Mag. pharm. Julia Haring

Investigation of the physicochemical properties of new-synthesized lipids for application as coating material for hot-melt-coating-technology

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Kurzzusammenfassung

Ziel dieser Masterarbeit war es die physikochemischen Eigenschaften von neu-synthetisierten Lipiden zu untersuchen, die später als Überzugsmaterial beim Hot-Melt-Coating (HMC) Verfahren verwendet werden sollten. Bei diesen neuartigen Lipiden handelte es sich um Polyglycerolester von Fettsäuren. Zuerst wurde die chemische Struktur der Lipide mithilfe von Kernresonanzspektroskopie und Massenspektrometrie aufgeklärt. Die Ergebnisse zeigten, dass es sich bei allen 3 neuen Lipiden um eine Mischung aus verschiedenen Polyglycerolen (PG) handelte, wobei jeweils ein PG die Mischung dominierte. Dynamische Differenzkalorimetrie- und Röntgenstrahlbeugungsversuche zeigten, dass diese neuen Lipide keinen Polymorphismus aufweisen. Sie kommen ausschließlich im Alpha-kristallinen Zustand vor, was die Lagerung bei verschiedenen Bedingungen erleichtert, weil die Stabilität dieser Lipide weder über die Zeit noch bei verschiedenen Temperaturen beeinflusst wird. HMC ist die praktischste Technik um Arzneistoffe zu überziehen, wenn Lipide als Überzugsmaterial dienen sollen. Als Modell-Arzneistoffe wurden Zitronensäure-Anhydrat und N-Acetylcystein verwendet. Diese wurden mit den neuen Lipiden und auch mit Tristearin überzogen, um die Coating-Eigenschaften der verschiedenen Lipide zu vergleichen. Dissolutionsversuche wurden direkt nach der Produktion dieser Mikrokapseln (Arzneistoff + Lipid) und nach einmonatiger Lagerung bei Raumtemperatur und bei Lagerung bei 40 °C durchgeführt. Mit den neuartigen Lipiden blieben die Mikrostruktur des Coatings, die Oberflächenspannung und die Benetzbarkeit sowie das Verhalten des überzogenen Materials auch bei Lagerung unter verschiedenen Bedingungen stabil. Dissolutionsergebnisse der Endprodukte, die mit PG6 überzogen waren, zeigten, dass das Dissolutionsprofil über die Lagerung stabil bleibt. Die Verwendung von Dynasan® 118 als Überzugsmaterial resultierte in einer Änderung des Dissolutionsprofils nach der Lagerung, weil es zu einer Änderung des polymorphen Zustands der Lipide kommt.

Abstract

The aim of this thesis was to investigate the physiochemical properties of new-synthesized lipids which should further be used as coating material for hot melt coating technology. These new lipids were polyglycerol (PG) esters of fatty acids. First the chemical structure of lipids was detected by using NMR spectroscopy and mass spectrometry. Results showed that each of these 3 new polyglycerols were a mixture of different PG esters but having one dominant PG-component. The DSC and X-ray diffraction measurements show no polymorphism of these new synthesized lipids. They only appear in the alpha crystalline state which enables storage at different conditions because the stability is not influenced over time or at different temperatures. Hot melt coating is the most suitable technique when lipid excipients are used as coating material. Citric acid anhydrous and N-Acetylcysteine were used as model drugs and coated with these new lipids and also with tristearin to compare the coating properties of these different lipids. The release profiles of the microcapsules (API + lipid) have been investigated directly after production of microcapsules and after one month storage at room temperature and 40 °C, respectively. With the new-synthesized lipids the microstructure of coatings, their surface tension and wettability and the performance of coated material remained stable after storage under different conditions. Dissolution results of the final products coated with PG6 revealed that the dissolution profil remains stable after storage. Using Dynasan® 118 as coating material resulted in the alteration of dissolution profile after storage, due to the changes in the polymorphism of the lipid.

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1 Introduction

A main topic in the pharmaceutical field is the long term stability of pharmaceutical formulations, containing lipid-based excipients. Within the frame of a previous work, new formulations have been developed containing mixtures of tristearin or tripalmitin and polysorbat 65 (as a β-inducing emulsifier). The objective of this development was to provide stable immediate release coating formulations for taste masking of N-acetyl cysteine via hot melt coating. By adding of proper amounts of polysorbat 65 to the system, it was possible to induce the β-form in short times and lower process temperatures (25-30 °C), which was beneficial for the hot melt coating (HMC) of the thermo-sensitive N-acetyl cysteine. Despite achieving the β-form during the HMC process, which was stable within the whole storage time, the release profile of the coatings containing tristearin and polysorbat 65 did not remain stable, resulting in the initial increased release profile during the storage under accelerated conditions (40 °C, 75% r.H.). Investigations of the mixtures revealed the biphasic systems of TAG and polysorbat 65 with a phase separation during the time with proportional correlation to time and temperature. This phase separation is most probably due to the crystal growth of triacylglycerides (TAGs) during the storage. Using tripalmitin and polysorbat 65, despite a latent phase separation, the release profile remained stable.

In order to further improve the stability of lipid-based formulations, new lipid-based structures; namely polyglycerol fatty acid esters have been synthesized.

This thesis is focusing on the characterization of these new coating materials. Moreover, two different active pharmaceutical ingredients (API) have been coated with the new lipids via hot melt coating. Drug release as a function of temperature and storage has been studied. Hot melt coating is of growing interest for coating of active ingredients [1]. As the name already says hot melt coating is a coating procedure at which a molten lipid is directly sprayed onto the surface of particles to be coated. HMC offers a lot of advantages compared to other coating techniques. First there are neither aqueous nor organic solvents required for a hot melt coating process, thus no evaporation of the solvent is necessary, making the process environmental friendly

and time and cost-saving. HMC is used for taste masking of bitter or sour API's and it is also applied to ensure stability of physical or chemical properties of active ingredients [1]. Coating material are often lipid excipients, which often occur in nature, generally recognized as safe (GRAS) status [1].

In the current work, NAC and citric acid anhydrous were used as core materials and coated with lipid excipients. As lipid excipients one of the new-synthesized lipids, PG6 stearat and also pure tristearin (Dynasan® 118) were used and the coating properties were compared. Samples were withdrawn of each HMC batch at 30%, 40% and 50% of coating amount. The influence of the coating amount, temperature, storage time and also coating material on the drug release was investigated. The coating amount is an important parameter, with a significant influence on taste masking and drug release. In this case the aim of a pharmaceutical formulation was to ensure a good taste masking with lowest amount of coating material. For the new synthesized lipids a chemical structure analysis was conducted and also the physical properties, such as melting and recrystallization point, polymorphic behaviour and wettability/stability studies and the viscosity behaviour were determined. Chemical structure analysis was performed by calculating the hydroxyl and the saponification value drawing all possible arrangements of the fatty acids at their glycerol backbone and by interpretating NMR and MS spectra. The physical characterization of the solid state behaviour to observe polymorphism was conducted with DSC measurements and also with x-ray-diffraction measurements. Furthermore, the wettability of samples has been analyzed by contact angle measurements at time 0 and 16 weeks of storage at RT and also at 40 °C. As a good taste masking is necessary to obtain patient compliance, taste masking studies were perfomed by conducting pH-measurements of the final products.

2 General Information

2.1 Lipids

Prehistoric humans have already used fats and oils in their diets. Fats play a major role in alimentation, because they are rich in calories and they provide, apart from essential fatty acids, also vitamins A, E, K , phytosterols, beta-carotene, lutein and other precious micronutrients. The subgroups of lipids are fats and oils [2]. Fats and lipids are used in food, cosmetics or pharmaceuticals as principal part of end products, or also as matrices in which cosmetics and pharmacological fine chemicals are dispersed. The molecular structures of fats and lipids are so- called “long-chain-compounds” like paraffins or fatty acids [3]. Lipids, which are organic compounds, are soluble in non-polar organic solvents such as hexane and isobutanol and they are not soluble in water. The terms „oil“ and „fat“ are used interchangeably, the usage of these terms is depending on the physical state of the material at room temperature. A fat or oil is defined as a triacylglycerol (TAG)-mixture which is liquid or solid at 25°C, respectively. From chemical view, fats contain usually over 95 % of TAG molecules and 1-5 % other components, such as phospholipids, glycolipids, free fatty acids, monoacylglycerols (MAGs) and diacylglycerols (DAGs). A TAG molecule is built up of a glycerol backbone with three fatty acids that are esterified to the three alcohol groups at special position referred to the stereospecific numbering as sn-1, sn-2 and sn-3. These fatty acids can be different in their chain lengths, saturation, branching and the attendance of trans or cis double bounds. TAGs are normally grouped into three classes. Monoacid TAGs have identical fatty acid present in position sn-1, sn-2 and sn-3. Examples are tristearin and tripalmitin. TAG molecules that have two or three various kinds of fatty acids are known as diacid or triacid TAGs, whereas both of which are referred to as mixed-acid TAGs. The main influencing structural variables in a TAG molecule which have an effect on the physical properties are the chain lenght of the fatty acid, the position, number and configuration of double bounds (saturated, unsaturated, cis / trans) and last but not least the stereospecific position of the fatty acid on the glycerol backbone.

Physical properties are for example melting and crystallization behaviour, solid fat content, polymorphism, micro and nanostructure, oil binding capacity and mechanical properties. The most established TAGs consists of fatty acids with a chain length between four and twenty-two carbons with zero to six double bounds. A typical characteristic of a fat that consists of a mixture of different TAG molecules is that the substance does not exhibit a typical melting point. Instead of that the material shows rather a melting range that is determined by the chemical nature and molecular interaction of this extensive mixture. This can be explained by the great variety of molecular species present and due to the extensive phase behaviour between and among TAG molecules. TAGs are able to crystallize into different solid state structures. This depends on the crystallization conditions such as temperature, shear and time which causes polymorphism. Understanding the arrangement and interaction of TAGs is important if an appreciation of the macroscopic functionality of fats is desired. An interesting point of fats is that they act like elastic solid as far as a deformation stress exceeds a certain value. This value is called yield value. This is the point where the product beginns to flow like a viscous fluid. This phenomen is called plasticity. Plasticity arises from the fact that the crystallized material builds a fat crystal network that leads to liquid oil. Some of the sensory characteristic of fat and fat-structured food products such as spreadability, mouth feel, texture and flavor are intensively affected by the physical characteristics of the fat crystal network. The mechanical and organoleptic properties and also the stability, i.e. the shelf life of materials like chocolate, butter, margarine and spreads are strongly determined upon crystallization of the substance. If there is an understanding of fat crystallization and its structural relationship available then it is possible to order and control engineer material properties. It is necessary to understand the structural organization that is present in a material and to allocate it to the macroscopic properties. Otherwise it costs efforts to replace ingredients, optimizing functionality and enhance health in a rational manner. Finally this will also lead to a more focused, less frustrating and not that expensive endeavor [2].

2.2 Polymorphism of lipids

Crystallization of fats and lipids leads to specific properties, which might be declared with polymorphism on the one hand and molecular interaction on the other hand. Every long-chain compound shows polymorphism, especially fats and lipids. Polymorphism influences the crystallization rate, the size of crystals, crystallinity and also the crystal morphology. Polymorphism itself is affected by the molecular structure and by many external factors such as temperature, type of solvent, crystallization rate, pressure, impurities. An example for molecules showing polymorphism are TAGs, which are glycerin fatty acid esters and which possess the usual three polymorphs. The typical characteristics of polymorphism of fat crystals are shown in Fig. 1. The chemical property of a TAG molecule is determined by its nature and the composition of three fatty acid sections, defined as R_1, R_2 and R_3 (Fig. 1 a). α, β' and β forms are three polymorphs, which are based on subcell structures. They define cross-sectional packing modes of the zigzag aliphatic chain which are applied to single-crystal structure of tricaprin β form. The chain length structure creates a repeating sequence of the acyl chains which are involved in a unit cell lamella along the long-chain axis.

A double chain length structure is built when the chemical properties of the three acid sections are the same or very similar, as indicated in tricaprin β-form. On the contrary, when the chemical properties of one or two of the three chain sections are very different from each other, a triple chain length structure is formed. The chain length structure assumes a critical role in the mixing-phase behavior of different types of the TAGs in solid phases [3].

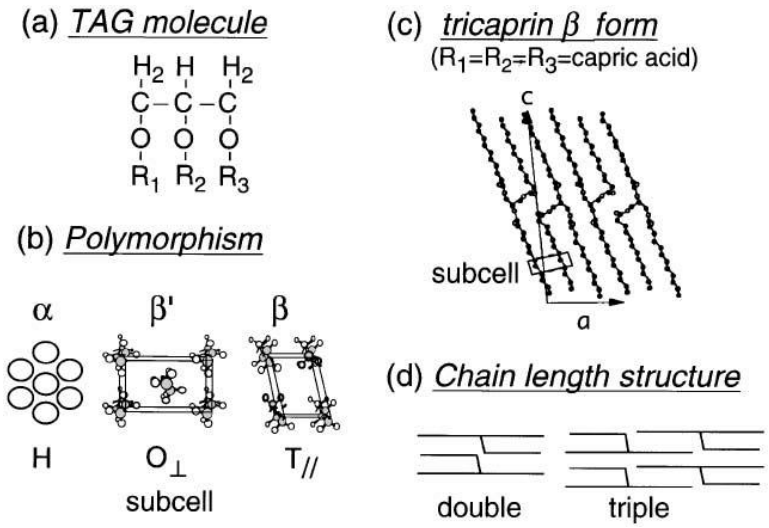


Fig. 1. a) TAG molecule b) polymorphs and subcell structures c) tricaprin β-form d) chain length structure [3]

2.3 Manufacturing process - Coating

Coating is already known for a long time and was conducted for the first time by Rhazes (850-923 AD) with the slime of plant seeds. Coated dosage forms consist of a core, i.e. a tablet or a granule, which is covered by a smooth consistently and often coloured layer. The conventional coating processes occur in rotating pans and fluid bed coaters.

Reasons for coating of tablets and granules are:

- covering of an unpleasant taste or smell (was the primary reason for coating)
- protection of the API's against environmental influences, like atmospheric-oxygen or air-moisture)
- better capability of resistance against mechanically exposures
- protection of the API's against inactivation or destruction due to gastric acid (enteric coated tablets)
- protection of the patients against API's that irritate the oral mucosa or the gastric mucosa
- facilitating of the swallowing of the tablets due to smooth surfaces
- psychological effect of the colour, the polish and the shape to the attitude of the patient (aesthetic reasons)

- better possibilities to distinguish between different pharmaceutical products due to different colours of the coating (to avoid that a patient swallows the wrong tablet)
- possibility is given to produce coated tablets with controllable drug release (delayed release or graduated release)

The primary core material that is used for the coating process are tablets, granules, pellets and API crystals. Only such cores that possess enough physical stability to resist the mechanically exposure of the coating pan or the fluid bed are used for the coating process. On the other hand the dissolution profile, i.e. the dissolution in the gastrointestinal tract, of the coated granules, tablets etc. should also be adequate [4].

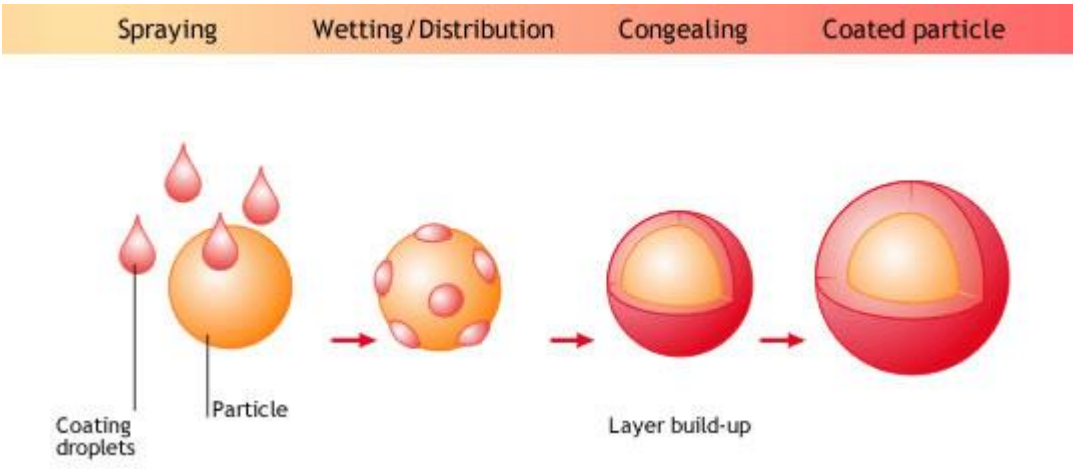


Fig. 2. Coating on particles [5]

2.3.1 Coating in the fluid bed

Coating in fluidized beds is the preferred coating technique due to the constitutional advantages of the technology. The advantages are high flowability of the particulate material, the uniformity of the temperature, more equable coating due to a good blending of the solids and reduced process time because of high heat transfer. The coating quality is determined of two factors, which are coating mass uniformity and the morphology of the coating [6]. The coating material will be sprayed on the surface of the core. While particles are flowing around in the fluid bed they are sprayed with a liquid at the same time. The coating material can be applied as a solution, a melt or as

a suspension. There are 3 methods, how the spraying of the coating material can be applied: top spray, tangential spray and bottom spray [7].

2.3.2 Hot melt coating (HMC)

Hot-melt coating has its roots in textile and paper industry in the 1940s. At the beginning hot melt coating was just used for coating large materials, like paper, foil and textiles and not for coating of single particles or dosage forms. For hot melt coating the coating material (mainly lipid based excipients) is applied in a molten state and because of that no solvent is necessary. The main lipid excipients used for hot melt coating are waxes, vegetable oils, polyoxylglyceride, fatty acids and partial glycerides. The selection of excipient is based on the required quality attribute of the coated formulation, such as drug release profile, taste masking, etc. as well as physicochemical characteristics of excipient, such as thermal or rheological behaviour and polymorphism.

There are many advantages of HMC compared to typical coating techniques:

- reduction of costs and time due to avoidance of intensive solvent treatment, thus there is no evaporation of the solvent needed
- reduction of microbial contamination because there is no water used in the process [8].

2.4 Lipid analysis

2.4.1 Chemical structure analysis

2.4.1.1 Evaluation of the saponification number (SAP)

The saponification number represents the number of esterifications in 1 g of the substance. The exact definition of the SAP says: “The saponification value IS is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the free acids and to saponify the esters present in 1 g of the substance [9].”

2.4.1.2 Evaluation of the hydroxyl value (HV)

With the hydroxyl value (HV) the calculation of the number of free OH-groups (OH-groups that are not esterified) is possible. The exact definition of the HV says: “The hydroxyl value IOH is the number that expresses in milligrams the quantity of potassium hydroxide required to neutralise the acid combined by acylation in 1 g of the substance [10].”

2.4.1.3 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance (NMR) signals were detected for the first time in 1946. They were observed by two research groups, the one group of F. Bloch, W.W. Hansen and M.E. Packard and the other one of E.M. Purcell, H.C. Torrey and R.V. Pound, independently from each other [11]. Henceforward NMR spectroscopy has become an important tool for chemists, biochemists, physicists and also for medical scientists. The common field in which NMR spectroscopy is used, is the determination of molecular structures. The nuclids that are of main interest in NMR spectroscopy are protons (^{1}H) and carbon-13 (^{13}C), because these two nuclids give the most important resonances for identifiying the structures of organic molecules [11]. From a NMR spectrum three types of spectral parameters can be obtained, namely the chemical shift, indirect spin-spin couplings and intensities. The chemical shift is induced by the magnetic shielding of the nuclei by their surrounding electrons. Due to the dependence of the resonance frequencies on the magnetic flux density, absolute line positions are never specified in an NMR spectrum. Therefore a dimensionless quantity, the δ-value was defined. The

δ-value indicates the position of the signal relative to that signal of a reference compound and it is also related to the measurement frequency. In general for the ^{1}H and ^{13}C NMR spectroscopy tetramethylsilane (TMS) is used as reference compound. Between neighboring nuclear dipoles interaction occurs. For defininig the strength of this interaction a spin-spin coupling constant J exists. The indirect spin-spin coupling does not depend on the external magnetic field and hence the coupling constants J are given in Hz and not in ppm. The signal intensities can only be measured for the ^{1}H NMR spectrum and it is not possible to measure the intensities of a ^{13}C NMR spectrum [11].

2.4.1.4 Mass spectrometry

For an accurate determination of the mass of molecules and atoms, mass spectrometry (MS) is used. MS measures the mass-to-charge ratio (m/z) of gas phase ions and therefore the mass of a molecule can be given if the charge is known. The m/z ratio is a dimensionless value. Charging of molecules facilitates the calculation of their masses, which are estimated indirectly from their interactions with electric and magnetic fields. If the mass of a molecule is known the identification of the „unknown“ molecule is much easier. Therefore MS is an appropriate method for analysing the composition of complex samples with great exactness [12]. Generally a mass spectrum contains information on the molecular mass of an analyte and also of the masses of its structural fragments [13]. At the beginning MS was just a field within physics, because biological molecules were too large and labile and thus they were not easy to ionize and volatize. This problem was solved with introduction of the so-called „soft“ ionization techniques such as MALDI (matrix-assisted laser desorption / ionization) and ESI (electrospray ionization) [12].

In the graphic form of a mass spectrum the abscissa (x-axis) is representing the mass of ions, exactly speaking the mass-to-charge ratio (m/z), whereas the ordinate (y-axis) is representing the relative intensity of these ions’s peaks. Atomic mass units (amu) are appropriated as units to measure masses of ions. The intensity is demonstrated in percent relative to the base peak in the spectrum or even to the entire abundance of all the ions in the spectra [13].

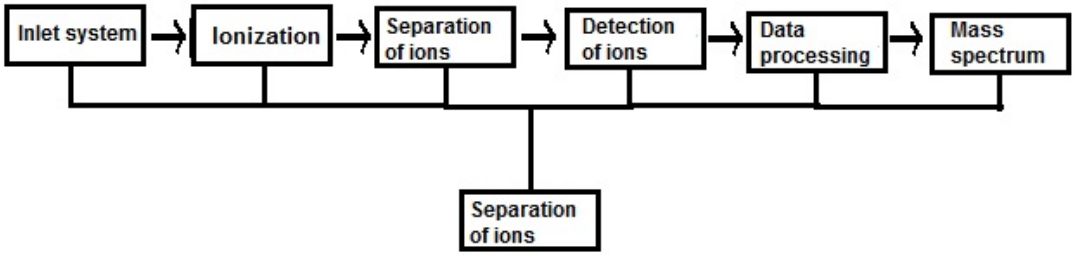


Fig. 3. principal scheme of a mass-spectrometer [13]

2.4.1.4.1 Ionization

For MS gas-phase ions are necessary. It is distinguished between „hard“ ionization techniques and „soft“ ionization techniques. Hard ionization techniques such as electron ionization and chemical ionization were bordered to gas-phase molecules. The first soft ionization techniques were plasma desorption (PD), fast atom bombardment (FAB) and secondary ion MS (SIMS), whereas nowadays these techniques are substituted by even softer techniques such as ESI and MALDI [12].

