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Stimuli-responsive polymer thin films by initiated chemical vapor deposition for controlled drug delivery

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Abstract

Stimuli-responsive polymer thin films can change their chemical and physical properties by small changes of their surroundings. A thermo-responsive hydrogel for example, is a polymer that exhibits a temperature dependant thickness change if exposed to a liquid environment. This can be used for various new applications in nanotechnology and nanomedicine, where organic polymer thin films are considered to be an alternative approach for controlled drug delivery. The studied active pharmaceutical ingredients phenytoin, clotrimazole and indomethacin were dissolved in tetrahydrofuran and then drop casted on glass substrates. With a technique called initiated chemical vapor deposition (iCVD) a co-polymer thin film of *N-Isopropylacrylamide* (*NIPAAm*) cross-linked with *Di(ethylene glycol) divinyl ether* (*DEGDVE*) was synthesized on top of the drug layer. Dissolution testing was performed in a buffer solution at various temperatures to simulate the human body environment. A high-performance liquid chromatograph was used for the chemical analysis of the dissolution experiments. It was found that the drug delivery is affected by applying a thermo-responsive *p(NIPAAm-co-DEGDVE)* layer on top of the drug and then vary the temperature of the dissolution medium.

Similar dissolution studies were performed on other hydrogel films consisting of *2-Hydroxyethyl methacrylate* cross-linked with *Ethylene glycol dimethacrylate* deposited by iCVD. Those were also investigated by Fourier transform infrared spectroscopy and spectroscopic ellipsometry to measure the thickness increase upon swelling in water and the distance between two cross-links, called mesh size.

Kurzfassung

Dünnschichten aus Stimuli-responsiven Polymeren können ihre chemischen und physikalischen Eigenschaften durch kleine Veränderungen ihrer Umwelt anpassen. Thermo-responsive Hydrogele zum Beispiel, weisen eine temperaturabhängige Änderung der Dicke auf, wenn sie Flüssigkeiten ausgesetzt werden. Das kann für verschiedene neue Anwendungen in der Nanotechnologie und Nanomedizin eingesetzt werden, wo organische Polymer-Dünnfilme als ein alternativer Ansatz für die kontrollierte Arzneimittelabgabe angesehen werden. Die pharmazeutischen Inhaltsstoffe Phenytoin, Clotrimazol und Indomethacin wurden in Tetrahydrofuran aufgelöst und auf Glassubstrate aufgebracht. Mittels initiierter chemischer Gasphasenabscheidung (iCVD) wurde ein vernetzter Copolymer-Dünnfilm aus *N-Isopropylacrylamid* (*NIPAAm*) und *Di(ethylenglykol) divinylether* (*DEGDVE*) auf der Arzneimittelschicht synthetisiert. Die Auflösungstests wurden in einer Pufferlösung bei verschiedenen Temperaturen durchgeführt, um die Freisetzung im menschlichen Körper zu simulieren. Für die chemische Analyse der Proben des Auflösungstests wurde ein Hochleistungs-Flüssigkeitschromatograph verwendet. Dabei wurde festgestellt, dass die Arzneimittelabgabe beeinflusst werden kann, indem eine Thermo-responsive *p(NIPAAm-co-DEGDVE)* Schicht auf das Medikament aufgetragen und die Temperatur des Auflösungsmediums verändert wird.

Ähnliche Auflösungstests wurden mit Hydrogelfilmen, bestehend aus *2-Hydroxyethylmethacrylat* und *Ethylenglykoldimethacrylat* durchgeführt. Sie wurden mittels iCVD hergestellt und mit Fourier-Transformations-Infrarotspektroskopie und spektroskopischer Ellipsometrie untersucht. Der Abstand zwischen den Querverbindungen im Polymer Netzwerk wurde durch die Analyse des Schwellverhaltens bestimmt.

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CHAPTER 1

Introduction

Many possible administration routes are nowadays available to cure medical conditions, like injection, oral tratment, inhalation or transdermal patches. The route depends on factors like the patient compliance, the drug stability in different environments (e.g. stomach pH $\sim$ 1-2) and if there is self-administration possible or not. Sometimes it is desirable to control the drug delivery to achieve local medication with a tuneable release of the drug. An attempt to tackle this problem is through polymer encapsulation of the drug. Polymeric coatings would stabilize the solid state of the drug making it less vulnerable to environmental impact by creating a diffusion membrane around the medication.[2]

Stimuli-responsive polymers are currently getting a lot of attention since they have the promising property to show a big response to a small external stimulus such as temperature, pH or light.[5] They are also researched for biomedical applications because they can exhibit nice biocompatibility and nontoxicity. Another big advantage is the possibility to deposit them with initiated chemical vapor deposition (iCVD) whereby a thin film is directly synthesized on a substrate through surface polymerization. This solvent free technique allows coating after drug loading, without harming the drug layer, on a variety of substrates whereby the chemical composition of the polymer layer can be easily controlled.[8]

Polymer encapsulation of medication in drug delivery systems can be beneficial for many therapies, because it enables controlled drug release and minimizes the toxic side effects.[17] Using a thermo-responsive coating, such as the

tested *p(NIPAAm-co-DEGDVE)*, McInnes et. al. show in [17] that this material can be used to deliver drugs on-demand at elevated temperatures. This is possible because *p(NIPAAm)* undergoes a reversible coil-to-globule transition at a lower critical solution temperature (LCST) of about 32 $°C$, i.e. lying near body temperature.[24]

In this thesis the iCVD process is used to coat different drugs with a thermo-responsive polymer thin film layer, which is schematically shown in figure 1.1. The pristine drug layer and polymer coating are investigated, especially the temperature dependant thickness change of the *p(NIPAAm-co-DEGDVE)* hydrogel layer in liquid environment. Model drug delivery systems are then investigated by dissolution experiments, where the drug release is of high interest.

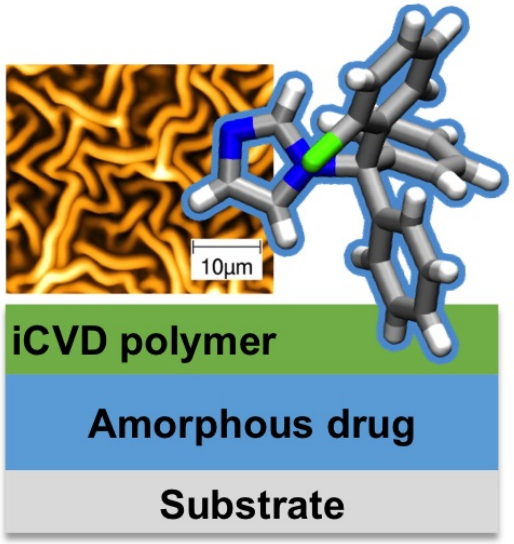


Figure 1.1: Schematic drawing of a polymer layer encapsulating a drug. Reprinted from [6].

CHAPTER 2

Fundamentals

2.1 Polymers

A polymer is a macromolecule consisting of many, rather simple molecules built together to a long, chain-like structure in the process of polymerization. These small parts of the chain are called monomers. They are connected to each other by covalent bonds and provide a backbone, to which afterwards atoms or groups of atoms can join. A generel example of a monomer and its corresponding polymer is given in figure 2.1. Comparing the polymer network with smaller molecules gives rise to very different physical properties. The strong intermolecular bonds and the high molecular weight lead to higher melting points or properties like viscoelasticity.[21]

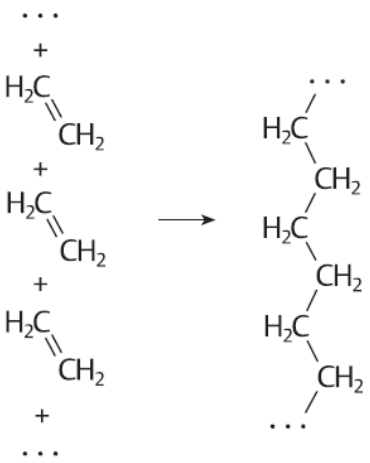


Figure 2.1: Schematic example of ethylene monomers (left) forming the polymer polyethylene (right). Adapted from [19].

A possibility to categorize polymers is if whether they are homopolymers or heteropolymers. Homopolymers only consist of one type of repeating unit building up the chain. In comparison to that a heteropolymer, also known as co-polymer, can consist of more than one monomer unit. The different types of combinations of co-polymers are depicted in figure 2.2.[13] Through co-polymerization, the mechanical properties of the polymer can be changed.

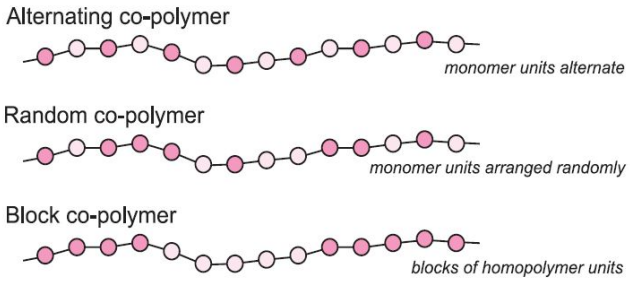


Figure 2.2: Different types of co-polymerization. Adapted from [21].

Mechanical properties can be enhanced even further by adding a cross-linker, which is another monomer that connects the linear chains, forming a cross-linked polymer network. In figure 2.3 this is shown for one of the

polymers used for this work. With the cross-linking agent the stability of polymer thin films gets highly increased.

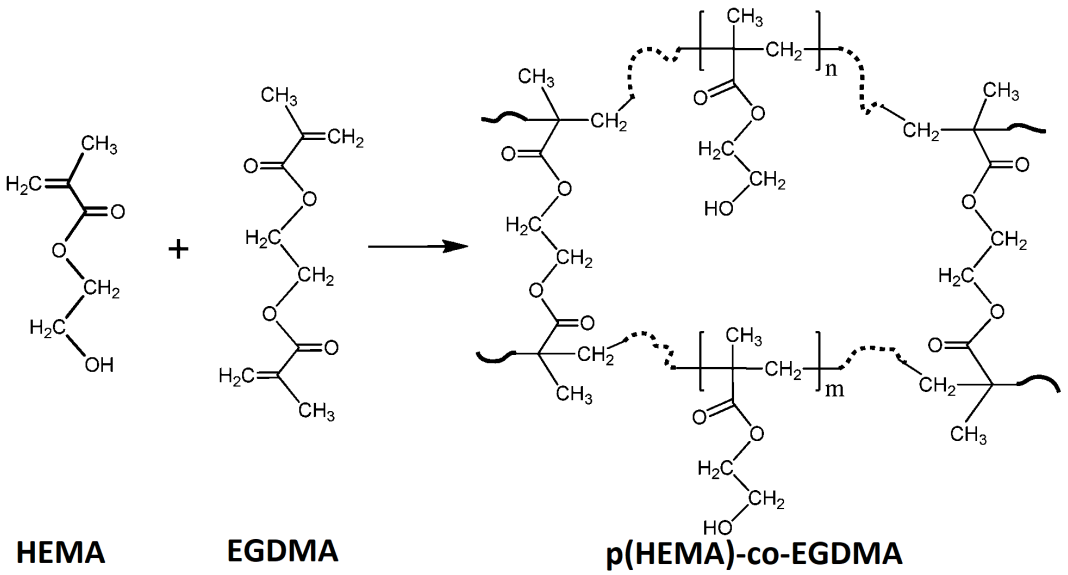


Figure 2.3: The Monomer *HEMA* (left) and the cross-linker *EGDMA* (middle) form the cross-linked polymer *p(HEMA-co-EGDMA)*(right). Reprinted from [26].

As already mentioned cross-linking plays an important role concerning the stability of a polymer, especially when working with hydrogels. [7] A hydrogel in general is a cross-linked, hydrophilic polymer network that can uptake water and thereby increase its volume. This process is called swelling. The latter is very much affected by the mesh size, i.e. the average length between two cross-links. Considering an application in nanomedicine for example, this parameter greatly contributes to the process of drug delivery, because it is easier for drug molecules to diffuse through a big mesh than a small one. To calculate this mesh size, the Flory-Rehner theory suggests to link it to the swelling behavior of the polymer network in water. Therefore measurements of the dry polymer layer (d_0) and of the swollen layer (d) have to be performed. The mesh size (ξ) can be calculated from equation 2.1, where l is the $C-C$ bond length, $\overline{M}$ is the average molecular weight between two cross-links, M_m is the molecular weight of the monomer and c_n is the characteristic monomer ratio.

$$\xi = \left(\frac{d_0}{d}\right)^{-1/3} l \left(\frac{2\overline{M_c}}{M_m}\right)^{1/2} c_n^{1/2} \tag{2.1}$$

2.1.1 Smart hydrogels

To achieve more versatile applications for polymer thin films, there are stimuli-responsive hydrogels that can perform swelling, controlled by an external stimulus, for example termperature, pH or light. One of these smart hydrogels that is currently getting a lot of attention, is the temperature-sensitive *Poly(N-isopropylacrylamide)* (*p(NIPAAm)*), which is also investigated in this work. By changing the temperature to the corresponding volume-phase transition temperature (VPTT) the hydrogel performs a volume change.[5] This behavior is illustrated in figure 2.4.

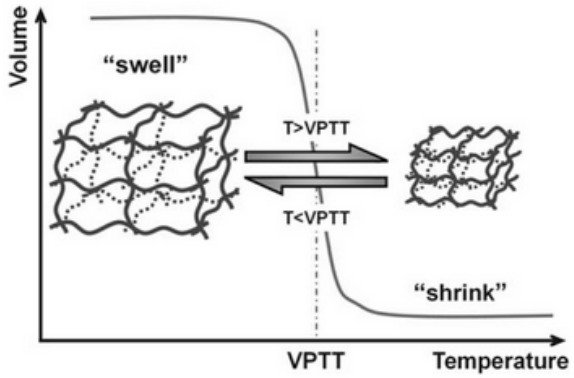


Figure 2.4: Schematic illustration of the volume change of a hydrogel by changing the temperature. Reprinted from [5].

