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Ph.D. Thesis

Formulation and Investigation of in Situ Gelling Lyotropic Liquid Crystalline Systems

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PUBLICATIONS RELATED TO THE SUBJECT OF THESIS

PAPERS

I. **A. Fehér**, E. Csányi, I. Csóka, Anita Kovács and I. Erős: Thermoanalytical investigation of lyotropic liquid crystals and microemulsions for pharmaceutical use.
J. Therm. Anal. Cal. 82 (2005) 507-512 IF: 1.478

II. A. Kovács, I. Csóka, M. Kónya, E. Csányi, **A. Fehér** and I. Erős: Structural analysis of w/o/w multiple emulsions by means of DSC.
J. Therm. Anal. Cal. 82 (2005) 491–497 IF: 1.478

III. **A. Fehér**, E. Csányi, I. Erős: In situ forming lyotropic liquid crystalline systemscontaining metronidazole-benzoate.
J. Drug Del. Sci. Tech. 15 (5) (2005) 343-346 IF: 0.585

ABSTRACTS

1. **A. Fehér**, E. Csányi, I Erős: Drug containing lyotropic liquid crystals for the treatment of periodontitis. 14th Surfactants in Solution Symposium, Barcelona, Spain, Abstracts 235 (2002)

2. E. Csányi, M. Makai, **A. Fehér**, I. Erős: Lyotropic liquid crystals as drug delivery for poorly soluble drugs, 14th Surfactants in Solution Symposium, Barcelona, Spain, Abstracts 237 (2002)

3. **A. Fehér**: Periodontitis kezelésére szánt spontán képződő folyadékkristályos rendszerek előállítása. VI. Clauder Ottó emlékverseny, Budapest, Hungary, Abstracts 16 (2002)

4. **A. Fehér**, E. Csányi, I. Erős: In situ képződő folyadékkristályos rendszerek fogászati alkalmazása. XIV. Országos Gyógyszertechnológiai Konferencia, Hévíz, Hungary, (2002)

5. E. Csányi, **A. Fehér**, I. Erős: Tenzidekből felépülő kolloid rendszerek a gyógyszertechnológiában. XIV. Országos Gyógyszertechnológiai Konferencia, Hévíz, Hungary, (2002).

6. **A. Fehér**, E. Csányi, I. Erős: Liotróp folyadékkristályos rendszerek hatóanyagfelszabadulásának és reológiai tulajdonságainak jellemzése. Congressus Pharmaceuticus Hungaricus XII, Budapest, Hungary, (2003)

7. E. Csányi, **A. Fehér**, E. Urbán, I. Erős: Side-specific drug delivery systems for the treatment of periodontal diseases. 6th Central European Symposium on Pharmaceutical Technology and Biotechnology, Siófok, Hungary, Eur. J. Pharm. Sci. 25S1 S70 (2005)

8. **A. Fehér**, E. Csányi, I. Erős: Thermal and rheological analysis of lyotropic liquid crystalline systems. 6th Central European Symposium on Pharmaceutical Technology and Biotechnology, Siófok, Hungary, Eur. J. Pharm. Sci. 25S1 S101 (2005)

9. A. Kovács, I. Csóka, M. Kónya, E. Csányi, **A. Fehér**, I. Erős: Structural analysis of w/o/w multiple emulsions by means of DSC. 6th Central European Symposium on Pharmaceutical Technology and Biotechnology, Siófok, Hungary, Eur. J. Pharm. Sci. 25S1 S135 (2005)

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1 INTRODUCTION

In the physical sciences, a phase is a set of states of a macroscopic physical system that have relatively uniform chemical composition and physical properties (i.e. density, crystal structure, index of refraction, and so forth). The most familiar examples of phases are solids, liquids, and gases [1]. In special circumstances – at very high or at very low temperature – less familiar phases of matter can occur including plasmas and quark-gluon plasmas, Bose-Einstein condensates and fermionic condensates, strange matter, superfluids and supersolids [2-4]. Nevertheless, it is not required to reach the million degrees of fervor of starcores or the nearly absolute zero temperature of cosmic space in order to generate a specific state of matter.

In case of certain substances an unique state of matter called liquid crystal phase exists which is located between solid and liquid state of aggregation. Within solids, particles (atoms, ions, molecules) have a fixed spatial ordering often localized in a regular crystal lattice. These crystalline solids exhibit short as well as long-range order with regard to both position and orientation of the corpuscles. In case of liquids short range order of particles can be observed at the most. Particles are not strongly bonded to their neighbours and are able to move according to each other. The liquid crystalline phases – also called mesophases – represent intermediate states between the ordered structure of solids and amorphous properties of liquids. For instance, a liquid crystal may flow like a liquid, but have the molecules in arranged and oriented in a crystal-like way. Liquid crystals show at least orientational long-range order and may show short range order, whereas positional long-range order disappears [5, 6].

2 LITERATURE SURVEY

2.1 Formation of liquid crystals

There are three main routes to govern the formation of liquid crystalline phases: these are the molecular shape, microsegregation effects and chirality. Materials which tend to form liquid crystals generally consists of molecules – often called mesogens – characterized by an anisometric molecular shape. Majority of mesogens that belong to the rigid-rod class orient

along their long axis to form calamitic mesophases. The second type of mesogens possess a planar, disk-like molecular structure that form discotic mesophases which contain mesogens oriented in the direction of their short axis [6]. Nevertheless, this orientation of constituents is not restricted only to molecular level: polymers, colloidal suspensions, micrometer-sized objects such as anisotropic colloids, and even some viruses can organize themselves in liquid crystalline phases [7, 8].

We can distinguish two possibilities on the effect of which a crystalline solid with its ordered stucture can turn into a disordered liquid: these are the melting and the dissolution. According to these processes the transient liquid crystalline state can appear by two ways. In case of thermotropic liquid cystals the formation of mesophases occurs solely by a variation in thermal energy and do not require any interaction with a solvent. Contrarily, for the formation of lyotropic liquid crystals presence of a solvent is required. They are formed by mesogens that are not the molecules themselves but their solvates as well as by associates of solvated molecules. However it should be noted that in case of lyotropic liquid crystals this strict diffrerentiation is not possible, because not only the solvent and its concentration but also the temperature can affect on the stucure of the system [5, 6].

2.2 Lyotropic liquid crystals

2.2.1 Constituents of lyotropic liquid crystals

Lyotropic liquid crystals are usually formed from water and one or two surfactants and possibly oils within a definite concentration and temperature range [9-11]. In the lyotropic phases, solvent molecules fill the space around the compounds to provide fluidity to the system. In contrast to thermotropic liquid crystals, these lyotropics have another degree of freedom: the concentration that enables them to induce a variety of different phases. Lyotropic liquid crystals are formed in consequence of interaction occuring between the amphiphilic molecules and a solvent. These amphiphilics – also called surfactants – have two distinct parts differing very much in their solubility. One part is hydrophilic, with a high solubility in polar solvents, while the other one is hydrophobic, with a high solubility in hydrocarbon. This part of the molecule consists of long linear or ramified hydrocarbon chains with or without benzene cycles. This is a good reason to imagine such a molecule as a little

sphere representing the hydrophilic "head" continued with a long zigzag "tale" representing the hydrophobic chain. Conforming to the nature of the hydrophilic part, the amphiphilic molecule may belong to one of the following types: ionic, nonionic, or zwitterionic [12].

2.2.2 Formation of micelles

Due to this molecular structure surfactants have affinities for both aqueous and nonpolar phases and show interesting behaviour in presence of solvents. At low concentrations, they dissolve in water. At some critical concentration, however, they reach their solubility limit and begin to aggregate into micellar structures, but the solution did not yet show liquid crystalline behaviour and is called "isotropic micellar solution" (IS) [12]. The micelle structure – the first model of which was worked out by Hartley in the 1930's and 1940's – allows the molecules to keep their polar region in the aqueous phase on the surface of the micelle and the nonpolar portion in the nonpolar interior of the micelle [l3]. In case of nonpolar solvents the molecules are arranged in an interchanged position, and the formed structure is called reversed micelle. Micelles generally consist of 20-100 surfactant molecules and their characteristic size is 10-20 nm. However it should be noted, that the structure of micelles is not stationary, but rather dynamic. The hydrocarbon chains of surfactant molecules can take on a lot of conformation, the average life-time of which is 10^{-10} s. Water and surfactant molecules undergo a fast rotational and translational diffusion which takes 10^{-12} s. A surfactant monomer resides 10^{-6} – 10^{-3} s in a micelle before dissolving in the solvent as monomer or taking part in formation of an otther aggregate and the life-time of a micelle is between 10^{-2} and 10 s [6, 14].

The limiting monomer solubility is called the critical micelle concentration (cmc). At concentrations below the cmc, the amphiphile will exist as monomers. At concentrations above this level, the excess amphiphile will aggregate to form micelles [13, 15]. The sudden appearance of micelles at the cmc and the almost constant free monomer concentration upon further increase of total amphiphile concentration resemble a phase transition, where the micelles play the role of a condensed phase while the free monomers the vapor [16]. The factors which are involved in the spontaneous formation of micelles are the hydrophobic effect and the interactions between the polar head groups. The former causes the nonpolar

portion of the molecule to be separated from water and sequestered in the interior of the structure and the latter determines how closely the molecules may be packed [17, 18].

2.2.3 Thermodynamic approach of micelle formation

According to the second law of thermodynamics, in a closed system enthropy (order) never increases. The association of the disordered monomers into ordered aggregates involves entropy loss. Hence it would appear that micelle formation is entropically disfavored. How can be then explained this counter-intuitive favorable entropic process? At first glance it seems that entropy should disfavor micelle formation, since a single multimolecular aggregate of lower positional entropy (fewer possible microstates for amphiphile dispersal) is formed from many surfactant molecules. However, this is only one part of this process. The entropy change of water should be taken into account as well. Placing a hydrocarbon in water, a literal cavity in the water must be created that will accommodate the hydrocarbon. Water molecules still want to maintain their hydrogen bondings. Hence they are forced into a more ordered structure around the hydrocarbon group to maintain their hydrogen bondings, characterized by fewer microstates. Creation of this more ordered cavity must be entropically disfavored. Transferring the hydrocarbon from water to a micelle will dissipate the cavity, and lead to more available microstates for the released solvent, bulk water. This is the entropic contribution that favors micelle formation. The effect, in which the spontaneous formation of micelles is preferred by the increase in entropy when water structured around the nonpolar part of the surfactant is freed as the nonpolar part sequesters into the micelle is called the hydrophobic effect, mentioned before [6, 18, 19].

2.2.4 Role of the molecular geometry

The preferred aggregation geometry (spherical micelles, cylindrical aggregates, planar bilayers) and the equilibrum size distribution are determined by the molecular characteristics of the amphiphiles, as well as by the total concentration, temperature, pH, electrolyte content and other thermodynamic variables. In concentrated solutions, interactions occur between the aggregates which also influence their sizes and shapes; lyotropic specific structures appear with a structural order even up to three dimensions and the solution becomes an anisotropic

one, more or less viscous. In this case, the micellar solution turned into a lyotropic liquid crystal [12, 16].

As mentioned before, in the micellisation process molecular geometry plays an important role and it is essential to understand how a surfactant molecule can pack. As described previously, two opposing forces control the self-association process: the hydrophobic effect (i.e. pulling surfactant molecules out of the aqueous environment), and head group interactions that work in the opposite sense [20, 21]. These two contributions can be considered as an attractive interfacial tension term due to hydrocarbon tails and a repulsion term depending on the nature of the hydrophilic group. This basic theory was constructed and quantitatively described by Mitchell and Ninham [22] and Israelachvili [23], resulting in the concept, that aggregation of surfactants is controlled by a balanced molecular geometry.The geometric approach considers three critical geometric terms:

- the minimum interfacial area occupied by the head goup, a_0;
- the volume of the hydrophobic tail(s), v;
- the maximum extended chain length of the tail in the micelle core, l_c.

This defines the critical packing parameter P_c as the ratio of volume to surface area:

$$P_c=v/(a_0 l_c) \tag{1}$$

The parameter v varies with the size of the hydrophobic part of surfactant molecule, so it depends on the number of hydrophobic groups, chain unsaturation, chain branching and chain penetration by other compatible hydrophobic groups. The value of a_0 is mainly governed by electrostatic interactions and head group hydration. The critical packing parameter is a useful quantity since it allows to predict the shape and size of the aggregates. The possible aggregation characteristics of surfactants cover a wide range of geometric arrangement, and the main types are presented in Figure 1.