2.4.1.4.2 Electrospray Ionization

The Introduction of Electrospray Ionization (ESI) was done by Dole and his coworkers in 1968 and was then coupled to MS in 1984 by Yamashita and Fenn. In ESI the dissolution solvent for the sample is polar and volatile [13]. Polar solvents are for example water, methanol or acetonitrile [14]. For ESI a large potential difference has to be applied on a liquid in a conducting capillary (needle) and a counterelectrode, producing even smaller droplets by the Coulomb fission [12]. The dissolved sample is transferred through a needle which is located at high positive or negative potential relative to a nozzle surface. Due to the high electric potential (1-4 kV) between the needle and the nozzle the fluid is induced to form a Taylor cone, which is abundant on positive or negative ions at the tip of the needle. A spray of charged droplets is emanated from the Taylor cone by the electric field. The droplets diminish due to evaporation, while a warm flow of nitrogen gas is passing across the front of the ionisation source. At atmospheric pressure ions are formed (atmospheric pressure ionisation). The ions run through a cone-shaped nozzle (counterelectrode), from there into an intermediate vacuum region and then through a small orifice into the

high vacuum of the mass analyzer. ESI has been used in combination with all common mass analyzers [13].

A schematic representation of an ESI source is shown below (Fig. 4).

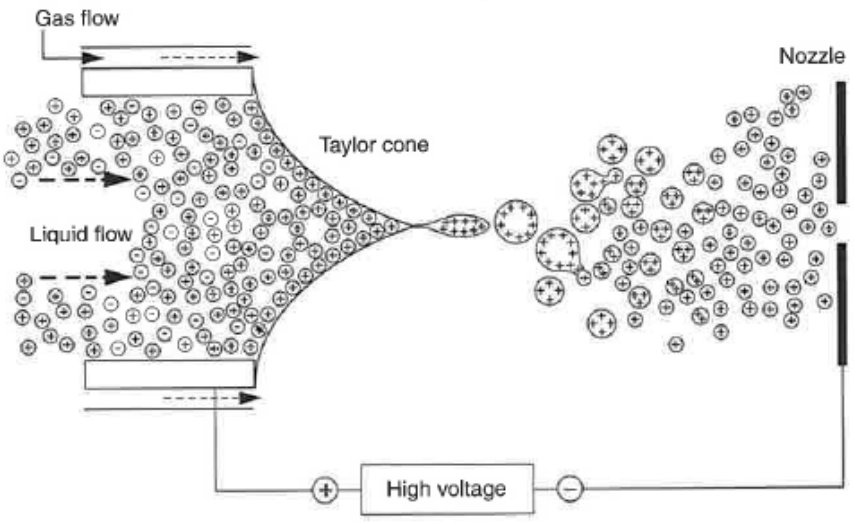


Fig. 4. schematic representation of an ESI source [13]

2.4.1.4.3 Mass analyzers

A mass analyzer is a technical equipment that separates atoms, molecules or clusters in accordance with their mass. All mass analyzers that are used nowadays are based on electromagnetism so that ions are required to receive separation. Hence an ion source has to be connected with the analyzer. The analyzer separates ions that are coming from the ion source in accordance to their mass to charge ratio (m/z). There exists a couple of mass analyzers and they can be categorized into different classes like magnetic or pure electric, scanning or nonscanning (pulse based) and trapping or nontrapping analyzers. Examples for analyzers are time-of-flight (TOF), magetic/ electric sector, quadrupole mass filter (Q), quadrupole ion trap (QIT) and Fourier transform ion cyclotron resonance (FTICR) [13]. For this thesis the main interest was in TOF analysator.

2.4.1.4.4 Time-of-Flight

A TOF mass analyzer separates ions in accordance to the time difference between a start signal and the pulse that is created when an ion beats on the detector.

The first publisher of the principle of a TOF analyzer was Stephens in 1946. For TOF-MS a well-defined start signal is necessary [13].

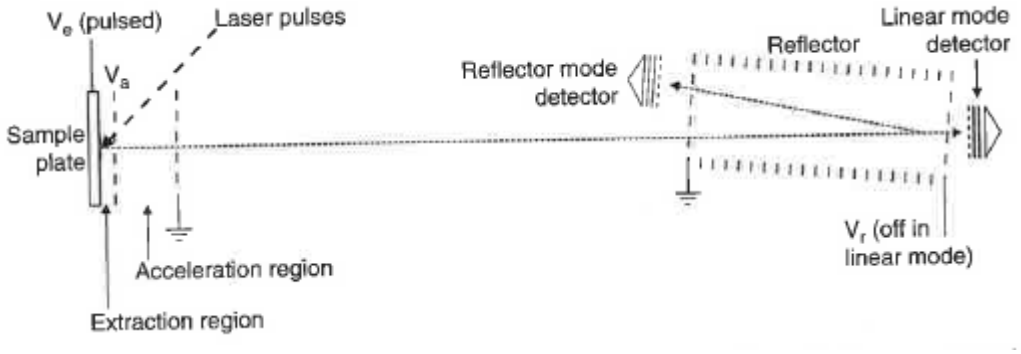


Fig. 5. MALDI-TOF mass spectrometer [13]

2.4.2 Determination of the physical behaviour of the lipids

2.4.2.1 Differential Scanning Calorimetry

The field of calorimetry is based on the principles of thermodynamics. Calorimetry measurements are measurements of energy changes within a material. Materials can be either exothermic, i.e. they are liberating heat, or endothermic, which means that they are heat consuming materials. Calorimetry means the determination of energy changes and also quantitative measurements are possible if the accurate mass of the sample is known. In pharmaceutical sciences one of the most used application in the field of calorimetry is the differential scanning calorimetrie (DSC). With DSC it is possible to heat or cool a sample in a controlled manner. The main component of a calorimeter is a container which is isolated from its exterior surroundings. In this way the heat exchange that happens between the system and the environment can be determined. “Environment” means the calorimeter and its contents and the “system“ means either a chemical reaction or a physical change of the sample. There are two ways in which the system can behave. On the one hand an endothermic process can occur, which means that the system is absorbing energy from its environment.

On the other hand an exothermic process can occur, that means that the system is loosing energy to its environment.

If an exothermic change is occuring the temperature of the environment will increase, thus the environment receives energy, which got lost by the system. The whole energy of an isolated system has to stay constant, because the only thing that happens is a heat exchange. It follows the first law order of thermodynamics, which says that energy neither can not get lost or neither can be created, it just can be transformed into other forms of energy. So the energy exchange of the system has to be equal. In contrast if an endothermic change is occuring, the temperature of the environment will decrease, since the environment is loosing energy, which is absorbed by the system [15]. Some fundamental thermal events like melting and crystallization are described below. Melting belongs to the first order endothermic process in which the sample needs a net quantity of heat. In a DSC diagramm an endothermic peak can be seen. The broadness of the peak is an evidence for the purity of the crystalline compound that is undergoing a melting process. At a melting process less pure or less perfect smaller crystals are melting first and after that purer larger crystals are melting. The melting temperature is defined as the temperature at which the three-dimensionally ordered crystalls change their state into the disordered liquid state. Melting-process-describing-temperatures are the onset of melting (T_o) and the peak melting temperature (T_m) [15].

Crystallization is the process at which a melt is cooled down or if a melt is heating above the glass transition temperature of amorphous materials. The temperature at which crystallization occurs is defined as the crystallization temperature (T_C). In a DSC diagramm an exothermic peak can be seen. The energy that is released when the molecules, atoms, etc. reorganize into a three-dimensional solid state is in relation to the crystall lattice energy. Some materials can crystallize into other molecular arrangements. This phenomen is called polymorphism [15].

2.4.2.2 X-Ray Diffraction

In the study of polymorphism x-ray diffraction has been used as a very essential tool for a very long time. This method facilitated the understanding of the polymorphic nature of TAGs. X-rays can be seen as electromagnetic waves with wavelenghts of about 1 Å and also as photons with an energy of about 10 keV [2]. In the crystallographic lattice x-rays are diffracted off of various planes. The intensity is depending on the position and the kind of atoms within the unit cell. If a x-ray hits a crystal it gives characteristically diffraction patterns which are used for structure analysis. Every crystalline substance possesses an own diffraction pattern, which is determined by its crystalline structure [4].

2.4.2.3 Contact angle

Contact angle measurements are usually used for undertaking wettability studies. The contact angle is the angle that is built due to the intersection of the liquid-solid interface and the liquid-vapor (gas) interphase. This interface in which all 3 phases do co-exist is the so-called „three-phase-contact line. Geometrically the contact angle is received when a tangent line is applied from the 3-phase point along the liquid-vapor interface in the profile of the droplet. The contact angle shows the degree of wetting when an interaction between a solid and a liquid occurs. Receiving a small contact angle, i.e. a contact angle <<90°, indicates a high wettability of a surface, wheras a large contact angle, i.e. a contact angle >> 90°, is corresponding to low wettability. A small contact angle is detected when the the liquid spreads on the surface, whereas a large contact angle can be detected when the liquid beads on the surface. Spreading means that the liquid drop turns into a flat puddle. Beading means that after getting in touch with the surface a compact liquid droplet is built [16].

Using water as the liquid compound information of the hydrophilic or hydrophobic behaviour of a substance can be received. The more hydrophilic a substance is the lower the contact angle. The higher the contact angle the more hydrophobic is the investigated substance.

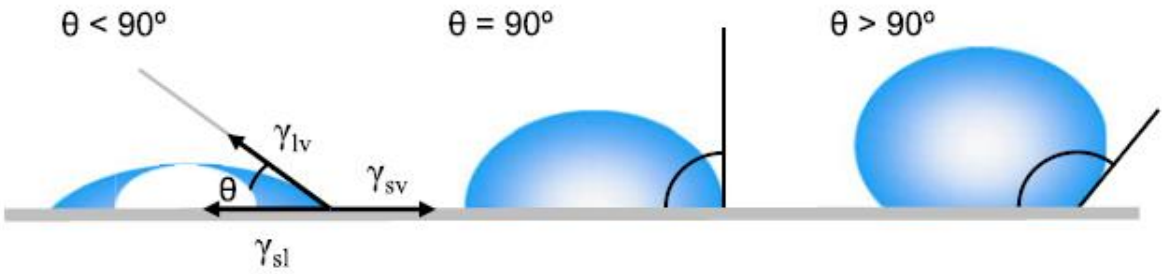


Fig. 6. Contact angles of different samples by sessile liquid drops on a smooth solid surface [16]

2.4.3 Analytical Methods for evaluation of the coated material – Dissolution

For drug characterization and qualtiy control of certain dosage forms dissolution tests are one of the most conducted tests. Dissolution tests are important, especially if dissolution is the rate-limiting step relating to drug absorption. From the quality control point of view, a dissolution test is established to verify product quality and also batch-to-batch consistency. Dissolution tests are also used in the period of product development and stability testing due to its importantance of the development specification for the product [15].

3 Material & Methods

3.1 APIs and coating materials

3.1.1 Active pharmaceutical ingredients

3.1.1.1 Citric acid anhydrous

Citric acid anhydrous, which is odorless, appears as colorless crystals and has a strong acidic taste. Its empirical fomula is $C_6H_8O_7$ with a molecular weight of 192.12 g/mol and a melting point of 153°C. Synonyms are acidum citricum anhydricum or E330. Citric acid is often used in pharmaceutical formulations and also in food products with the main reason of adjusting the pH of solutions. Anhydrous citric acid is mostly used in the preparation of effervescent tablets whereas citric acid monohydrat is mainly used for the prepartion of effervescent granules. It is listed as generally recognized as safe (GRAS). In Europe citric acid anhydrous is accepted as food additive [17]. Citric acid anhydrous was purchased from Jungbunzlauer, Wulzeshofen, Austria.

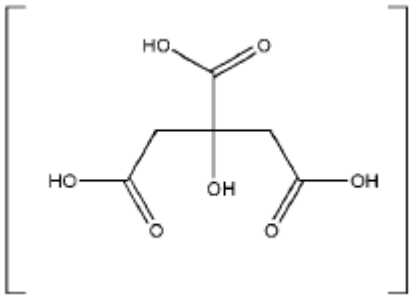


Fig. 7. Citric acid anhydrous [17]

3.1.1.2 N-Acetylcysteine

N-Acetylcysteine (NAC) is also known as acetylcysteine or N-acetyl-L-cysteine [18]. It appears as a white crystalline powder or as transparent crystals and is easy soluble in water. Indicated as mucolytic the daily recommended dose of NAC for adults is 600 mg and for children at a age from 2-12 years it is 300 mg as a daily dose distributed into 3 single doses per day [19]. Its empirical formula is $C_5H_9NO_3S$ with a molecular weight of 163.20 g/mol and a melting point of 105°C. It is used as a mucolytic agent. NAC leads to a reduction of the viscosity of the bronchial exudate by dissolving of the disulphide bonds in the glycoproteins of the mucus. It is also used as antidote after a paracetamol intoxication [18].

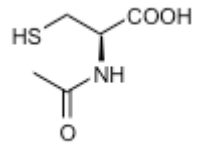


Fig. 8. N-Acetylcysteine

3.1.2 Coating material - Lipids

3.1.2.1 Tristearin

Tristearin with the trade name Dynasan® 118, is also known as stearic acid triglyceride or as glyceryl tristearate. Its molecular formula is $C_{57}H_{110}O_6$ and its molecular weight is 891.48 g/mol. It appears as white powder or colorless crystals and is odorless and also tasteless. Its melting point is 55°C for the alpha polymorph and about 73 °C for the beta polymorph. Tristearin is not soluble in water. If tristearin is heated above 55 °C it solidifies and melts again at 72°C [20]. Tristearin is a triglyceride in which all 3 hydroxyl-groups of the glycerol are esterified with stearic acid. Tristearin is a very hydrophobic substance as it can be seen in the chemical structure. There are no hydroxyl-groups, that would make the substance more hydrophilic. Dynasan® 118 was purchased from Cremer Oleo GmbH, Witten, Germany.

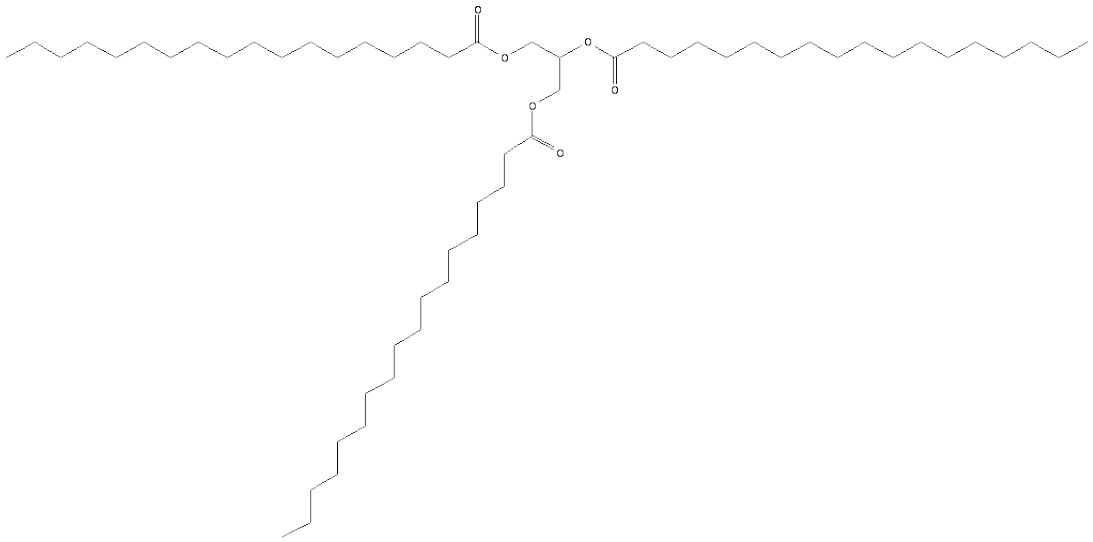


Fig. 9. Structure of tristearin [21]

3.1.2.2 New synthesized lipids

The new lipids are PG3, PG4 and PG6. PG is the abbreviation for polyglycerol. In general these new lipids are consisting of a polyglycerol molecule (so called glycerol-backbone), which is the alcoholic compound, and also of fatty acids. One molecule of glycerol consists of 3 C-atoms. The esterfication of the OH-groups of the glycerol with the fatty acid leads to the lipid structure.

All of the new-synthesized lipids were purchased from Cremer Oleo GmbH, Witten, Germany.

3.1.2.2.1 PG3

PG3 is a lipid that possess 3 molecules of glycerol and which is esterified with palmitic and stearic acid. The ratio of the esterification with palmitic and steric acid had to be find out and also the arrangement of the fatty acids was not known at the beginning.

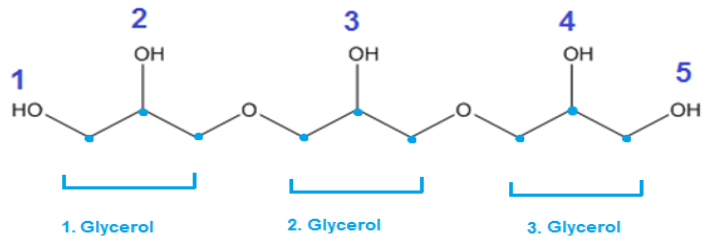


Fig. 10. PG3 molecule with its possible esterification positions

As it is shown above (Fig. 10) a PG3 molecule consists of 3 molecules of glycerol and it possesses 5 possible esterification positions (OH-positions).

Appearance: snow-white lipid

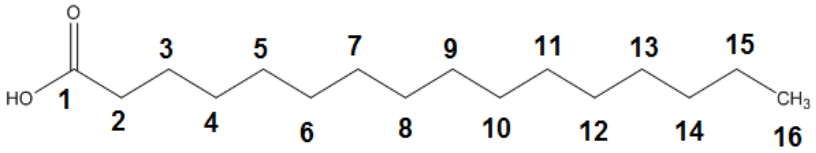


Fig. 11. Palmitic acid

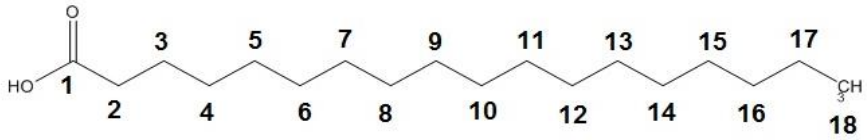


Fig. 12. Stearic acid

In the figures above (Fig. 11 & Fig. 12) palmitic fatty acid (C-16-FA) and stearic fatty acid (C-18-FA) are shown. As already mentioned the ratio of these FA's in a PG3 molecule is not known.

3.1.2.2.2 PG4

PG4 is a lipid that possesses 4 molecules of glycerol and is esterified only with stearic acid (C-18-FA).

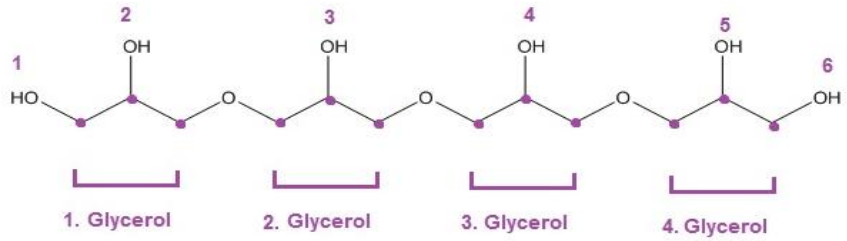


Fig. 13. PG4 molecule with its possible esterification positions

As it is shown above (Fig. 13), a PG4 structure consists of 4 molecules of glycerol and it possesses 6 possible esterification positions for the esterification with stearic acid. The structure of stearic acid is already shown above (Fig. 12).

Appearance: dark-yellow lipid

3.1.2.2.3 PG6

PG6 is a lipid that possesses 6 molecules of glycerol and is also only esterified with stearic acid (C-18-FA).

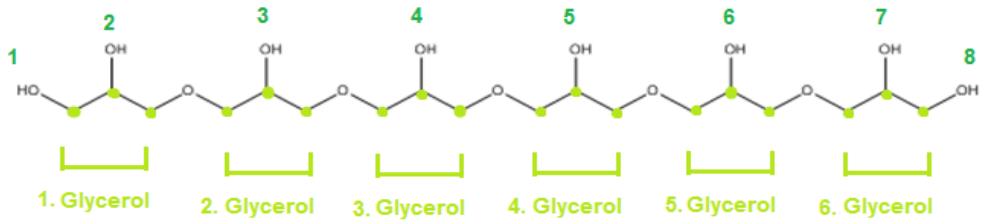


Fig. 14. PG6 molecule with its possible esterification positions

As it is shown above (Fig. 14) PG6 consists of 6 molecules of glycerol and it possesses 8 possible esterification positions.

Appearance: light-yellow lipid (same colour as butter has)

3.2 Methods

3.2.1 Manufacturing methods – Hot melt coating

Before starting a HMC process the parameter settings had to be choosen carefully. The parameters which had to be taken in mind are listed in the table below (Tab. 1).

Tab. 1. Consideration of parameter settings for the HMC-process

API	Lipid	Spraying pressure [bar]	Spraying rate [g/min]	Air flow rate [m^3/h]	Inlet temperature [°C]	Coating amount [%]
Citric acid anhydrous	Dynsasan 118	0,7	8	40	25	30; 40 & 50
NAC	PG6	1,4	7,9	35	48	30; 40 & 50
Citric acid anhydrous	PG6	1,4	7,53	40	48	30; 40 & 50
NAC	Dynasan 118	0,7	8	35	25	30; 40 & 50

After setting the parameters the HMC device was heated to 90 -100 °C. The lipid was molten in a pot and lead from the HMC device to the nozzle by a liquid pump.

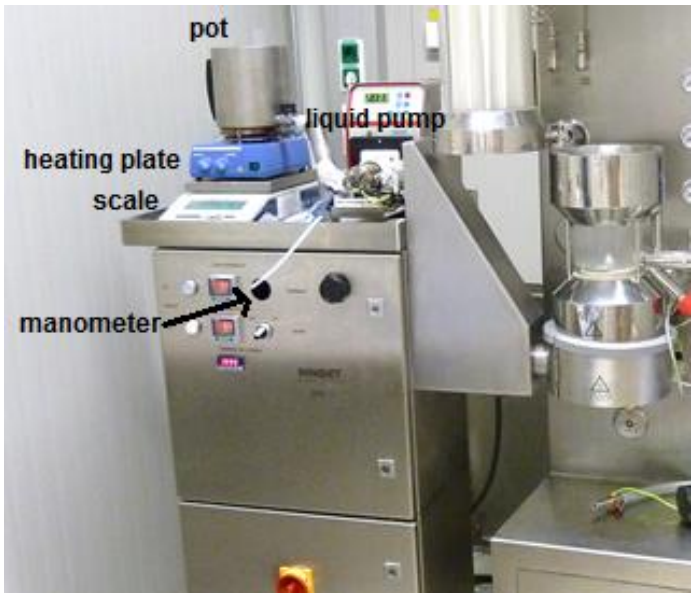


Fig. 15. HMC device

The nozzle, which is one of the most important parts of this process, was a three media nozzle, having one opening for spray liquid and two openings for guiding and atomizing air.

Fig. 16. Nozzle for the HMC process

The API was directly soaking into the fluid bed through an opening, which is shown in the picture below (Fig. 17). HMC was performed in a 1 liter glass container (Ventilus 2.5, Innojet Herbert Hüttlin, Germany). The considered coating amount of the end product was 50 % (w/w). Samples with coating amounts of 30 % and 40 % were drawn during the process.