For *p(NIPAAm)* this VPTT is actually a lower critical solution temperature (LCST), describing the temperature below which the material is fully miscible in water and above which phase separation takes place. The physical background of this is that the change in entropy for mixing is negative due to strong inter-molecular bonds, preventing the polymer from random mixing in water. The mixing below the LCST is not due to entropy, but due to the enthalpy of formation of the hydrogen bonds with water. The LCST for *p(NIPAAm)* is about 32 $^{\circ}C$, which makes it a promising candidate for biomedical application as it lies close to body temperature.[20]

In the hydrated, swollen state the polymer backbone has a flexible coil structure, where the hydrophilic sites point in the direction of the surface. Thereby water molecules can form H-bonds with the hydrophilic sites, increasing the thickness of the layer. By heating up the environment and exceeding the LCST the coil structure collapses and the polymer backbone forms a globule shape.[22] This is called a coil-to-globule transition. The hydrophilic elements of *p(NIPAAm)* now form hydrogen bonds with each other and the water is repelled out of the system, reaching the shrunken state. This behavior concerning the film thickness is plotted in figure 2.5.

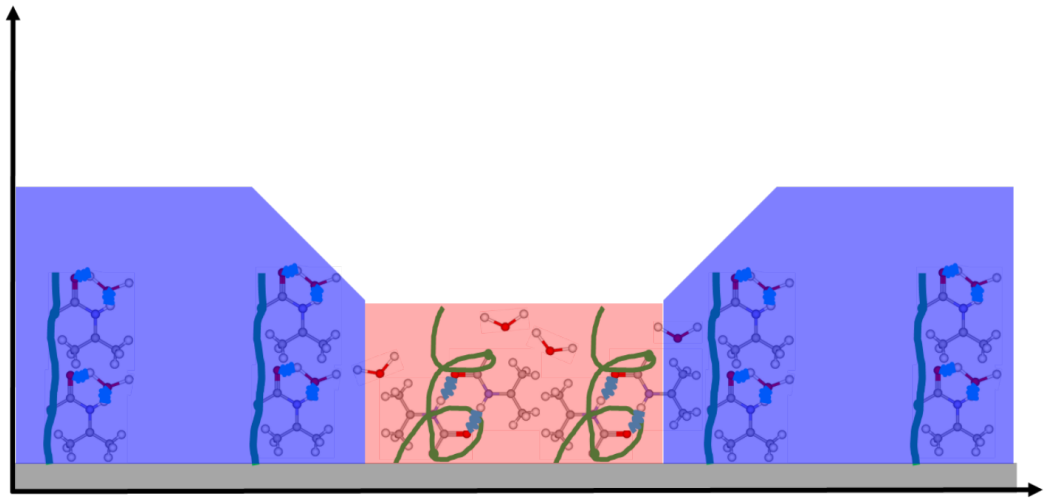


Figure 2.5: Graphical interpretation of a hydrogel film on a substrate, where the thickness difference between hydrated, swollen state (left and right) and dehydrated, shrinken state (middle) is described by a coil-to-globule transition. Reprinted from [24].

As hydrogels are of interest in being implemented in sensors and actuators, the stimuli-responsive swelling of such a film is crucial. The study of swelling is therefore very important, whereby the kinetics of the process determines the response time of an external stimulus, which is influencing the sensing properties.[24]

2.2 Theory of initiated chemical vapor deposition (iCVD)

There are a variety of deposition methods to create thin polymer films. The most common ones are solution based techniques such as spraying, dipping or spin coating. They are infact easy and fast to perform but show quite some limitations concerning substrates that can be used. A rather new approach for thin film deposition is a technique called initiated chemical vapor deposition (iCVD). Since it is solvent-free, iCVD allows to coat a variety of substrates with conformal coatings in the nanometer scale.[16]

In iCVD free radical polymerization, gas phase monomers are converted directly into thin solid macromolecular films.[13] These films are mostly amorphous but can also exhibit crystalline structure. Since most of the organic monomers suitable for this process arise as liquids or solids, they need to be evaporated by heating the substance up. This enables the flow of the precursor molecules in to the reaction chamber. In the chamber prevail vacuum condition to minimize contamination through organic impurities. In the first step of figure 2.6, where the iCVD reaction process is shown, the initiator (I) is thermally decomposed by heated filaments after entering the chamber, forming primary radicals (R). These radicals and the monomer precursor (M) are adsorbed from the vapor phase onto the substrate surface. Polymerization takes place at the surface through radical addition via initiation, propagation, and termination events to form the final polymer (P) coating (see figure 2.6).[16] The temperature of the filaments is at about 250 $°C$ which is sufficient to break the labile oxygen-oxygen bond of the initiator but low enough to prevent decomposition of the monomers (for which a temperature of over 500 $°C$ would be needed). By flowing in not just one but a mixture of monomers, co-polymerization can be achieved as the film grows. In the iCVD process alternating and random co-polymerization takes place.

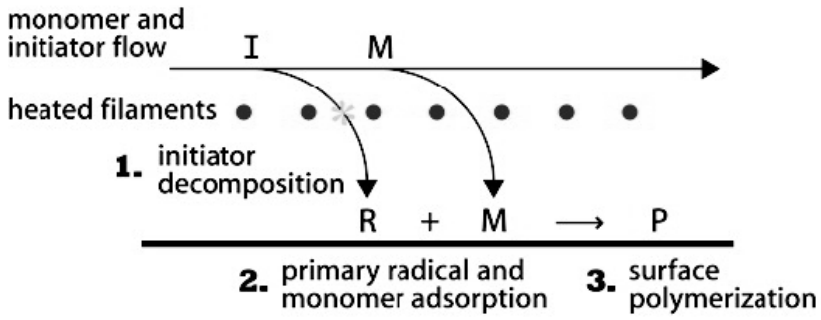


Figure 2.6: Illustration of the three major steps for the iCVD process. Adapted from [16].

The iCVD process is exploiting the advantages of chemical vapor deposition, which are conformal coating, nanometer scale thickness control, the absence of solvents and the chemical tunability and combines it with the full retention of the functional groups.[16] The initiator radicals only react with the vinyl bonds of a monomer thus leaving the other groups unharmed. This is of great importance if the specific functionality of the polymer is desired which is the case for stimuli-responsive hydrogels.

In the iCVD process the monomer flow rate as well as the temperatures of the substrate and the filament contribute to the deposition kinetics. Low substrate temperatures for example enhance the absorption of monomer units thus increasing the deposition rate. However, it is the ratio between the monomer partial pressure (P_m) and the saturation pressure at the substrate temperature (P_{sat}) that is the dominant parameter.[8] This ratio is also called monomer saturation ratio and it not only determines the concentration of monomer absorbed on the surface but also the degree of conformality and the film growth rate. If this ratio approaches unity, a liquid would be in equilibrium with the monomer vapor. Because this is undesirable in the iCVD process a ratio below one is typically used which decreases the deposition rate of the film but results in more homogeneous and higher molecular weight films.[13]

2.3 Dissolution testing

In pharmaceutical technology drug dissolution testing is an important tool to provide drug release information of oral solid dosage forms. It not only serves as control mechanism for quality but also to predict the in-vivo drug release profiles.[9] This is important because in the human body the active pharmaceutical ingredient (API) has to be dissolved in order to be absorbed by the blood to show its therapeutic effect. Dissolution is the process in which a solid matter is put into a liquid medium where its original components dissolve to form a solution. The liquid medium hereby is called a solvent.

Typically the API is filled into a capsule or directly pressed into a tablet form thus serving as oral dosage form. A new approach is to use stimuli-responsive hydrogels as polymeric coating of the drug to achieve controlled drug delivery. The hydrogel coating acts as a shield from environmental influences and at the same time releases the drug only at a specific stimulus. This can be again for example be temperature, pH or light as explained in chapter 2.1.1. To test the amount released by such a hydrogel-model drug delivery system, the samples are immersed into a relevant dissolution medium. To mimic the human body it is preferably to use a buffer solution with a low pH value, as it would be in the stomach region ($pH \sim$ 1-2). The drug release is then evaluated by a photometric measurement, whereby in this thesis a specific chromatographic apparatus is used, explained below.

2.3.1 High performance liquid chromatography (HPLC)

Chromatography in general is a process where a mixture of substances is separated into their components, also called analytes, by distributing them between two phases in the chromatographic bed. The mobile phase acts as a carrier of the analytes and flows through a stationary phase, which is mostly a solid with a high surface area. If the mobile phase is a liquid this process is called liquid chromatography. If in this liquid chromatography technique high pressures are used to pump the mobile phase through the stationary phase to achieve a faster performance one is speaking of high performance (or pressure) liquid chromatography (HPLC). The separation process is explained and depicted below.[18]

(a) A mixture with the components • and ▲ is applied to the chromatographic column.

(b) The component • has more affinity with the mobile phase, ▲ with the stationary phase.

(c) A new equilibrium is achieved by addition of fresh eluent: sample molecules are partly adsorbed by the stationary phase whereas previously adsorbed ones appear again in the mobile phase.

(d) Separation arises as this process is repeated since the component • is traveling through the column faster than component ▲

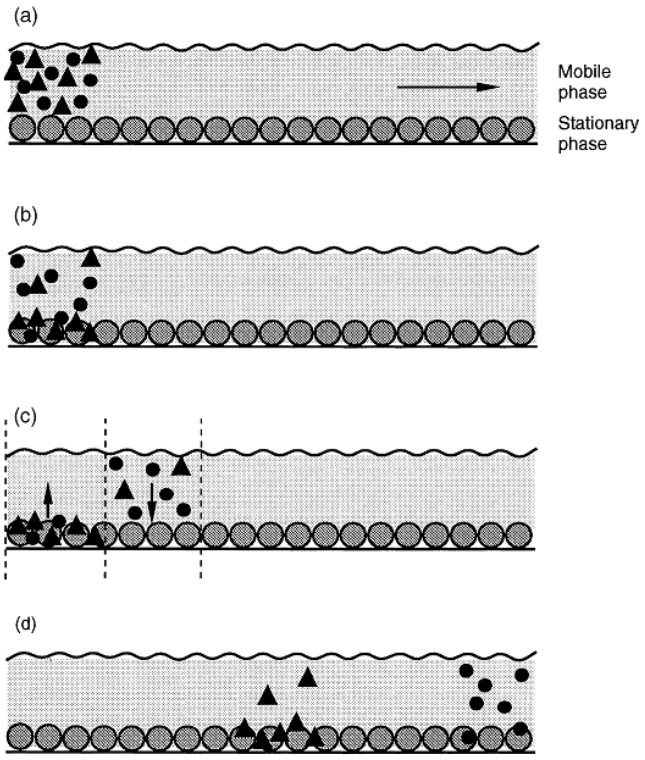


Figure 2.7: Graphical representation of the separation process in the chromatographic column of a HPLC apparatus. Reprinted from [18].

CHAPTER 3

Experimental

3.1 Deposition of drug films

In this work two different approaches to the deposition of the drug layer onto either silicon or glass substrates were performed. Since they are both solution based techniques it was really important to clean the substrates properly before applying the drug layer. Therefore they were sonicated (*ELMA Transsonic 310/H*) in acetone for 15 minutes. Afterwards they were rinsed with deionized water and dried under an air stream. For large impurities it was tried to carefully clean the sample mechanically with a dust free tissue.

3.1.1 Spin coating

Spin coating (*CHEMAT SPIN COATER KW-4A)* is a deposition method were thin films are achieved by spreading a liquid solution over a spinning substrate. The angular speed of spinning and the concentration and amount of the solution influence the thickness of the film. The solvent for the solution is typically a volatile one so it just evaporates during the process leaving the desired substance on the substrate.

For the depositions in this work an angular speed of 2000 rpm for 60 seconds was found to give layers of about 150 nm when dropping 200 μL of a 30 mg/mL solution on to the substrate. This concentration was achieved by first weighting out 90 mg (*SARTORIUS Entris*) of the particular drug and then adding 3 mL of the organic solvent tetrahydrofuran (THF). The vial

with these two components was then vortexed for a couple of seconds. This enhanced the degree of dissolution of the drug within the solvent significantly.

The huge advantage of spin coating is that the thin films deposited are typically very flat and homogeneous. The main problem though is that because of the fast rotation some of the substance is spinning off the substrate, so it is impossible to determine the precise amount of deposited drug.

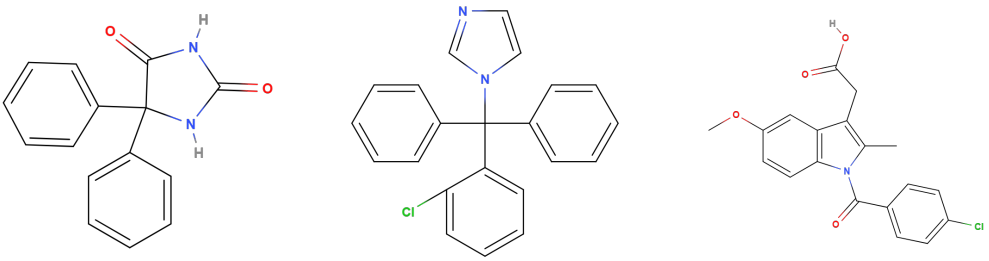
3.1.2 Drop casting

Another rather easy solution based deposition technique is drop casting. The solution is dropped on a substrate and left untouched until the solvent is evaporated. Therefore the substrate must be positioned very horizontally to avoid that the liquid drops off the edge. The films achieved with this simple approach may not be as homogeneous as with spin coating but the control over exact amount of drug deposited is given, which is essential for the dissolution experiments performed within this work.