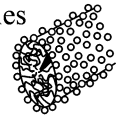
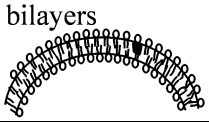
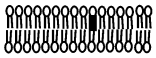
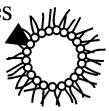
Critical packing parameter $P_c=v/a_0l_c$	Critical packing shape	Structures formed
$P_c<1/3$	Cone 	Spherical micelles 
$1/3<P_c<1/2$	Truncated cone 	Cylindrical micelles 
$1/2<P_c<1$	Truncated cone 	Flexible bilayers 
$P_c\cong1$	Cylinder 	Planar bilayers 
$1<P_c$	Negative curvature 	Oil soluble micelles 

Figure 1. Schematic representation of the geometric shape of solvated surfactant molecules and structures formed.

2.3 Lyotropic liquid crystalline structures

When the volume fraction of a surfactant in a micellar solution is increased above a certain threshold, a series of regular geometries is commonly encountered. Because of the repulsive electrostatic or hydration forces occuring between the micellar surfaces, the only way to maximise the separation of micelles is to change their shape and size. This explains the sequence of surfactant phases observed in the concentrated regime. The main structures are: lamellar, hexagonal and several cubic phases, but nematic, cholesteric and some intermediate

phase can also occur [6, 12]. The different structures can be classified on the base of their position order.

2.3.1 The hexagonal phase

Increasing the surfactant concentration – generally up to 30-50 % – the viscosity of the system compared to the micellar solution rises abruptly, which indicates the appearance of the hexagonal phase (H) [6, 12]. This mesophase generally exist in wide concentration and temperature range. Its structure composed of close-packed positionally ordered rod-like aggregates arranged in a hexagonal pattern, and the cross-section of aggregates can shape circle, oval, square, rectangle or hexagon. The length of these micelles depends on the properties of the system, but very short aggregates characterized by a 2:1 length/diameter ratio can also arrange in hexagonal structure.

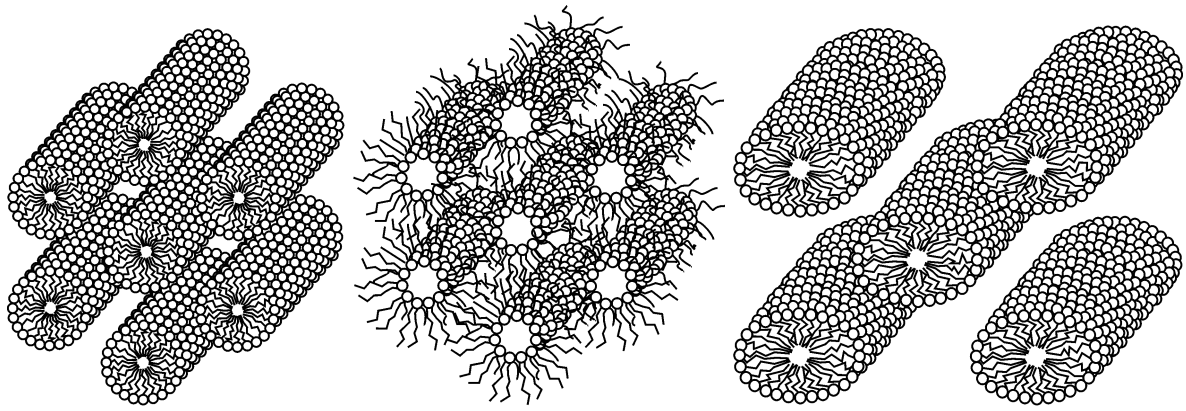


Figure 2. Structure of the normal-, inverted hexagonal and rectangular ribbon phase.

According to the solvent polarity beside the nomal hexagonal phase (H_1), in case of which the hydrophilic head groups are located on the outer surface of the cylinder and the hydrophobic tail is in the inside of micelles, "inverted" hexagonal phase also exists. This inverted phase consists of micelles with internally located hydrophilic groups. Since all the space between adjacent cylinders is filled with hydrophobic groups, the cylindrical micelles are more closely packed than those found in the H_1 phase. As a result, H_2 phases occupy a much smaller region

of the phase diagram and are much less common. The high viscosity of the hexagonal phase is due to its two-dimensional order [24]. Because of this arrangement the hexagonally packed aggregates can move freely only along their lengthwise direction [25, 26]. Nearly related structures are the so-called ribbon phase, in case of which the columnar micelles are located in a rectangular arrangement (Figure 2) [27, 28].

2.3.2 The lamellar phase

If the surfactant concentration of a hexagonal phase is increased above a certain threshold, a sharp decrease in the viscosity of the system can be observed generally. This phenomenon indicates the occuring phase change: the hexagonal arrangement turns into a layer structure. This mesophase is denoted lamellar phase (L), the existence of which covers usually the widest range considering both concentration and temperature intervals. The theoretical model of the lamellar phase involves infinitely extended bilayers composed of surfactant molecules. Ideally, the lateral extension of the bilayers is restricted only by the walls of the vial. Their thickness are between one- and two-fold of the length of the constituent amphiphilics. The space between the surfactant-layers is filled out by the solvent. Water and aqueous solution can be included in the polar layers, resulting in an increase of layer thickness. Analogously, affinic molecules can be included in the nonpolar layers. Under special circumstances an extreme dilution can be achieved resulting a hyper-swollen state, characterized by a layer distance in magnitude of 1μm. On the bases of recent examinations water-filled pores can be found in the inside of bilayers as well [6, 29, 30]. This lamellar structure is considered to be one-dimensional as there is only one parameter that can be quantified, that of the repeat distance between the bilayers. The layers can slide over each other readily, their movement is restricted only in perpendicular direction to the plane of the layers. This property explains the lower viscosity of lamellar phase compared to the hexagonal arrangement. On the bases of molecular ordering three different subgroup can be distinguished within the lamellar phase (see Figure 4). In a fluid lamellar phase (L_α), which is the least ordered of the lamellar phases movement within the bilayer is not restrained as the alkyl chains are melted and fluid-like. The hydrocarbon tails are thus able to „wiggle” about with movement driven by trans-gauche isomerization. Collisions with neighbouring molecules then occur as the molecules are able to

undergo rapid rotational and translational motions as well as thermally activated lateral diffusion in the bilayer. In contrast it was found that the interchange between adjacent sheets is extremely rare with even the flipping of a molecule across a bilayer requiring days to take place. Below the chain melting transition temperature one finds that the mobility of the amphiphiles within the bilayers is reduced thereby providing the opportunity for lateral ordering to take place. Lamellar phases other than the fluid case arise: a crystalline (L_C), and a gel type (L_β) occur with the latter appearing at a temperature lower than that of the L_α but higher than that of the L_C. The structures of these phases are summarised in Figure 3.

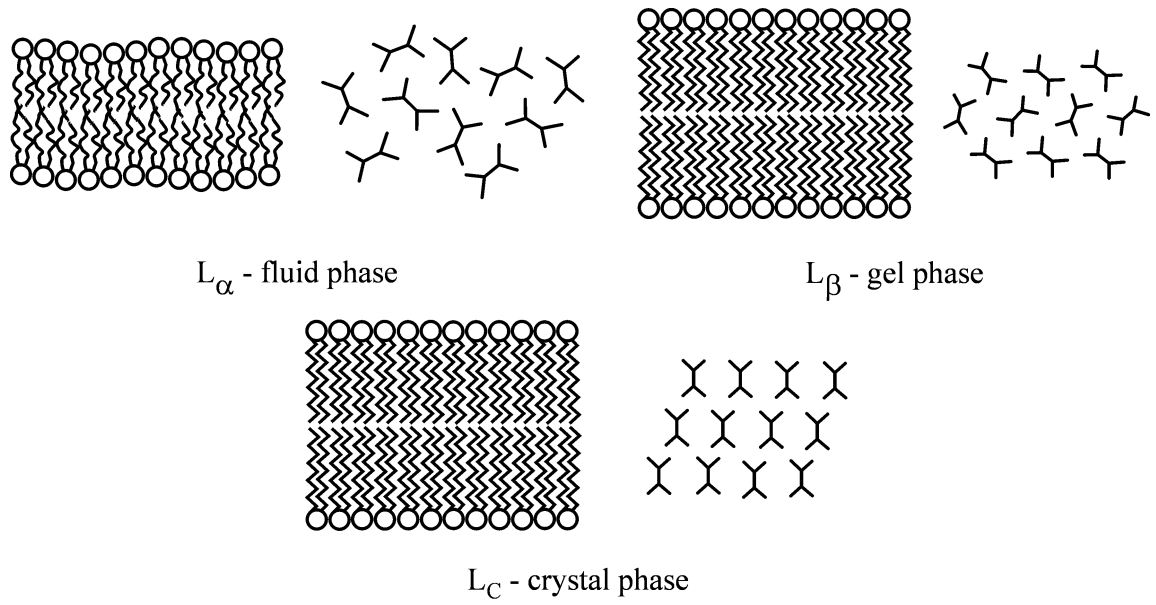


Figure 3. Schematic representation of the three main types of lamellar phases.

In case of L_β phases the headgroups are still completely disordered but the hexagonally packed chains show a reduced degree of freedom, are partially ordered and are essentially confined to the all trans configuration (there is an average of one gauche link per chain). It should be noted that although there is no lateral diffusion in the phase, there is sufficient rotation (approx. 30^o) about the long axis of the chains to make the lattice positions equivalent. When considering the gel phase a further distinction must be made between chains which align normal to the lamellar planes (L_β) and the situation when the chains are tilted

with respect to the lamellar layers ($L_{\beta'}$ and $L_{\beta I}$). And finally, the L_C phase (commonly called the crystal phase) has the chains of each amphiphile "frozen" onto specific lattice sites in this way it is the most ordered of the three lamellar phases [6].

2.3.3 The cubic phase

In certain cases an optically isotropic, highly viscous phase can occur between the isotropic micellar solution and the hexagonal or lamellar phase; or between the lamellar and hexagonal phase: this liquid crystalline structure is the cubic phase. On the one hand this mesophase may consist of discrete aggregates, e. g. spherical or short rod-like micelles arranged in a cubic grid. This cubic pattern can show three different packing forms: simple cubic, cubic body-centered and cubic face-centered lattice (Figure 4). The observed high viscosity is due to the three-dimensional order of the aggregates.

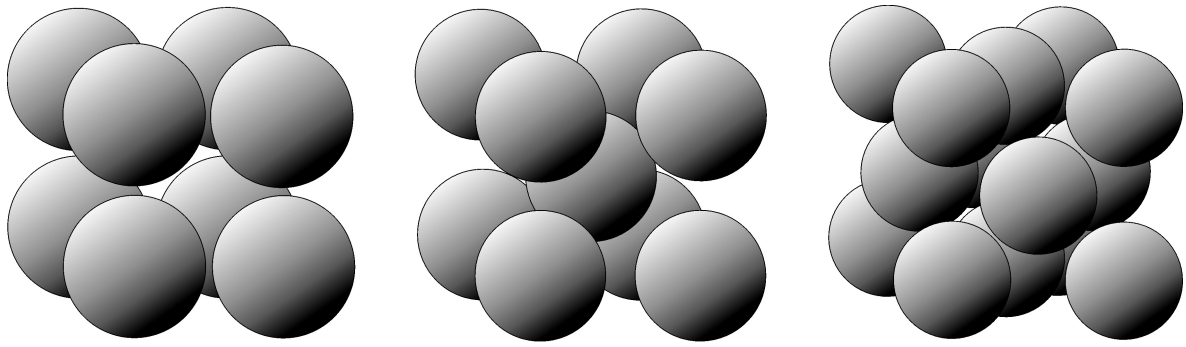
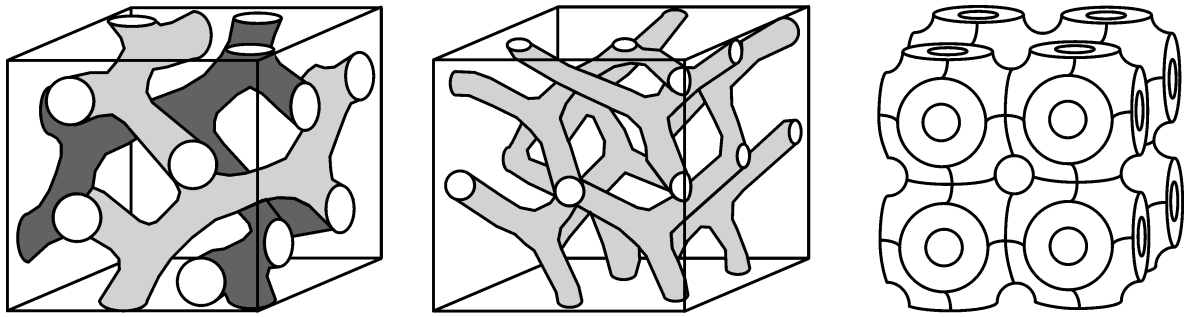


Figure 4. Structure of simple-, body-centered and face-centered cubic lattice.