Fig. 17. Fluid bed

HMC coating processes were performed for the API's citric acid anhydrous and NAC, used as core material, with Dynasan® 118 and PG6 as coating material. HMC batches were performed with an Innojet® Laboratory System Ventilus® V-2.5/1 with an Innojet® Hot-melt-Device IHD-1 (Herbert Hüttlin, Steinen, Germany).

3.2.2 Lipid analysis

3.2.2.1 Chemical structure analysis

For identifying the exact structures of the new-synthesized lipids the chemical structures were drawn with the software ChemBioDraw Ultra 14.0. For each of the new lipids the saponifaction number as well as the the hydroxyl value was calculated to estimate the number of esterifications for each lipid. By receiving the number of esterifications of each glycerol backbone all possible arrangements of the fatty acids were drawn with the software and the ^{1}H NMR spectrum was simulated and compared with the experimental ^{1}H NMR spectrum.

The 2 main methods were:

^{1}H NMR	Proton nuclear magnetic resonance
MS	Mass spectrometry

3.2.2.2 Physical behaviour of the new lipids

To obtain the knowledge of the physical solid state behaviour of the new synthesized lipids the 2 main methods were:

DSC	Differential scanning calorimetry
SWAX	Small and wide angle X-ray scattering

3.2.2.2.1 Differential scanning calorimetry

DSC measurements make it possible to observe the polymorphism in a sample. DSC measurements were conducted with DSC 204 F1 Phoenix from NETZSCH (Selb, Germany). A sample amount of about 5 mg was accurately weighted using an analytical balance (Mettler Toledo, XP 205, Vienna, Austria) and put into a perforated aluminium pan.

3.2.2.2.2 X-Ray diffraction

A small and wide angle compact Kratky camera with line-focus optics (HECUS X-Ray Systems, Graz, Austria) was used. The camera was fixed on a sealed-tube X-ray generator (Seifert, Ahrensburg, Germany) and was used at 30 kV and 0.4 mA. The measurements were conducted with a high brilliance microfocus x-ray source Cu, that has a wavelength of λ =1.54 A. The SAXS spectra were performed by using a linear position sensitive detector (PSD-50 M, HECUS X-ray systems, Graz, Austria) in the angular range of $0.06° < 2\theta < 8°$. By using an independent detector the WAXS spectra were recorded in the angular range of $17° < 2\theta < 27°$. The samples were put into a glass capillary and given into the capillary rotation unit.

3.2.2.2.3 Rheology - Viscosity measurements

Viscosity measurements of the two coating materials PG6 and Dynasan® 118 were perfomed in order to achieve good parameter settings for the HMC process. Knowing the viscosity of the coating materials allows the adjustment of a perfect setting of the spray pressure during the hot melt coating process. For viscosity measurements the Rheometer „Physica – Modular Compact Rheometer, MCR 300 by Anton Paar (Graz, Austria) was used. Measurements were conducted with a cone-plate system CP-50-2. The sample was directly molten on the plate and the viscosity was measured at various temperatures.

3.2.2.2.4 Contact angle measurements with obtaining the aging-effect

The lipid was taken out from its origin container with a spatula and was given into a 50 mL beaker glass (Schott Duran, Germany). The beaker glass was then put into a drying oven (Heraeus, Hanau, Germany) setted at 90°C until the whole lipid was molten. The molten lipid was carefully poured over an object slide 24*40 mm, (ROTH, Karlsruhe, Germany) and waited until the lipid resolidified. The aim was to get a flat and smooth surface, suitable for the contact angle measurements.

The solidified lipid slides were then given into a plastic container. For each lipid 2 containers, one for storage of lipids at room temperature (RT) and the other one for storage of the lipids at 40°C were used. The containers were put in bags and sealed under vacuum (Profi Cook PC-VK 1015, Kempen, Germany) and stored under two different conditions; RT and drying chamber at 40°C (WTC binder, Tuttlingen, Germany). At different time periods the contact angle of the prepared lipid slides was measured. For a better overview the conditions for the contact angle measurements are listed in the table below (Tab. 2).

Tab. 2. Conditions for contact angle measurements

Sample	Temperature	Contact angle [°] ± SD	
PG3	RT 40 °C	time 0	16 weeks
PG4	RT 40 °C	time 0	16 weeks
PG6	RT 40 °C	time 0	16 weeks

Contact angle measurements were conducted with the EasyDrop instrument (KRÜSS GmbH, Germany). For measuring the contact angle a drop of ultrapure water, with a volume of 3 µL, was put on the smooth surface of the lipid. After a waiting time of 3 minutes the contact angle was measured. All measurements were conducted 10 times (n=10).

3.2.2.3 Analytical methods for evaluation of the coated material

3.2.2.3.1 Dissolution

Dissolution trials were used for determining the amount of drug released [%] after a certain time. In dependency of time dissolution results were categorized into immediate release, delayed release and controlled release. In dependency of drug formulations different dissolution methods are available in many pharmacopoeias, such as the USP and EuPh. For this study the USP apparatus 2, paddle method was used.

3.2.2.3.1.1 Reference solution

For every dissolution trial fresh reference solutions had to be prepared. The preparation of the reference solutions requires solely the pure API for constructing a calibration curve. For the dissolution trials for citric acid products a stock solution with the concentration of 0.555 mg / mL was prepared. Different concentrated reference solutions were provided by diluting the stock solution (Tab. 3).

Tab. 3. Concentration of the reference solutions of citric acid anhydrous

Concentration [%]	Concentration [mg / mL]
100	0.555
80	0.444
50	0.278
25	0.139
12.5	0.069
6.25	0.035

For the dissolution trials of NAC products the stock solution of 0.666 mg / mL was prepared and diluted (Tab. 4).

Tab. 4. Concentrations of the reference solutions of NAC

Concentration [%]	Concentration [mg / mL]
100	0.666
80	0.533
50	0.333
25	0.166
12.5	0.083
6.25	0.042

After preparing the reference solutions their absorbance was measured with a UV-Vis spectrometer. The concentration values were put against the absorbance values in a diagram. A trendline with the coefficient of determination [R^2] was received.

3.2.2.3.1.2 Content assay

The HMC material was milled in two cycles, total 5 minutes, using a cryomill (RETSCH, Haan, Germany).

The milled coated material equal to a concentration of 0.5 mg citric acid/ mL was weighted into a 250 mL measuring flask. The flasks were filled with 100 mL ultrapure water with a 100 mL volumetric pipette (BRAND, Wertheim, Germany). The measuring flask was given into an ultrasonic bath (ELMA, Singen, Germany) for 10 minutes at 40 °C and was shaken carefully but strongly every few minutes for dissolving the whole core material. After the ultrasonic bath the sample was cooled down at RT. Some milliliters of the sample were filled into a labelled eprouvette through a filter by using a syringe for the following detection via UV-Vis spectrometer. All samples were done in triplicates [n=3].

3.2.2.3.1.3 Dissolution trials

For preparing the dissolution tester (ERWEKA, Heusenstamm, Germany), 900 mL of ultrapure water, used as dissolution medium, was poured into each of the six dissolution vessels. The dissolution tester was heated up to 37 °C ± 0.5 °C before starting the dissolution trials. The amount of coated particles that is equal to the daily dose of 500 mg citric acid or 600 mg NAC, which was calculated on the basis of the theoretical API content was prepared for each vessel. The dissolution conditions for the different coating materials can be seen in the tables below (Tab. 5 & Tab. 6).

Tab. 5. Dissolution conditions for PG6 as coating material

Equipment	Dissolution Tester Erweka
Rotational speed	100 rpm
Medium	Ultrapure water
Volume	900 mL
Temperature	37°C ± 0,5 °C
Sampling points	1, 5, 15, 30, 45, 60, 90 min
Sampling	Automatic sampler

Tab. 6. Dissolution conditions for Dynasan 118 as coating material

Equipment	Dissolution Tester Erweka
Rotational speed	100 rpm
Medium	Ultrapure water
Volume	900 mL
Temperature	37°C ± 0,5 °C
Sampling points	1, 5, 15, 30, 45, 60, 90, 120, 150, 180, 210, 240, 270, 300, 330, 360 min
Sampling	Automatic sampler

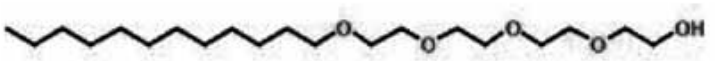
The concentration of the drug in the dissolution medium at 37 °C was determined by using a Perkin Elmer LAMBDA 25 UV-VIS spectrophotometer (Boston, MA, US), at the wavelenghts of 213 nm and 216.8 nm for citric acid anhydrous and NAC, respectively. The spectrophotometer was directly linked to the Erweka Dissolution Tester. The absorption of the samples at various time points was measured and the amount of drug released [%] of the different samples at this time points was calculated.

3.2.2.3.2 pH- measurements

For conducting pH measurements with the final products a pH meter from Carl Roth GmbH + Co KG (Karlsruhe, Germany) was used. The pH meter was calibrated with 2 buffer solutions with the pH of 4.01 and 7.00 to obtain a two point calibration. Due to the point that both APIs having carboxylic groups and sour taste, the aim of the pH measurements was to correlate the taste masking of coated API to the changes in the pH after one minute application. 100 mL of ultrapure water were given into a 250 mL beaker glass and due to the poor wettability of the lipid-coated products, 1 % (w/w) of Brij® 30 (Croda GmbH, East Yorkshire, UK) was added to the ultrapure water as surfactant. The amount of coated product that is equal to a theoretical daily dose of 500 mg of citric acid and 600 mg for NAC, was given to the liquid mixture and stirred with a magnetic stirrer. A countdown-timer was setted every 5 seconds over 5 minutes and the pH value was noted. Reference solutions were prepared for the pure APIs. Solutions with the following concentrations of 5; 3.75; 2.5; 1; 0.5 and 0.1 mg/ mL of citric acid anhydrous were prepared and used as reference. For NAC solutions with the following concentrations were prepared: 6; 4.5; 3; 1 and 0.1 mg/mL. For evaluating the data the pH of the product after 60 seconds was compared with the respective reference solution to obtain the concentration of released API after 1 minute.The lower the pH, the faster the drug release, but also the worse the taste masking.

3.2.2.3.2.1 Brij® 30

Brij® 30 belongs to the group of polyoxyethylene alkyl ethers, which are non-ionic-surfactants. They are consisting of polyoxyethylene glycol ethers of n-alcohols. Another name for Brij® 30 is Polyoxyl-4-lauryl ether [17]. Polyoxyethylene alkyl ethers are used as emulsifying agents, penetration enhancers, solubilizing agent as well as wetting agent [17]. The HLB-value of Brij® 30 is 9.7 [17] [22].



$[C_{12}H_{25}(OCH_2CH_2)_4OH]$

Fig. 18. Molecular structure and formula of Brij® 30 [22]

4 Results & Discussion

4.1 Manufacturing Results

The aim of the HMC studies was to compare the coating properties of PG6 with tristearin (Dynasan® 118). The inlet temperature was selected below the melting point of the α-form for both tristearin and PG6. The viscosity measurements were used to choose the best setting of atomizing air pressure for the pre-defined spray rate of approximately 8 g / min. The more viscous the coating material the higher the spraying pressure should be to achieve a certain spray rate. The lower the spraying pressure, the larger are the droplets of the sprayed lipid. Choosing the lower atomizing air pressure for viscose coating material can easily lead to agglomeration. Due to the very electrostatic behaviour of NAC a lower air flow rate was setted for this process to achieve a rather good fluidization. In comparison to a HMC-process with citric acid anhydrous as core material it was possible to choose a higher air flow rate and still have a good fluidizing process.

Tab. 7. Final settings for the HMC process

API	Lipid	Spraying pressure [bar]	Spraying rate [g/min]	Air flow rate [m^3/h]	Inlet temperature [°C]	Coating amount [%]
Citric acid anhydrous	Dynsasan 118	0,7	8	40	25	30; 40 & 50
NAC	PG6	1,4	7,9	35	48	30; 40 & 50
Citric acid anhydrous	PG6	1,4	7,53	40	48	30; 40 & 50
NAC	Dynasan 118	0,7	8	35	25	30; 40 & 50

4.2 Results of lipid analyis

4.2.1 Chemical structure analysis

4.2.1.1 Calculation of the number of esterifications with the SAP

4.2.1.1.1 SAP of PG3

SAP= 162 mg KOH / g fat

MW (KOH)= 56.11 [g/mol]

$$n = \frac{m}{M} \qquad \textbf{(Equ. 1)}$$

$$n = \frac{0.162\ [g]}{56.11\ [\frac{g}{mol}]} = 0.0028871 \text{ mol KOH} \qquad \textbf{(Equ. 2)}$$

➔ 0.0028871 mol KOH are needed for the saponification of 1 g fat of PG3.

MW (PG3 + 1 FA) = 492.69 [g/ mol]

$$n = \frac{1\ [g]}{492.69\ [\frac{g}{mol}]} = 0.0020296 \text{ mol} \qquad \textbf{(Equ. 3)}$$

MW (PG3 + 2 FA) = 745.14 [g/mol]

$$n = \frac{1\ [g]}{745.14\ [\frac{g}{mol}]} = 0.0013420\ [mol] \times 2 = 0.002684 \text{ mol} \qquad \textbf{(Equ. 4)}$$

MW (PG3 + 3 FA) = 997.63 [g/ mol]

$$n = \frac{1\ [g]}{997.63\ \left[\frac{g}{mol}\right]} = 0.0010023\ [mol] \times 3 = 0.0030072\ mol \qquad \textbf{(Equ. 5)}$$

➔ According to the SAP PG3 is esterified with 2 and / or 3 fatty acids.

4.2.1.1.2 SAP of PG4

SAP= 148 mg KOH / g fat

MW (KOH)= 56.11 [g/mol]

$$n = \frac{0.148\ [g]}{56.11\ \left[\frac{g}{mol}\right]} = 0.002638\ mol\ KOH \qquad \textbf{(Equ. 6)}$$

➔ 0.002638 mol KOH are needed for the saponification of 1g fat of PG4.

MW (PG4 + 1 FA) = 580.891 [g/ mol]

$$n = \frac{1\ [g]}{580.8919\ \left[\frac{g}{mol}\right]} = 0.00172\ mol \qquad \textbf{(Equ. 7)}$$

MW (PG4 + 2 FA) = 847.27 [g/mol]

$$n = \frac{1\ [g]}{847.27\ \left[\frac{g}{mol}\right]} = 0.00118\ [mol] \times 2 = 0.00236\ mol$$ **(Equ. 8)**

MW (PG4 + 3 FA) = 1113.74 [g/mol]

$$n = \frac{1\ [g]}{1113.74\ \left[\frac{g}{mol}\right]} = 8.979 \times 10^{-4}\ [mol] \times 3 = 0.00269\ mol$$ **(Equ. 9)**

➔ According to the SAP PG4 is esterified with 3 fatty acids.

4.2.1.1.3 SAP of PG6

SAP= 148 mg KOH / g fat

MW (KOH)= 56.11 [g/mol]

$$n = \frac{0.148\ [g]}{56.11\ \left[\frac{g}{mol}\right]} = 0.002638\ mol\ KOH$$ **(Equ. 10)**

➔ 0.0028638 mol KOH are needed for the saponification of 1 g fat of PG6.

MW (PG6 + *1 FA*) = 728.96 [g/ mol]

$$n = \frac{1\ [g]}{728.96\ \left[\frac{g}{mol}\right]} = 0.001372\ mol$$ **(Equ. 11)**

MW (PG6 + 2 FA)= 995.43 [g/mol]

$$n = \frac{1\ [g]}{728.96\ \left[\frac{g}{mol}\right]} = 0.00100459\ [mol] \times 2 = 0.002009\ mol \qquad \textbf{(Equ. 12)}$$

MW (PG6 + 3 FA)= 1261.9 [g/mol]

$$n = \frac{1\ [g]}{1261.9\ \left[\frac{g}{mol}\right]} = 0.00079246\ [mol] \times 3 = 0.002377\ mol \qquad \textbf{(Equ. 13)}$$

MW (PG6 + 4 FA) = 1528.37 [g/mol]

$$n = \frac{1\ [g]}{1528.37\ \left[\frac{g}{mol}\right]} = 0.00065429\ [mol] \times 4 = 0.002612\ mol \qquad \textbf{(Equ. 14)}$$

➔ According to the SAP PG6 is esterified with 4 fatty acids.

4.2.1.2 Calculation of the free OH-groups with the hydroxyl value (HV)

4.2.1.2.1 HV of PG3

HV= 148 mg KOH / g fat

MW (KOH)= 56.11 [g/mol]

$$n = \frac{0.148\ [g]}{56.11\ \left[\frac{g}{mol}\right]} = 0.0026377\ mol\ KOH \qquad \textbf{(Equ. 15)}$$

MW (PG3 + 4 OH-groups)= 492.71 [g/mol]

$$n = \frac{1\ [g]}{492.71\ \left[\frac{g}{mol}\right]} = 0.0020296\ [mol] \times 4 = 0.008118\ mol \qquad \textbf{(Equ. 16)}$$

MW (PG3 + 3 OH-groups) = 745.14 [g/mol]

$$n = \frac{1\ [g]}{745.14\ \left[\frac{g}{mol}\right]} = 0.00134203\ [mol] \times 3 = 0.00402\ mol \qquad \textbf{(Equ. 17)}$$

MW (PG3 + 2 OH-groups) = 997.58 [g/mol]

$$n = \frac{1\ [g]}{997.58\ \left[\frac{g}{mol}\right]} = 0.0010024\ [mol] \times 2 = 0.002004\ mol \qquad \textbf{(Equ. 18)}$$

➔ According to the HV PG3 consists of a mixture of molecules with 2 and 3 free hydroxyl groups. That means when 2 free OH-groups are existing the molecule is esterified with 3 FA's and with 3 free OH-groups the molecule is esterfied with 2 FA's.

4.2.1.2.2 HV of PG4

HV= 167 mg KOH / g fat

MW (KOH)= 56.11 [g/mol]

$$n = \frac{0.167\ [g]}{56.11\ \left[\frac{g}{mol}\right]} = 0.0029763 \text{ mol KOH} \qquad \textbf{(Equ.19)}$$

MW (PG4 + 5 OH-groups) = 580.81 [g/mol]

$$n = \frac{1\ [g]}{580.81\ \left[\frac{g}{mol}\right]} = 0.001721173\ [mol] \times 5 = 0.0086086 \text{ mol} \qquad \textbf{(Equ. 20)}$$

MW (PG4 + 4 OH-groups) = 847.27 [g/mol]

$$n = \frac{1\ [g]}{847.27\left[\frac{g}{mol}\right]} = 0.00118026\ [mol] \times 4 = 0.004721 \text{ mol} \qquad \textbf{(Equ. 21)}$$

MW (PG4 + 3 OH-groups) = 1113.74 [g/mol]

$$n = \frac{1\ [g]}{1113.74\ \left[\frac{g}{mol}\right]} = 8.9787 * 10^{-4}\ [mol] \times 3 = 0.0026936\ mol \qquad \textbf{(Equ. 22)}$$

➔ According to the HV PG4 consists of a mixture of molecules with 3 and 4 free hydroxyl groups. That means when 3 free OH-groups are existing the molecule is esterified with 3 FA's and with 4 free OH-groups the molecule is esterfied with 2 FA's.

4.2.1.2.3 HV of PG6

HV= 172 mg KOH / g fat

MW (KOH)= 56.11 [g/mol]

$$n = \frac{0.172\ [g]}{56.11\ \left[\frac{g}{mol}\right]} = 0.003066\ mol\ KOH \qquad \textbf{(Equ. 23)}$$

MW (PG6 + 7 OH-groups) = 728.96 [g/mol]

$$n = \frac{1\ [g]}{728.96\ \left[\frac{g}{mol}\right]} = 0.00137182\ [mol] \times 7 = 0.0096027\ mol \qquad \textbf{(Equ. 24)}$$

MW (PG6 + 6 OH-groups) = 995.43 [g/mol]

$$n = \frac{1\ [g]}{995.43\left[\frac{g}{mol}\right]} = 0.001004\ [mol] \times 6 = 0.0060275\ mol \quad \textbf{(Equ. 25)}$$

MW (PG6 + 5 OH-groups) = 1261.9 [g/mol]

$$n = \frac{1\ [g]}{1261.9\left[\frac{g}{mol}\right]} = 7.924558 * 10^{-4}\ [mol] \times 5 = 0.003962\ mol \quad \textbf{(Equ. 26)}$$

MW (PG6 + 4 OH-groups) = 1528.37 [g/mol]

$$n = \frac{1\ [g]}{1528.37\left[\frac{g}{mol}\right]} = 6.5429 * 10^{-4}\ [mol] \times 4 = 0.002617\ mol \quad \textbf{(Equ. 27)}$$

➔ According to the HV PG6 consists of a mixture of molecules with 4 and 5 free hydroxyl groups. That means when 4 free OH-groups are existing the molecule is esterified with 4 FA's and with 5 free OH-groups the molecule is esterfied with 3 FA's.

4.2.1.3 ^{1}H NMR

4.2.1.3.1 ^{1}H NMR spectrum of PG3

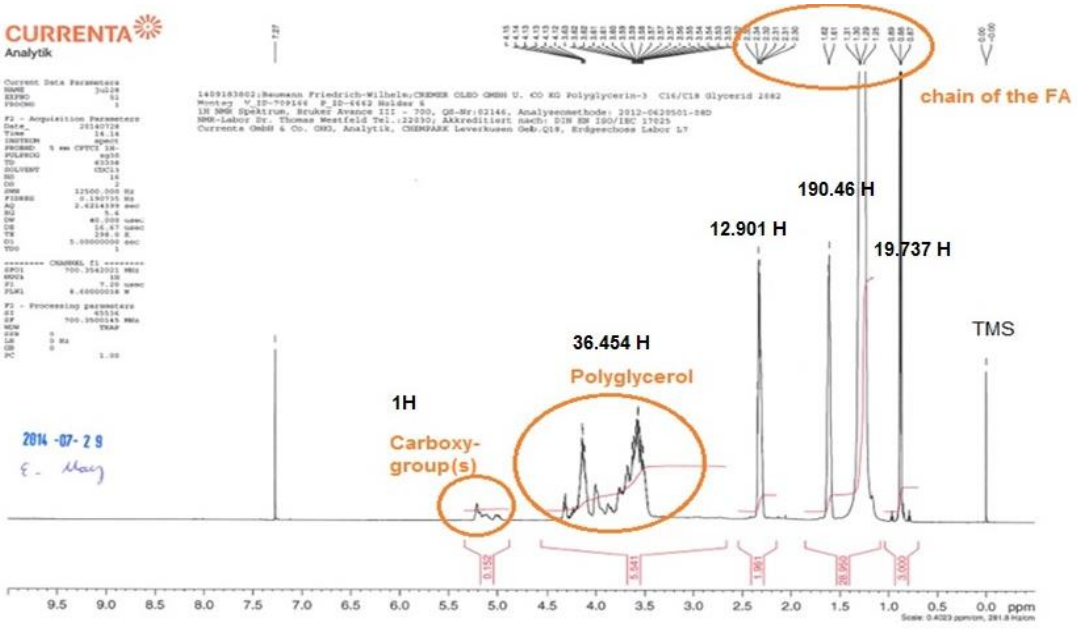


Fig. 19. ^{1}H NMR spectrum of PG3

In the lower range (0.8 ppm - 2.35 ppm) the chain of the fatty acid is apparent in the ^{1}H NMR. In the range of 3.52 ppm – 4.15 ppm the glycerol-backbone is apparent in the ^{1}H NMR. The highest chemical shift is apparent in the range of 5.0 ppm – 5.40 ppm, which is the influence of the ester.