3.1.3 Active pharmaceutical ingredients (API)

Three different model drugs were used for the determination of drug release in the dissolution testing. All the APIs were used without any further purification. Their chemical structure can be found in figure 3.1 (exported from the MolView open-source project [1]). These three model substances were chosen because of their property to be stable in the vacuum conditions necessary for the iCVD deposition that was later on performed on top of the drug films. As a result they can easily be processed without sublimation of the drug layer itself.

- phenytoin (IUPAC name 5,5-diphenylimidazolidine-2,4-dione) is an anti-seizure medication.
- clotrimazole (IUPAC name 1-[(2-chlorophenyl)-diphenylmethyl]imidazole) is used in anti-fungal treatments but is also currently under investigation for a malaria medication.
- indomethacin (IUPAC name 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methylindol-3-yl]acetic acid) is an anti-inflammatory pain killer.



(a) phenytoin (b) clotrimazole (c) indomethacin

Figure 3.1: Chemical structures of the drug molecules. Reprinted from [1].

3.2 Producing polymer thin films using initiated chemical vapor deposition (iCVD)

As already in chapter 2.2 explained, the polymer deposition technique chosen for this work was iCVD. Therefore a custom build reactor setup was used as depicted in fig 3.2. The chemicals for the iCVD process were liquid or solid in their original state and has to be evaporated to grant a gas flow. This evaporation process was achieved by a heater (*OMEGA MCS-2110K-R*) and controlled by a thermocouple, which were both connected to the chemical jar. The flow rate was controlled by a needle valve, leading the gas into a line system that fed into the vacuum chamber of the reactor. It was of great importance to set the temperatures long enough before starting the deposition to ensure that a thermodynamic equilibrium prevailed in the system. The line system where the vapors travel through was held at 100 $°C$ to avoid condensation. For some depositions a fixed nitrogen flow rate was installed serving as a patch flow. Since the initiator for the process was kept at room temperature it had its own line leading into the chamber.

The substrate temperature was controlled by a chiller (*THERMO SCIENTIFIC ACCEL 500LC*) connected to the bottom of the reactor, where temperatures in the range of 20 $°C$ to 50 $°C$ could be applied during the deposition. Another important step to set up the iCVD process was the in-situ thickness measurement during the deposition. Therefore a pristine silicon wafer (*Siegert Wafer, Germany*) was put in the middle of the reactor where it was illuminated by a He-Ne Laser ($\lambda = 633\ nm$, ThorLabs). The Laser shined through the glass lid and reflected off the silicon wafer into the detector where a change of film thickness was recorded through a change of intensity. It was preferable to position the substrates for coating near to this reference. The technique should produce the same film thickness all over the reactor but experience showed that there was typically more thickness deposited near the inlet of the monomer vapors.

After placing the substrates on the reactor bottom the connected rotary vane pump (*PFEIFFER VACUUM DUO 65*) was creating a base pressure in the range of 1 $mTorr$. After reaching this base pressure the leak rate of the system was measured. A *LabView* program was used, which drove a pressure control unit (*MKS 600 series pressure controller 651CD2S1N*) connected to an electrically adjustable valve that linked the pump with the vacuum chamber of the reactor. The leak rate was calculated by closing this valve and monitoring the pressure increase in time. The next step was to adjust the flow rates of the individual chemical components necessary for the iCVD process, starting with the most volatile one. Opening the needle valve to one of the jars con-

taining the tested chemical allowed a vapor flow in to the reactor. Now again the pump was disconnected and the increase in pressure was recorded by the *LabView* software, which calculated the flow rate in standard cubic centimeter per minute (*sccm*).

When the flow rates were set up, all the lines were opened creating a mixture of monomer, cross-linker and initiator vapor in the reactor chamber. The deposition was performed at a constant working pressure of either 200 $mTorr$ or 350 $mTorr$ depending on the other deposition parameters, explained below. As soon as a constant working pressure was achieved the filament array of nickel-chromium wires (*Goodfellow, UK*) was heated up to about 250 $°C$ by driving a current through them, provided by a power supply (*HEINZINGER PTN 350-5*). As soon as this temperature was reached the decomposition of the initiator began and thus the deposition of the layer started.

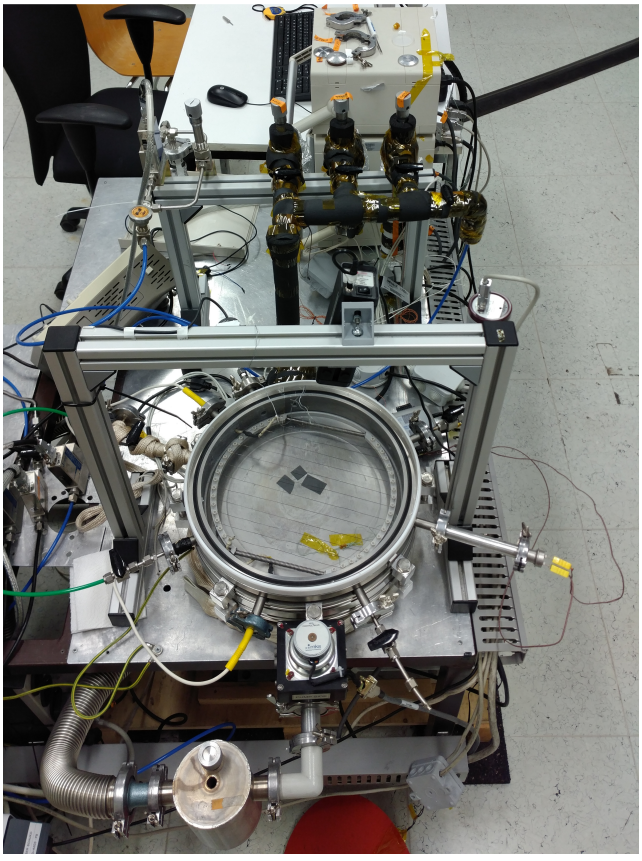


Figure 3.2: Picture of the custom build iCVD reactor standing in the 3^{rd} floor lab at the institute of solid state physics at the TU Graz.

3.2.1 Chemicals

The following chemicals were used as monomer, cross-linker and initiator in the iCVD process:

- N-isopropylacrylamide (*NIPAAm*, 99%, Aldrich, Germany)
- di(ethylene glycol) divinyl ether (*DEGDVE*, 99%, Aldrich, Germany)
- tert-butyl peroxide (*TBPO*, 98%, Aldrich, Germany)

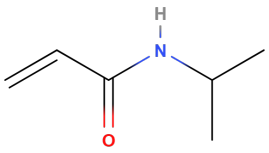
The essential parts in the chemical structure of the monomer (*NIPAAm*, 3.3a) are not only the carbon double bonds where the polymerization takes place but also the hydrophilic sites. Especially the NH group is important for the swelling, which makes this polymer a hydrogel. For the cross-linker (*DEGDVE*, 3.3b) there are two carbon double bonds present at the end of the chain which enables them to polymerize on both sides and interconnect polymer chains.

As initiator a peroxide was used where the labile oxygen bond is broken up by heated filaments. All the chemical structures are shown in figure 3.3 (exported from the MolView open-source project[1]).

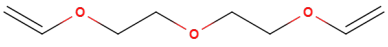
3.2.2 *p(NIPAAm-co-DEGDVE)* depositions

This deposition series was executed with the monomer *NIPAAm* and the cross-linker *DEGDVE* producing a *p(NIPAAm-co-DEGDVE)* thermo-respon-sive hydrogel. The base pressure for all depositions was at about 1 $mTorr$ while the leak rate was kept between 0.01 and 0.03 *sccm*. Also a substrate temperature of 30 $°C$, a filament temperature of about 220 $°C$ and a working pressure of 200 $mTorr$ was established throughout the deposition series. The temperature for the monomer and the cross-linker was kept at 85 $°C$ and 70 $°C$ respectively, reaching a flow rate of 0.25 $\pm$ 0.05 *sccm* for *NIPAAm* and 0.80 $\pm$ 0.15 *sccm* for *EGDMA*. The initiator *TBPO* was kept at room temperature with a flow rate of 0.50 $\pm$ 0.05 *sccm*. The thickness of the grown layers was ranging from 200 nm to 600 nm.

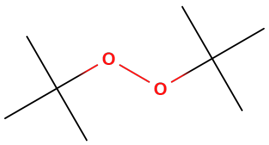
Silicon substrates with the dimension of (2 x 2) cm and (5 x 2) cm were installed. For the dissolution experiments also glass slides of (2.5 x 2.5) cm with a drug layer were coated with the polymer layer.



(a) *NIPAAm*



(b) *DEGDVE*



(c) *TBPO*

Figure 3.3: Chemical structures of the monomer and the corresponding cross-linker for a *p(NIPAAm-co-DEGDVE)* thin film, with *TBPO* as initiator. Reprinted from [1].

3.3 Thin film characterization using atomic force microscopy (AFM)

Morphological characterization was performed by atomic force microscopy (*FlexAFM Nanosurf*). This is a scanning technique where the sample surface is probed by a cantilever with a tip (*TAP 300, Budget sensors*) that is guided over the sample in xy-direction by piezoelectric elements. The deflection of the cantilever in z-direction, induced by the sample, is measured by a Laser that is reflected off the cantilever itself and detected by a segmented photodetector. All measurements in this work were performed in non-contact mode where the cantilever is oscillating at a specific frequency. When approaching the sample the forces from the surface, such as van-der-Waals forces, are interacting with the cantilever thus decreasing its resonance frequency. By adjusting the distance between the tip and the surface the frequency is held constant and a topographic image of the sample can be constructed.

The AFM used for the measurements was connected to a Easyscan 2 controller and mounted on an attenuation table. For specific measurements there were two different heads available for the AFM which contained the cantilever and the tip. The small one was used for probing sizes below 10 μm and the big one for above 10 μm. The investigated sample was placed in the middle of the AFM while the head with the tip was aimed to a region of interest. After a quick setup by hand, the provided software was used to drive the tip close to the surface of the sample. Before the acquisition the software also defined the parameters used for the measurement, as for example the free vibration amplitude, the size of the image and time frame of the measurement, thus the resolution. Another important parameter was the setpoint, which basically defines the force applied by the tip to the sample. This of course had to be set differently for every sample that was investigated by AFM. Recorded images were afterwards processed using the freely available software package Gwyddion.

3.4 Spectroscopic ellipsometry

Since this work is mainly about thin films it is very important to have a suitable thickness analysis tool. A tecchnique that provides a lot of information for polymer layers is spectroscopic ellipsometry (*J.A. Woollam M-200*), which uses polarized light to characterize thin film materials. The incident linear polarized beam interacts with the surface layer of the sample, changing the polarization, which is shown in figure 3.4. This change in polarization is described by the raw measurement of the ellipsometer, represented by the amplitude ratio ($tan(\Psi)$) and the phase difference (Δ) as followed:

$$tan(\Psi)exp^{i\Delta} = \frac{\widetilde{r_p}}{\widetilde{r_s}} \tag{3.1}$$

Thereby $\widetilde{r_p}$ and $\widetilde{r_s}$ are the Fresnel reflection coefficients for the p- and s-polarized light respectively. Because the signal depends on the thickness as well as the material properties, ellipsometry can be an universal tool for contact free determination of thickness and optical constants of films of all kinds.

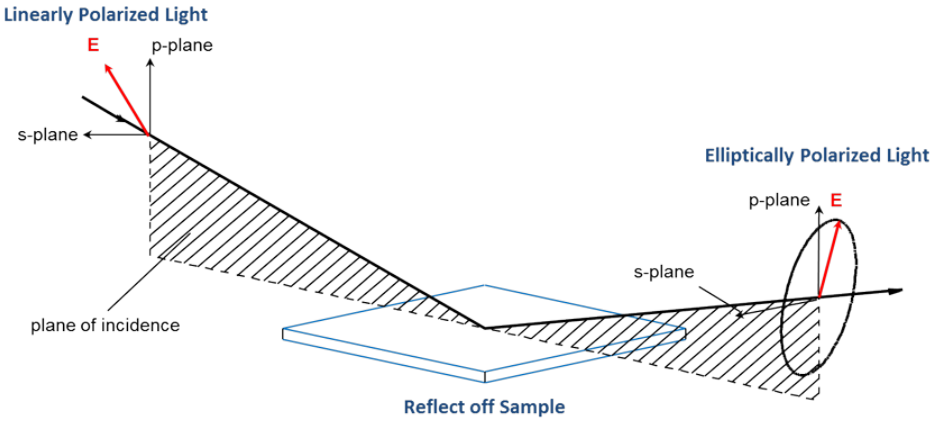


Figure 3.4: Schematic drawing of the light path occuring during an ellipsometry measurement. Reprinted from [15].

For measurements in ambient air the ellipsometer was a very easy to handle and fast analysis tool. The specimen was placed in the middle of the basic stage so it got hit by the incident beam. This setup is depicted in figure 3.5. A short alignment procedure was guided by the provided software (*CompleteEASE ®, J.A.Woollam*) to ensure a good illumination of the detector. The acquisition was performedd at three angles (65, 70 and 75°) in the wavelength range from

370 to 1000 nm. For the analysis the Cauchy-Model was used, which estimated the refractive index (n) of the film by fitting the coefficients A, B and C.

$$n(\lambda) = A + \frac{B}{\lambda^2} + \frac{C}{\lambda^4} \tag{3.2}$$

The fitting was performed with the provided software where the experimental data was compared to a model consisting of three layers. On top of the bulk silicon was a 1.7 nm native silicon oxide layer as prepared from the manufacturer. Again on top to describe the polymer thin film a Cauchy layer was used, with the coefficients from equation 3.2.