According to the solvent polarity normal- (I_1), and reversed (I_2) micellar cubic phase can arise. The location of I_1 phase is between the isotropic micellar solution and H_1 phase, while the I_2 phase is located between the reversed isotropic micellar solution and H_2 phase. On the other hand besides cubic phases consisting of discrete aggregates, bicontinuous cubic phases also exist. They are thought to be rather extended, porous, connected structures in three dimensions. They are considered to be formed by either connected rod-like micelles, similar

to branched micelles, or bilayer structures. They can form normal (V_1) or reversed (V_2) phases and are positioned between H_1 and L_α and between L_α and H_2 phase respectively [31, 32]. In the V phases three interesting bicontinous network can be formed (see Figure 5).



(a) (b) (c)

Figure 5. Cubic phases with bicontinous structure.

In case of network „a" both surfactant and water form short cylinders that couple together three by three and form two independent interdigitating branches [12, 33]. For the network „b" it is supposed that two tetrahedral interwoven ramifications are arranged in a double diamond structure. In network „c" long water cylinders joined six by six are arranged in a cubic pattern [12, 34]. Although unlike the other liquid crystalline phases, in case of cubic phase the orientation of anisotropic constituents practically does not occur, however because of their three-dimensional positional order the system is characterized as liquid crystalline structure.

2.4 In situ forming lyotropic liquid crystalline systems

In situ gelling drug delivery systems undergoing transitions from a low to a high viscous state gained significant interest among formulators within the pharmaceutical field as drug delivery vehicles for dermal, nasal, ocular, oral, buccal, vaginal, rectal and parenteral administration [35-41]. Factors inducing gelation include alterations in the pH, ionic strength, solvent concentration and temperature [42, 43]. In situ gelling systems possess several advantageous properties. In certain cases the systematically applied active agent can not achieve the required concentration and/or retention time at the site of action [44, 45]. This drawback can

be markedly reduced by incorporating the active ingredient into topically applied in situ gelling drug delivery systems. Due to their low initial viscosity these preparations can be easily injected into site of application where they undergo a sol-gel transformation by meeting physiological conditions [42]. The increased viscosity ensures the desired retention time while the local release of drug the adequate concentration. In this way site-specific action [46–48], prolonged delivery periods, decreased drug dosage with concurrent reduction in possible undesirable side effects common to most forms of systemic delivery, and improved patient compliance and comfort can be achieved [24, 44, 46-52].

In the first sections of my thesis the formation of lyotropic liquid crystals and brief description of certain mesophases were discussed. Figure 6 shows the arising lyotropic liquid crystalline phases depending on the concentration and polarity of surfactant. It can be seen that the decrease of surfactant concentration – with coinciding increase in solvent content – leads to the formation of dimensionally more ordered structures chraracterized by higher viscosity values. On the bases of this behaviour in situ gelling lyotropic liquid crystal preconcentrates characterized by low viscosity can be prepared which are not liquid crystals yet but they are able to form liquid crystalline structure upon contact with body fluids at the site of administration.

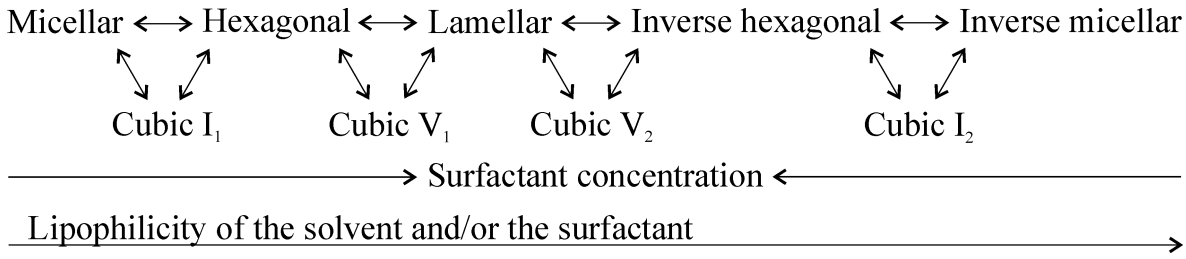


Figure 6. Transformations of lyotropic liquid crystalline mesophases.

The preconcentrates – depending on the site of application – interacts with different body fluids such as plasma, tear fluid, saliva etc. and undergoes a phase transition into a mono- or multiphasic system of liquid crystals. An example of this is oily solutions of reverse micellar phospholipids, which solubilize additional drug and transform into different liquid crystalline phases by absorbing water upon mucosal application. The diffusion of the drug – depending on the stucure – can be reduced by factor 10-1000 in comparison with a liquid vehicle such as

a solution in this way the diffusion within the liquid crystalline phase is the rate-controlling step [24, 53-55]. This feature permit of preparation of sustained formulations, which coincides with reduced application frequency and consequently better patient compliance [24]. This principle can be used for ophthalmological administration as well as for nasal, buccal, rectal, vaginal or even parenteral subcutaneous application [24, 35-41, 56]. However, the peroral administration of such reverse micellar solutions either directly or encapsulated within soft gelatin capsules is not recommended, because the sustained release effect is limited by interindividual variations in digestion [24, 57].

3 EXPERIMENTAL AIMS

The aims of my experimental work can be summarized as follows:

- On the bases of preliminary experiments selection of suitable components (surfactants and oils with good physiological tolerance), the mixtures of which spontaneously form lyotropic liquid crystalline phase in aqueous environment;
- determination of the optimal surfactant-oil ratio by constructing of triangular phase diagrams;
- investigation of water absorption of the water-free liquid crystal preconcentrates;
- examination of the effect of different water content on the liquid crystalline structure by means of direct and indirect methods:
    - direct method: polarization microscopy,
    - indirect methods: rotational and oscillatory rheology, thermoanalytical (DSC) measurements;
- incorporation of drug into the water-free preparations;
- in vitro drug release studies:
    - diffusion cell method,
    - modified Kirby-Bauer disk diffusion method;
- determination of the relationship between the applied components, the liquid crystallines structures and the results of the drug release studies.

4 MATERIALS AND METHODS

4.1 Materials

4.1.1 Polyoxyl 35 Castor Oil

Appearance: yellow oily liquid with a faint characteristic odour.

Solubility: very soluble in water, producing a practically odourless and colourless solution; soluble in alcohol and in ethyl acetate; insoluble in mineral oils.

Definition: a mixture of the triricinoleate ester of ethoxylated glycerol with smaller amounts of macrogol ricinoleate and the corresponding free glycols.

It is produced by reacting 1 mole of glycerol ricinoleate with about 35 moles of ethylene oxide. Polyoxyl 35 Castor Oil is tolerated extremely well, as tests with single and repeated oral doses and exposure tests on the skin and mucous membranes have shown. It is used as emulsifying and solubilising agent and as a solvent in vehicles for various intravenous injections [58, 59]. The HLB value lies between 12 and 14.

4.1.2 Polyoxyl 40 Hydrogenated Castor Oil

Appearance: white to yellowish paste or pasty liquid with a faint odour. Congealing temperature 20-30 ^{o}C.

Solubility: very soluble in water, producing an odourless solution, soluble in alcohol and in ethyl acetate; insoluble in liquid paraffin.

Definition: a mixture of mainly the trihydroxystearate ester of ethoxylated glycerol, with smaller amounts of macrogol trihydroxystearate and the corresponding free glycols.

It is produced by reacting 1 mole of glycerol trihydroxystearate with about 40 to 45 moles of ethylene oxide. Polyoxyl 40 Hydrogenated Castor Oil is well tolerated by tissues, as tests with single and repeated oral doses and exposure tests on the skin and mucous membranes have shown.The HLB value lies between 14 and 16. Polyoxyl hydrogenated castor oils are used as surfactants, emulsifying and solubilizing agent [58, 59].

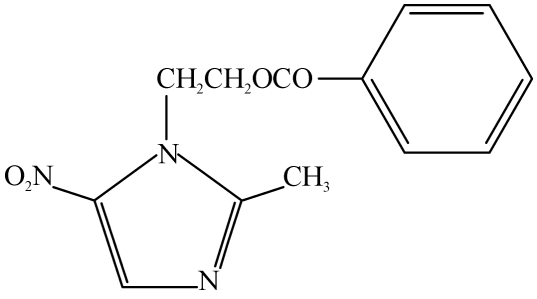
4.1.3 Miglyol 810 (fractionated coconut oil, neutral oil)

Appearance: colourless or slightly yellowish, oily liquid.
Solubility: practically insoluble in water, miscible with alcohol, with methylene chloride, with light petroleum and with fatty oils.
Definition: Mixture of triglycerides of saturated fatty acids, mainly of caprylic acid (octanoic acid, $C_8H_{16}O_2$) and of capric acid (decanoic acid, $C_{10}H_{20}O_2$) [60].
Neutral oil is used as a basis for the preparation of oral suspensions of drugs unstable in aqueous media and as a source of medium-chain tiglycerids [61].

4.1.4 Isopropyl myristate

Appearance: clear practically colourless, almost odourless oily liquid; congeals at about 5 ^{o}C.
Solubility: insoluble in water, in glycerol and in propylene glycol; freely soluble in alcohol. Miscible with most organic solvents and with fixed oils.
Definition: 1-Methylethyl tetradecanoate together with variable amounts of other fatty acid isopropyl esters.
Isopropyl myristate is resistant to oxidation and hydrolysis and does not become rancid. It is absorbed fairly readily by the skin and is used as a basis for relatively nongreasy emollient ointments and creams. It is also used as a solvent for many substances applied externally [58].

4.1.5 Metronidazole benzoate



$C_{13}H_{13}N_3O_4$
M_r: 275.3

Appearance: white or slightly yellowish, crystalline powder or flakes.
Solubility: practically insoluble in water, freely soluble in methylene chloride, soluble in acetone, slightly soluble in alcohol [60].
Definition: 2-(2-Methyl-5-nitro-1H-imidazol-1-yl)ethyl benzoate.
Metronidazole benzoate – official in Ph. Eur. 4, BP and USP – is a prodrug, which dissolves and hydrolyses to form the active metronidazole [24, 59, 60, 62]. Metronidazole is a 5-nitroimidazole derivative and has well-established bactericidal activity against obligate anaerobic bacteria in vitro, including the Gram-negative *Bacteroides* spp., *Fusobacterium* spp., and *Veillonella* spp., and the Gram-positive organisms *Clostridium* spp., *Eubacterium* spp., *Peptococcus* spp., and *Peptostreptococcus* spp. It has activity against some strains of *Campylobacter* spp. and *Helicobacter pylori* as well [58]. Besides the antibacterial effect Metronidazol is also active against several protozoa including *Balantidium coli*, *Blastocystis hominis*, *Entamoeba histolytica*, *Giardia intestinalis* and *Trichomonas vaginalis*. The mode of action of metronidazole is not entirely clear, but is thought to involve reduction by bacterial nitroreductases to an unstable intermediate which interacts with DNA, effectively preventing further replication [44, 58, 63].

4.2 Methods

4.2.1 Construction of phase diagrams, selection of suitable surfactants and oils

In order to find appropriate components for the formulation of lyotropic liquid crystals, safe and physiologicallly compatible oils and nonionic surfactants were chosen. The ternary phase diagrams of oil, surfactant, and water were constructed using water titration method to obtain the components and their concentration ranges that can result in large existence area of lyotropic liquid crystalline mesophases. Each surfactant – after melting, if required – was mixed with oil at room temperature (25 ^{o}C). For each phase diagram, the ratio of oil to the surfactant was varied as 9:1, 8:2, 7:3, 6:4, 5:5, 4:6, 3:7, 2:8, 1:9 (w/w). Given quantities of water was added dropwise to each oil-surfactant mixture under vigorous stirring. After equilibrium, the samples were checked visually and by means of polarization microscope and determined as being lyotropic liquid crystals, microemulsions, macroemulsions, or gels. On the basis of these preliminary experiments Cremophor EL and Cremophor RH40 were chosen as surfactants, isopropyl myristate and Miglyol 810 as oily components.

