are quite large. However, pore morphology and water absorption varies between the phases where the hexagonal phase has significantly lower water content in the non-swollen state (25 % compared to 75% in the cubic phase), and the cubic phase have a higher water swelling capacity, which is known to affect the drug-diffusion through the hydrogel matrix[31]

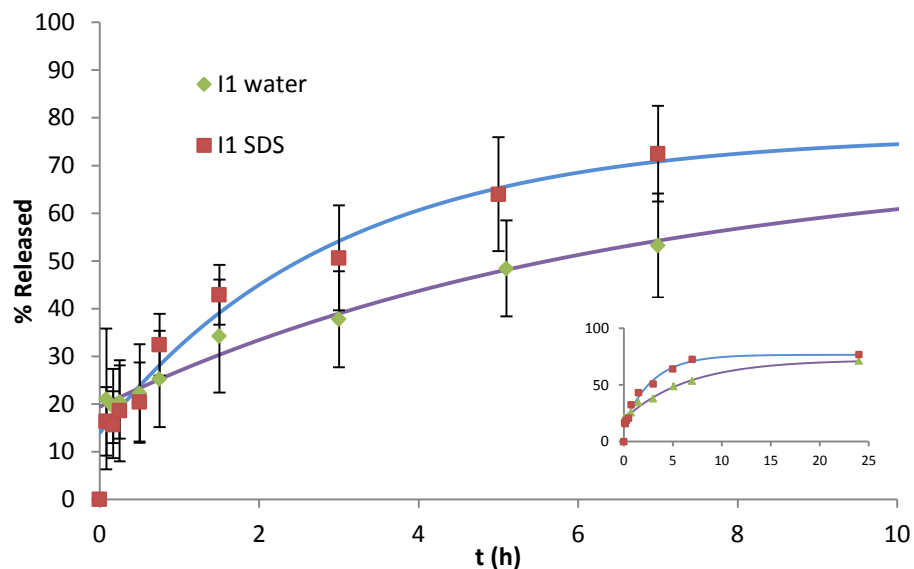


Figure 17. Release profiles for Ibuprofen from F127 Cubic (I_{1A}) hydrogels in water (green rhombs) and 1 % SDS solution (red squares) for the first 10 hours with first-order release models. Inserted figure shows the release profiles over 24 hours.

Figure 18 demonstrates the release behaviour of Ibuprofen from PEG-DA particles, showing a small burst release (10 %) followed by a controlled release reaching equilibrium concentration of 74 % after 20 hours. The release rate of Ibuprofen from PEG-DA particles are considerably slower than for the F127 hydrogels, probably because the small size of the particles gives a large surface area and by so slower diffusion through the pores.

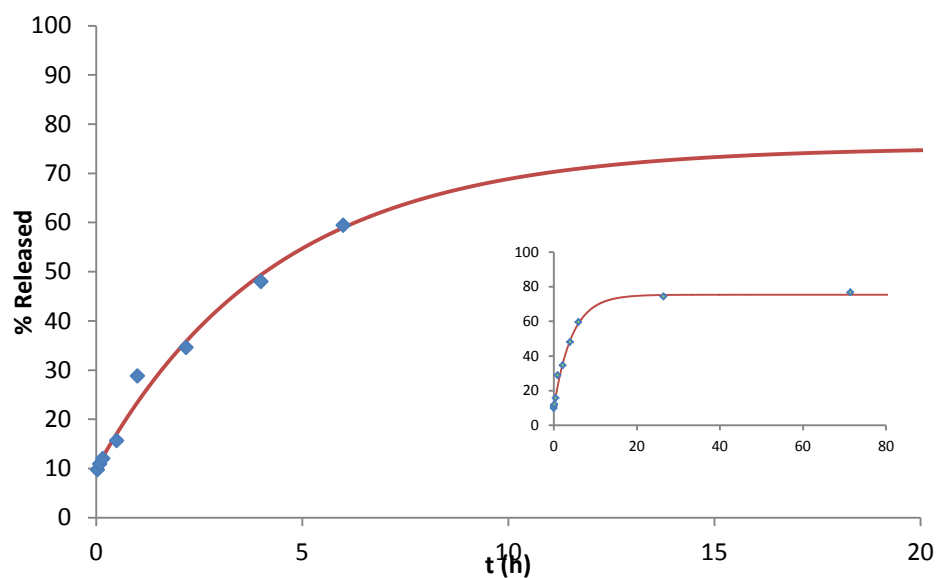


Figure 18. Release profile for Ibuprofen from PEG-DA hydrogel particles for the first 20 hours, with first-order release model. Inserted figure shows the release profile for 72 hours.

The release profile of ^{14}C Alendronate from the cubic ($\text{I}_{1\text{B}}$) F127 hydrogel is presented in Figure 19. An initial burst release of 30 % is observed followed by a sustained release with equilibrium concentration of 92 % reached after one hour. The magnitude of the burst could be explained by the hydrophilic nature of ALN and by presence of drug solution at the surface of the hydrogel.