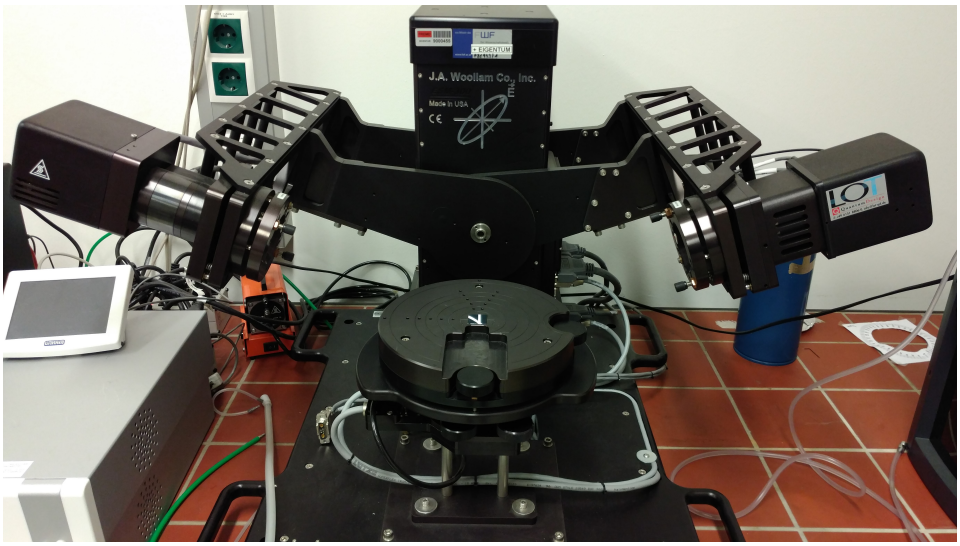


Figure 3.5: The ellipsometry setup with the basic stage attached for measurements in air.

Since the swelling behavior of hydrogels is crucial for the investigation, there was also an experimental setup where the sample was put in a liquid environment. This was done by installing another stage for the ellipsometer and equipping it with a so called liquid cell (see figure 3.6). For the sample to fit for the liquid cell it has to be a bit bigger than the o-ring of the cell, hence they has the size of (5 x 2) cm. The cell was screwed tightly on top of the tested sample. Because of the structure of this attachment the measurement was only performed for a fixed incident angle of 75°. The alignment was done with the liquid cell attached but without any liquid inside. The substance that served as environment for the experiment was filled into a syringe prior the

experiment. During the acquisition the syringe was led to a nozzle where the substance was injected into the liquid cell, surrounding the polymer film of the sample. An example of such a swelling behavior is schematically plotted in figure 3.7.

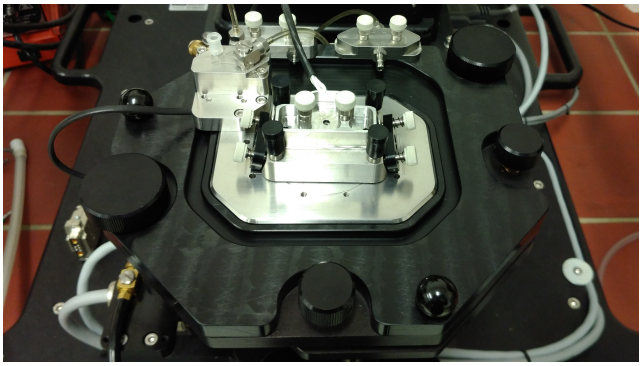


Figure 3.6: Close up picture of the liquid cell that can be attached on to the stage of the ellipsometer for in-situ thickness measurements of the hydrogel layer in liquid environment.

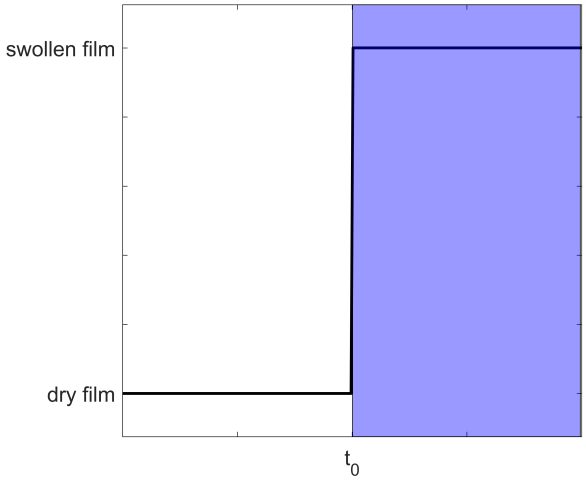


Figure 3.7: Schematic drawing of a swelling experiment of a hydrogel thin film. At t_0 the liquid is applied to the liquid cell where the thickness of the film increases from the dry to the swollen state.

A heating element connected to the setup enabled the investigation the temperature response of the hydrogel thin films. Therefore a temperature ramp was applied, starting at room temperature and going up to 50 $°C$. Since we also wanted to investigate the temperature response below room temperature the whole liquid cell was stored in the fridge for a couple of minutes. When it reached a cooler temperature the cell was installed in the ellipsometer again and the acquisition was started. For this heated swelling an schematic example is plotted in figure 3.8.

For the analysis of a swelling experiment we used a model called effective medium approximation (EMA). Within this model the analyzed sample is treated as composite material of polymer and voids, where the latter is present as spherical inclusions within the polymer matrix. These voids can be filled with liquid and thereby induce the thickness increase of the hydrogel. The model mixes the optical constants of both the liquid used for the experiment and the Cauchy layer as applied before and retaining a percentage value for each composite material for the measurement.

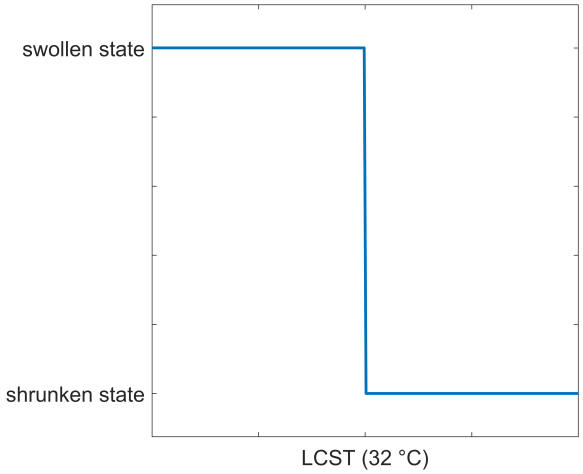


Figure 3.8: Schematic drawing of the sharp thickness transition at the LCST (about 32 $°C$ for *p(NIPAAm)*) of a hydrogel thin film. There is a change from the hydrated, swollen state below the LCST to a dehydrated, shrunken state at higher temperatures.

3.5 Dissolution experiments

With a dissolution experiment, the amount of drug released over time in a specific dissolution medium was measured. For the dissolution experiments in this work a custom build setup was used, as depicted in figure 3.9. For every dissolution three samples were prepared with the same conditions to obtain a small statistic. To simulate the environment of the human body the setup contained heating plates with temperature control (*VELP Scientifica AREX Digital Pro* and *Heidolph EKT 3001*) which were set to 37 $°C$. For various approaches this temperature was changed to 25 $°C$ to also test the thermo-responsive features of the hydrogels. Since there was only one thermometer available per heater, the three jars were placed in a water bath, which was set to the corresponding temperature. The heating plates were mounted on a moving table (*AL-Labortechnik OS 10 control*) set to about 100 rpm to retain a nice mixing within the jars.

As a dissolution medium a buffer solution of NaH_2PO_4 was used. To achieve pH values in the acidic regime trifluoroacetic acid was added while controlled via a pH meter (*SI Analytics Lab 860*). Again this was done to mimic the conditions in the human body since there is acidic environment inside the stomach for example. In contrast to achieve a more neutral pH value $NaOH$ was added to investigate if the dissolution varied with pH. 50 mL of this buffer solution was filled into every specimen jar. These jars should be closed at any chance to minimize the evaporation during the experiment.

For the evaluation of the drug release with HPLC (see chapter 4.4) also a internal standard (ISTD) had to be prepared. Therefore one of the drugs was dissolved in acetonitrile (ACN) with a predefined concentration, which was added to the collected dissolution probes. When evaluating the measurements with HPLC, the predefined concentration was monitored as well and helped eliminating experimental errors that might occurred during the process.

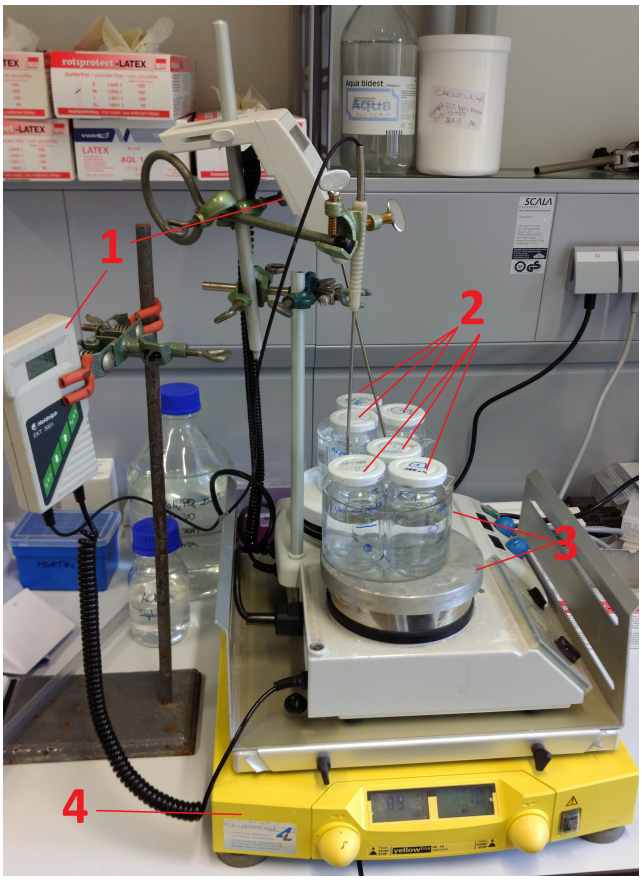


Figure 3.9: Picture of the custom build setup for the dissolution testing. Six specimen jars (2) were placed into temperature controlled (1) water baths, standing on heating plates (3), which were mounted on a moving table (4) to facilitate mixing of the dissolution medium.

3.5.1 Sample preparation

The sample preparation started with cutting glass slides (*Roth, Germany*) into pieces of (2.5 x 2.5) *cm*, serving as substrates. These were subsequently cleaned in an acetone ultra sonic bath, rinsed with deionized water and dried under an air stream.

The next step was to prepare the solutions for drop casting to create the drug layers. Therefore the drugs were weighted out and dissolved in tetrahydrofuran as listed in table 3.1. A concentration of the dissolved drug in the 50 mL of buffer solution in the range of the detection limit of the HPLC had to be achieved. From these solutions 350 μL were drop casted on the substrates as explained in chapter 3.1.2.

Table 3.1: Drug solution concentrations for dissolution testing.
c_{THF}...concentration of drug in tetrahydrofuran (THF)
M...weight of drug deposited by drop casting 350 μL of the c_{THF} solution
c_{buffer}...concentration of the dissolved drug in 50 mL buffer solution

drug	c_{THF} [mg/mL]	M [mg]	c_{buffer} [μg/mL]
phenytoin	2.5	0.875	17.5
clotrimazole	28.6	10	200
indomethacin	0.286	0.1	2

3.5.2 Measurement procedure

The dissolution experiment started by putting the prepared samples into the specimen jars which were already prepared with the dissolution medium at the right temperature. Since this could not be done simultaneously for all three samples there was a gap of 20 seconds between the insertions. The first dissolution probe was then acquired after 2 minutes by taking 500 μL out of the specimen jar into a small vial. Also added were 500 μL of the internal standard (ISTD). Then 500 μL of pure buffer solution was filled back into the specimen jar to keep the amount of dissolution medium constant. This of course had to be considered when calculating the release of the drug. For every dissolution experiment a total of 21 probes were acquired over a total time frame of 28 hours.

When doing the dissolution testing some small problems emerged. Even though it was tried to always keep the specimen jars closed, evaporation occurred, assisted by the elevated temperature. Also condensation was observed

on the lid of the jars. When removing them for the accumulation of a probe sometimes a bit of solution dropped off. Nevertheless the amount of dissolution medium could be kept at about 50 mL for all times.

3.5.3 Chemical analysis using high performance liquid chromatography (HPLC)

To perform a HPLC (*Agilent Technologies 1260 Infinity Quat Pump VL*) measurement the samples obtained from the dissolution had to be further processed. At first the vials with the samples were concentrated by evaporation of the buffer solution and solvents present. That was achieved by heating the open vials up to 95 $°C$ and simultaneously streaming nitrogen into the vial opening (*TECHNE Sample Concentrator*). Remaining was not only the desired drug in its solid form but also the internal standard and the salt residue from the buffer solution. After cooling down to room temperature, 100 μL of acetonitrile (ACN) was added to again dissolute the drug and internal standard. For a good mixing 20 seconds of vortexing was applied. These 100 μL were then transferred into a new vial which was ready to be installed into the HPLC.