4.2.2 Preparation of samples

Once the liquid crystal region was identified, the formulations at desired component ratios were prepared with or without metronidazole benzoate. The preparation of drug-loaded liquid crystals was performed by dissolving the drug into the oil-surfactant mixture, adding the required weight of water, and stirring to form liquid crystal. In order to study the effect of water on the properties of the samples compositions with various water content (from 10 % to 90 %) were also prepared, meanwhile the oil : surfactant ratio was kept at the constant value of 1:4. The resulting formulations were tightly sealed and stored for 1 week before investigation at ambient temperature, and their physical stability was measured by observing periodically the occurrence of phase separation.

4.2.3 Investigation of water absorption of the water-free liquid crystal preconcentrates

The water absorption mechanism of the samples was examined with the instrument used for determining the Enslin number. The instrument consists of a glass filter and a pipette attached to it with a rubber hose in a flexible way. The pipette is fixed horizontally at the same height as the glass filter. 1 g of waterfree sample was placed on the G1 glass filter of the instrument filled with bubble-free water, then the quantity of the absorbed water was measured as the function of time. The duration of the measurement was 2 hours.

4.2.4 Polarization microscopy

The structure of the samples was examined with a polarization microscope (Leica Q500 MC image analyzer system) between crossed polarizers at room temperature. The magnification was 40 fold.

4.2.5 Rheological investigations

Rheological measurements were carried out with a RheoStress 1 HAAKE rheometer. A cone-plate measuring device was used in which the cone angle was 1 degree, and the thickness of the sample was 0.048 mm in the middle of the cone. The measurements were performed at

25 ^{o}C. The samples were kept in a space saturated with water vapour during measurement in order to prevent evaporation. The linear viscoelastic range was determined in the first step by examining the complex modulus (G*) as the function of shear stress (τ) at a given frequency (f=1 Hz). Based on these experiments, the value of shear stress was set at 2.5 Pa during the dynamic test as this value was always within the linear viscoelastic range, then the values of the storage (G') and loss (G") moduli were examined as the function of frequency. Besides the oscillation tests, flow curves and viscosity curves of the different samples were also determined. In the course of this shear rate was increased from 0.1 to 100 1/s (up curve) and then decreased from 100 to 0.1 1/s (down curve) in the CR mode. The shearing time was 300 s in case of both segments.

4.2.6 Subzero temperature DSC measurements

The measurements were carried out with a Mettler-Toledo DSC 821e instrument. Samples (5-10 mg) were weighed in aluminium pans and immediately sealed by press. The reference was an empty pan. The samples were cooled at a heating rate of –5 ^{o}C/min to –40 ^{o}C. They were kept 2 minutes at this temperature and then samples were heated to 25 ^{o}C. The heating rate was 5 ^{o}C/min. The heat flow was measured as a function of the temperature.

4.2.7 In vitro drug diffusion studies - diffusion cell method

In vitro drug release studies were performed by means of a vertical diffusion cell method (Hanson Microette System, Hanson Research Corporation). 0.4 g of sample was placed as a donor phase on the Porafil membrane filter, the pore diameter of which was 0.45 μm. The effective diffusion surface area was 1.767 cm^2. Alcohol of 10% w/w was used as acceptor phase to ensure sink conditions. Measurements were performed at 25°C for 6 h. The quantitative measurement of metronidazole benzoate was carried out with a UV spectrophotometer (Unicam Helios α) at a wavelength of λ = 318 nm.

4.2.8 Modified Kirby-Bauer disk diffusion method

Agar cup diffusion method was adopted. These tests were carried out using cultures of *Fusobacterium varium* ATCC 27725. In each petri-dish six holes with a diameter of 9 mm were made and filled with an accurately weighed 0.2 g sample. The petri-dishes were kept under anaerobic condition at 37 ^{o}C for 72 hours. After incubation, the zones of inhibition around the samples were measured by means of a metric ruler to the nearest millimeter. The experiment was replicated three times in case of all samples.

5 RESULTS AND DISCUSSION

5.1 Ternary phase diagrams of the ivestigated systems

On the basis of the preliminary experiments it was concluded that neutral oil and isopropyl myristate are the most suitable oils, in the case of applying Cremophor EL and Cremophor RH40 as surfactants.

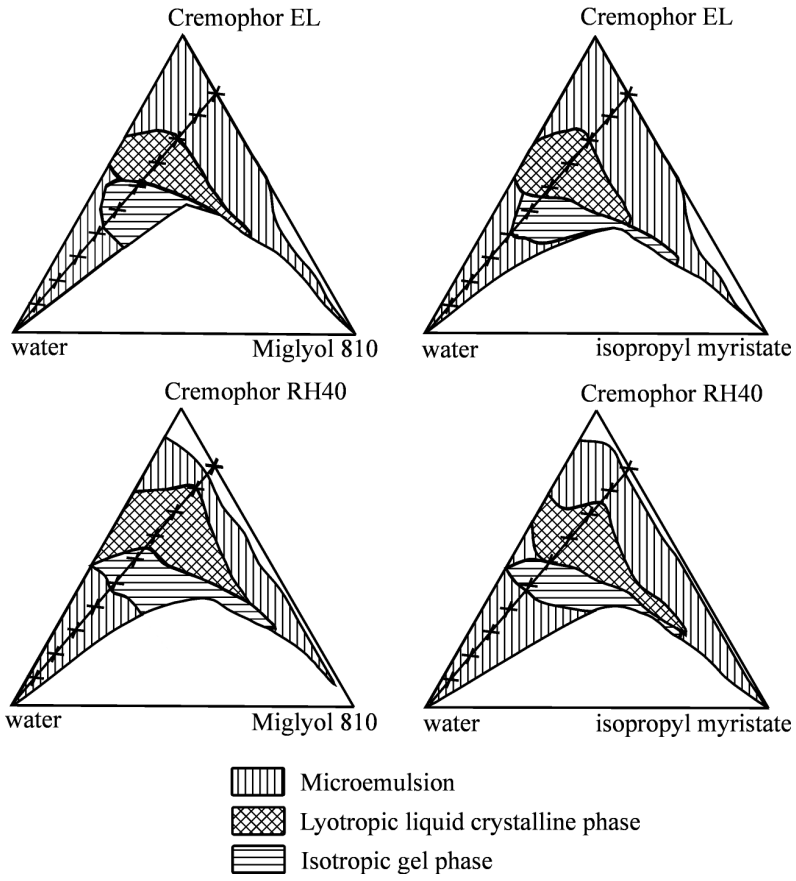


Figure 7. Ternary phase diagrams of the investigated systems.

Figure 7 shows the phase diagrams of the studied ternary systems. It can be seen that the material quality of the surfactants has a more remarkable effect on the size and location of high-viscosity lyotropic liquid crystalline and isotropic gel areas, than the oil components have. In order to examine the effect of water concentration, compositions characterized by 4:1 surfactant:oil ratio were selected, because in this case a wide variety of phase transformations caused by water addition can be observed.The straight lines starting from the side of ternary diagrams representing the oil-surfactant mixture show this alteration of the initial water-free compositions occurring at different water content. The compositions of the studied systems and their designation are presented in Table I.

Table I. Compositions of the studied systems.

System	Surfactant		Oil
I.	Cremophor EL (polyethoxylated 35 castor oil)	80 %	Miglyol 810 20 %
II.	Cremophor EL (polyethoxylated 35 castor oil)	80 %	isopropyl myristate 20 %
III.	Cremophor RH40 (polyethoxylated 40 hydrogenated castor oil)	80 %	Miglyol 810 20 %
IV.	Cremophor RH40 (polyethoxylated 40 hydrogenated castor oil)	80 %	isopropyl myristate 20 %

Sample number	Surfactant	Oil	Water
1	80 %	20 %	0 %
2	72 %	18 %	10 %
3	64 %	16 %	20 %
4	56 %	14 %	30 %
5	48 %	12 %	40 %
6	40 %	10 %	50 %
7	32 %	8 %	60 %
8	24 %	6 %	70 %
9	16 %	4 %	80 %
10	8 %	2 %	90 %

5.2 Water absorption of the water-free liquid crystal preconcentrates

The knowledge of the mechanism of water-uptake process may be important, because the direction of the flow caused by water absorption is opposed to drug liberation in this way it can influence the degree of drug release. The water absorption of water-free compositions was investigated using different surfactants and oils (system I-IV/1). Figure 8 shows the amount of absorbed water when Cremophor EL and Cremophor RH40 was applied as surfactants and isopropyl myristate as oily component. It can be seen that the sytem I/1 which has Cremophor EL as surfactant absorbed significantly more water (0.725 ml/g) during the 2 hours of examinaton than the system III/1 (0.555 ml/g) which contains Cremophor RH40. This difference can be caused by the distinctions of the appllied surface active agents. In order to verify the effect of the surfactants exerted on water absorption, samples containing the two surfactants in different proportions (2:1; 1:1; 1:2) were prepared and the water absorption of these samples were also investigated (Figure 8).

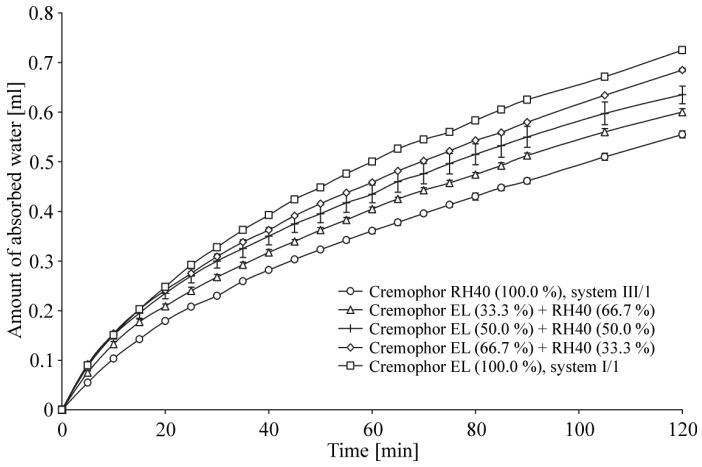


Figure 8. Water absorption of the water-free compositions containing Cremophor EL, Cremophor RH40 in different proportions and isopropyl myristate.

Plotting the amount of absorbed water as the function of square root time (Figure 9) data give straight lines the slopes of which show the rate of the water absorption process. These kinetic parameters are summarized in Table II.

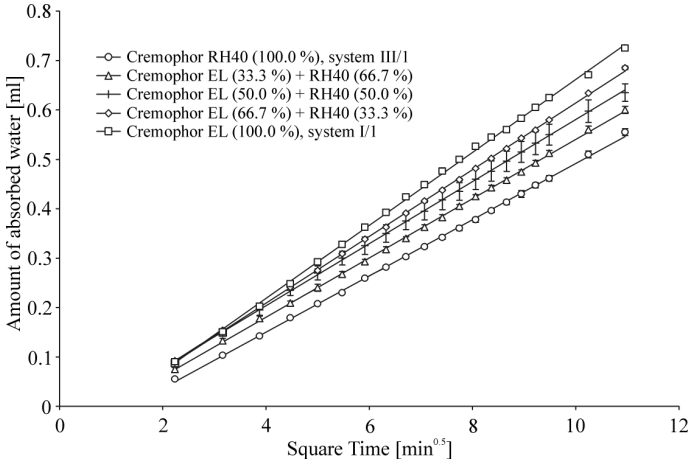


Figure 9. Water absorption as the function of square root time.

Table II. Kinetic parameters of the water absorption.

Surfactant	Equation of the straight line	R^2
Cremophor RH40	y = 0.057x - 0.0779	0.9994
Cremophor EL:RH40 = 1:2	y = 0.06x - 0.0599	0.9997
Cremophor EL:RH40 = 1:1	y = 0.0628x - 0.0477	0.9997
Cremophor EL:RH40 = 2:1	y = 0.0676x - 0.0615	0.9998
Cremophor EL	y = 0.0739x - 0.0771	0.9993

It can be seen how the rate of water absorption alters with increasing Cremophor EL content. Similar result was obtained in case of systems containing Miglyol 810 (data not shown).