The number of protons in the experimental spectrum involving the intensity are listed below:

0.152 / 0.152 = 1 H

5.541 / 0.152 = 36.454 H

1.961 / 0.152 = 12.901 H

28.950 / 0.152 = 190.46 H3.000 / 0.152 = 19.737 H

1 H	either at 5.46 ppm or 5.07 ppm (Carboxy)
36.454 H	including glycerol backbone and free OH-groups at 2.55 ppm
12.901 H	part of the FA‘s next to the carboxy-group at 2.32 ppm (2 H) → 12.901/2 = 6,45 FA‘s
19.737 H	terminal part of a FA (-CH3-group → 3 H)
	19.737 / 3 = 6.57 FA‘s
190.46 H	main part of the FA’s
	190.46 H / 24 H = 7.93 FA‘s
	190.46 H / 28 H = 6.8 FA‘s
	The main part oft he FA‘s is depending if there is a C-16 chain (24 protons from 1.26 ppm to 1.66 ppm) or a C-18 chain (28 protons from 1.26 ppm to 1.66 ppm).

Due to the highest chemical shift in the range of 5.0 ppm – 5.40 ppm the ^{1}H NMR spectrum indicates that there should also be a 1_2_PG3-C-16_C-18 arrangement in the mixture.

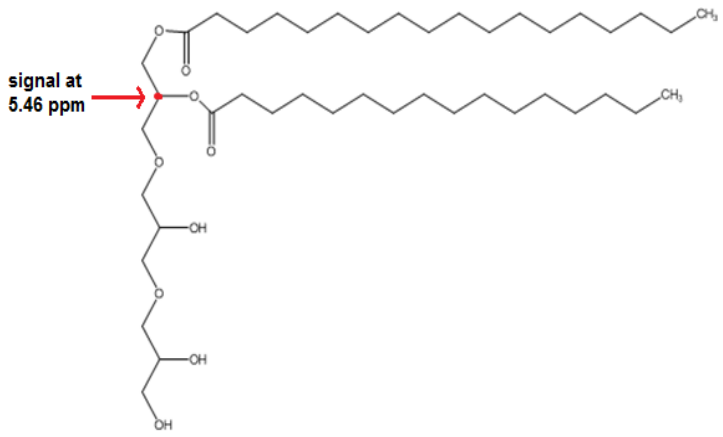


Fig. 20. PG3 molecule esterified with palmitic and stearic acid at position 1 and 2

4.2.1.3.2 ^{1}H NMR spectrum of PG4

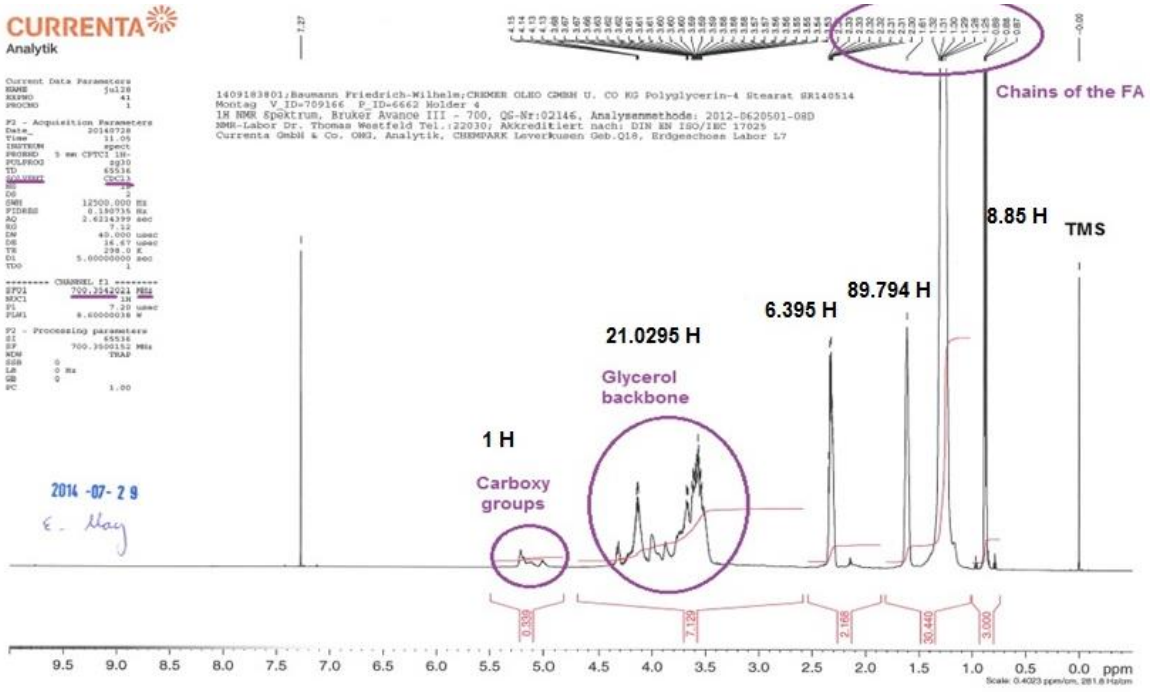


Fig. 21. ^{1}H NMR spectrum of PG4

In the lower range (0.8 ppm - 2.35 ppm) the chain of the fatty acid is apparent in the NMR. In the range of 3.53 ppm – 4.15 ppm the glycerol-backbone is apparent in the NMR. The highest chemical shift is apparent in the range of 5.0 ppm – 5.40 ppm, which is the influence of the ester.

The number of protons in the experimental spectrum involving the intensity are listed below:

0.339 / 0.339 = 1H

7.129 / 0.339 = 21.0295 H

2.168 / 0.339 = 6.395 H

30.440 / 0.339 = 89.794 H

3.000 / 0.339 = 8.85 H

1 H	either at 5.46 ppm or 5.07 ppm (carboxy-group)
21.0295 H	including glycerol backbone and free OH-groups at 2.55 ppm
6.395 H	part of the FA‘s next to the carboxy-group at 2.32 ppm (2 H) → 6.395/2 = 3.198 FA’s
89.794 H	main part of the FA‘s
	89.794 H / 28 H = 3.207 FA‘s
8.85 H	terminal part of a FA (-CH3 → 3 H)
	8.85 / 3 = 2.95 FA‘s

Due to the highest chemical shift in the range of 5.0 ppm – 5.40 ppm the ^{1}H NMR spectrum indicates that there should also be a 1_2_3_PG4-C18 arrangement in the mixture.

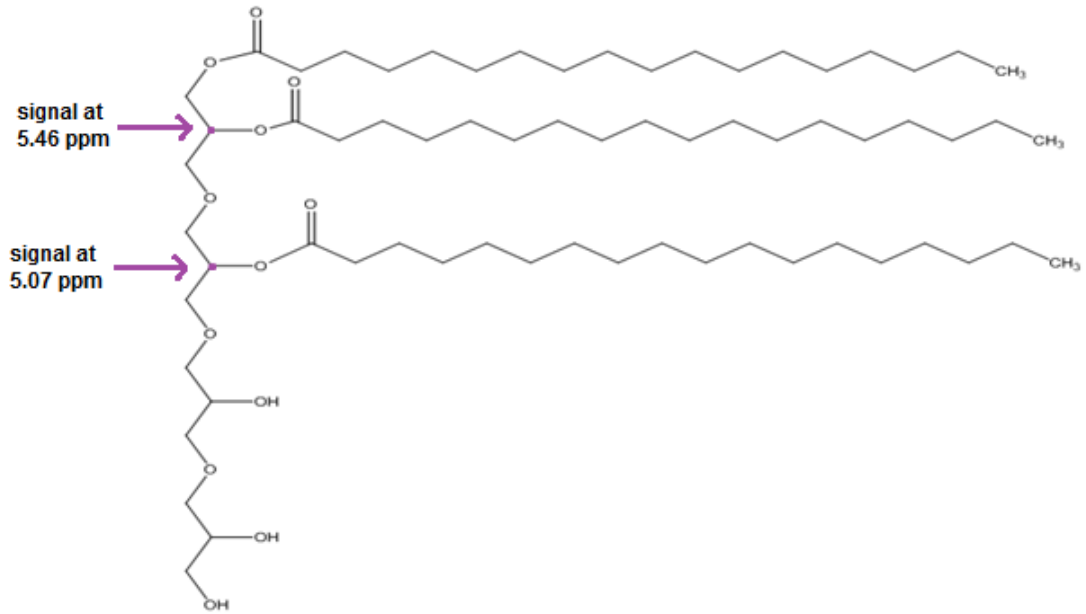


Fig. 22. PG4 esterified with stearic acid at the position 1, 2 and 3

4.2.1.3.3 ^{1}H NMR spectrum of PG6

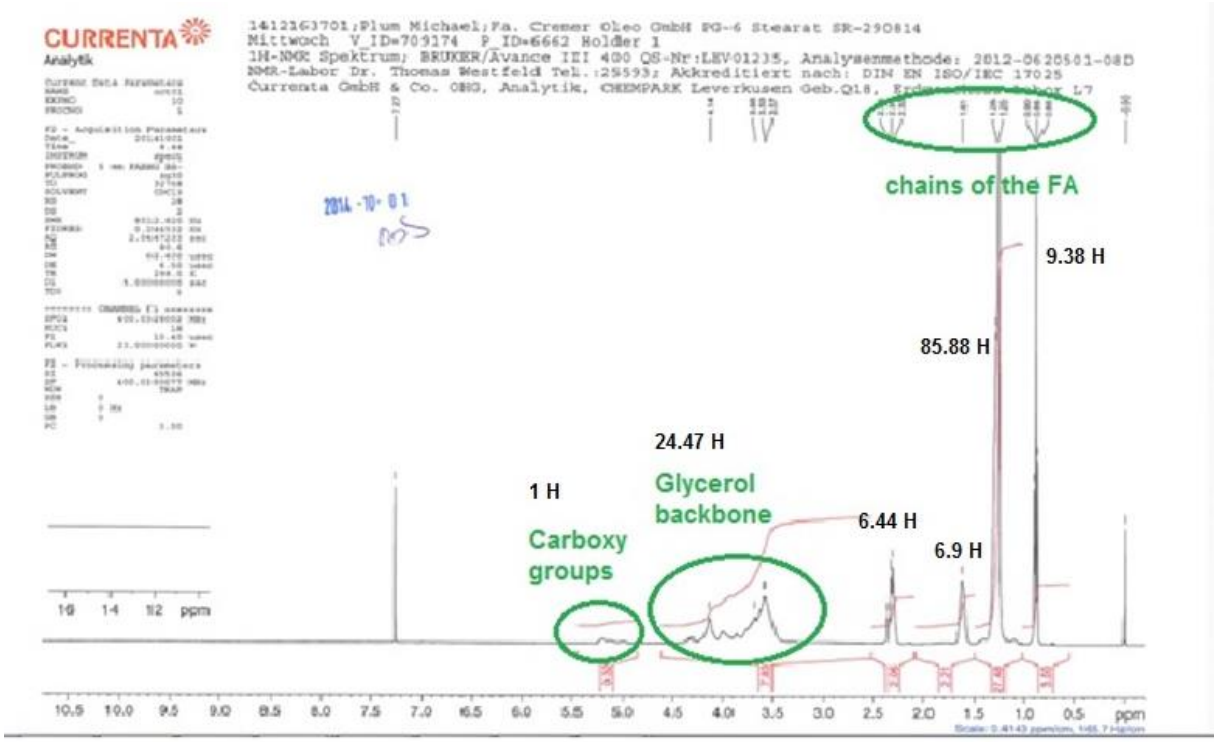


Fig. 23. ^{1}H NMR spectra of PG6

In the lower range (0.8 ppm - 2.35 ppm) the chain of the fatty acid is apparent in the NMR. In the range of 3.53 ppm – 4.15 ppm the glycerol-backbone is apparent in the NMR. The highest chemical shift is apparent in the range of 5.0 ppm – 5.40 ppm, which is the influence of the ester. The number of protons in the experimental spectrum involving the intensity are listed below:

0.32 / 0.32 = 1 H

7.83 / 0.32 = 24.47 H

2.06 / 0.32 = 6.44 H

2.21 / 0.32 = 6.9 H

27.48 / 0.32 = 85.88 H

3.00/ 0.32 = 9.38 H

1 H	either at 5.46 ppm or 5.07 ppm (carboxy-group)
24.47 H	including glycerol backbone and free OH-groups at 2.55 ppm
6.44 H	part of the FA‘s next to the carboxy-group at 2.32 ppm
	(2 H) → 6.44/2 = 3.22 FA‘s
85.88 H	main part of the FA‘s
	85.88H / 28 H = 3.067FA‘s
9.38 H	terminal part of a FA (-CH3 → 3 H)
	9.38 / 3 = 3.127 FA‘s

Due to the highest chemical shift in the range of 5.0 ppm – 5.40 ppm the 1H NMR spectrum indicates that the following arrangements might be possible:

1.) PG6 _1_2_3_4_stearic acid

2.) PG6 _1_2_3_5_stearic acid

3.) PG6 _1_2_3_6_stearic acid

4.) PG6_1_2_3_7_stearic acid

5.) PG6_1_2_3_8_stearic acid

6.) PG6_1_2_4_8_stearic acid

7.) PG6_1_2_5_8_stearic acid

8.) PG6_1_2_6_8_stearic acid

9.) PG6_1_2_7_8_stearic acid

It is again shown that a mixture of various arrangements of the fatty acids might be possible.

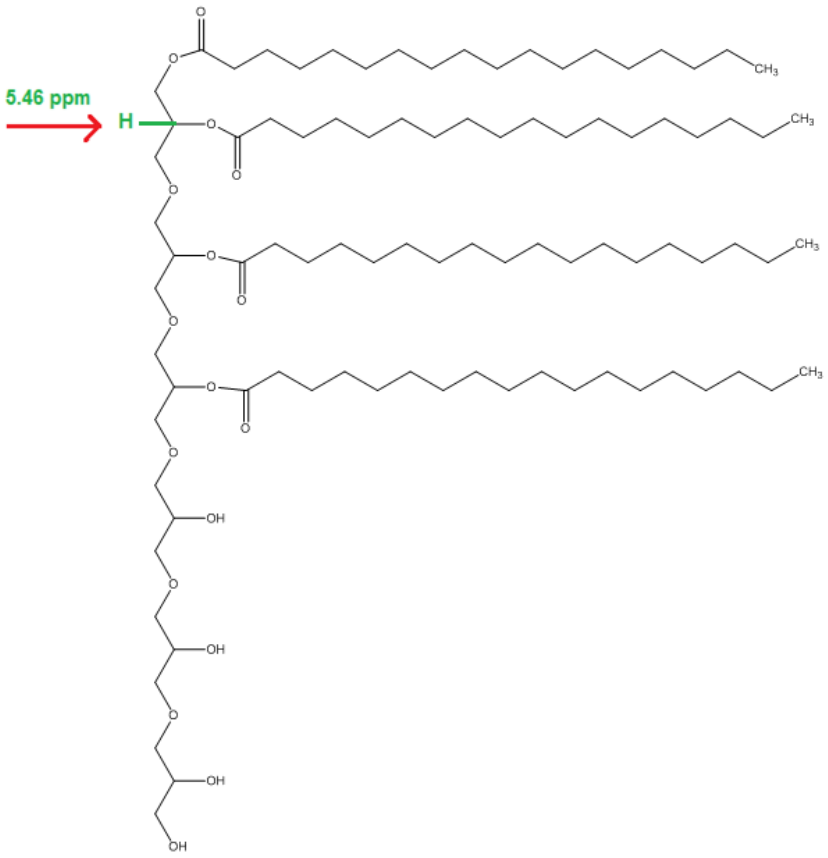


Fig. 24. Example of a possible structure in the mixture of PG6

4.2.1.4 Mass spectrometry

4.2.1.4.1 Mass spectrum of PG3

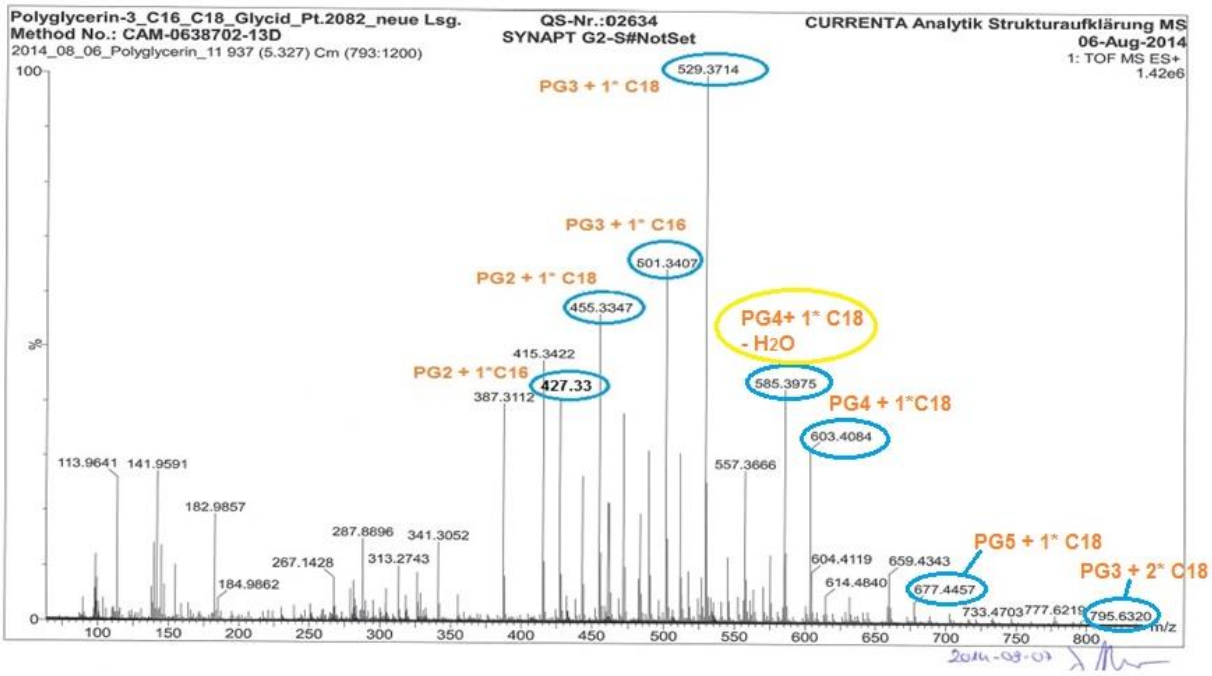


Fig. 25. Mass spectrum of PG3

TOF MS ES+: Electrospray ionization (positive mode) with time-of-flight mass analyzer

Due to the absence of a M+ signal in the MS-spectrum it is not possible to read the molecular weight of the structure out of the spectrum.

Due to ESI quasi-molecular-ions [M+H+] might be available. In this case the quasi-molecular-ion is Na^+. The molecular weight (MW) of Na^+ is 22.99 g/mol. This means that fragment-signals in the spectrum show their own MW plus the MW of Na^+. The basis signal, which represents the most common fragment, which hits the detector appears at 529.3714 m/z. This basis signal at 529.3714 m/z represents the molecular weight of a PG3 molecule with a C-18-fatty acid (m/z: 506.38) plus the MW of Na^+.

Other representative signals for a PG3 lipid, which were detected as Na^+-adducts are listed below:

501.3407 m/z PG3 + 1 * C-16 FA (1 esterification)

455.3347 m/z PG-2 + 1 * C-18 FA (1 esterification)

427.30 m/z PG-2 + 1 * C-16 FA (1 esterification)

795.6320 m/z PG3 + 2 * C-18 FA (2 esterifications)

585.3975 m/z PG4 + 1 * C-18 FA minus H_2O (1 esterification & dehydration)

603.4084 m/z PG4 + 1 * C-18 FA (1 esterification)

677.4500 m/z PG-5 + 1 * C-18 FA (1 esterification)

4.2.1.4.2 Mass spectrum of PG4

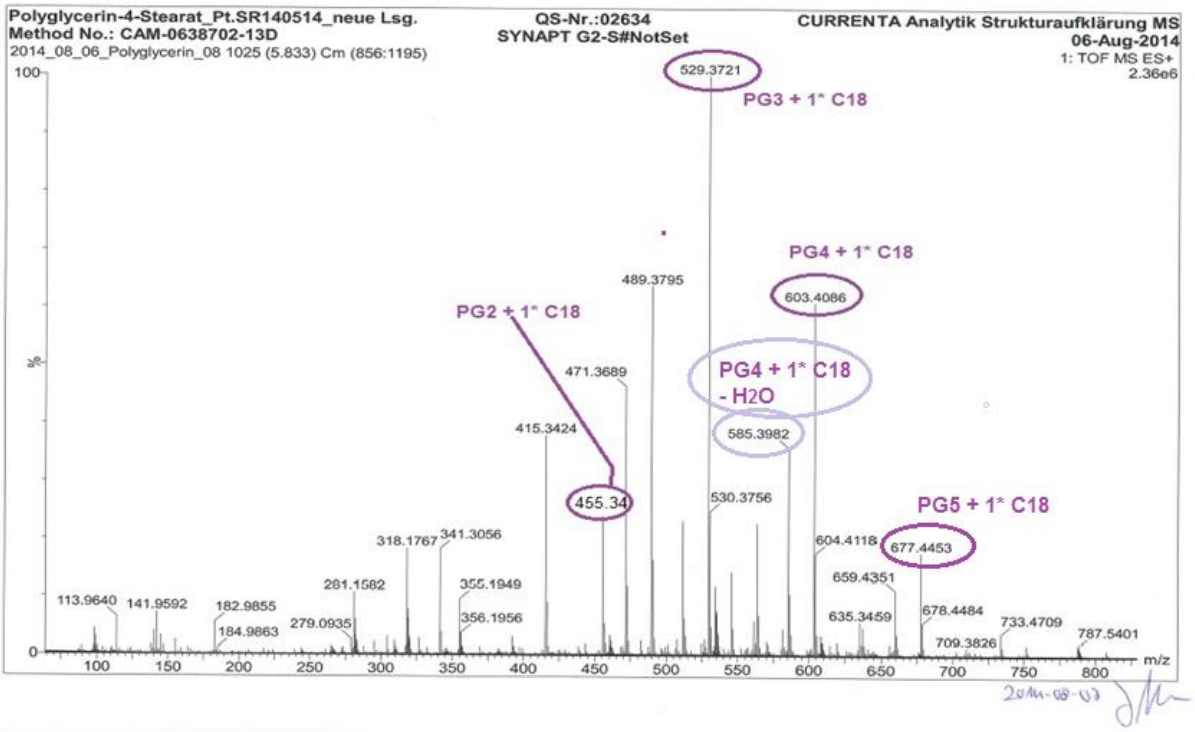


Fig. 26. Mass spectrum of PG4

TOF MS ES+: Electrospray ionization (positive mode) with time-of-flight mass analyzer

Due to the absence of a M+ signal in the MS-spectrum it is not possible to read the molecular weight of the structure out of the spectrum.