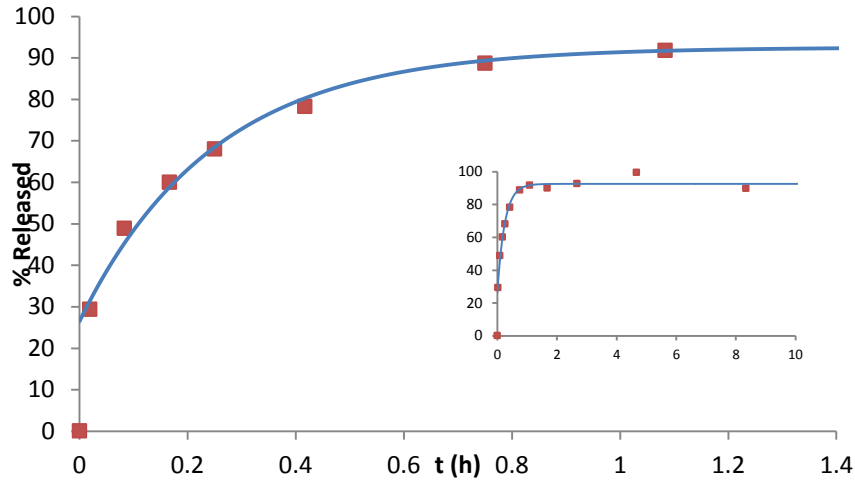


Figure 19. Release profile for ^{14}C -Alendronate from the F127 Cubic ($\text{I}_{1\text{B}}$) hydrogel for the first 1, 4 hours, with first-order release model. Inserted figure shows the release profile for 10 hours release.

For all release profiles shown in the Figures above, an initial burst-release could be observed (around 10-35 %), which is a rapid initial release of drug that commonly exist in controlled release systems and is probably due to drug molecules weakly adsorbed on the surface of the hydrogel[32]. Following the burst- release was a slow sustained release of drug, implying a drug-release from the pores of the hydrogels where the interaction between drug and gel is stronger and the release mainly is diffusion controlled.

Kinetic modeling was applied to the drug- release data to evaluate release mechanisms and kinetics. Models investigated were the zero-order model and the first-order model, earlier described in Section 2.5. For the existing release data, the initial burst was not taken into account in the modeling. The model that gave the best fitting to existing data was the first-order model, and in order to give the best fit, two parameters were added to the equation resulting in the following expression:

$$F_t = 100(1 - A \cdot e^{-K_1 t}) + C \quad (8)$$

Where parameters A, C and K_1 were fitted to respective release data using MatLab. A summary of values obtained from the kinetic modelling is presented in Table 3.

The first parameter A was added to the expression to adjust the model to the release data points. According to the original expression in Section 2.5.2, the equilibrium release has to reach 100 % to follow first-order kinetics. For the hydrogels studied this does not occur, where the observed equilibrium release lies between 71-83 % for Ibuprofen, and around 92 % for Alendronate. In order to account for this lower plateau value, the constant C was added to the first-order expression. The kinetic modeling gave good correlation to data with R-square values of 0.96-0.99 (Table 3).

Table 3. Parameters for each hydrogel retrieved from the first-order model fitted to release data.

Sample	F127 Hexagonal (H_1)		F127 Cubic (I_{1A})		PEG-DA particles	F127 Cubic (I_{1B})
Release media	Water	SDS (1wt. %)	Water	SDS (1wt. %)	Water	Water
Drug	Ibuprofen		Ibuprofen		Ibuprofen	C^{14} Alendronate
r^2	0.96	0.99	0.99	0.99	0.99	0.98
$K_1 (h^{-1})$	0.18	0.28	0.15	0.34	0.23	4.04
A	0.39	0.64	0.53	0.63	0.65	0.66
C	-27.5	-11.4	-28	-23.5	-24.7	-7.5

A reasonable explanation for this behavior is that at equilibrium concentration in the release media, the drug absorbed in the hydrogel matrix has a partitioning coefficient between the surrounding media and the gel. This means that some drug will remain inside the gel even after a long time and will not be released before the hydrogel eventually disintegrate. The partitioning coefficient depends on the drugs polarity and charge, size and interaction with the hydrogel pores [31]. Hence, Ibuprofen which is quite hydrophobic will prefer to distribute in the hydrophobic domains of the porous hydrogels resulting in a lower equilibrium concentration. The hydrophilic drug Alendronate on the other hand shows weaker interactions with the pores, and an equilibrium concentration closer to 100%.

5. Conclusions

This project aimed at forming two novel meso-ordered hydrogels and to evaluate them as controlled release systems. Meso-ordered hydrogels based on LLCs formed by F127 triblock copolymers with hexagonal and cubic phases were successfully formed, and retention of the structure after polymerization was detected with SAXS. Ordered mesoporous PEG-DA hydrogel particles with a size of 209-242 nm were formed using a w/o emulsion based technique.

Ibuprofen and ^{14}C labeled Alendronate were successfully loaded into the hydrogels. For F127 hydrogels loaded with Ibuprofen, XRD measurements revealed a disruption of the drugs crystallinity, implying that when absorbed in the hydrogel the drug exist in an amorphous phase.

The F127 hydrogels served as controlled release systems with an initial burst effect followed by sustained drug release. Clear differences in drug release rates were observed between Ibuprofen and Alendronate, which were explained by difference in polarity, where the more hydrophilic drug Alendronate was released faster. Altering the release media by addition of SDS increased the release rate and final concentration of Ibuprofen by enhancing its solubility. PEG-DA hydrogel particles presented a controlled release behavior with significantly slower release compared to F127 hydrogels.

Drug-release kinetics for the hydrogels best corresponded to the first-order model with good correlation with data with r^2 -values varying between 0.96-0.99.

6. Future work

This project has shown possibilities to use mesoporous hydrogels in applications as controlled release systems.

It would be of interest to further study the release properties from the PEG-DA hydrogel particles, the effect of the small particles size and its high porosity and surface area.

When it comes to release behavior it would be interesting to examine release performance of drugs with different chemical properties. Also, it would be interesting to study the effect of altering the surrounding release media by studying drug-release in simulated body fluid.

In vivo studies would be a great complement to this work to see how the different hydrogels work as drug-delivery systems in a more complex environment and to evaluate the therapeutic response.

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