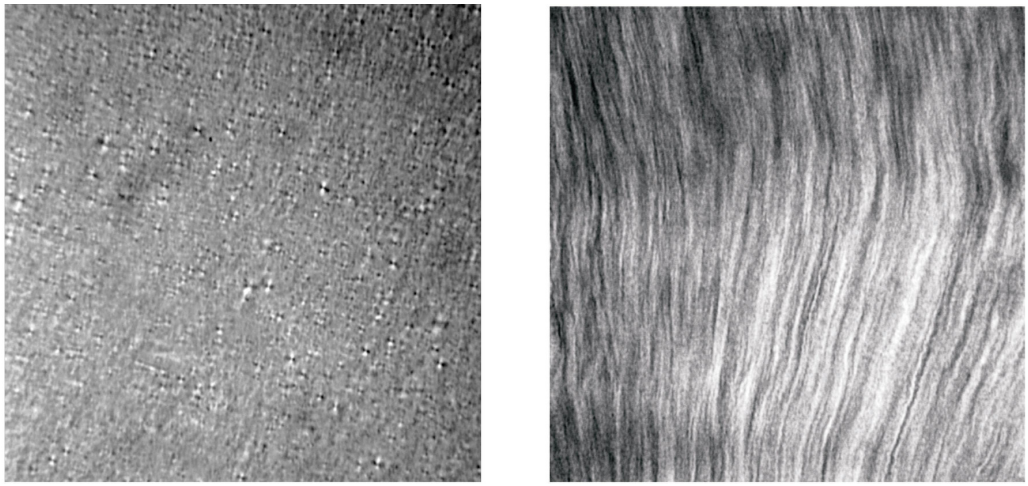
5.3 Polarization microscopy

The behaviour of light in crystalline materials depends on its direction related to the crystal structure. In case of certain crystals the incident light coming from a definite course or courses (along the optical axle(s) of the crystal) can pass through the material without any change of direction. If the included angle between the optical axle and the electric field (E) of light propagating perpendicular to the optical axle is accurately 45 degree, the vibration plane of the electic field will turn 90 degree [64]. Certain crystals possess another interesting property, called birefringence or double refraction. These materials refract the unpolarized incident light in two different directions, thus splitting an incident ray into two rays. It is

found that the two refracted rays (the ordinary ray and the extraordinary ray) are both polarized and that their directions of polarization are perpendicular to each other. It can be seen that due to these features crystals may alter the vibration plane of the linearly polarized light. The above mentioned phenomena can be observed not only in case of crystalline solids, but also in case of liquid crystals showing similar ordered structure to the real crystals. Unlike isotropic materials which are invisible between crossed polarizers of a polarization microscope, placing a thin sample of lyotropic liquid crystals into the same experimental arrangement, a picture – called texture – can be seen which shows the effective positional birefringence of the system [6, 65, 66]. Because the degree of double refraction varies with the size and shape of aggregates, the effective birefringence of a sample depends strictly on its inner structure. For instance in case of lamellar mesophase the texture is generally „streaky" or mosaic-like. Alternatively, lamellae can eradicate all edges by folding into vesicles – essentially spherical globules. These are typically multiwalled (liposomes), exhibiting characteristic „Maltese cross" textures in the polarization microscope. (Single-walled (sometimes giant) vesicles are also found; these are not lamellar mesostructures.) [24, 10, 67-75]. Hexagonal mesophase containing dense packing of cylindrical micelles arranged in a two dimensional hexagonal lattice is often identified by a characteristic angular or fan-like texture in polarization microscope, due to the focal conic domains of columns [67, 71]. Distinctly from the lamellar and hexagonal structure, the cubic mesophase – arranged in three dimensional lattice – is optically isotropic because its properties are independent of direction. As a consequence of this behaviour in case of cubic phase no texture can be seen in the polarization microscope. On the basis of these knowledges polarization microscopy can be used for the examination and identification of lyotropic liquid crystalline mesophases.

In the course of polarization microscopic examinations samples with different water content (along the marked dilution line) were investigated, and their liquid crystalline textures were identified. On the grounds of results it can be seen that samples with 10 % - 40 % water content showed anisotropic properties. Below this conentration limit optically isotropic samples were described as reversed micellar solutions – and in case of water containing systems as w/o microemulsions. However, in case of Cremophor RH40 containing water-free samples the surfactant and the oil were inmiscible in each other thus they formed a biphasic mixture. On the addition of small amount of water these systems turned into w/o microemulsion as well. Within the optically anisotropic liquid crystalline regime at lower

water content – at about 20 % - samples possessed lamellar structures. Above this water concentration a phase transformation occured: the lamellar phase turned into hexagonal one.



(a) (b)

Figure 10. Polarizationsmicrograph of sample II/3 (a) with 20 % water content, and II/4 (b) with 30 % water content at magnification of 40x.

Table III. Structures of the investigated systems

water content [w/w%]	**Cremophor EL**		**Cremophor RH40**	
	Miglyol 810	**Isopropyl myristate**	**Miglyol 810**	**isopropyl myristate**
0	rev. mic. sol.	rev. mic. sol.	biphasic	biphasic
10	w/o microem.	w/o microem.	lamellar	w/o microem.
20	lamellar	lamellar	lamellar	lamellar
30	hexagonal	hexagonal	hexagonal	hexagonal
40	isotr. gel ph.	hexagonal	isotr. gel ph.	hexagonal
50	isotr. gel ph.	isotr. gel ph.	isotr. gel ph.	isotr. gel ph.
60	o/w microem.	o/w microem.	o/w microem.	o/w microem.
70	o/w microem.	o/w microem.	o/w microem.	o/w microem.
80	o/w microem.	o/w microem.	o/w microem.	o/w microem.
90	o/w microem.	o/w microem.	o/w microem.	o/w microem.

In Figure 10 polarizationsmicropgraphs of samples II/3 and II/4 can be seen characterized by 20 % and 30 % water content respectively. In case of 20 % water content Maltese crosses – typical pattern of lammellar mesophase – can be observed, while at 30 % water content fan-like texture of hexagonal phase can be seen. With increasing water content – generally between 40 % and 50 % - an isotropic gel phase arose which changed to o/w microemulsion on the effect of further addition of water. The structures of the investigated samples are summerized in Table III.

5.4 Rheological investigations

Rheology is the study of how matters deform and flow under the influence of external forces. This deformation is strongly influenced by the inner structure, in this way rheological investigations are usefool tools to describe different materials [76]. In the course of rheological investigations flow- and viscosity curves, and viscoelastic properties of samples with different water content were determined.

In case of flow- and viscosity curves shear stress and viscosity values were measured as the function of increasing and decreasing shear rate. The alteration in the flow behaviour on the effect of water addition is presented on system I containing Cremophor EL as surfactant and Miglyol 810 as oil. In case of systems II-IV similar phenomena were observed. However it should be noted that system III/1 and IV/1 were biphasic mixtures of the surfactant and oil thus these samples showed non-Newtonian flow behaviour.

Considering the I/1 (water-free) and I/2 (10 % water) samples (Figure 11) linear relationship can be observed between the shear stress shear rate values. This Newtonian – ideal viscous – flow behaviour is characteristic among others for real and micellar solutions and microemulsions [77-83]. In case of I/2 sample the greater slope of flow curve refers to higher viscosity value. The viscosity increase with increasing water content can be attributed to cluster formation by intermicellar interactions causing reverse micelles to connect and form temporary continuous and infinite structures [79].

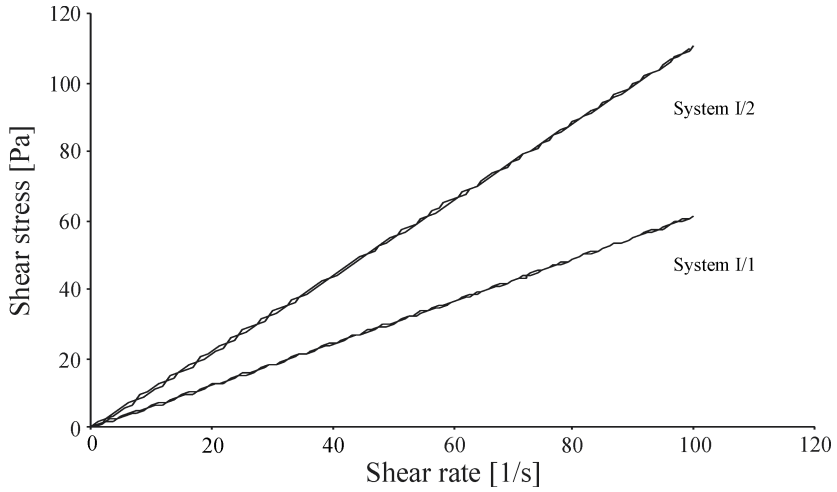


Figure 11. Flow curves of samples I/1 (water-free) and I/2 (10 % water).

With increasing water concentration it can be seen that due to the appearance of coherent structure a non-Newtonian – thixotropic – flow behaviour occurs. It means the reduction in structural strength during the shear load phase and the more or less rapid, but complete structural regeneration during the subsequent rest phase [84-87]. Thixotropic materials – similar to pseudoplastic liquids – show shear-thinning behaviour when a gradually increasing shear is applied. However, in case of pseudoplastic liquids thinning under the influence of increasing shear depends mainly on the particle/molecular orientation or alignment in the direction of flow surpassing the randomizing effect of the Brownian motion. Contrarily for thixotropic systems it is typical that they not only show this potential for orientation but additionally for a time-related particle/molecule interaction. This phenomenon leads to formation of bonds creating a three-dimensional network strucure which is often called a „gel". When this gel is subjected to shear over an extended period of time the network will be disrupted and the viscosity drops with shear time until it asymptotically reaches the lowest possible level. This minimum viscosity level describes the „sol"-state of the system [84-87]. Due to the time consuming structural regeneration, considering the flow curve of a thixotropic material one can find that the up curve is not directly underneath the down curve. The hysteresis between these two curves forms a loop – the area of which defines the magnitude of thixotropy. This area has the dimension of energy related to the volume of the sample sheared which is proportional to the invested work necessary to break down the thixotropic structure [88].

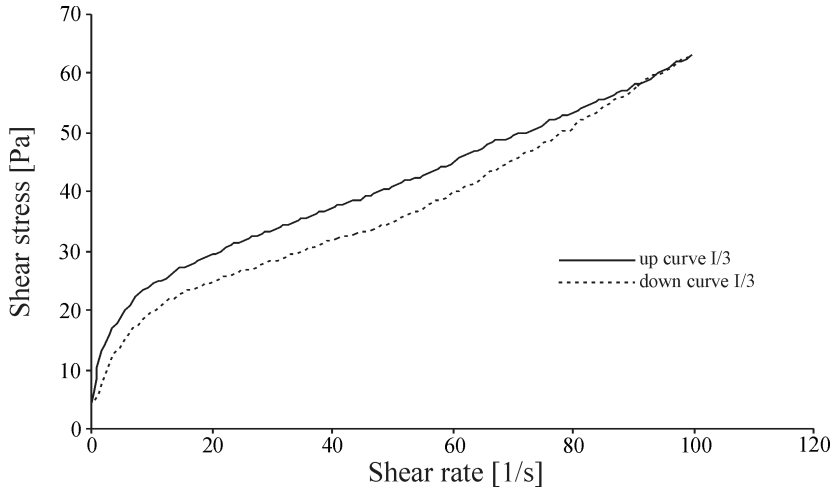


Figure 12. Flow curve of sample I/3 (20 % water).

Figure 12 shows the flowcurve of sample I/3 with 20 % water content. On the basis of polarization microscopic examinations this sample possess lamellar stucture. Indeed, one can see the thixotropic area between the up- and down curves which refers to the presence of liquid crystalline structure. Compared to system I/4 (Figure 13) with 30 % water content and hexagonal structure, the ratio of shear stress to shear rate – often called apparent (shear) viscosity - is relatively low because the lamellae can easily orient in the direction of flow [89].

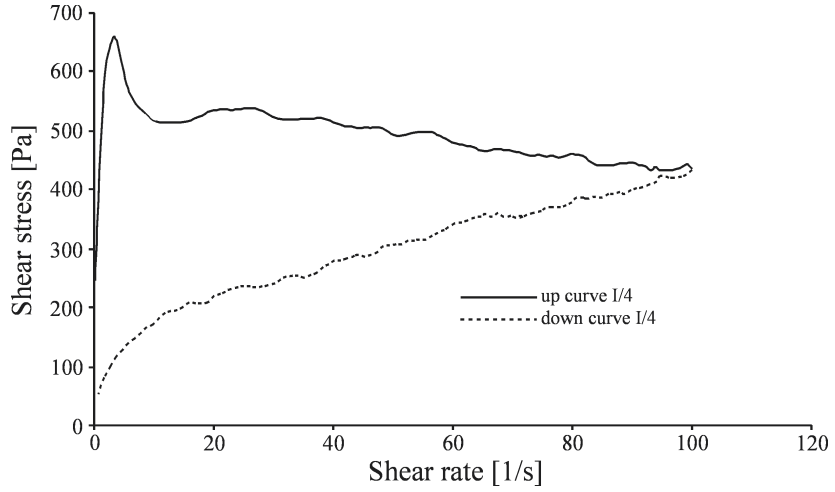


Figure 13. Flow curve of sample I/4 (30 % water).