Due to ESI quasi-molecular-ions [M+H+] might be available. In this case the quasi-molecular-ion is Na^+. The MW of Na^+ is 22.99 g/mol. This means that fragment-signals in the spectrum show their own MW plus the MW of Na^+.

The basis signal, which represents the most common fragment, which hits the detector appears at 529.3714 m/z. This basis signal at 529.3714 m/z represents the molecular weight of a PG3 molecule with a C-18-fatty acid (m/z: 506.38) plus the MW of Na^+. Other representative signals for a PG4 lipid, which were detected as Na^+-adducts are listed below:

603.4086 m/z	PG4 + 1 * C-18 FA (1 esterification)
585.40 m/z	PG4 + 1 * C-18 minus H_2O (1 esterification & dehydration)
455.3400 m/z	PG-2 + 1 * C-18 FA (1 esterification)
529.3714 m/z	PG3 + 1 * C-18 FA (basis signal & 1 esterification)
677.4453 m/z	PG-5 + 1 * C-18 FA (1 esterification)

4.2.1.4.3 Mass spectrum of PG6

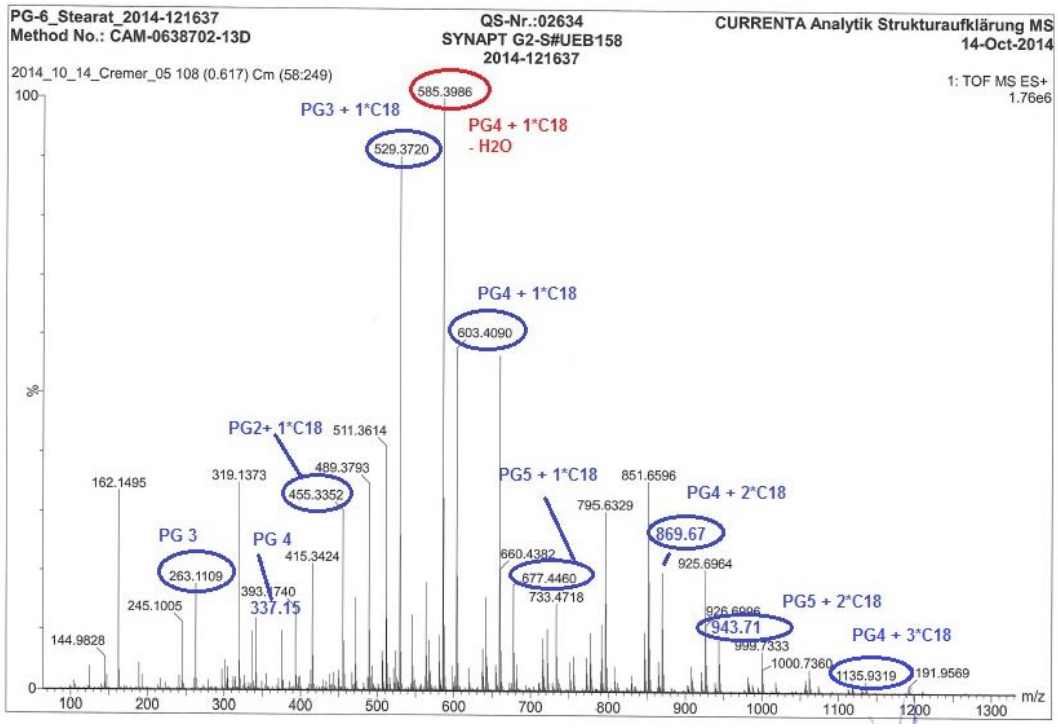


Fig. 27. Mass spectrum of PG6

TOF MS ES+: Electrospray ionization (positive mode) with time-of-flight mass analyzer

Due to the absence of a M+ signal in the MS-spectrum it is not possible to read the molecular weight of the structure out of the spectrum.

Due to ESI quasi-molecular-ions [M+H+] might be available. In this case the quasi-molecular-ion is Na^+. The MW of Na^+ is 22.99 g/mol. This means that fragment-signals in the spectrum show their own MW plus the MW of Na^+.

The basis signal, that represents the most common fragment, which hits the detector appears at 585.3986 m/z. This basis signal at 585.3986 m/z represents the molecular weight of a PG4 molecule with a C-18-FA (m/z: 580.891) plus the MW of Na^+ minus the loss of water (dehydration).

Other representative signals for a PG6 lipid, which were detected as Na^+-adducts are listed below:

263.1109 m/z	PG3 molecule (unesterified)
337.15 m/z	PG4 molecule (unesterified)
455.3352 m/z	PG-2 + 1 * C-18 FA (1 esterification)
529.3720 m/z	PG3 + 1 * C-18 FA (1 esterification)
603.490 m/z	PG4 + 1 * C-18 FA (1 esterification)
677.4460 m/z	PG-5 + 1 * C-18 FA (1 esterification)
869.67 m/z	PG4 + 2 * C-18 FA (2 esterifications)
943.71 m/z	PG-5 + 2 * C-18 FA (2 esterifications)
1135.9319 m/z	PG4 + 3 * C-18 FA (3 esterifications)

In the mass spectra of PG3, PG4 and PG6, there was no solely structure found, so a mixture of various polyglycerols has to be considered.

4.2.2 Physical behaviour of the lipids

4.2.2.1 DSC

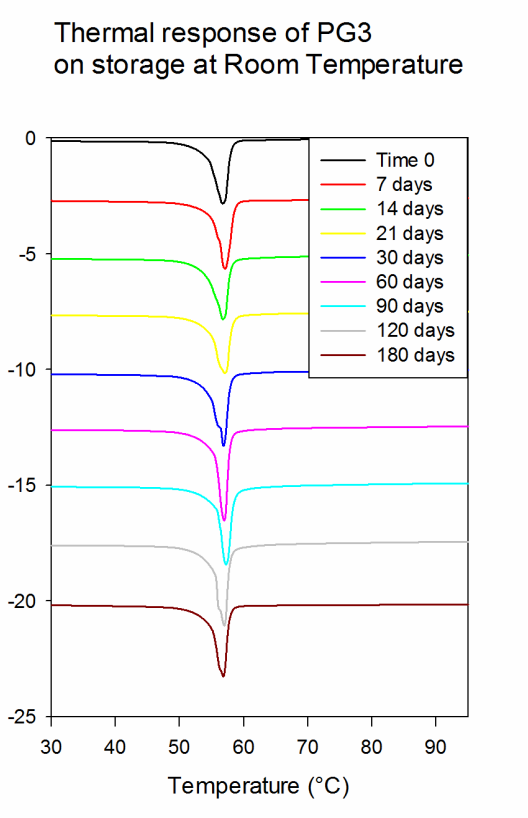


Fig. 29. DSC results of PG3 on storage at room temperature

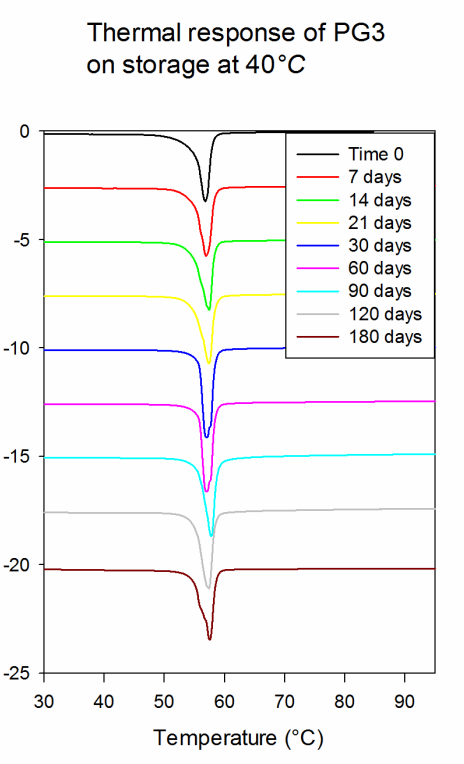


Fig. 28. DSC results of PG3 on storage at 40 °C

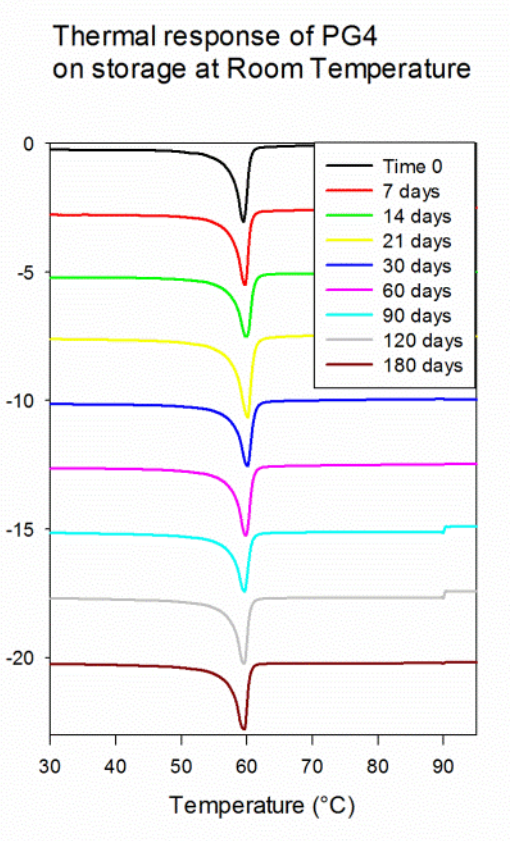


Fig. 31. DSC resulst of PG4 on storage on room temperature

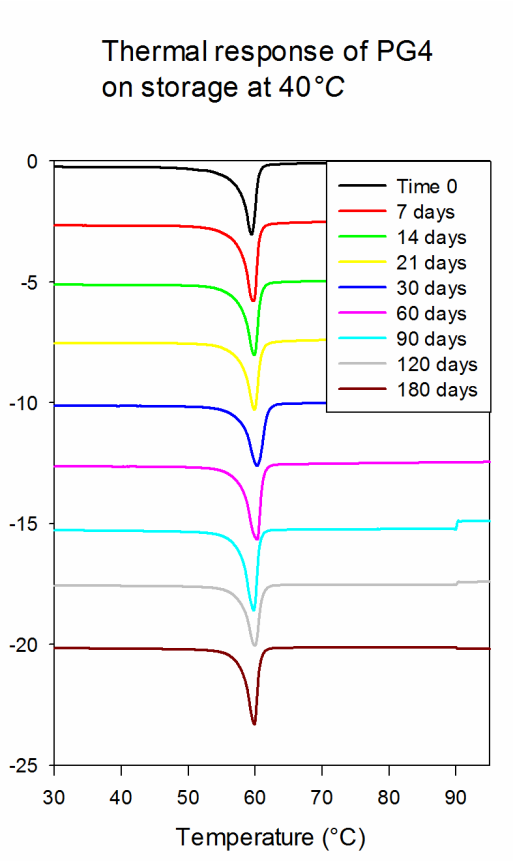


Fig. 30. DSC results of PG4 on storage at 40°C

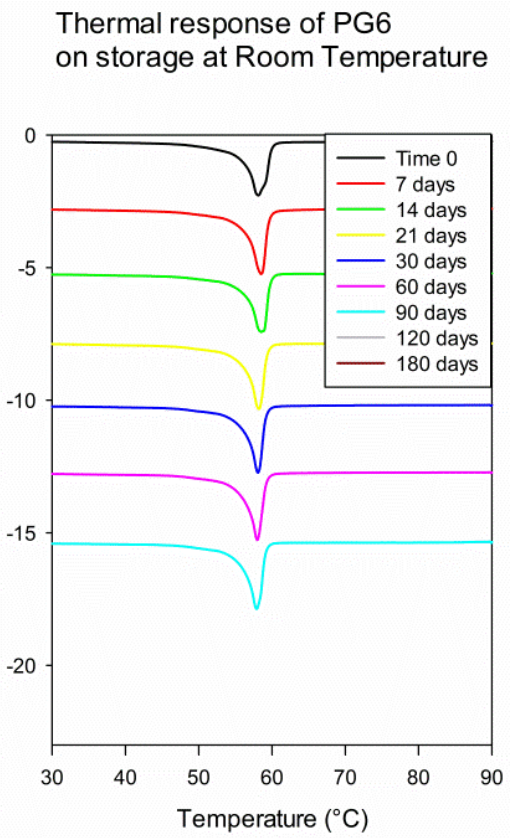


Fig. 33. DSC results of PG6 on storage at RT

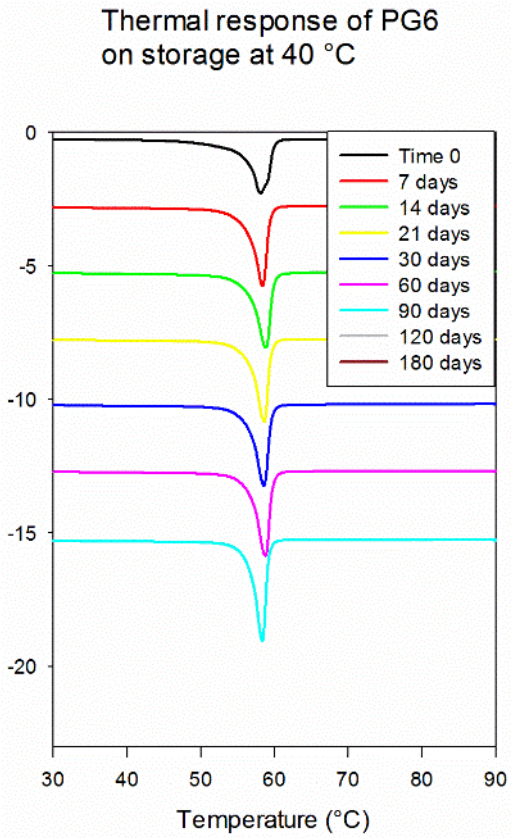


Fig. 32. – DSC results of PG6 on storage at 40 °C

The thermograms of PG3, PG4 and PG6 stored at 40 °C and 25 °C for 6 months are shown in the figures above (from Fig. 28 to Fig. 32). The signals in the DSC diagramms show always the endothermic behaviour, i.e. the melting point of PG's. As it can be seen always only one melting point exists, indicating the stable polymorphism of these lipids. The melting point of PG3, PG4 and PG6 are 56 °C, 59 °C and 57.5 °C, respectively. Comparing this data with the thermograms of tristerarin, shows that tristearin posseses the instable α-form, the metastable β' and the stable β-form [23].

4.2.2.2 X-Ray- diffraction

PG3

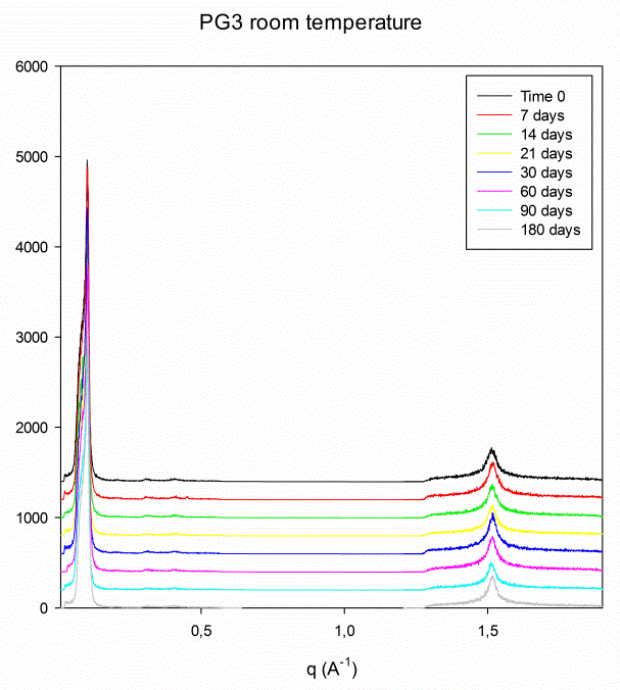


Fig. 34. SWAXS of PG3 at RT

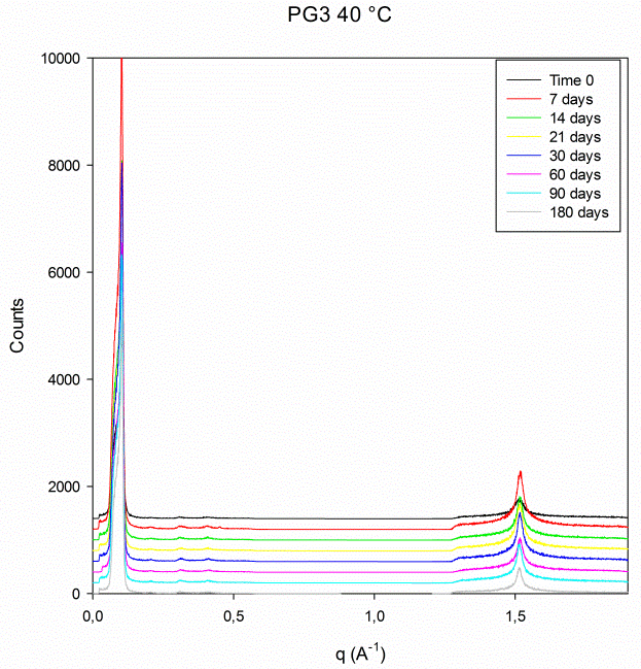


Fig. 35. SWAXS of PG3 at 40 °C

PG4

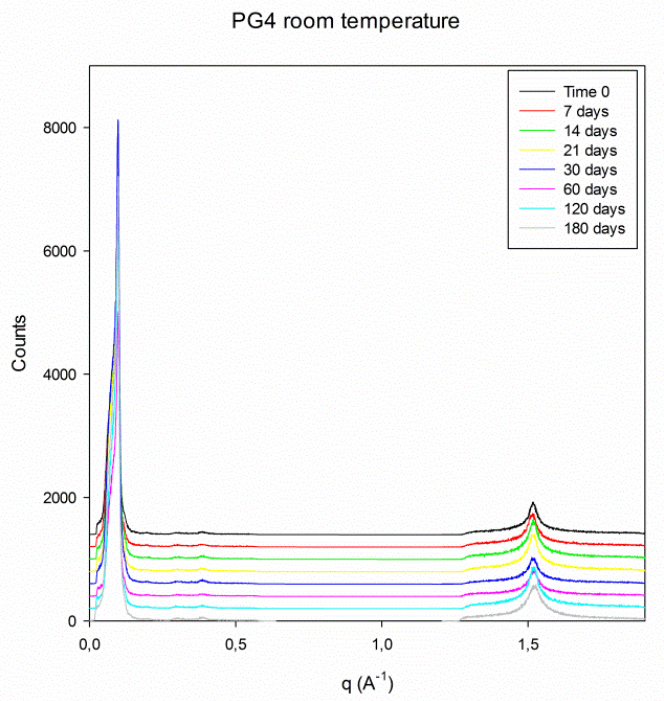


Fig. 36. SWAXS of PG4 at RT

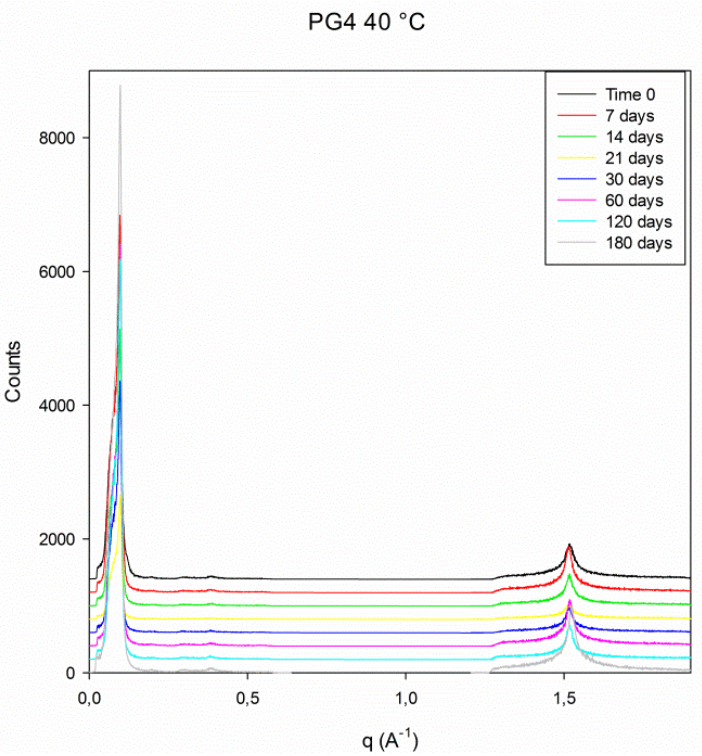


Fig. 37. SWAXS of PG4 at 40°C

PG6

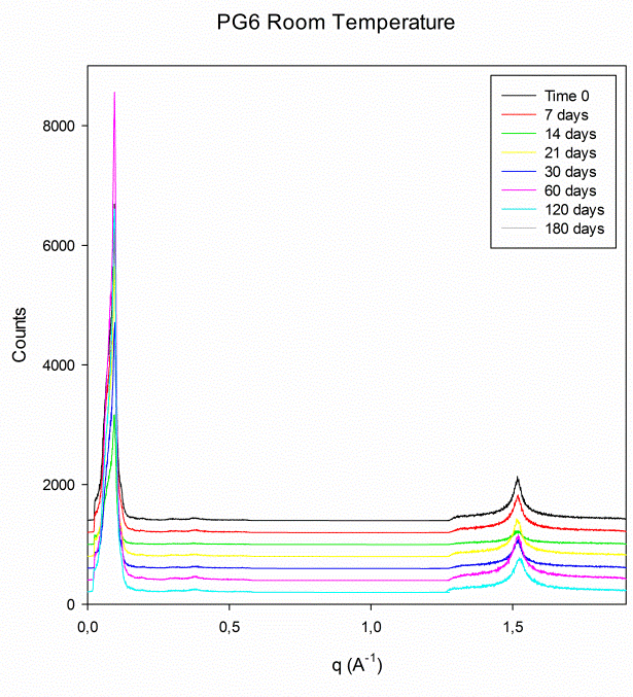


Fig. 38. SWAXS of PG6 at RT

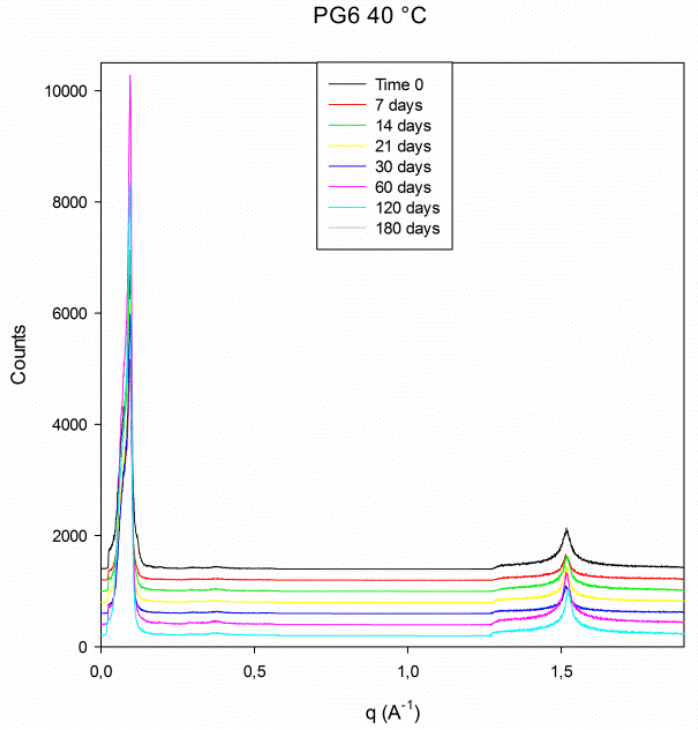


Fig. 39. SWAXS of PG6 at 40 °C

The SWAXS spectra of PG3, PG4 and PG6 at time 0 and after storage under different conditions is shown in the figures above (Fig. 34 to Fig. 39). For the WAXS measurement the scattering factor q was received, which is an inverse factor for Angstrom. With q the distance d between to carbon atoms of various chains was calculated: $q = \frac{2\pi}{d}$ [2]. WAXS measurements gave a lenght of 4.15 Angstrom for PG3, PG4 and PG6, respectively. Literature shows that characteristic d-spacings are associated with different polymorphs [2]. 4.15 Angstrom is a characteristic d-spacing value for the alpha polymorphic state with a hexagonal chain packing subcell [2]. SWAXS results indicate that the new synthesized lipids are just existing in the alpha crystalline state. It is also confirmed that they are stable after storage in different temperatures.