Necessary for the evaluation was furthermore the recording of a calibration curve. A solution of the probing drug in ACN was prepared with a concentration of 10 $\mu g/mL$ for phenytoin, 1.6 $\mu g/mL$ for clotrimazole and 5 $\mu g/mL$ for indomethacin respectively. This concentration represented the 100 % solution and is consecutive diluted to solutions with 80 %, 60 %, 40 %, 20 %, 10 % and 1 %. 1 mL of each of these solutions was mixed with 500 μL ISTD of the corresponding dissolution experiment in a vial which was then build in to the HPLC. The calibration curve was later on used to match a detected signal to the predefined concentration of the probes. By starting with the lowest concentration the effect of carrying over was minimized.

Before starting a measurement with HPLC the lines where the liquid mobile phase was traveling through the system, which were typically stored in 90 % methanol (10 % Milli-Q water), was washed out by pumping a solution of 90 % Milli-Q water (10 % methanol) through. The chromatographic column (*phenomenex Luna 5U C8 100A*), which contained the 5 μm small silica globes as stationary phase, was build into the apparatus. A blank run, without any chemical injection, was performed through the column to get it ready for the actual measurements.

A schematic diagram of such a HPLC process is shown in figure 3.10. At first the HPLC solvent (mobile phase) was filled into the pumping cycle of the apparatus. As liquid mobile phase in our experiments a gradient was used by

mixing 20 % ACN with 80 % Milli-Q water with a pH of 3. This mobile phase was pumped together with the sample, which was collected by the injector, through the chromatographic column with a pressure of 117 *bar*. The sample interacted with the stationary phase as explained in chapter 2.3.1. At the end of the column the analytes were detected by a UV-VIS detector, working at a wavelength of 210 *nm*.

To retain a value for the concentration of the sample, the signal detected by the HPLC was then recalculated using the concentration of the predefined ISTD and the calibration curve.

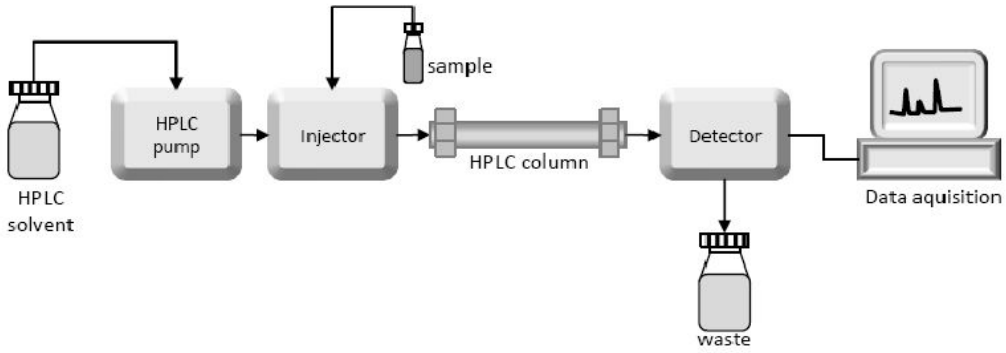


Figure 3.10: Schematic diagram of the HPLC instrumentation. Reprinted from [12].

CHAPTER 4

Results and Discussion

4.1 Characterization of the pristine drug films

In the course of work for this thesis various drug layers were prepared on model surfaces, like glass substrates or silicon wafers. For all tested substances the deposition was either performed by spin coating or drop casting depending on the demands of the further investigation. All the materials were dissolved in tetrahydrofuran (THF) at given concentrations, so that the final amount of material (drug load) could be easily adjusted.

The preparation of phenytoin on any surfaces resulted always in a drug layer consisting of crystalline phenytoin which means that the molecules assemble in a way that a three-dimensional long range order exists. An example is depicted in figure 4.1. X-ray investigations (data not shown) revealed that these samples constisted solely of the stable bulk form of phenytoin. Samples consisting of phenytoin in the amorphous state, where there is no long range order were never observed.[10]

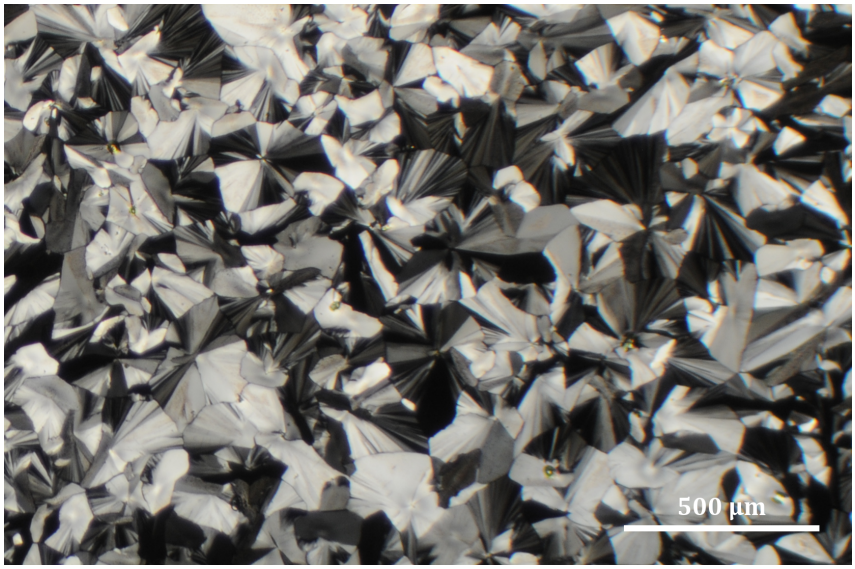


Figure 4.1: Light microscopy image (2.5x magnification) with a polarizer of a spin coated phenytoin layer representing a crystalline state.

On the other hand clotrimazole and indomethacin are both materials for which the deposition technique has a strong impact on the solid state form. In general, having high concentration and low processing speeds both materials assemble in crystalline forms. Using low concentration and fast processing the formation of layers in the amorphous state either from clotrimazole and indomethacin is observed.[11] Since there the molecules assemble randomly without long range order such a film does not provide any morphological relevant information so that images of these films are not provided. In terms of intrinsic dissolution, the lack of a regular lattice means that lower energy is required. On the downside, often the stability of the drug formulation (medication) can not be guaranteed as eventually crystallization might occur. A way of stabilizing the amorphous state can be achieved using a coating of a polymer which will be demonstrated below.[2]

4.2 Characterization of the polymer layers on silicon substrate

For the polymer *p(NIPAAm-co-DEGDVE)* in this work the monomer *NIPAAm* was responsible for the hydrogel characteristics while *DEGDVE* was used as cross-linker. The ratio was estimated to be 60:40 (*NIPAAm:DEGDVE*) for all samples, as experiments from other members of the group showed the most promising swelling ability while remaining stable in a liquid environment. Prior the iCVD deposition the flow rates for each chemical was calculated using P_m/P_{sat} calculation to achieve this ratio in the final polymer thin film.

Before depositing the *p(NIPAAm-co-DEGDVE)* onto drug layers, the behavior of this polymer on a bare substrate was investigated. For this, depositions identical to those later performed for the drug dissolution experiments were prepared using iCVD. Typically a layer thickness of 200 nm was used as a good trade-off between deposition time and overall material on the sample. As the polymer layer grew very conform with the substrate morphology, it was indistinguishable from the bare substrate. An AFM image of such a film grown on a silicon wafer is presented in figure 4.2, where the σ_{rms} is approximately 1 nm, which is about the mean surface roughness of the substrate.

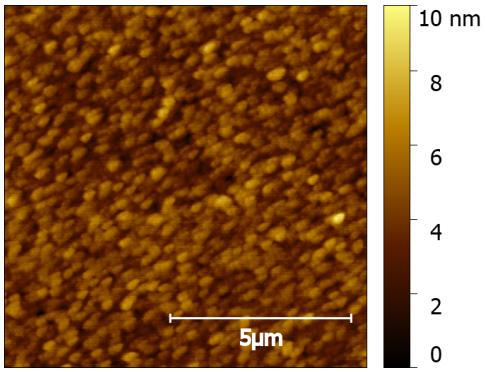


Figure 4.2: AFM image of a *p(NIPAAm-co-DEGDVE)* layer on top of a silicon substrate.

Having the very defined polymer layer allowed then to investigate the swelling of *p(NIPAAm-co-DEGDVE)* when immersed into a liquid environment. Further, the swelling experiments were performed as function of temperature by using in situ ellipsometry as shown in figure 4.3, where a buffer is used as environmental medium. At the beginning (t = 0 min) the initially

dry polymer film is already swollen into a hydrogel at a slightly cooled temperature of 20 $°C$. From this starting point a temperature ramp was driven to 50 $°C$ (ΔT/ Δt = 1 $°C$/min) and the thickness was monitored. From the highest swelling of 261 nm the layer thickness decreases, whereby the first 5 $°C$ temperature increase results in 12 nm thickness decrease observed after about five minutes. Further heating results than in continuous reduction of the layer thickness. A minimum swollen layer thickness of 198 nm is observed at 50 $°C$. It should be noted that this value is slightly larger compared to the initial dry polymer layer thickness of about 170 nm.

The mechanism that is responsible for this swelling behavior is a structural transition of the polymer chains at the lower critical solution temperature (LCST). For the tested *p(NIPAAm-co-DEGDVE)*, the LCST is at about 32 $°C$, where it undergoes a coil to globule phase transition. The hydrated, swollen state of the hydrogel at a temperature below the LCST is collapsing into a dehydrated, shrunken state below the LCST.

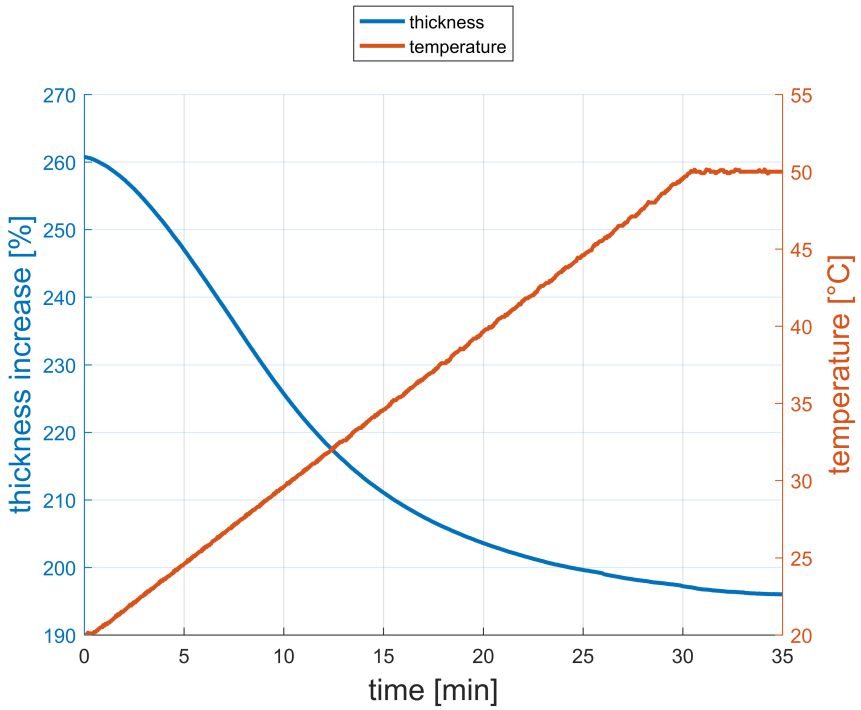


Figure 4.3: Heated swelling curve of *p(NIPAAm-co-DEGDVE)* in a buffer (NaH_2PO_4 at pH = 3) as liquid environment.

The heated swelling with pure buffer is representative for the beginning of the dissolution experiment were all of the drug is still present beneath the hydrogel coating. After some time though the drug is also in solution with the liquid environment meaning there is a change in the dissolution medium. To investigate if there is also a change in the temperature-responsive swelling behavior, a buffer solution containing a phenytoin concentration of about 15 $\mu g/mL$ was used (see figure 4.4). Here again, as in the experiment before, the same temperature ramp was driven, resulting in a similar course of the thickness decrease. For this sample the initial dry thickness of the polymer layer was about 190 nm, which is increased to about 226 nm as the slightly cooled liquid environment is applied. Then, again as in figure 4.3, the thickness of the swollen hydrogel is decreasing by increasing the temperature reaching a value of 203 nm at a temperature of 50 $°C$.

So comparing the two experiments it can be observed that there is a variation in the total amount of thickness increase from swelling when a drug is dissolved in the medium. The film shows swelling of about 50 % of its dry thickness in pure buffer, while close to 20 % swelling is monitored in the buffer-drug solution. The difference in layer thickness change on similar temperature difference may be very similar to the effect known as salting-out.[23] Hereby the monomer units start interacting with a species in the environment, which here might be H-bonding of hydrophilic sites with the phenytoin polar groups. As a result the interaction with the surrounding medium changes, thus lower swelling occurs. Likely, the ionic species of the buffer might strongly interact with the monomeric units and therefore might be competing for interaction sites with the drug molecules. Additional measurements would be required to gain further insight in the reason for the poorer swelling.