With increasing water concentration – up to 40 % and 50 % - further remarkeble growth of apparent viscosity values can be observed (Figure 14). These systems exhibit jelly like consistency, and they are transparent, clear, homogenous, optically isotropic and thermodynamically stable. To designate these gels, many terms are used inter exchangeably. Among them, the most common are microemulsion gel, transparent emulsion gel, clear resonant gel, cream gel, viscous isotropic phase, ringing gel.

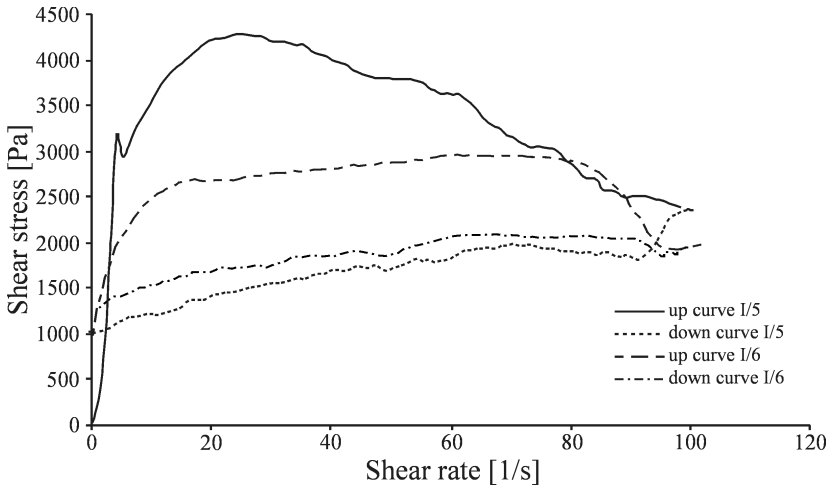


Figure 14. Flow curves of samples I/5 (40 % water) and I/6 (50 % water).

This last term refers to their highly elastic properties: if the gel is placed in a container and the container is tapped, the gel will vibrate with an audible resonance frequency. There is no agreement as to whether these gels have to be considered as microemulsions, solubilised systems, or a cubic liquid crystalline phase [90-96].

In Figure 15, taking into account the lower shear stress values it can be concluded that interactions between micelles is decreased at 60 % water concentration. This phenomenon is due to the dilution effect of solvent. Addition of further amounts of water leads to the formation of o/w microemulsion characterized by ideal viscous flow behaviour (Figure 16). The stiffness of structure can be followed up by comparing the thixotropic areas of different samples as well. These data are summarized in Table IV.

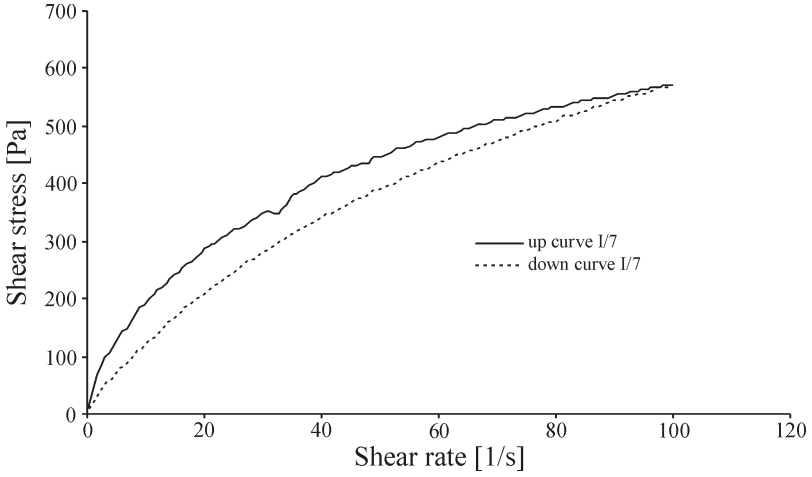


Figure 15. Flow curve of sample I/7 (60 % water).

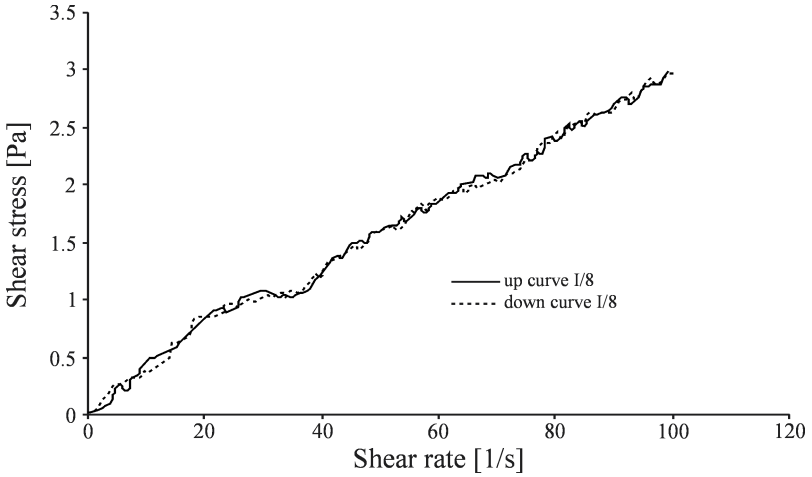


Figure 16. Flow curve of sample I/8 (70 % water).

It can bee seen that the oil component has considerable effect on the magnitude of thixotropic area: samples containig isopropyl myristate show less thixotropic properties. Nevertheless, it should be noted that on the basis of these data one can not conclude to the values of apparent viscosity (the highest value can be observed in case of sample I/5).

Table IV. Thixotropic areas of the investigated compositions.

water content [w/w%]	Cremophor EL		Cremophor RH40	
	Miglyol 810	isopropyl myristate	Miglyol 810	isopropyl myristate
0	ideal viscous	ideal viscous	1349	836.5
10	ideal viscous	ideal viscous	236.9	225.8
20	355.8	2491	1174	515
30	19210	3878	147400	4329
40	136800	5157	72560	62520
50	79890	44810	989.9	3924
60	5087	363.6	27.27	458
70	ideal viscous	ideal viscous	ideal viscous	ideal viscous
80	ideal viscous	ideal viscous	ideal viscous	ideal viscous
90	ideal viscous	ideal viscous	ideal viscous	ideal viscous

Besides the determination of flow curves the viscoelastic properties of samples were also examined by means of oscillatory tests. In course of this instead of applying a constant stress leading to steady-state flow the stress (within the linear viscoelastic range) is applied as a sinusoidal time function characterized by a given amplitude and frequency. The rheometer then measures the resulting time-dependent strain. Generally a material can respond to this type of deformation through two mechanisms: these are the elastic energy storage and the viscous energy dissipation. These parameters can be represented quantitatively by means of storage modulus (G') and loss modulus (G"). Storage modulus defines the energy stored per unit volume and its value is proportional to the extent of elastic property of the sample. Contrarily, loss modulus represents the energy dissipated per unit volume and its value is proportional to the viscous component of the sample [76, 88, 97-99]. Through determining these rheological parameters this method can be a powerful tool to describe the microscopic structure of a viscoelastic material. Németh et al. reported an oscillatory rheological method for the identification of pharmaceutically important lamellar phases [100].

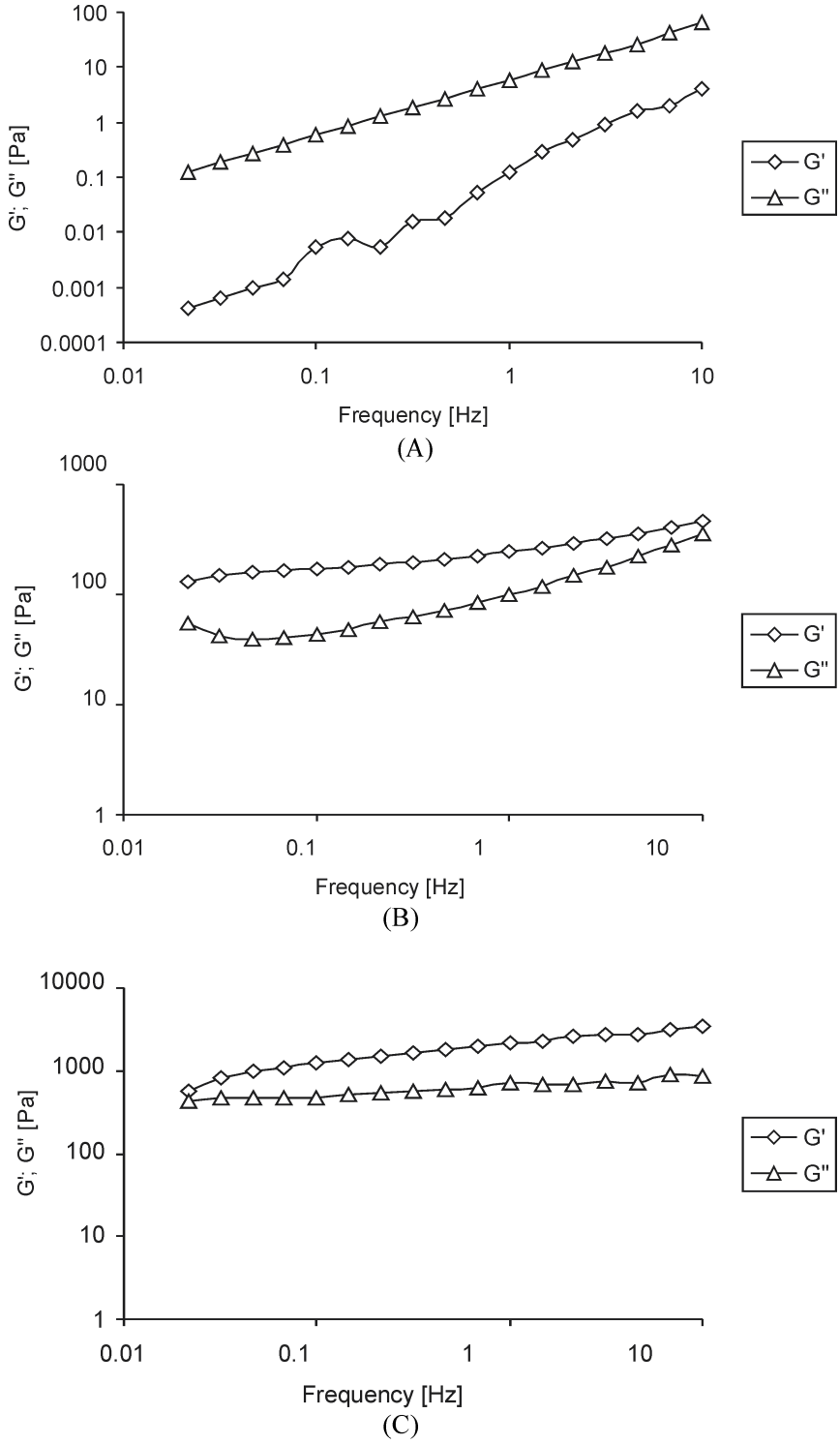


Figure 17. Oscillation test of sample II/2 (A) with 10 % water content, II/3 (B) with 20 % water content and II/4 (C) with 30 % water content.

According to their observations lamellar liquid crystals show more elastic than viscous behaviour, the storage moduli are higher by about one order of magnitude than the loss moduli and the loss moduli show a minimum value. Other phases like hexagonal phase, isotropic gel phase and inverse micellar phase show completely different curves as a function of the frequency. In Figure 17 results of oscillatory tests obtained from samples II/2, II/3 and II/4 can be seen. In the case of 10 % water content the G" value is higher than the G' value, that is the system shows plastic behaviour. At 20 % water content the value of G' will exceed with about one order of magnitude that of G", and the G" curve shows a minimum. This behaviour is in good correlation with Németh's observations. Similar rheological properties characterize each of our samples possessing lamellar structure. When the water concentration is 30 %, higher G' values can be observed, which indicates the presence of the more elastic hexagonal structure.