4.2.2.3 Rheology

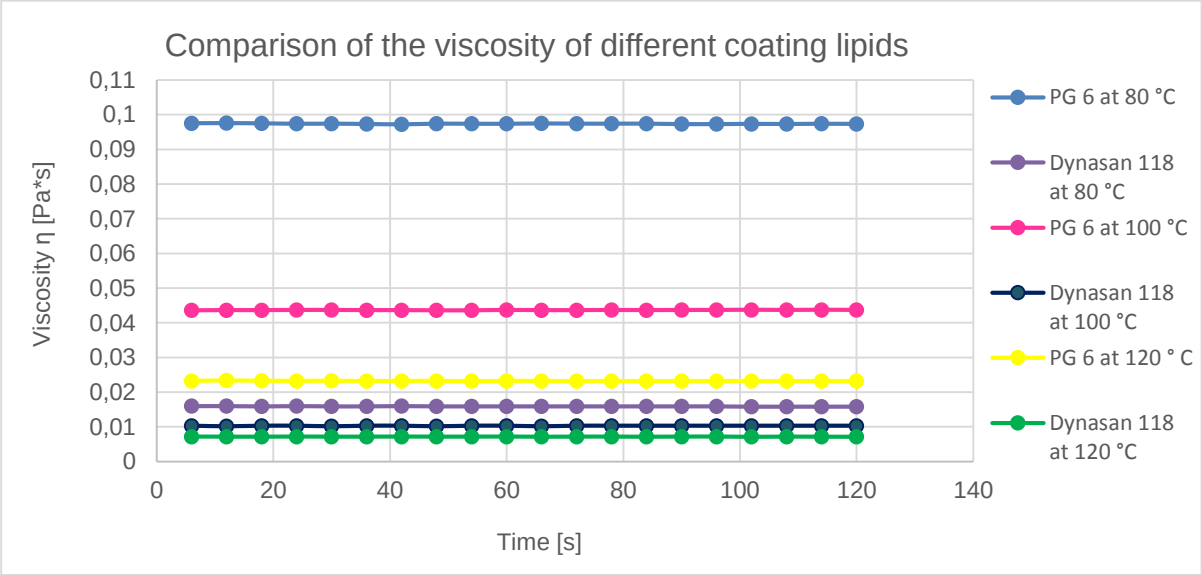


Fig. 40. Comparison of the viscosity of PG6 and Dynasan® 118 at different temperatures

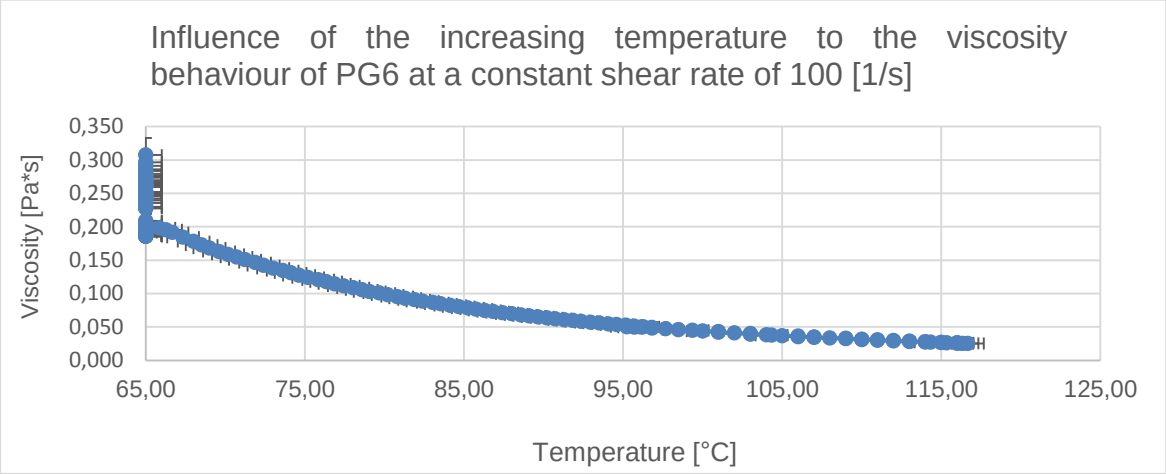


Fig. 41. Influence of increasing temperature to the viscosity behaviour of PG6 at a constant shear rate of 100 [1/s]

In the figure above (Fig. 40) the comparison of the viscosity behaviour of the coating materials Dynasan® 118 and PG6 at various temperatures is shown. At a temperature of 80 °C the viscosity of PG6 is almost 0.1 Pa*s whereas the viscosity of Dynasan® 118 at the same temperature is less than 0.02 Pa*s, which shows that the viscosity of PG6 is 5 times higher than the viscosity of Dynasan® 118. The viscosity is decreasing for both coating materials, when the temperature is increased. This measurements show that for the HMC processes the spraying pressure of PG6 has to be higher than the spraying pressure which is required for a coating process with Dynasan® 118.

In the second figure above (Fig. 41) it is shown that the temperature plays a major role on the viscosity of molten lipids. At a constant shear rate at 100 [1/s] and at a temperature of 65 °C the viscosity is 0.2 Pa*s. Increasing of temperature results in the decreased viscosity.

4.2.2.4 Contact angle measurements

In the table below (Tab. 8) a summary of the wettability of the 3 PG's in term of their contact angles over time and after storage at RT and at 40 °C are shown.

Tab. 8. Contact angle measurements of PG's considering the aging effect

n=10		Contact angle [°] ± SD	
Sample	**Temperature**	**Time 0**	**16 weeks**
PG3	RT	100.7 (± 1.37)	102.7 (± 1.14)
PG3	40 °C	100.7 (± 1.37)	95.31 (± 1.14)
PG4	RT	84.1 (± 1.74)	89.76 (± 1.02)
PG4	40 °C	84.1 (± 1.74)	89.65 (± 1.88)
PG6	RT	82.73 (± 1.06)	77.95 (± 0.89)
PG6	40 °C	82.73 (± 1.06)	82.66 (± 0.98)

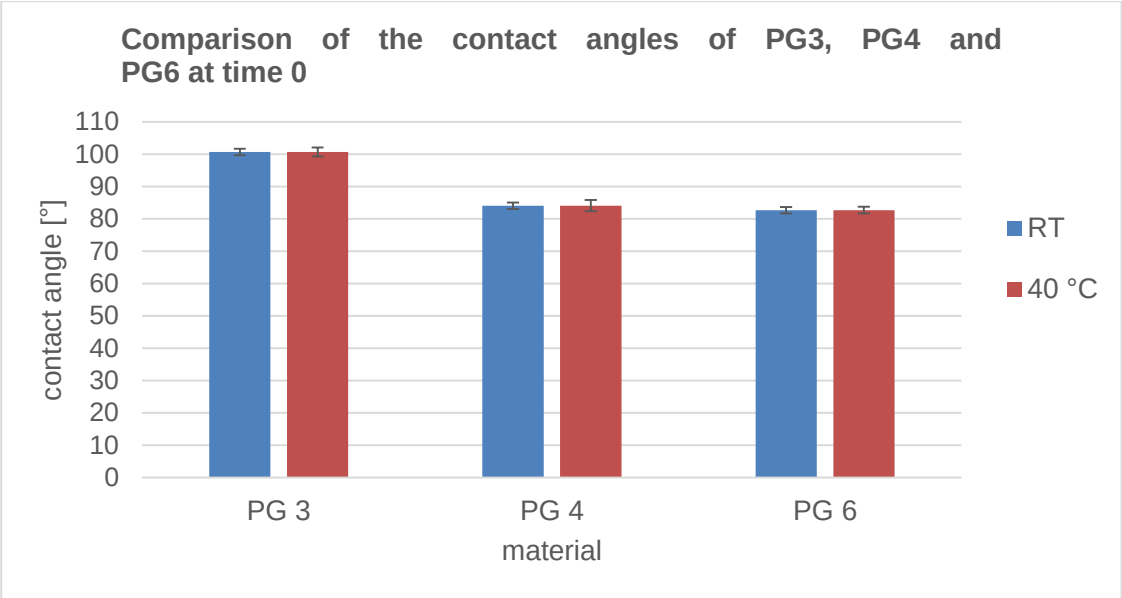


Fig. 42. Comparison of the contact angles of PG3, PG4 and PG6 at time 0

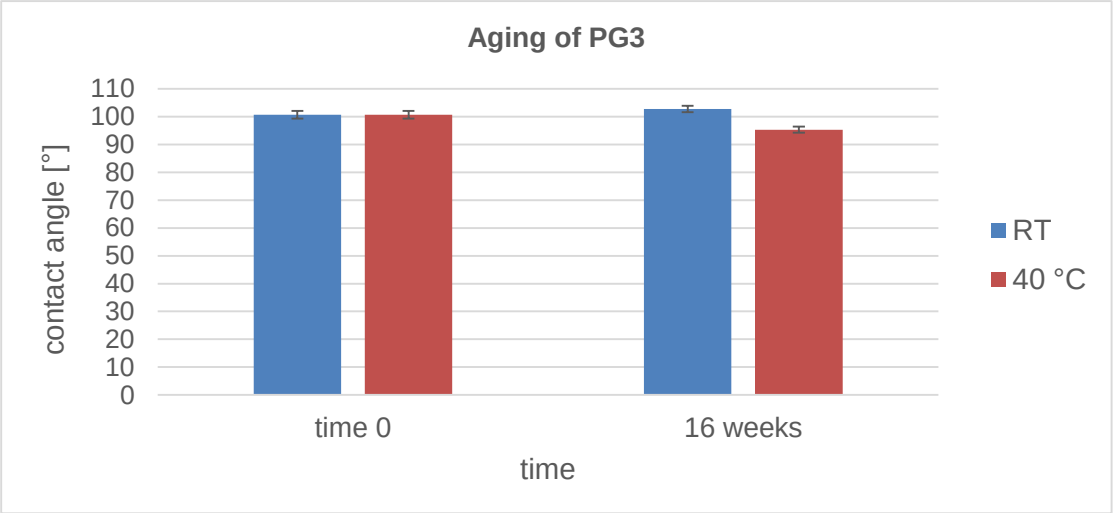


Fig. 43. Contact angle measurements of PG 3 after production and different storage conditions

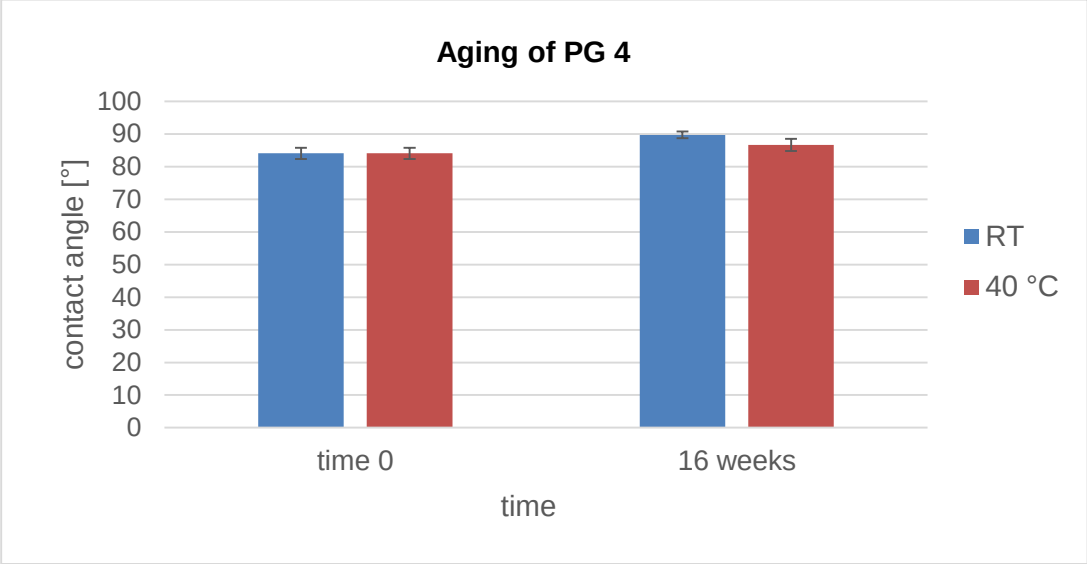


Fig. 44. Contact angle measurements of PG4 after production and different storage conditions

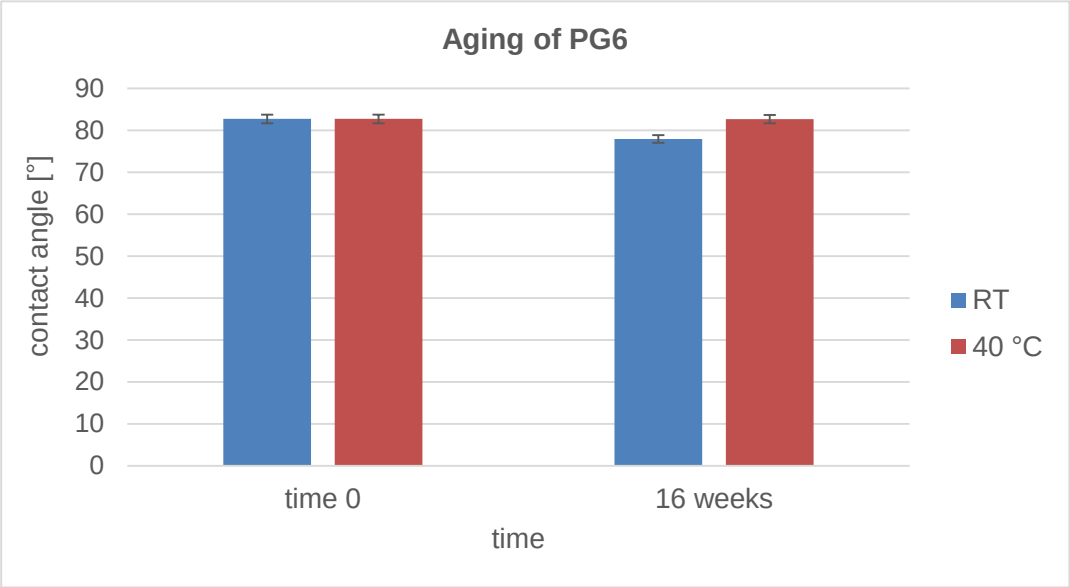


Fig. 45. Contact angle measurements of PG6 after production and different storage conditions

In the figures above (Fig. 43 to Fig. 45) the results of the contact angle mesurements are shown. For PG3 after 16 weeks the contact angle at RT was slightly higher than at time 0. A t-test was performed between PG3 time 0 and PG3 stored 16 weeks at RT,

with the result that there was a statistically significant difference between the input groups (P=0.002). Another t-test was also conducted for PG3 at time 0 and PG3 stored for 16 weeks at a temperature of 40 C°, with the result that there was a statistically significant difference between the input groups (P=0.001). The significant differences in the contact angle of PG3 after preparation and after storage are due to the very good reproducibility of the measurements. Considering the physical stability of the samples, these differences are negligible and do not refer to changes in the microstructure and the crystalline arrangement of the material.

Fig. 42 shows the comparison of the contact angles of PG3, PG4 and PG6 at time 0. As expected the contact angle for PG4 was lower than for PG3. At time 0 it was about 16° lower as it has been for PG3 at time 0. This behaviour can be explained by the chemical structure of the lipids. PG3 has 3 glycerol backbones, whereas PG4 has 4 glycerol backbones and therefore it has 3 OH groups more than PG3. OH- groups make a molecule more hydrophilic. Wettability studies have shown that contact angle measurements are acting as a parameter indicating the degree of wetting when a solid is interacting with a liquid [16]. In this case the solid phase was the lipid and as liquid phase a water drop was used. The smaller the contact angle the higher is the wettability and the more hydrophilic is the solid [16]. Large contact angles corresponding to low wettability [16], that means higher lipophilicity. To be allowed for making a statement for the stability a statistically test was also conducted for PG4 at time 0 compared with PG4 after 16 weeks at RT. The t-test showed that there was a statistically significant difference between the input groups (P= <0.001). As same as PG3, despite the statistical significance of the changes in the wettability of PG4 after storage under different conditions, these alterations are negligible and PG4 is physically stable.

In the figure above (Fig. 45) the contact angle of PG6 with water after production and after storage under different conditions is shown. A study t-test for PG6 at time 0 and PG6 after 16 weeks at RT was performed with a statistically significant difference between the input groups (P=<0.001). The contact angle of 82.73° (± 1.06) at time 0 stayed nearly the same after 16 week of storage at 40°C with 82.66° (± 0.98).

Over a storage time of 16 weeks under two different conditions, the new synthesized lipids showed a physical stability.

4.2.3 Analytical methods for evaluation of the coated material

4.2.3.1 Drug release of HMC products after production and storage at different conditions

4.2.3.1.1 Citric acid anhydrous coated with Dynasan® 118

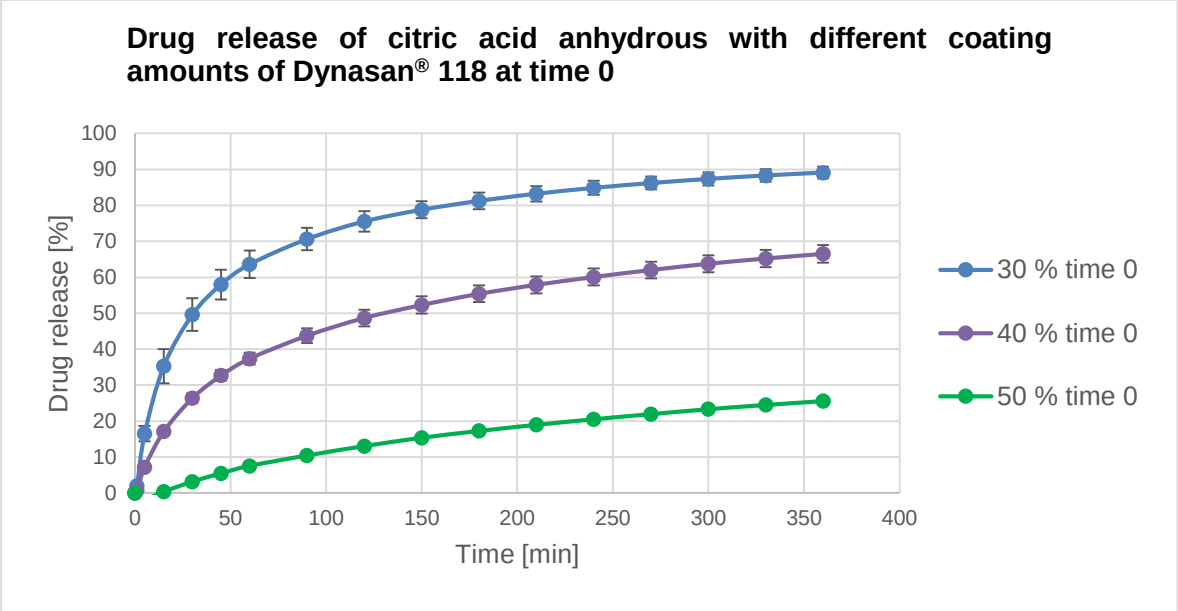


Fig. 46. Influence of the coating amount to the drug release of citric acid anhydrous coated with Dynasan® 118 at time 0

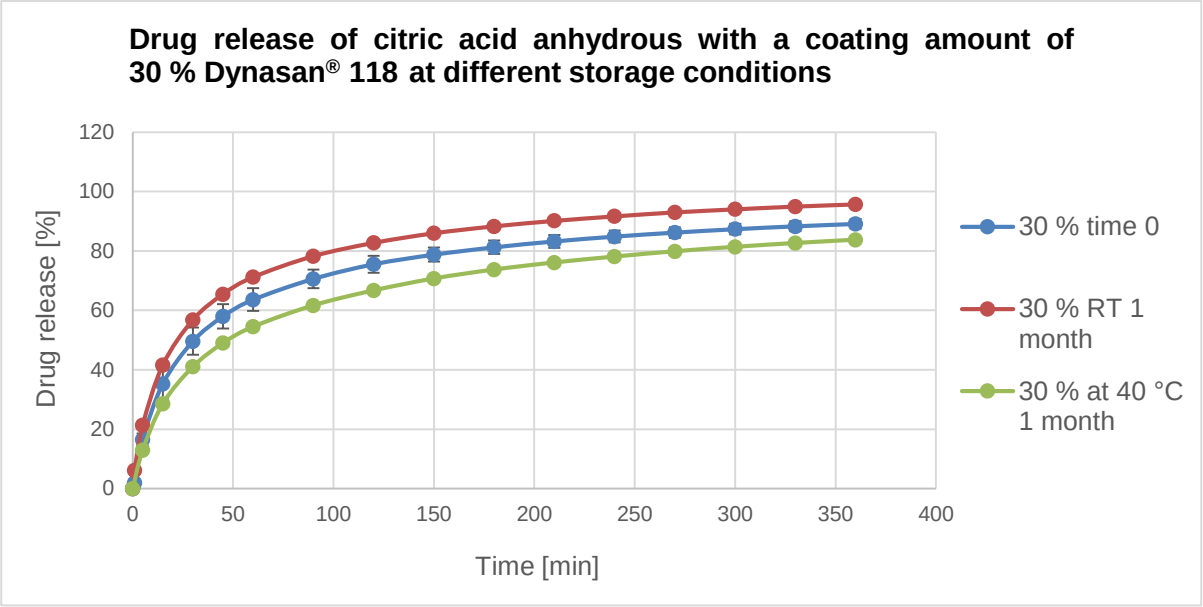


Fig. 47. Influence of the storage conditions to the drug release of citric acid anhydrous with 30 % coating of Dynasan® 118