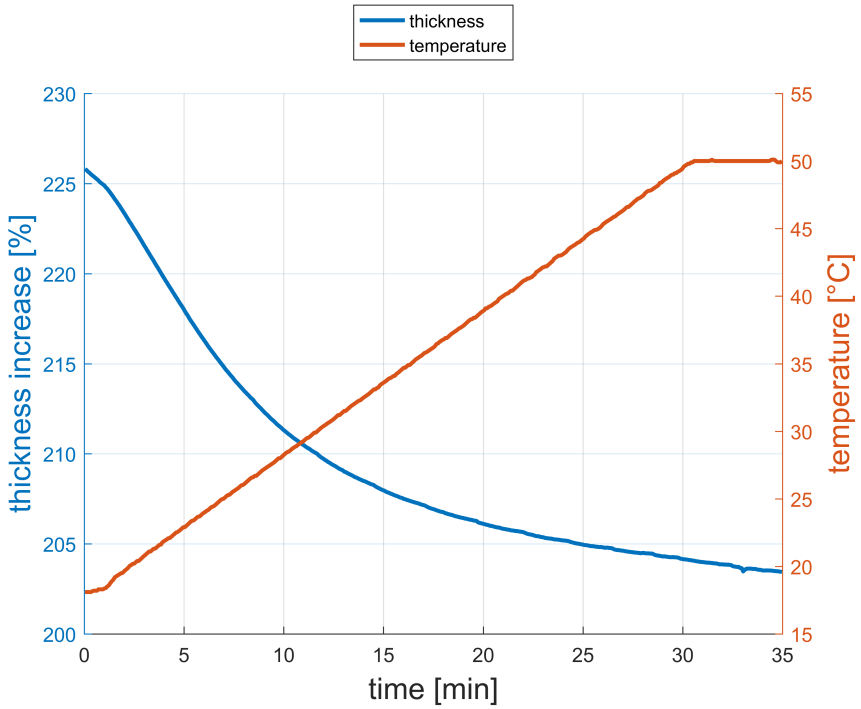


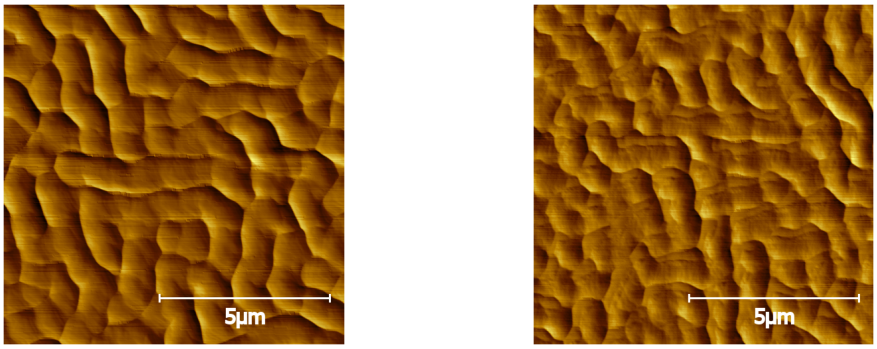
Figure 4.4: Heated swelling curve of *p(NIPAAm-co-DEGDVE)* with phenytoin dissolved in a buffer (NaH_2PO_4 at pH = 3) as liquid environment.

4.3 Impact of iCVD layer on top of drug film

As mentioned before, homogeneous films of phenytoin, clotrimazole and indomethacin were obtained by solution cast methods. Hereby phenytoin assembled in crystalline form and the others were amorphous. These layers were of high stability in the ambient environment but also in the vacuum conditions necessary for the iCVD process. The polymer layer could directly be deposited on top without sublimation or degradation of the films below. During the deposition of the iCVD layer the appearance of the phenytoin thin film remained the same. This is a first indication that the coating was conformal following the surface structure, as confirmed by AFM measurements (data not shown). Using the amorphous layers the situation changed. Shortly after starting the deposition, the appearance of the film, inspected by eye through the glass lid of the iCVD reactor, changed drastically. The initially reflective surface was exchanged by an opaque appearance, suggesting a change in the surface occurred.

A polymer layer deposited on top of an amorphous drug layer exhibits some interesting polymer-drug interaction at the interface, called wrinkling. Due to the different elastic modules of the soft polymer and the more rigid drug layer the initially flat surface shows a wrinkled structure. This wrinkeling is also a function of the amorphous spacer thickness, while it is completely absent on crytsalline films due to their larger elastic modulus.[3]

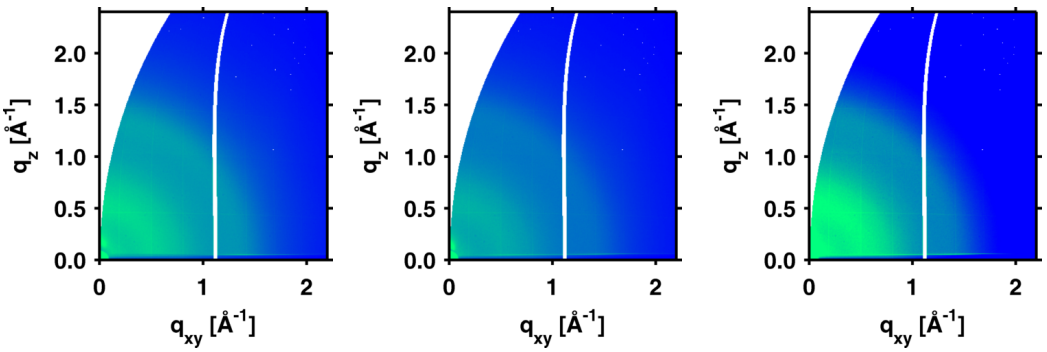
At first, we investigated the solid state of the drug upon iCVD. Recent literature results show that in fact a polymer coating can stabilize the amorphous state significantly.[2] To test this impact of the *p(NIPAAm-co-DEGDVE)* on the amorphous state, AFM measurements were performed to check the morphology of the samples before and after a heat treatment. Exemplarily, films of clotrimazole were spin coated on to silicon substrates. The initially amorphous drug film was then coated by a nominal 200 nm thick *p(NIPAAm-co-DEGDVE)* using iCVD. Afterwards the samples were either stored at ambient temperature, at 50 $°C$ or at 70 $°C$ for 48 hours. In both cases shown in figure 4.5) the surface hosts these wrinkles described above, indicating that there is an amorphous drug layer present thus the iCVD layer prevented the layer below from crystallizing.



(a) As prepared (b) Heated to 70 $°C$ for 48 h

Figure 4.5: AFM images of an amorphous clotrimazole layer coated with *p(NIPAAm-co-DEGDVE)*.

Another proof that the drug layer remained in the amorphous state can be taken from grazing incidence x-ray diffraction experiments, which were performed during a short research stay at the XRD1 beamline at the Elettra Synchrotron in Trieste. The measurements of the various samples are shown in figure 4.6, whereby a reciprocal space map representation was chosen.



(a) As prepared (b) Heated to 50 $°C$ (c) Heated to 70 $°C$

Figure 4.6: Reciprocal space map of three samples with a clotrimazole layer coated with *p(NIPAAm-co-DEGDVE)*.

In such an image, spots of high intensity results from diffraction of regular ordered materials. The direction along the q_z represents order parallel to the surface and the direction along q_{xy} represents order along the substrate surface. In fact, all the measurements reveal high intensity around $q_z = q_{xy} = 0$ $\mathring{A}$ resulting from the primary beam being reflected and most likely some diffuse

scattering on account of the wrinkeling. At arround $|q| = 0.5\ \mathring{A}$ a broad ring appears, which is prototypical for diffraction of a polymer with some degree of order. Adjacent polymer chains assemble likely at certain distances to another but with some random deviation. Further, the ring means that there is no preferred orientation with respect to the surface present thus spatial random order of this common distances exists. Another ring is located at $|q| = 1.4\ \mathring{A}$ which again results from the polymer.

Calculating the interplanar spacing by $d = 2\pi/|q|$ it follows a distance of 1.25 nm from the first ring and 0.48 nm from the second ring. A large distance is often associated with backbone-backbone spacing, if side chains are attached. As the *DEGDVE* has tendency to link and therefore separate adjacent backbones, the distance of 1.25 nm might be most likely being similar to the length of the cross-linker. The distance of 0.48 nm might reflect a close packing of polymer chains were the cross-linker is missing in between. In the reciprocal space map is no further diffraction information present which suggests that the diffraction capability of the drugs is low. In other words the very thin drug layer consists solely in the amorphous state even after the heat treatment. Since this heating process performed with an uncoated clotrimazole layer would transfer it into the crystalline state it can be concluded that the iCVD layer does not harm the solid state of the drug layer but even stabilizes it.

4.4 Dissolution testing

The main goal of the thesis was to investigate the drug release of a model drug coated with a hydrogel layer on top. Therefore samples with a drop casted layer of the different drugs were produced. While half of them were further processed by coating a nominal 200 nm thick *p(NIPAAm-co-DEGDVE)* thin film on top, the other half was kept as prepared. The first dissolution series was performed with a buffer solution of NaH_2PO_4 at pH = 3 as dissolution medium and a temperature of 37 $°C$. These parameters were chosen to mimic the human body conditions as in terms of temeprature and physiological digestive tract pH. The next dissolution series was performed with the same dissolution medium but with a temperature of 25 $°C$ thus beeing below the lower critical solution temperature (LCST) of the hydrogel coating. This temperature regime might not be found in the human body but gives a good insight on the thermoresonsive behavior of the coating and how it affects the drug release as such.

In figure 4.7 the dissolution results for samples hosting phenytoin with and without iCVD coating at the two different temperatures are summarized. Starting with the bare phenytoin sample the drug release shows a steady increase in dissolved drug amount (release in %) as time progresses. Having samples measured at two different temperatures (25 $°C$ and 37 $°C$) about 20 % of the drug load dissolved as 60 minutes past. Hereby the difference in temperature could not produce a significant difference within this short time. As time progresses further, the amount of phenytoin dissolved in the 37 $°C$ samples appears higher in the next 360 minutes. Close to the end of the experiment the phenytoin amount in the dissolution media were very similar for both temperatures and since the value of 100 % release is reached one can conclude that all of the drug dissolved from the sample surface.

The deposition of the iCVD layer changes the dissolution behavior at respective temperatures significantly. Inspecting the sample dissolved at 25 $°C$ reveals a nearly negligible drug release up to times of 180 minutes. Even after 360 minutes less than five percent is released and waiting up to 1440 minutes (24 hours) only results in a release value of about 20 %. From the data present it can not be concluded if the amount of drug release is at its equilibrium value or in other words if about 80 % remain caught in/under the hydrogel layer. At higher temperatures the drug release from the coated samples is remarkable different and a dissolution curve very similar to those without iCVD layer is obtained. Only after 24 hours it seems that a final value of about 65 % release is reached, meaning that 35 % of drug amount is lost in the dosage form.

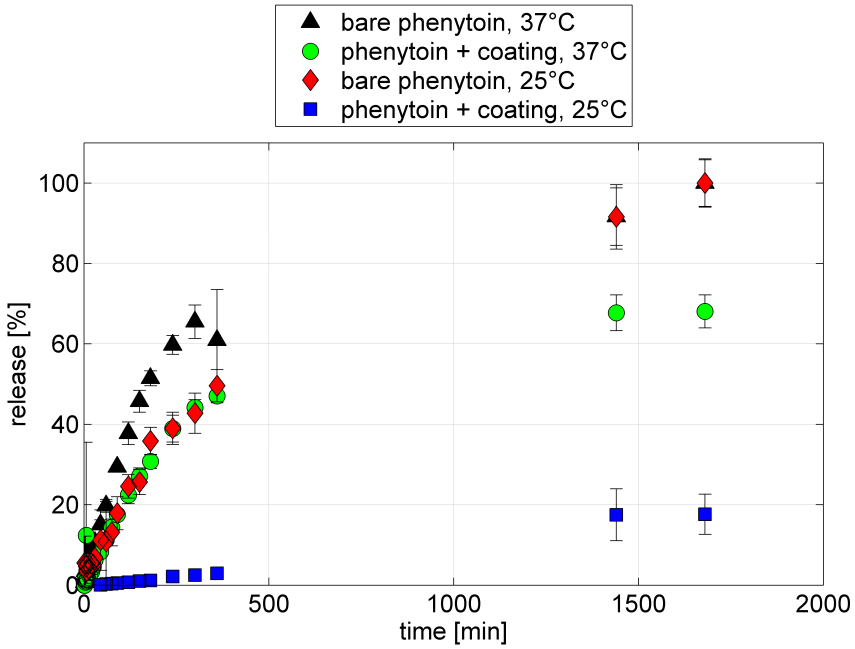


Figure 4.7: Drug release at different temperatures for a phenytoin layer coated with 200 *nm p(NIPAAm-co-DEGDVE)* compared to a pristine drug film. The dissolution medium was NaH_2PO_4 at pH = 3.

A very similar study was performed using clotrimazole with the results depicted in figure 4.8. Compared to bare phenytoin the drug release of bare clotrimazole is much faster, which is most likely a result of the solubility at lower pH values beeing much higher. Already after 180 minutes a drug release of about 100 % is reached, whereby the temperature again does not cause that much of a deviation. Another possible explanation would be the different solid state of the drug layer, since phenytoin was crystalline and clotrimazole was amorphous after the film preparation. As explained before the dissolution of an amorphous layer should be faster since there is less energy necessary for the molecules to disengage from the surface. The temperature reduction of the dissolution medium again results in reduced release rate so that it takes slightly longer for all drug molecules being released from the sample surface.

By putting an iCVD layer on top the drug release changes again drastically, which is even very similar to those of the phenytoin samples. Only the amount of drug remaining in the sample is lower at around 55 % at 37 $°C$ and just

10 % when the measurements are performed at 25 $°C$. The difference between these two types of samples (phenytoin and clotrimazole) might be a simple deviation in size whereby the clotrimazole molecule has a diameter of about 1 nm while phenytoin is smaller with just about 0.85 nm. Therefore it should be more difficult for the clotrimazole drug molecules to diffuse through the iCVD coating.