To be able to study the effect of water content exerted on rheological behaviour more precisely, the G' and G" values at a given frequency (1 Hz) were plotted against water content during the evaluation the results of the oscillatory test. Based on the figures obtained in this way (Figure 18) it can be seen that in the case of small water content the G" value is higher than the G' value.

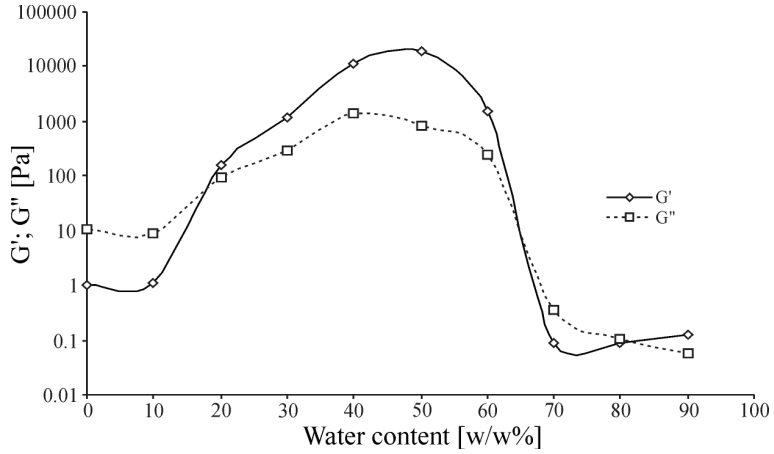


Figure 18. Values of storage (G') and loss moduli (G") at a given frequency (1Hz) as the function of water content.

It means that the system is pseudoplastic and can be characterized by viscous flow properties. When the water content is increased – approximately at 20 % – the value of G' will exceed

that of G", which indicates that the sample – due to the arising liquid crystalline structure – possesses elastic features. If the quantity of water is further increased up to about 70 %, the system shows plastic flow properties again, which is indicated by the greater value of the loss modulus. This phenomenon can be also observed by means of loss tangent which can be defined as the fraction of G" over G'. The smaller value of the loss tangent will result in a stronger elastic behaviour (Figure 19).

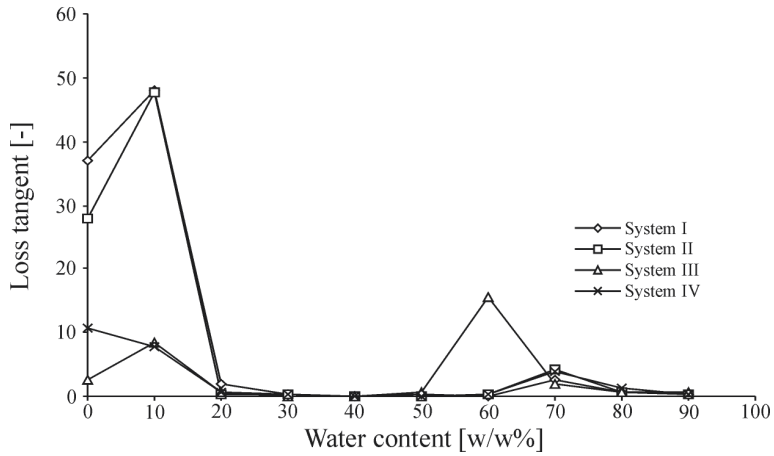


Figure 19. Values of loss tangent in the function of water content.

5.5 Subzero temperature DSC measurements

Considering the formation and structure of lyotropic liquid crystals it can be stated that one of the principal components of these surfactant-based systems is water, the behaviour of which is sensitive to the presence of adjacent interfaces of varying types. Different forms of water have been detected in surfactant-based microstructures such as microemulsions and liquid crystalline phases [101-102]. Castoro et al. and Atkas et al. classified water into two categories, free and bound water, based on a temperature of - 40 ^{o}C [103]. The part of water which achieves complete freezing at this reference temperature accepted by numerous researchers is termed free water, and the remaining unfrozen water is termed bound water. When the system is sufficiently diluted, free water is present in it, which is assumed to have similar physicochemical properties like pure water. Its presence can be detected by so-called subzero temperature differential scanning calorimetry (SZT-DSC) by the appearance of the melting peak on the DSC thermogram at about 0 ^{o}C. The disappearance of the melting

enthalpy of water (at the disappearance of free water) coincides with phase changes occurring in the liquid crystalline structure [103]. The knowledge of the proportion of varying types of water is important to get information of the structure, which has a strong effect on drug release from pharmaceutical formulations.

In the course of SZT-DSC experiments the sharp exothermic and the wider endothermic peak caused by freezing and melting of water were observed just above a definite water concentration (Figure 20 and Figure 21).

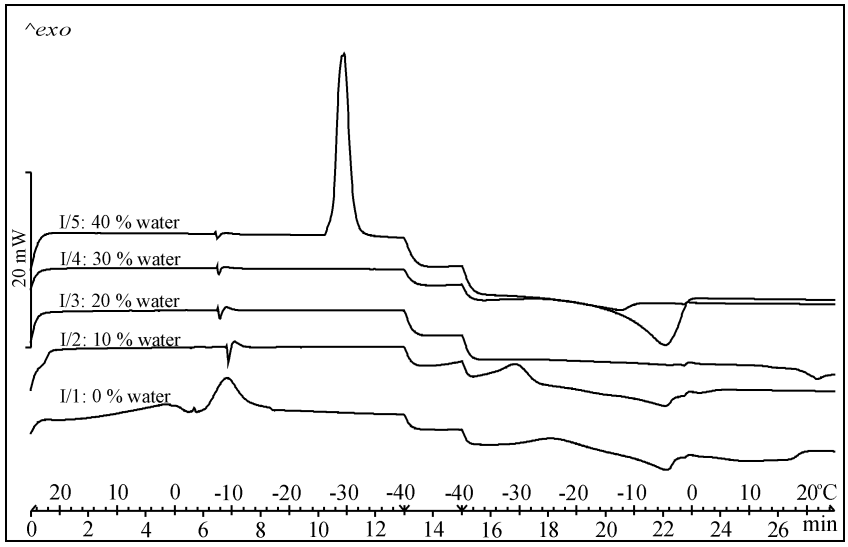


Figure 20. SZT-DSC thermograms of samples I/1-5.

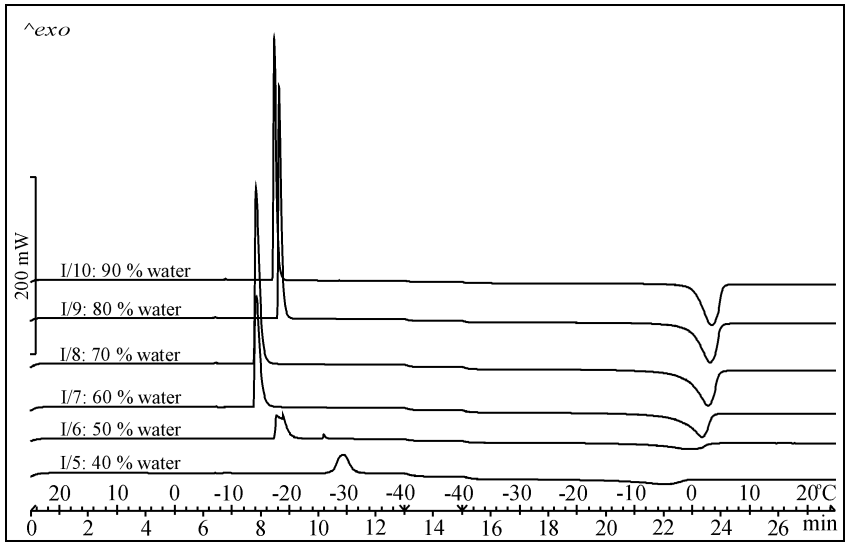


Figure 21. SZT-DSC thermograms of samples I/5-10.

This phenomenon is caused by the fact that under a certain water content there is a strong interaction between the surfactant and water molecules. This type of water is termed nonfreezable, or bound water and it has been interpreted as water forming a hydration layer in surfactant microstructures. When the saturation of surfactant molecules with water occurs, a new state of water appears which possesses similar features like the pure solvent. On the basis of thermograms the melting enthalpy of water ($\Delta H_{f(exp)}$) was calculated and plotted in the function of water concentration. In Figure 22 it can be seen that there is a linear relationship between the measured enthalpy change and the water content of samples. By means of equations describing this relationship the water concentration at which the melting enthalpy disappears and the melting enthalpy of systems containing 100 % water were calculated. The water contents obtained by the extrapolation to zero melting enthalpy values are in good accordance with lamellar-hexagonal phase transformations detected by polarization microscopic and rheological investigations. The calculated melting enthalpy was similar to the value which can be measured in the case of pure water (320 J/g) [103]. The above mentioned data are summarized in Table V.

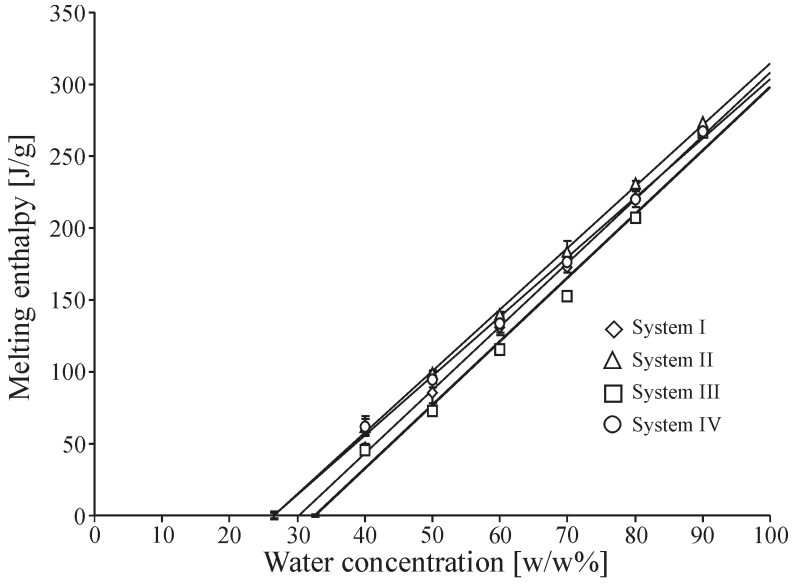


Figure 22. Variation of melting enthalpy in the function of water concentration.

Table V. Results calculated from the relationship between the water concentration and the measured melting enthalpy.

System	Equation	R^2	Water concentration at zero enthalpy [w/w%]	Calculated melting enthalpy of samples with 100 % water content [J/g]
I	y=4.4181x-133.31	0.9989	30.17	308.5
II	y=4.2827x-113.63	0.9985	26.53	314.64
III	y=4.4195x-143.97	0.9844	32.58	297.98
IV	y=4.1362x-109.85	0.9964	26.56	303.77

On the bases of the results the amount of free and bound water was calculated using the following equations:

$$W_f = \frac{\Delta H_{f(\exp)}}{\Delta H_f^0} \times 100 \tag{2}$$

$$W_t = W_f + W_b \tag{3}$$

where W_f is the weight percent of free water, W_t is the weight percent of total water content, W_b is the weight percent of bound water, $\Delta H_{f(\exp)}$ is the measured enthalpy change for the melting peak, and ΔH_f^0 is the melting enthalpy calculated by extrapolation to the 100 % water content [103]. Knowing the amount of bound water and the composition of samples, it was examined how the surfactant : bound water ratio varies with the increasing water content. On the basis of Figure 23 it can be stated that after the saturation of the surfactant and below 80 % water concentration the amount of bound water forming the hydration layer around the surfactant molecules is practically independent of the total water content of samples. Above 80 % water content the decrease of the amount of the bound water related to the surfactant concentration can be explained by the fact that these systems are situated at the border region of colloid systems and can be characterized by lower stability.