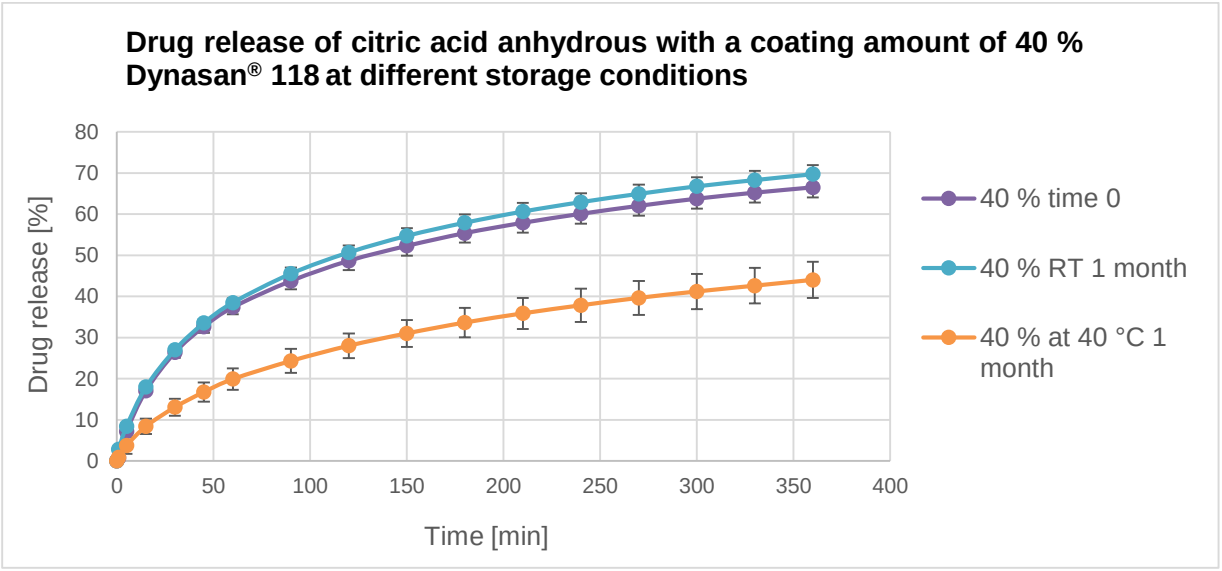


Fig. 48. Influence of the storage conditions to the drug release of citric acid anhydrous with 40 % coating of Dynasan® 118

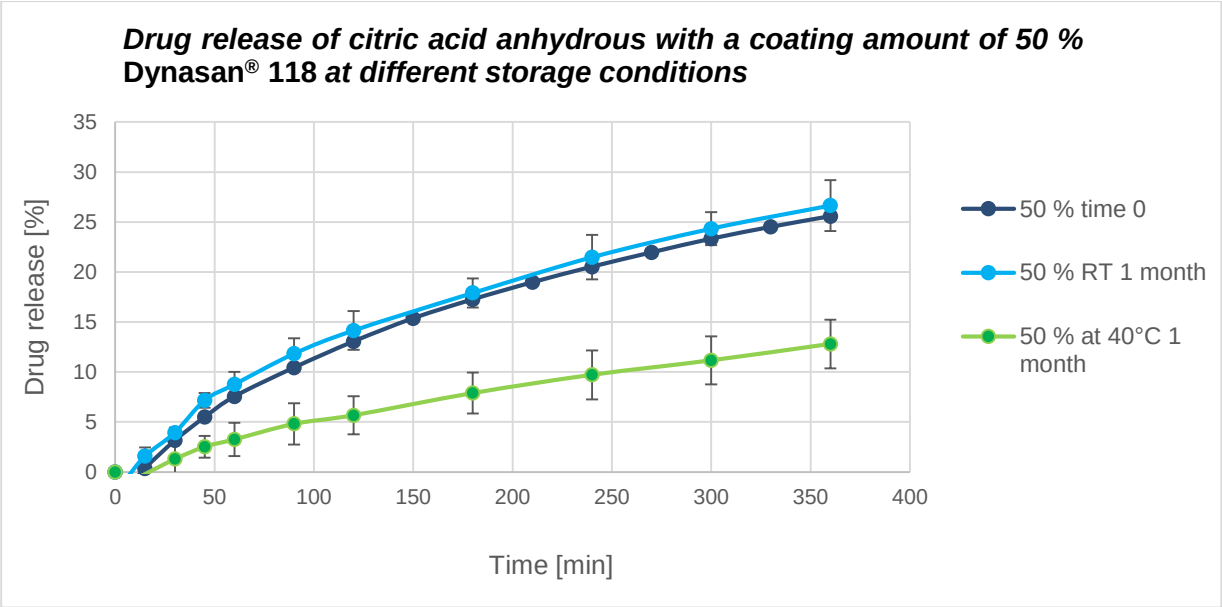


Fig. 49. Influence of the storage conditions to the drug release of citric acid anhydrous with 50 % coating of Dynasan® 118

4.2.3.1.2 Citric acid anhydrous coated with PG6

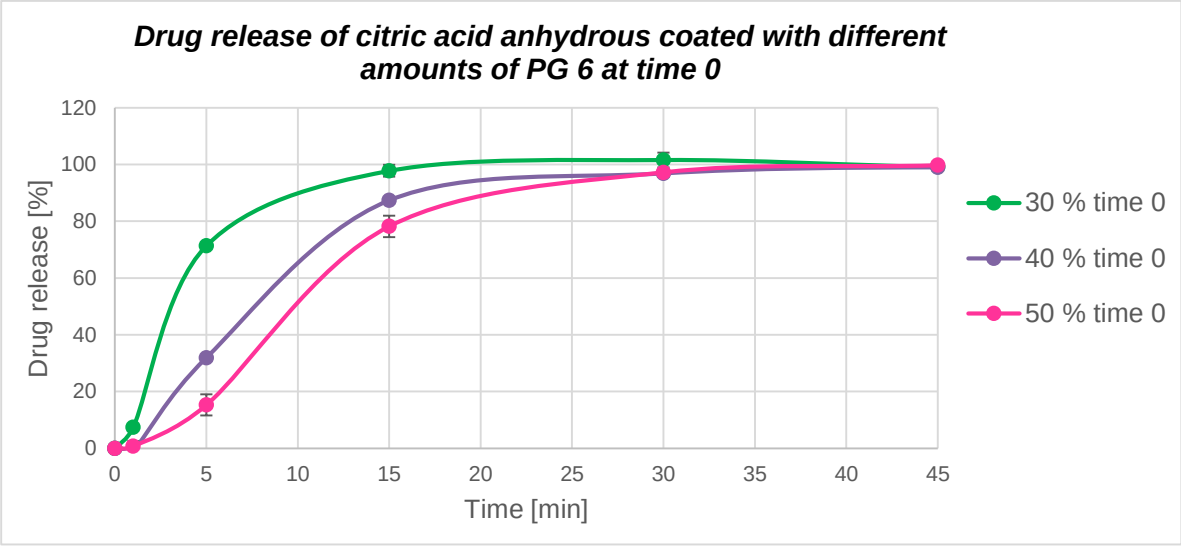


Fig. 50. Influence of the coating amount to the drug release of citric acid anhydrous coated with PG6 at time 0

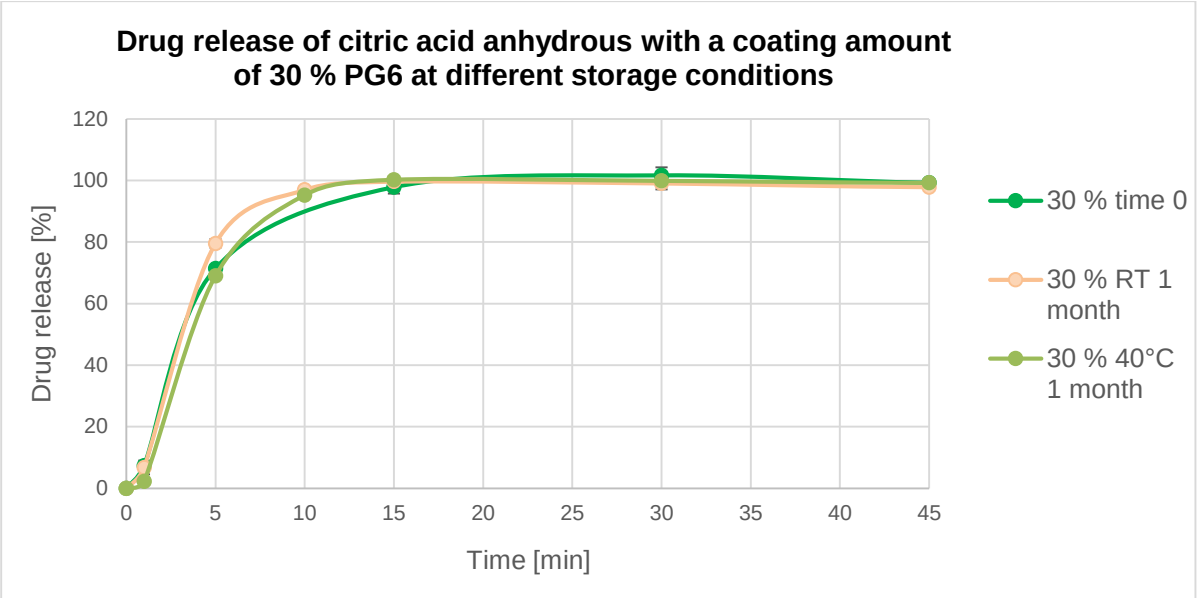


Fig. 51. Influence of the storage conditions to the drug release of citric acid anhydrous with 30 % coating of PG6

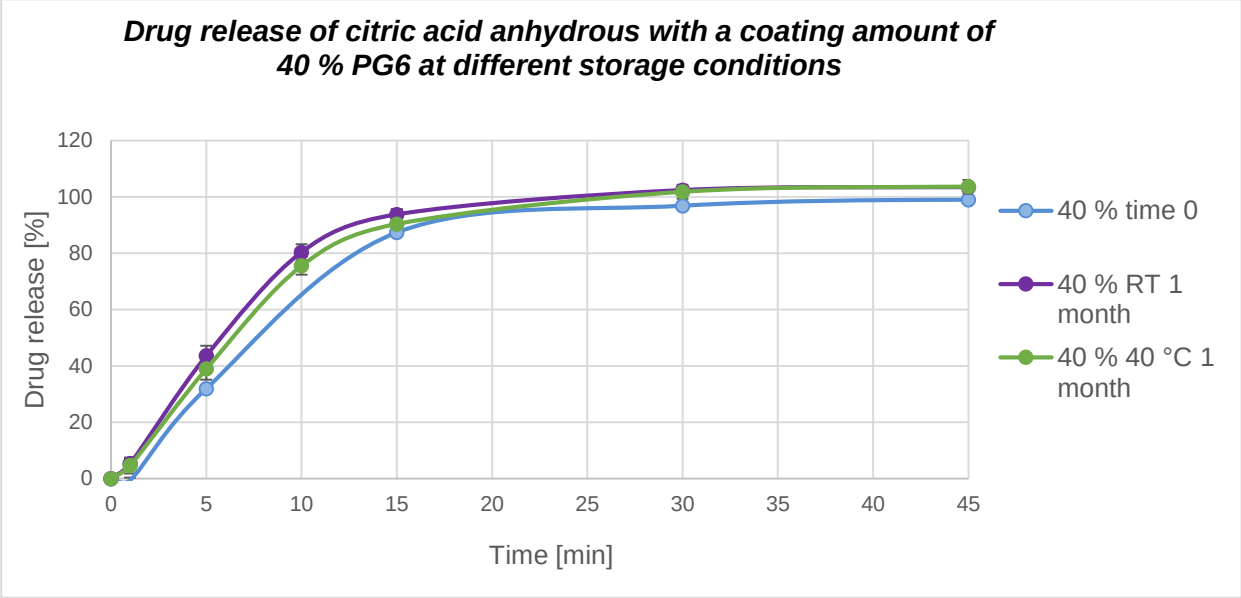


Fig. 52. Influence of the storage conditions to the drug release of citric acid anhydrous with 40 % coating of PG6

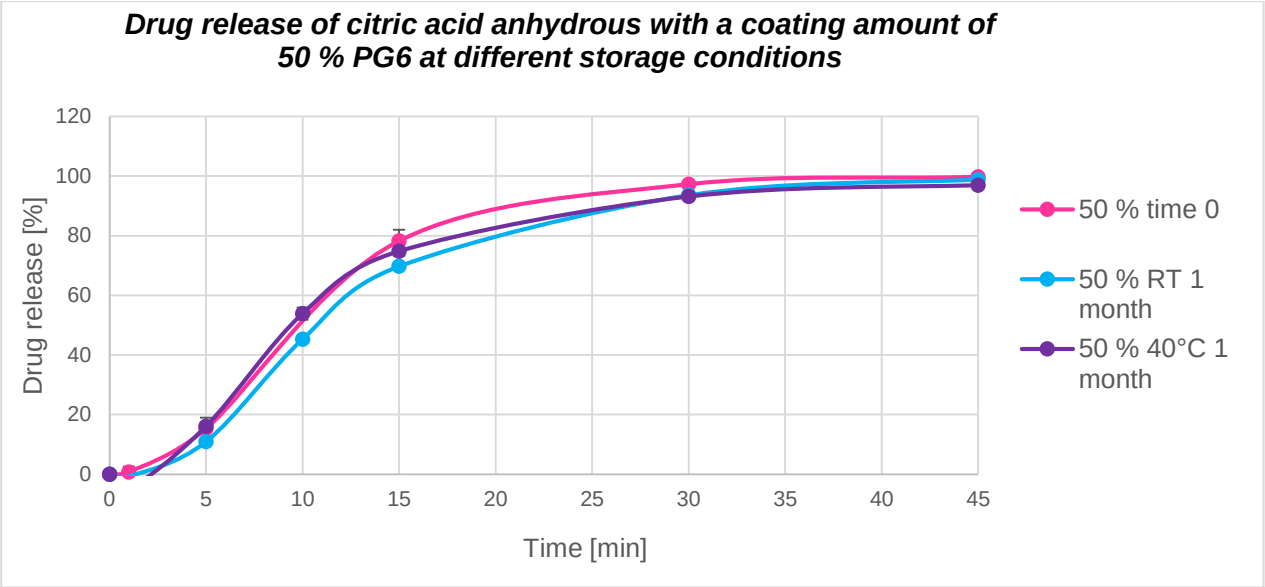


Fig. 53. Influence of the storage conditions to the drug release of citric acid anhydrous with 50 % coating of PG6

4.2.3.1.3 NAC coated with Dynasan® 118

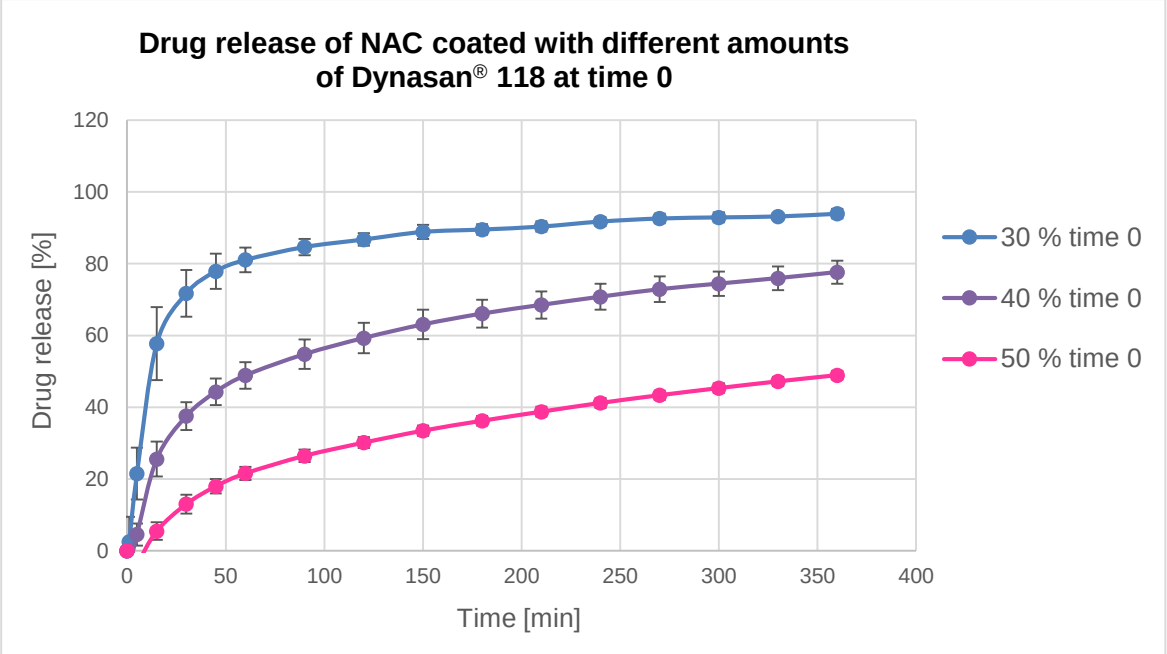


Fig. 54. Influence of the coating amount to the drug release of NAC coated with Dynasan® 118 at time 0

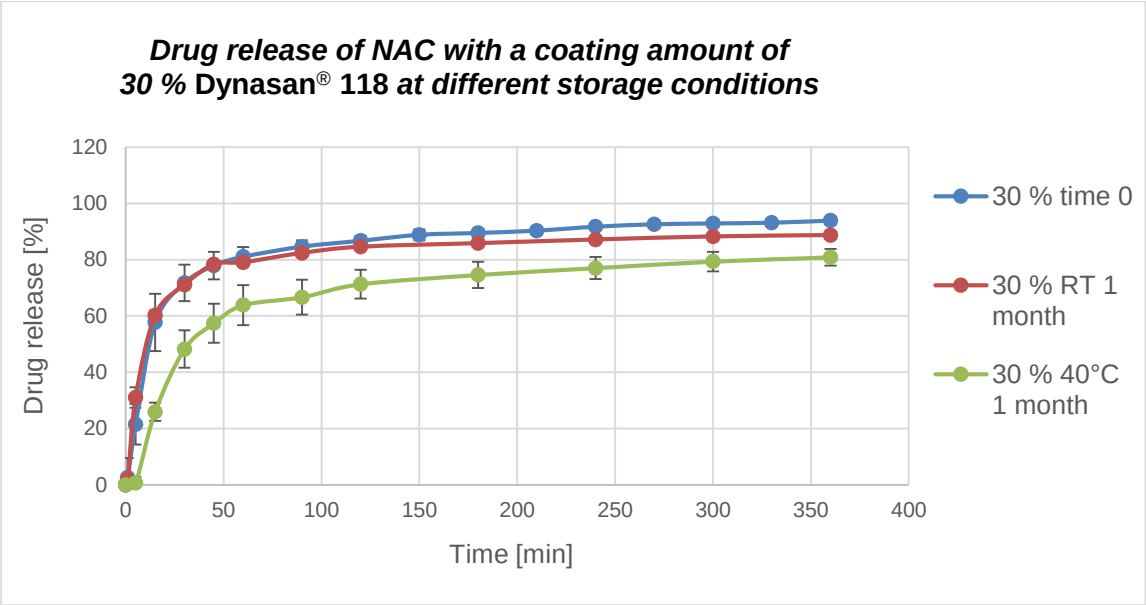


Fig. 55. Influence of the storage conditions to the drug release of NAC with 30 % coating of Dynasan® 118

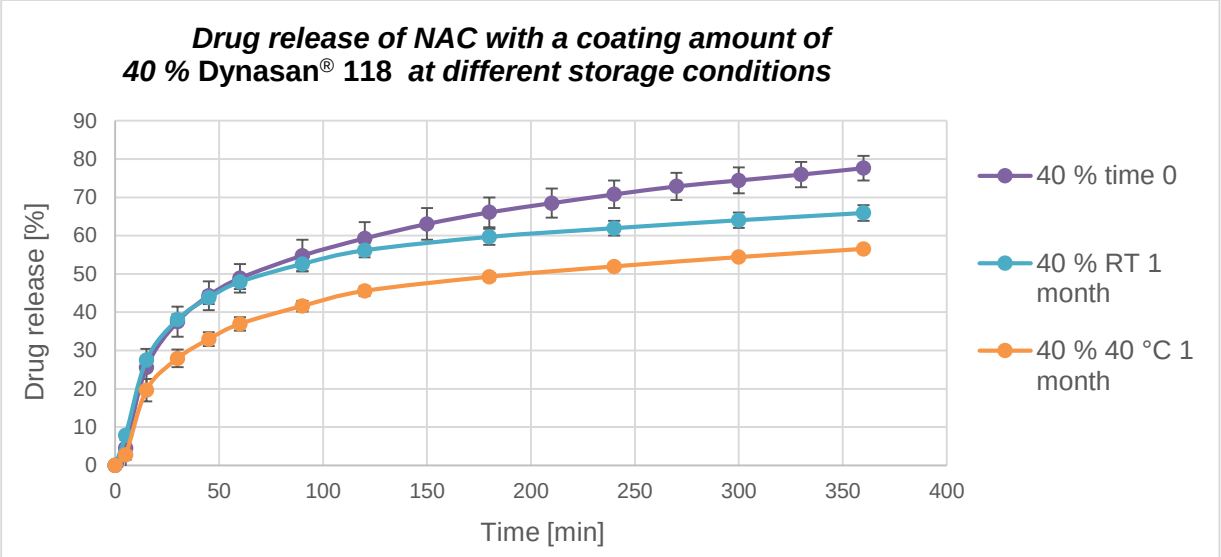


Fig. 56. Influence of the storage conditions to the drug release of NAC with 40 % coating of Dynasan® 118

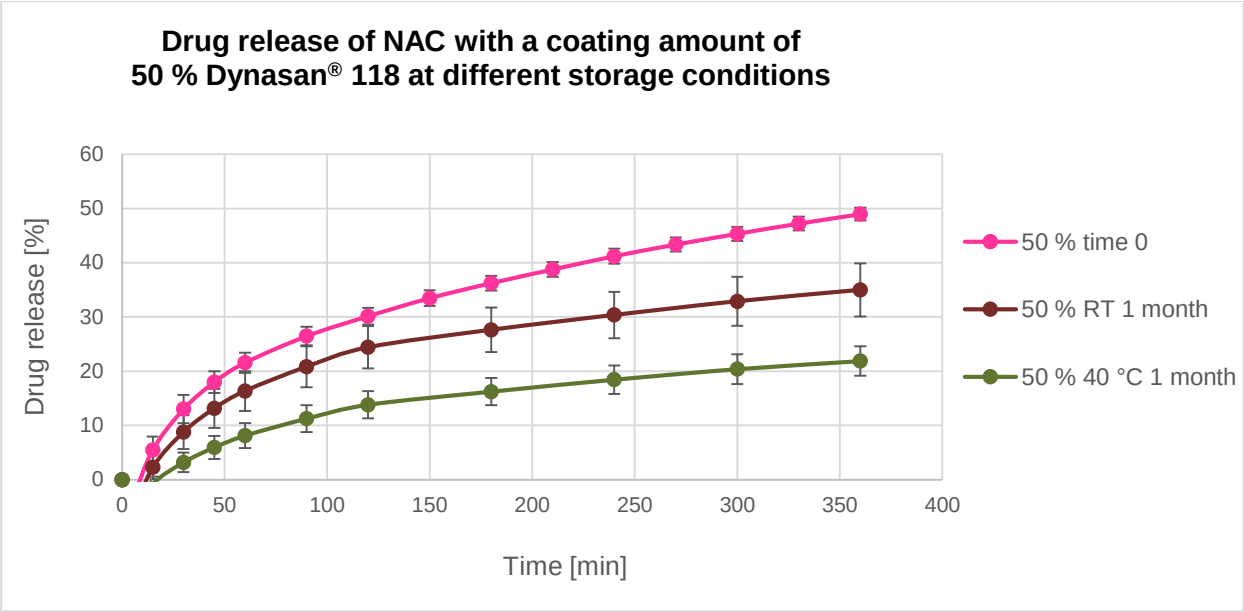


Fig. 57. Influence of the storage conditions to the drug release of NAC with 50 % coating of Dynasan® 118