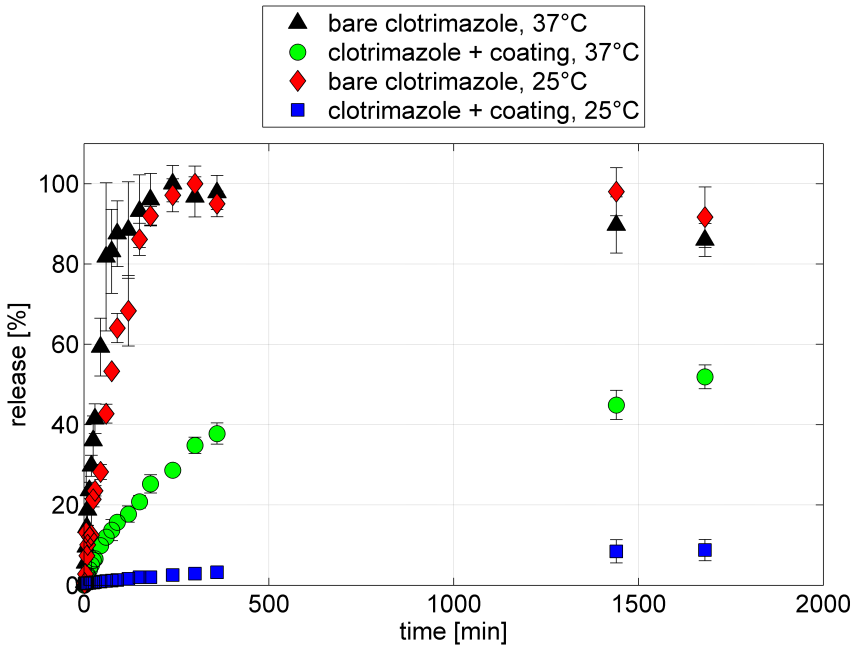


Figure 4.8: Drug release at different temperatures for a clotrimazole layer coated with 200 nm *p(NIPAAm-co-DEGDVE)* compared to a pristine drug film. The dissolution medium was NaH_2PO_4 at pH = 3.

Looking at figure 4.9, the indomethacin samples measured with a dissolution medium at pH = 3 show again a similar behavior for most of the samples. A rather fast release is observed when there is no coating of the drug layer while reduction in temperature reduces the dissolution rate. Though for both temperatures all of the indomethacin escapes the sample surface after 24 hours within the experiment.

Using a coating and performing the dissolution at 37 $°C$ reduces the release further, whereby a maximum amount of about 60 % of the drug can diffuse through the hydrogel layer. This is very comparable to the samples

measured above. Nevertheless, using a temperature of just 25 $°C$, the amount of indomethacin being released is drastically higher compared to the samples measured for phenytoin and clotrimazole. Although having a slightly slower release rate then the 37 $°C$ coated indomethacin sample, the total drug release remains at just above 60 %. A reason why much more indomethacin is able to dissolute might be given by the fact that among these three model drugs indomethacin has the lowest logarithmic acid dissociation constant of $pK_a = 3.8$. Therefore the low pH environment of the buffer dissolution medium has the most influence on the drug molecules. The low pH value might also have an impact on the secondary amino group of *NIPAAm* that could be responsible for a structural change within the polymer that enhances the transport through the layer.

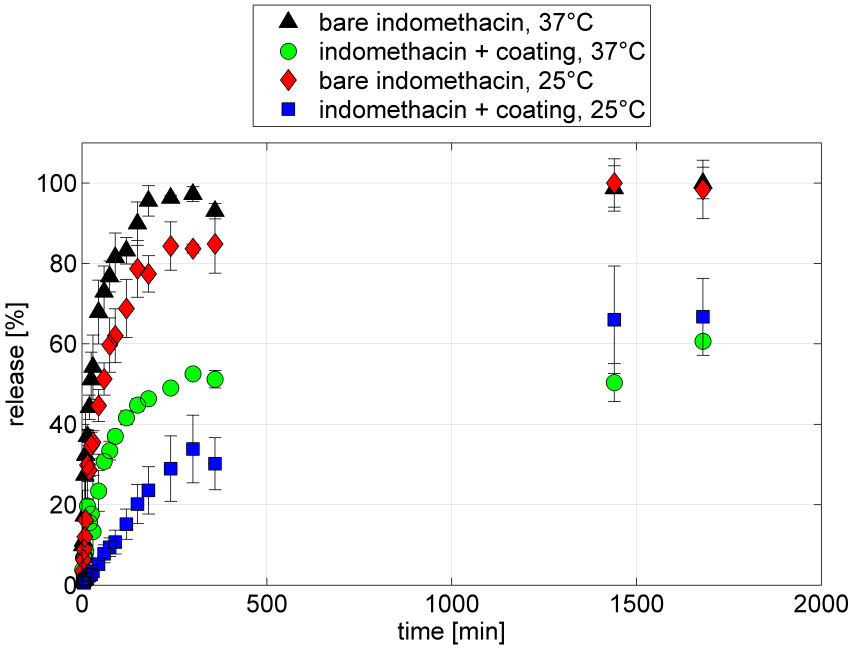


Figure 4.9: Drug release at different temperatures for an indomethacin layer coated with 200 *nm p(NIPAAm-co-DEGDVE)* compared to a pristine drug film. The dissolution medium was NaH_2PO_4 at pH = 3.

While a difference in the temperature response on the drug release for the iCVD coated samples is evident, the outcome is somehow unexpected. The initial expectation was based on the idea that the drug delivery is controlled by the diffusion of the drug molecules through the hydrogel network.[14] Thereby the hydrated, swollen state of the hydrogel layer, below the LCST at 25 $°C$, should enhance the release. Nevertheless the results indicate that the swollen hydrogel prevents the drug from getting released into the dissolution medium. Above the LCST at 37 $°C$ also a barrier function for the drug release is present, which reduces the dissolution rate compared to the uncoated samples at comparable temperatures. The de-swollen, collapsed polymer chains might also explain the final drug amount being caught in the drug formulation. The question that arises is why the collapse of the polymer (coil-to-globule transition) facilitates the faster release. Although we are not completely aware of the answer, we put forward the idea that not only the dissolution medium but also the drug molecules itself can form H-bonds with the hydrophilic sites of the polymer. The drug would thereby be trapped in the hydrogel network and does not dissolve.

Further dissolution experiments were implemented to investigate the influence of a change in the pH of the dissolution medium. Therefore, the dissolution testing was performed with the same buffer (NaH_2PO_4) but at pH = 7 and again for a temperature below (25 $°C$) and above (37 $°C$) the LCST. The results from this series for indomethacin as corresponding drug layer is plotted in figure 4.10. Starting with the pristine drug layers an even faster drug release in the first few minutes of the experiment are evident, compared to the results with the low pH buffer (see figure 4.9). It can be assumed that the change in pH induces to a change in the solubility of indomethacin thus leading to this burst release behavior.

Looking on the samples with the iCVD layer on top, the ones dissolved at 37 $°C$ show a decreased rate of release within the first minutes of the experiment. Already after 30 minutes a plateau value of the drug amount dissolved is adjusting at 40 % meaning that 60 % is kept in its film beneath the coating. For the sample with iCVD layer at 25 $°C$ the initial rate of release is comparable to the higher temperature curve but there is overall more amount of drug dissolving. A final value of about 75 % is reached within 120 minutes of the dissolution testing.

These results show a completely different release behavior than what has been experienced so far but now actually matches the expectation of our initial idea for the hydrogel release kinetic. In the case of the dehydrated, shrunken state of the hydrogel at higher temperatures the drug release is significantly lower than in the hydrated, swollen state at lower temperatures. At this pH

it looks like the swollen hydrogel network enhances the diffusion through the layer, letting more of the drug dissolve in the medium.

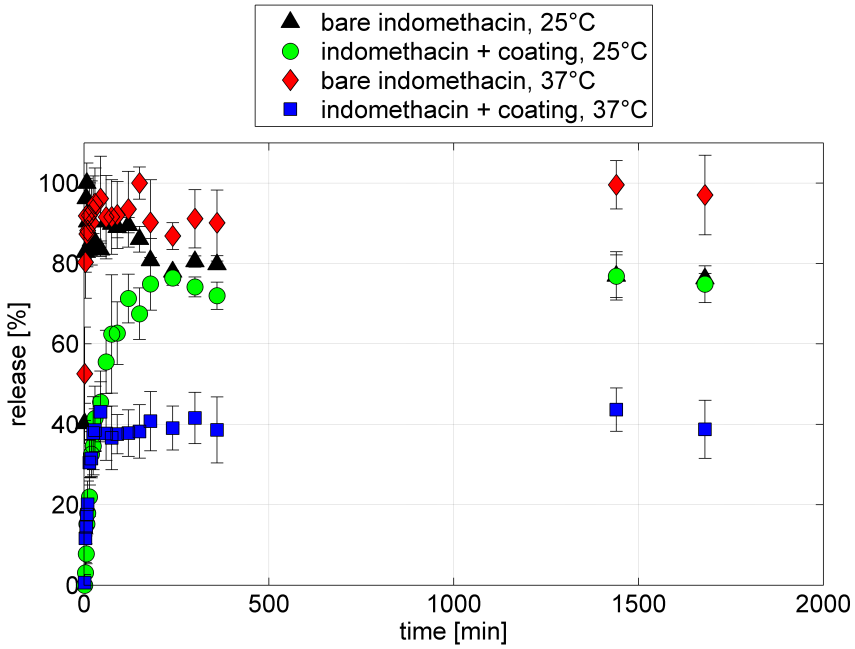


Figure 4.10: Drug release at different temperatures for an indomethacin layer coated with 200 *nm p(NIPAAm-co-DEGDVE)*. The dissolution medium was NaH_2PO_4 at pH = 7.

For the last dissolution experiment at 37 $°C$ with the neutral pH buffer we wanted to test the effect of aging on the dissolution. Therefore one of the three used samples for the dissolution was produced five months before the experiment and aged in air at room temperature. The dissolution testing was performed as for every other experiment but looking at figure 4.11 reveals an interesting result. Here the individual curves for the three coated samples are compared with the release of the bare indomethacin samples. Thereby the two samples coated with the deposition one day before the dissolution show the inhibiting trend as for comparable dissolution tests before. Although they show not completely the same final drug release, they are within the deviation of the experiment, since they where acquiredd in two different runs. The sample coated with the aged layer follows almost the drug release from the uncoated samples. This reveals that simply storing the samples in a petri dish in a cupboard for almost half a year is not sufficient, meaning that the coating

loses its impact on the controlled drug release. This could either be explained by the fact that the coating is degrading or that the drug molecules over this long time diffused through the iCVD layer to the sample surface.

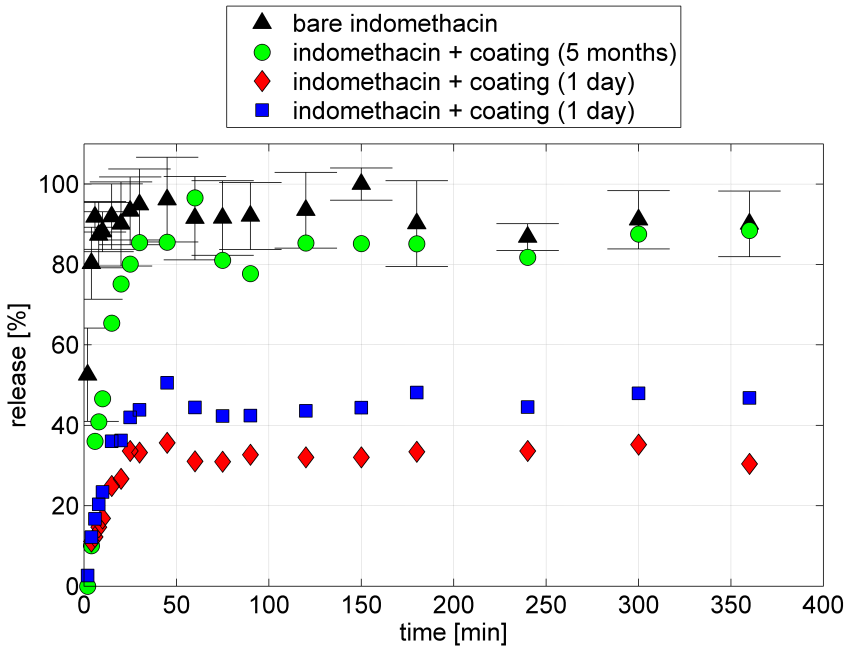


Figure 4.11: Drug release for an indomethacin film coated with two different 200 *nm p(NIPAAm-co-DEGDVE)* layers. The dissolution medium was NaH_2PO_4 at pH = 7 and 37 $^{\circ}C$.

CHAPTER 5

Conclusion

Using iCVD to deposit stimuli-responsive polymer thin films was proven to be a good choice since the polymer films synthesized were of great quality. The investigated swelling behavior when immersed in a liquid environment at different temperatures indicated the very fast response time of the thermo-responsive *p(NIPAAm-co-DEGDVE)*. The lower critical solution temperature of about 32 $°C$ was verified where the coil-to-globule transition significantly changed the thickness of the hydrogel layer. Compared to other hydrogels were the swelling is mostly governed by the change of the mesh size, it is a structural change of the polymer chains for *p(NIPAAm-co-DEGDVE)*.

When depositing on drug layers the iCVD technique showed uniform coatings throughout the experiments. A deposition of a polymer layer on top of an amorphous drug film lead to strong surface wrinkling due to different elastic moduli at the interface, which was absent when coating a crystalline drug film. Furthermore it was shown that the polymeric encapsulation of an amorphous drug layer enhanced its stability significantly. Keeping the amorphous state enhances the drug release, which can be important for applications in biomedicine where a fast drug treatment is preferred.

The custom build dissolution setup that has been used in combination with the high performance liquid chromatography provided very good results. It was shown that it is possible to control the drug release with stimuli-responsive polymer coatings. Against the initial expectation, for low pH values of 3 at low temperatures (25 $°C$) the drug release was almost inhibited while at higher

temperatures (37 $^{\circ}C$) it could be reduced compared with the pristine drug films. The results for indomethacin where a bit different than for the other tested drugs which can be ascribed to the low pK_a value of 3.8, thus the low pH value of the dissolution medium having a major impact. More experiments on this subject have to be performed to receive a better insight for this behavior. When changing the pH value to neutral the drug release reveals a faster, higher release in the hydrated, swollen state of the hydrogel at low temperatures, thus the release behavior expected. This can be an indication of the applicability of this drug delivery system as transdermal patches. In this application the pH environment is not that acidic while a cooling effect can be applied rather easy.

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CHAPTER 6

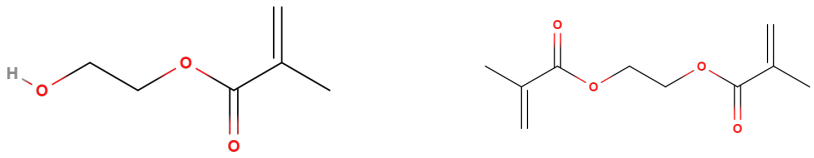
Appendix

6.1 Preparation and characterization of a different polymer thin film

In pre-experiments on hydrogels deposited by iCVD also the polymer *p(HEMA-co-EGDMA)* was studied in detail. Thereby the swelling was not investigated as function of the thermal-responsiveness, but the increase in thickness was evaluated in terms of chemical composition. For this iCVD deposition series the following chemicals were applied in the polymerization process:

- 2-hydroxyethyl methacrylate (*HEMA*, 97%, Aldrich, Germany)
- ethylene glycol dimethacrylate (*EGDMA*, 98%, Aldrich, Germany)

As aforementioned the most important part of the monomer (*HEMA*, figure 6.1a) are the hydrophilic sites, especially the OH-group, as they are responsible for the swelling in liquid environment. The carbon double bond at the end of the chain serves as polymerization site where the polymer backbone is created. The cross-linker (*EGDMA*, figure 6.1b) again shows the two prototypical polymerization sites on both ends of the chain, thus being able to connect other polymer chains and forming a network.



(a) *HEMA* (b) *EGDMA*

Figure 6.1: Chemical structures of the monomer and the corresponding cross-linker for a *p(HEMA-co-EGDMA)* thin film. Reprinted from [1].

Out of these components a hydrogel film of *p(HEMA-co-EGDMA)* was synthesized using iCVD. The flow rates of the chemicals are listed in table 6.1. Also a patch flow of nitrogen with a flow rate of 3 *sccm* was applied. The base pressure in the vacuum chamber before starting was at about 0.5 $mTorr$ while the leak rate was between 0.01 and 0.04 *sccm*for this series. The thickness of the layer is always given from the reference silicon wafer where also the in-situ laser interferometry was performed. The substrates for these depositions were either (2 x 2) cm or (5 x 2) cm big silicon wafers, whereby the latter were used in the swelling experiments to fit in the liquid cell. All substrates were treated with a carbon dioxide stream to clean them from dust before the installation into the reactor chamber.

A consistent substrate temperature of 20 $°C$, a filament temperature of about 237 $°C$ and a working pressure of 350 $mTorr$ was used for this series. The temperature of *HEMA* and *EGDMA* was kept at 75 $°C$ and 80 $°C$ respectively.

For the first 4 depositions a thickness of about 150 nm was desired whereas the deposition 5 was chosen to be thicker with around 400 nm. Deposition 2 had no flow rate of the monomer, creating a thin film of only polymerized cross-linker. For deposition 3 the thickness was not achieved as well as for the other ones, as you can see in table 6.1. A combination of the high flow rate of *HEMA* and its low volatility lead to film growth even though the valves were closed at the desired thickness.

Table 6.1: Samples prepared with the monomer *HEMA* and the cross-linker *EGDMA*.
f_{HEMA}...flowrate of *HEMA*
f_{EGDMA}...flowrate of *EGDMA*
f_{TBPO}...flowrate of *TBPO*
d...thickness of the film on the silicon wafer reference

deposition	f_{HEMA} [sccm]	f_{EGDMA} [sccm]	f_{TBPO} [sccm]	d [nm]
1	0.426	0.051	0.819	146.8
2	/	0.119	0.790	144.7
3	0.912	0.073	0.805	189.1
4	0.197	0.121	0.804	149.5
5	0.198	0.088	0.796	414.5

The produced *p(HEMA-co-EGDMA)* thin films were analyzed with Fourier-transform infrared spectroscopy (*Bruker IFS 66 v/S*). With this technique light of the wavelength range from 400 to 4000 cm^{-1} is shined on to the sample surface. The light interacts with the polymer film in a way that some energy is absorbed by the rotational and vibrational modes of the bonds inside the material.[25] Comparing the background corrected signal with the thickness normalized spectra of the homopolymer data from *p(HEMA)* and *p(EGDMA)* the copolymer composition can be calculated. This is done for all depositions (see table 6.2). In figure 6.2 you can see such a comparison of exemplary spectra for deposition 1. This is already zoomed into the region of interest containing the absorption from $C-O$ and $C=O$ stretching at about 1150 cm^{-1} and 1750 cm^{-1} respectively. Considering the chemical structure of the monomers present we know that *HEMA* has one $C=O$ and *EGDMA* has two. By simply looking at the spectrum we can already estimate that there has to be about three times as much *HEMA* in the film than *EGDMA*.

Table 6.2: Polymer composition of the films prepared with iCVD.

dep	*HEMA* [%]	*EGDMA* [%]
1	74	26
2	/	100
3	87	13
4	47	53
5	43	57

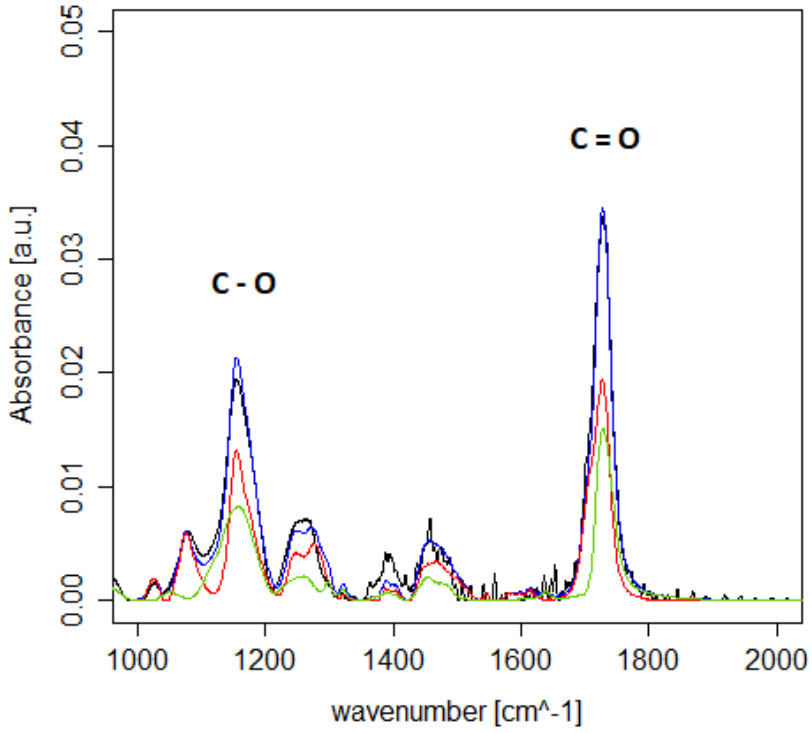


Figure 6.2: FTIR spectrum of deposition 1. The background corrected signal (blue) is compared with the thickness normalized spectra of the homopolymer data from *p(HEMA)* (red) and *p(EGDMA)* (green) retaining a cross-linking degree of 26%.

For these *p(HEMA-co-EGDMA)* films we want to especially investigate the swelling behavior, which is performed using deionized water as environment for all five depositions. From the acquired data the initial thickness of the film (d_0) and the thickness after swelling (d) are extracted and used to calculate the thickness increase in percent:

$$thickness\ increase\ [\%] = \frac{d - d_0}{d_0} * 100\ \% \tag{6.1}$$

For deposition 3 already after a few moments in the liquid environment the film started to delaminate from the substrate and dissolve. This is because its cross-linking ratio of only about 13 % provided not enough mechanical stability of the film. Therefore there is no swelling data for this deposition at all. In figure 6.3 the results of this works are plotted together with already published

data from the group.

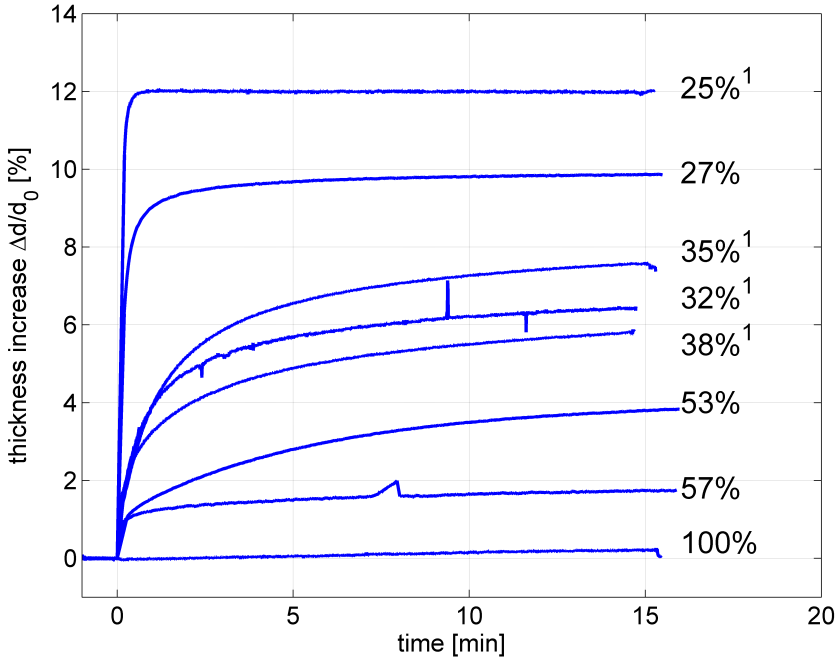


Figure 6.3: Thickness increase of the hydrogel thin films after swelling in water, where the percentages display the amount of cross-linking in the polymer network. [1] denotes already published measurements performed by another member of the group. [27]

As the degree of cross-linking gets higher, the hydrogel characteristic increase of the film thickness gets lower. The cross-linker creates a less flexible structure of the polymer network so that not as much water can be taken up by the film. This is actually also crucial for any application for drug release where the uptake of water strongly influences the drug transport through the polymer film. Also representing the swellability is the mesh size which can now be calculated with the results from the swelling measurements by using equation 2.1. As you can see in figure 6.4, the lower the cross-linking degree of the film is, the lower is also the mesh size. Again the evaluated mesh sizes are plotted together with the data already published from the group to complement the results.

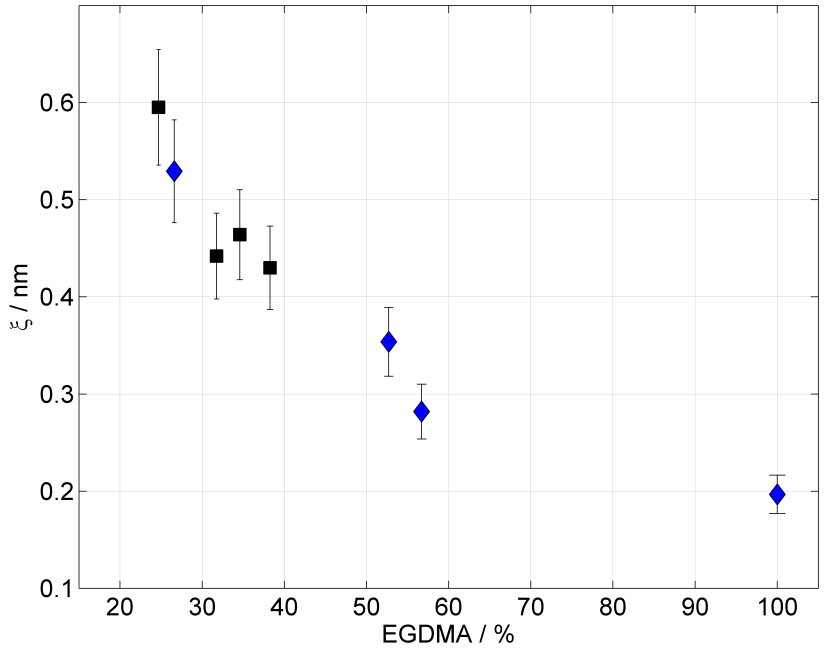


Figure 6.4: Mesh size for hydrogel films with different cross-linking degree. ♦ indicate the results from this work, while ■ represents the results from Unger et. al. [27].

The mesh size values obtained have to be interpreted with care. Since the diameter of water is about 0.27 nm it is almost the size of the polymer mesh, meaning it would not diffuse through. The hydrogel network though can not be compared to a rigid solid lattice. It is more a physical entanglement also hosting defects like incomplete vinyl conversions thus making the mesh size more of a mean measure of a distributed quantity.[4]

Dissolution tests performed with *p(HEMA-co-EGDMA)* layers deposited on drop casted indomethacin films are published elsewhere. [4]