4.2.3.1.4 NAC coated with PG6

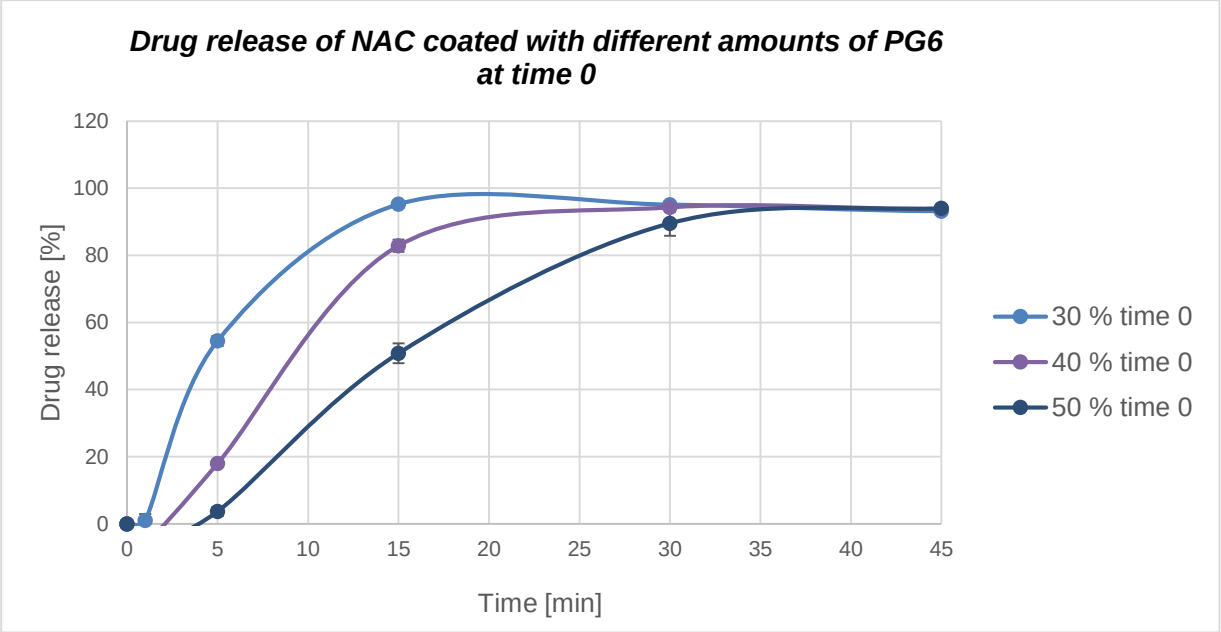


Fig. 58. Influence of the coating amount to the drug release of NAC coated with PG6 at time 0

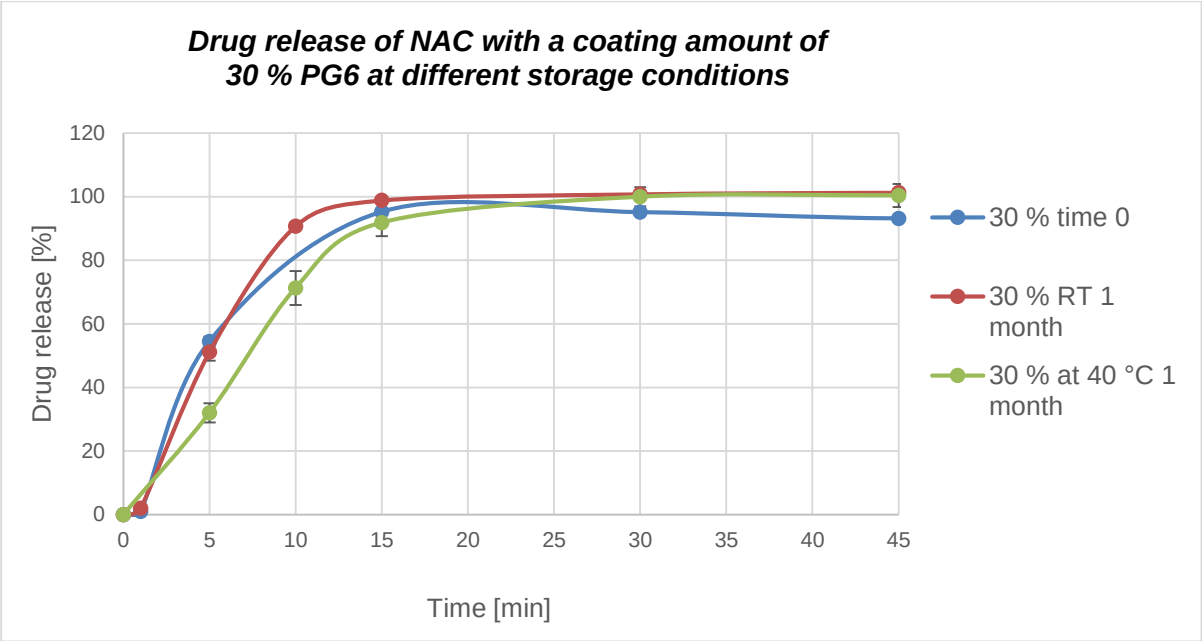


Fig. 59. Influence of the storage conditions to the drug release of NAC with 30 % coating of PG6

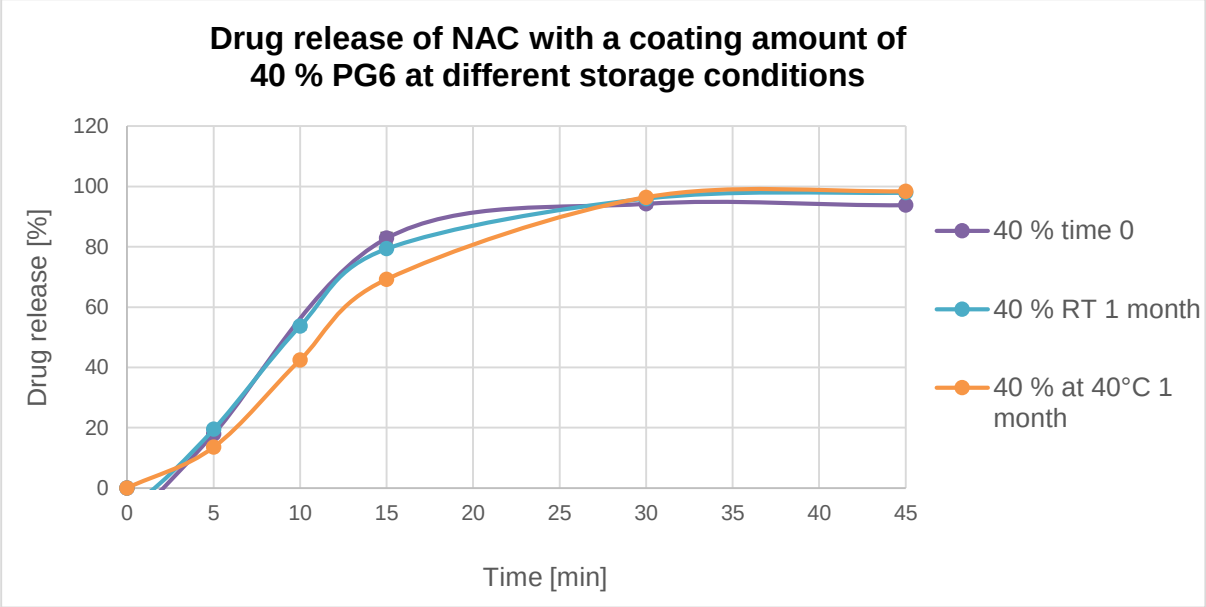


Fig. 60. Influence of the storage conditions to the drug release of NAC with 40 % coating of PG6

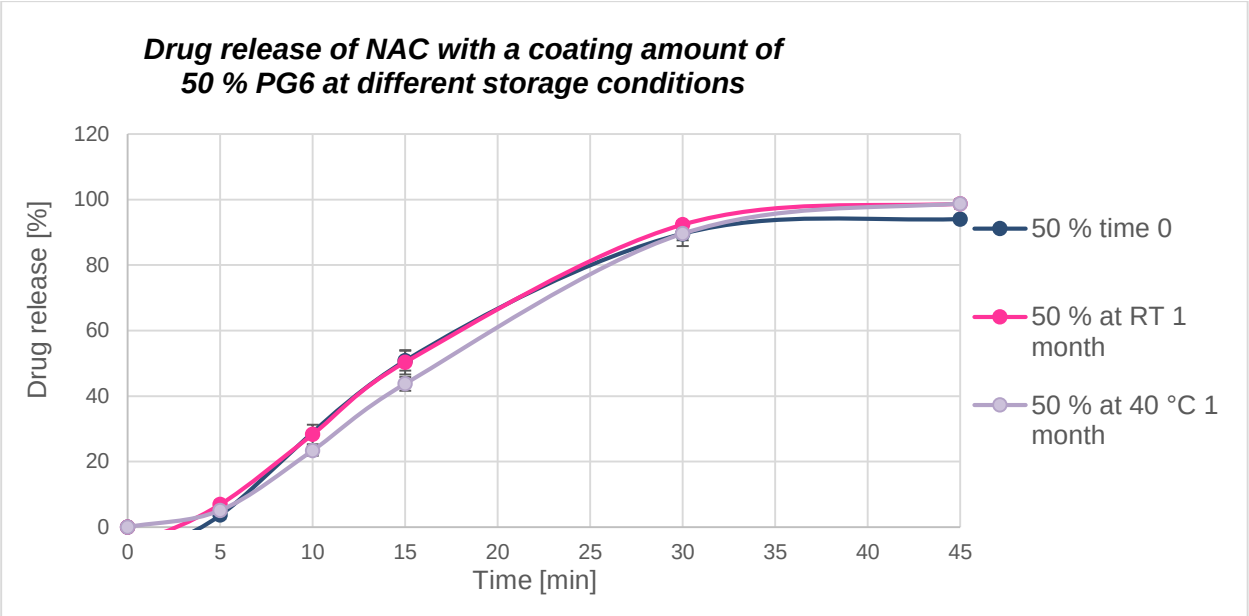


Fig. 61. Influence of the storage conditions to the drug release of NAC with 50 % coating of PG6

In the figures above (Fig. 46 to Fig. 61) the drug release profiles of the final products can be seen. In general it can be said that with a coating amount of 30 % the fastest drug release was achieved followed by 40 % coating amount and the slowest drug release was achieved with a coating amount of 50 % (Fig. 46; Fig. 50; Fig. 54; Fig. 58). Increased amounts of coating material result in increased thickness of the coating, which affects the diffusion of dissolution medium through the coating layer to the core material and the diffusion of the dissolved core material through the coating layer in the opposite direction. The diffusion of drug or water is an important topic in the pharmaceutical field in order to control the drug release [23].

By using PG6 as coating material (Fig. 50 to Fig. 53 and from Fig. 58 to Fig. 61) there was a drug release of 100 % within 30 minutes for both NAC and citric acid anhydrous. The drug release behaviour of citric acid anhydrous and NAC coated with PG6 shows nearly no difference between time 0 and storage for 1 month at RT. There occurs a very slight decelearation of the drug release of samples stored at 40 °C, independent to their coating thickness. The drug release from microspheres coated with polyglycerol esters of fatty acids is a process that is diffusion controlled within the micromatrix [24].

Compared with Dynasan® 118 as coating material a drug release of 100 % was not even achieved after 360 minutes (Fig. 46 to Fig. 49 and Fig. 54 to Fig. 57). In the figures above (Fig. 46 to Fig. 49) the release of citric acid anhydrous coated with Dynasan® 118 is shown. Comparing the results at time 0, 1 month RT and 1 month at 40 °C of storage shows the decelaration of release profiles of samples stored at 40°C, independent to their coating thickness. There is nearly no difference between time 0 and storage for 1 month at RT. This different behaviour after storage at RT and 40 °C is related to the polymorphic behaviour of Dynasan® 118. As described above, the inlet temperature during the hot melt coating process was selected below the melting temperature of α-form of tristearin, to get the instable α-form at the surface of coated particles. Thermal energy is a critical parameter, which accelerates the transformation of instable α-form to the well arranged stable β-form [3]. This transformation results in the alteration of release profile, which is considered as instability during the storage time [25]. As triglycerides are structures with a glycerol backbone esterified with 3 fatty acid molecules the arrangement of the fatty acids, which are long aliphatic chains, in various subcell structures indicates the polymorphism of triglycerides [25]. This final product was produced in the alpha crystalline state at 25 °C and after storage of 1 month at a temperature of 40 °C a change in drug release was occuring as it can be observed in the drug release profile. Normally the blooming effect was expected, which is leading to morphological changes due to storage and this might lead to a change in drug release [26].

4.2.3.2 pH measurements for taste masking trials

4.2.3.2.1 pH measurements of citric acid anhydrous

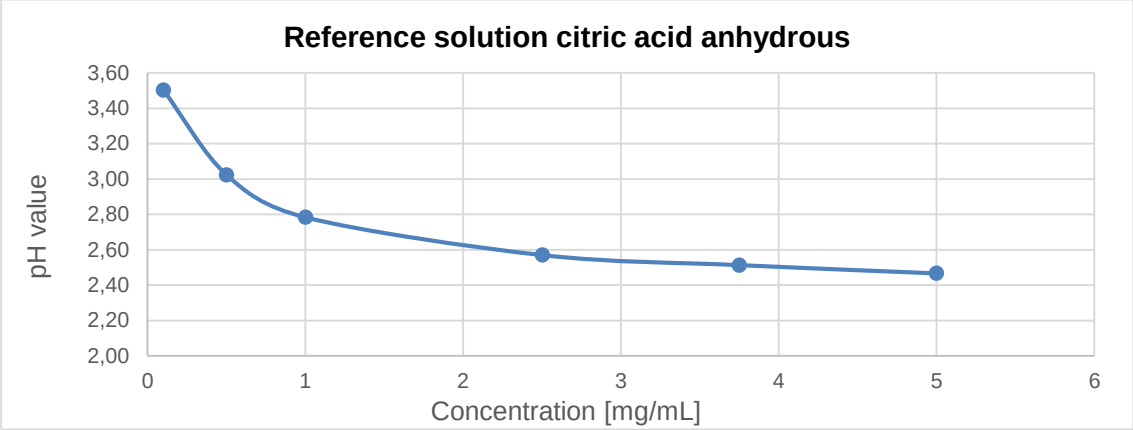


Fig. 62. Reference solution for citric acid anhydrous

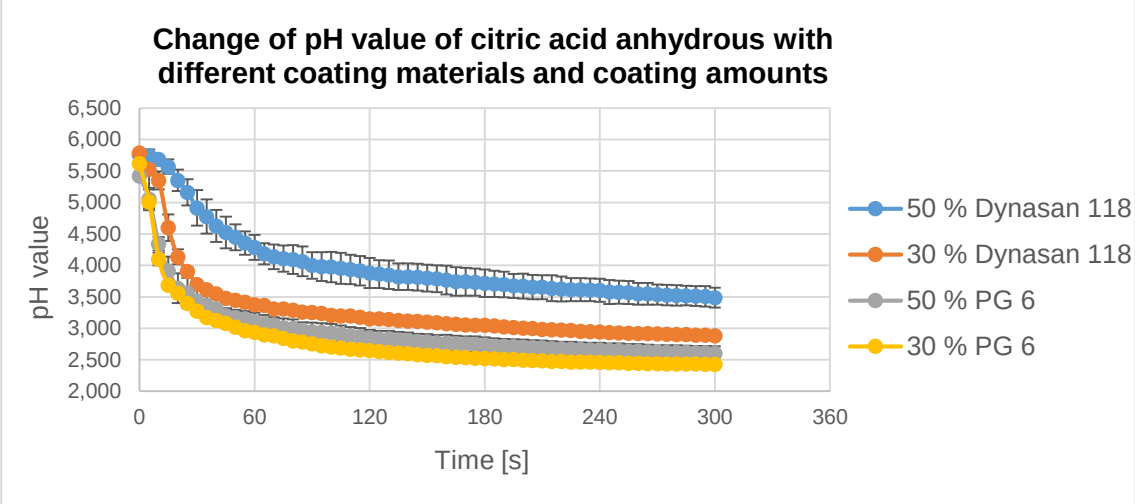


Fig. 63. pH measurements of citric acid anhydrous with different coating materials

Tab. 9. Concentration and pH of citric acid anhydrous after 60 seconds

Sample	pH after 60 s	Concentration of citric acid anhydrous [mg/mL] after 60 s
50 % Dynasan® 118	4.290	0.000
30 % Dynasan® 118	3.380	0.196
50 % PG6	3.110	0.410
30 % PG6	2.940	0.615

4.2.3.2.2 pH measurements of NAC

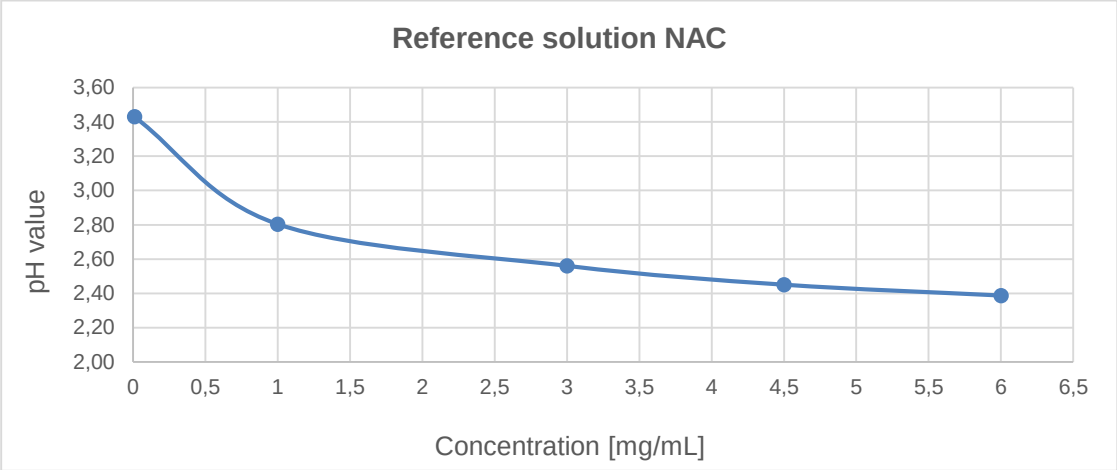


Fig. 64. Reference solution for NAC

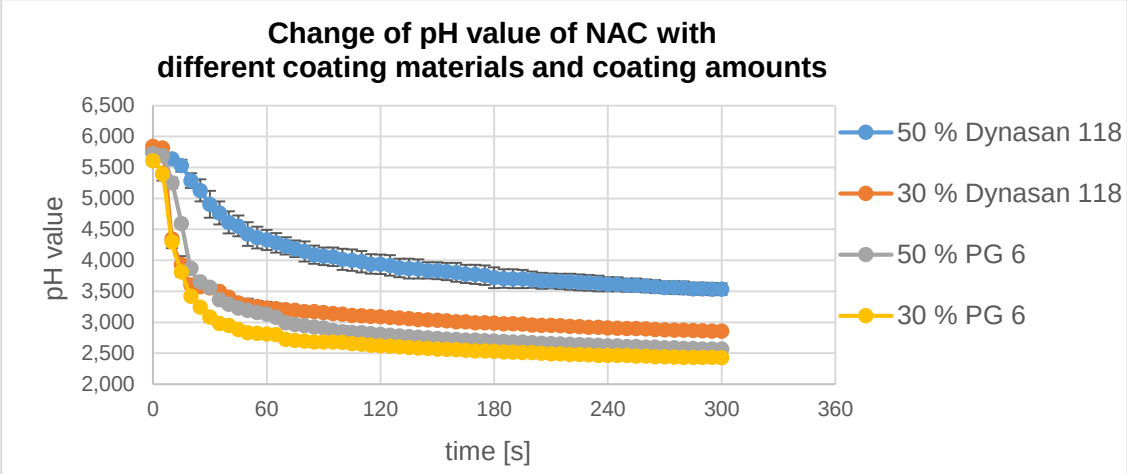


Fig. 65. pH measurements of NAC with different coating materials

Tab. 10. Concentration and pH of NAC after 60 seconds

Sample	pH after 60 s	Concentration of NAC [mg/mL] after 60 s
50 % Dynasan® 118	4.330	0.000
30 % Dynasan® 118	3.230	0.275
50 % PG6	3.130	0.396
30 % PG6	2.810	0.976

In the figures above (Fig. 62 to Fig. 65) the results of the taste masking trials of the final products are shown. The different reference solutions were used for estimating the concentration of citric acid anhydrous and NAC, respectively, after 60 seconds. Hence the pH value of the final products after 60 seconds was compared with the reference solutions of the pure API and so the concentration of the API could be deduced. The higher the pH after 60 seconds, the lower was the API concentration and the better was the taste masking of the HMC-process. Of course it has to be kept in mind that the hydrophilicity of the coating material and the applied coating amount have a significant impact on the degree of released API from the coating and thus on the alteration of the pH value. As it can be seen in the figures (Fig. 63 and Fig. 65) there is a significant difference between the release profile of a product with a coating amount of 30 % and 50 % of Dynasan® 118 as a lipophil coating material, whereas there is no significant difference between 30 % and 50 % coating amount of PG6. Achieving a good taste masking is important for patient compliance [27].

5 Conclusion

This thesis dealed with the stability problems of pharmaceutical formulations, that are occuring over storage due to the instability of coating materials. With the new-synthesized lipids it could be shown that no instability is occuring even after storage at 40 °C. It was evidenced by DSC measurements results and x-ray diffraction results that the new-synthesized lipids do not show polymorphism and that they only exist in the alpha crystalline form and only have one melting point. Contact angle measurements confirmed the stability of these lipids, as the contact angle was not changing a lot between time 0 and after 16 weeks of storage at room temperature and also at 40 °C. Dissolution results of the final products coated with PG6 revealed that the dissolution rate is the same even after storage. Concerning the chemical structure of the new synthesized lipids it came to the result, considering NMR and MS data, that PG3, PG4 and PG6, respectively, are a mixture of various polyglycerols. It is not only one chemical structure, the molecule consists of different amounts of various polyglcerol esters of fatty acids.

6 List of abbreviations

AMU	Atomic mass units
API	Active pharmaceutical ingredient
DAG	Diacylglycerol
DSC	Differential scanning calorimetrie
ESI	Electrospray Ionisation
FA	Fatty acid
FAB	Fast atom bombardment
GRAS	Generally recognized as safe
HMC	Hot melt coating
HV	Hydroxyl value
MAG	Monoacylglycerol
MALDI	Matrix assisted laser desorption/ ionization
MS	Mass spectrometry
NAC	N-Acetylcysteine
NMR	Nuclear magetic resonance
PD	Plasma desorption
PG	Polyglycerol
RT	Room temperature
SAP	Saponification value
SWAX	Small and wide angle x-ray scattering
TAG	Triacylglycerol
TMS	Tetramethylsilane
TOF	Time-of-flight

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