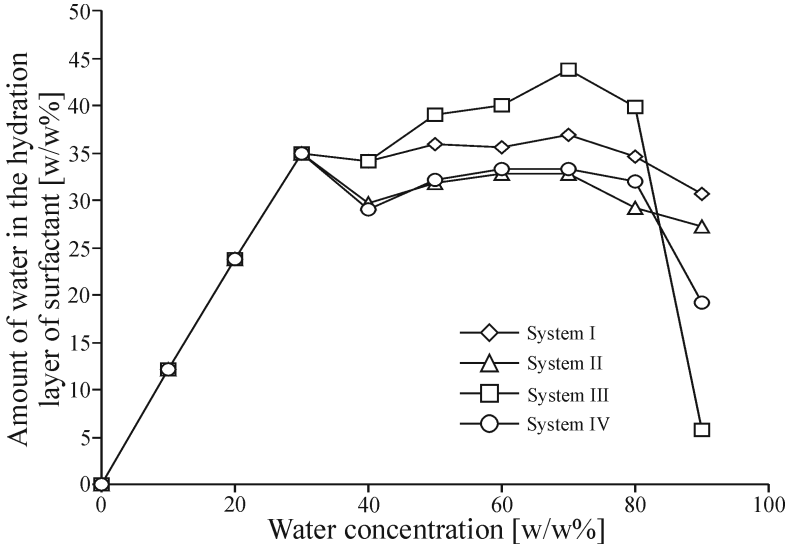


Figure 23. Water content of the hydrated surfactant in the function of water concentration.

5.6 In vitro drug diffusion studies - diffusion cell method

On the bases of polarization microscopic, rheological and SZT-DSC studies it may be concluded that the investigated surfactant-oil mixtures are promising compositions for the formulation of in situ gelling drug delivery systems. Preparations based on low-viscous isotropic phase turning into high-viscous lyotropic liquid crystalline phase in aqueous environment are in the focus of interest in the ophtalmologic, dermal, nasal, oral, buccal, vaginal, rectal and parenteral fields of application [35]. Wihtin the oral cavity these in situ gelling systems can be used for the topical administration of different forms of periodontal disease. Periodontitis is an inflammatory disease of the gingival tissue induced by bacteria residing in the plaque biofilm on the subgingival tooth surface. The inflammation leads to pocket formation in the gum tissue, attachment loss, bone destruction, and ultimately, possible tooth loss. In industrial countries, periodontitis affects 30-50% of the adults. Due to its high prevalence, the disease is a serious public-health concern [104-106]. As pathogenic micro-organisms play such a major role in the development and sustaining of periodontal disease, it is evident that patients need to be treated with systemic or local antibiotics as a supplement to traditional mechanical therapy. However, systemic treatment has numerous side effects, the necessary high dose may cause hypersensitivity reactions and gastrointestinal intolerance, and

it may also facilitate the development of bacterial resistance. Moreover, if not administered in a high dose, the drug cannot provide the therapeutic concentration in the pocket for the necessary duration. These disadvantages can be lowered substantially with the local delivery of the antimicrobial material. The local concentration of the drug may be increased if it is incorporated into a therapeutic system which can be injected directly into the periodontal pocket and the viscosity of which is increased drastically upon the effect of water (saliva) [44]. In order to study the applicability of our compositions for the treatment of periodontal disease, metronidazole benzoate was dissolved in the water-free samples, and the drug release was investigated in vitro by means of a vertical diffusion cell method. The applied drug is widely used for the treatment of periodontitis caused by anaerobe bacteria growing in the periodontal pocket [44, 107-108]. In spite of its rather poor solubility properties, the drug could be dissolved in a concentration of 3.5% in the oil-surfactant water-free mixtures. For comparison, oily and aqueous systems using neutral oil, isopropylmyristate and hydroxyethylcellulose mucilage (Cellosize QP 300, 3% w/w) were also prepared that contained the drug in a concentration of 3.5% in a suspended form. The drug release of these compositions was also examined. Composition of the ivestigated systems can be seen in Table VI. The amount of released drug as the function of time can be seen in Figure 24.

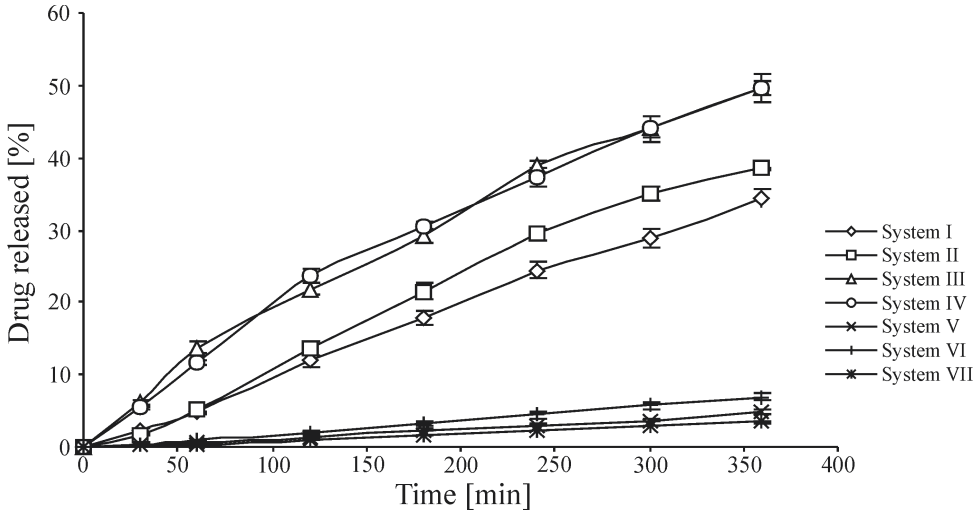


Figure 24. Amount of released drug as the function of time in case of different compositions.

In order to characterize the kinetics of drug liberation from different preparations, the results were plotted in a double logarithmic diagram as well (Figure 25).

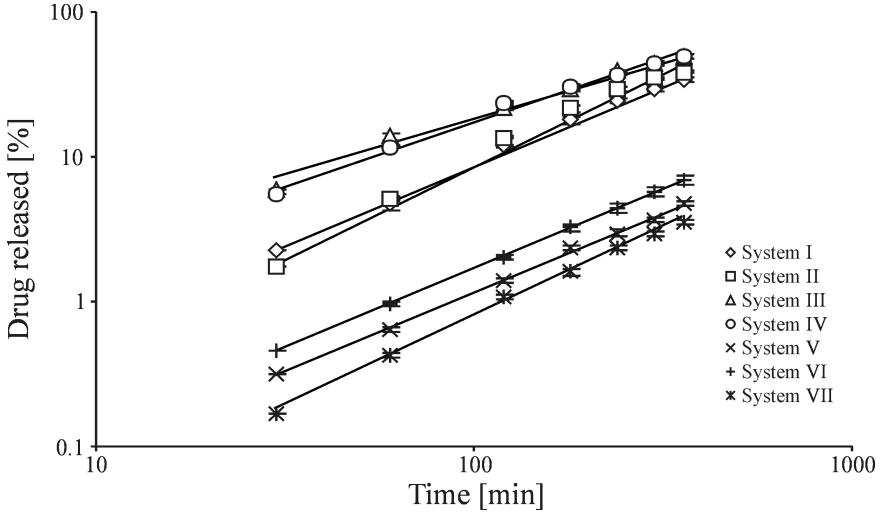


Figure 25. Amount of released drug as the function of time in case of different compositions plotted in a double logarithmic diagram.

Metronidazole benzoate release from the systems of different compositions can be described with good correlation by the following semi-empirical equation:

$$M_t/M_\infty = kt^n \qquad (4)$$

where M_t is the amount of released solute at time t, M_∞ is the released solute at infinite time t_∞, k is a constant characteristic of the system, n is an exponent characteristic of the mode of transport of the solute. The value of release exponent n is the slope, while value of k is the axial section of the straight lines obtained in the double logaritmical diagram. These kinetic parameters are summarized in Table VI. On the basis of these results, it may be seen that in the case of systems I-IV the release exponent for the formulations ranged from 0.8251 to 1.2593, indicating that drug release was non-diffusion controlled. This is not surprising when the behaviour of these formulations is considered in aqueous environment. Drug release was the result of two independent mechanisms, i.e., Fickian diffusion and water absorption. In the case of systems V-VI the release exponent is approximately 1.0, consequently the drug release rate is independent of time. This behaviour corresponds to zero-order release kinetics.

In the case of system VII non-Fickian type of drug release can be observed as a result of the interaction of Fickian diffusion and dynamic swelling [109-111].

Table VI. Composition of the invetigated system and kinetic parameters of drug release.

System	Vehicle	n	k	Corr. Coeff.
I	Cremophor EL:Miglyol 810= 4:1	1.1054	0.0592	0.9962
II	Cremophor EL:isopropyl myristate= 4:1	1.2593	0.0279	0.9867
III	Cremophor RH40:Miglyol 810= 4:1	0.8251	0.4078	0.9885
IV	Cremophor RH40:isopropyl myristate= 4:1	0.8778	0.3070	0.9883
V	Miglyol 810	1.093	0.0076	0.9986
VI	isporopyl myristate	1.0993	0.0107	0.9999
VII	hydroxyethylcellulose mucilage	1.2227	0.0028	0.9976

Considering Figure 24 it can be stated that despite the counter-flow caused by water absorption in the case of in situ gelling systems, metronidazole benzoate was released to an extent about one order of magnitude greater than from oily and aqueous suspensions. Compositions containing Cremophor EL showed 34.39 (SD=±1.21) and 38.46% (SD=±0.19) release, while from systems containing Cremophor RH40, 49.59 (SD=±3.98) and 49.68% (SD=±1.01) of the drug liberated depending on the oil used. It can be concluded that unlike the oils, the type of surfactant has significant effect ($p<0.05$) on drug liberation. I have tried to find connection between the composition of samples, the results of rheological examinations and drug release studies. The extent of drug release and the values of structural viscosity of the characteristically most viscous samples with 50 % water content are summarized in Table VII.

When the amount of the released drug was plotted according to structural viscosity, a linear relationship could be observed (Figure 26). Drug release was markedly influenced by the chemical entity of the surfactant applied, as they formed compositions with different rheological behaviour.

Table VII. The composition of in situ gelling systems and the results of the drug release studies and the values of structural viscosity.

System	Surfactant	Oil	Visvosity values [Pas]*	Drug released [%]
I	Cremophor EL	Miglyol 810	59.18	34.39
II	Cremophor EL	isopropyl myristate	48.82	38.46
III	Cremophor RH40	Miglyol 810	28.59	49.59
IV	Cremophor RH40	isopropyl myristate	26.87	49.68

*At D = 50 s-1 and 50% water content (Pas).

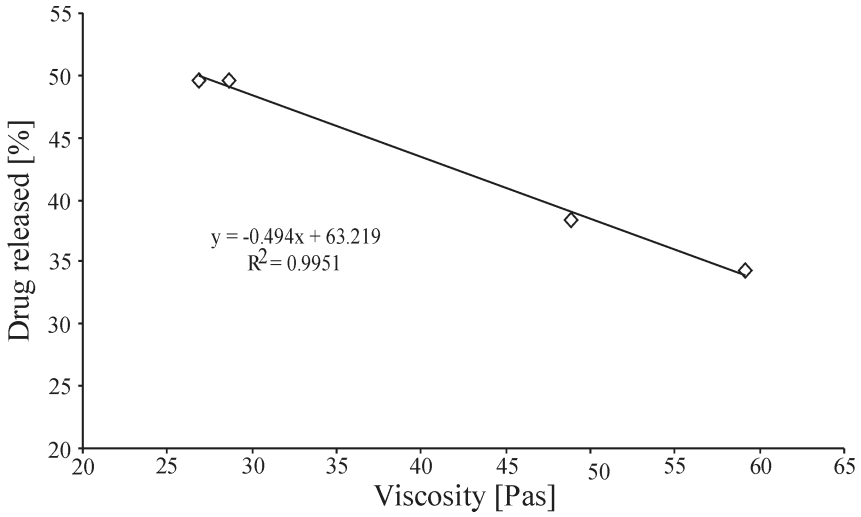


Figure 26. Relationship between the viscosity and the amount of released drug.

5.7 Modified Kirby-Bauer disk diffusion method

Different types of pathogens are associated with the periodontal disease but the microflora found in periodontitis is composed mainly of Gram negative anaerobic bacteria. Moreover, studies have shown that the various clinical forms of periodontitis are associated with different microbiota [44]. In the course of this modified disk diffusion experiment American Type Culture Cell line of *Fusobacterium varium* (ATCC 27725) was used. The genus *Fusobacterium* was created for small Gram negative rods that are spindle shaped. Fusobacterium species play important role in the development of different forms of

periodontal diseases, such as gingivitis, adult periodontitis, HIV-periodontitis and periimplantitis [112].

In order to get measurable inhibition zones, the suitable metronidazole benzoate concnetration of samples was determined in the first step. The active agent was incorporated in samples with medium (30 w/w%) water content in a concentration of 100, 200, 400, 600, 800 and 1000 μg/g, then the occuring inhibition zones were measured (Figure 27).

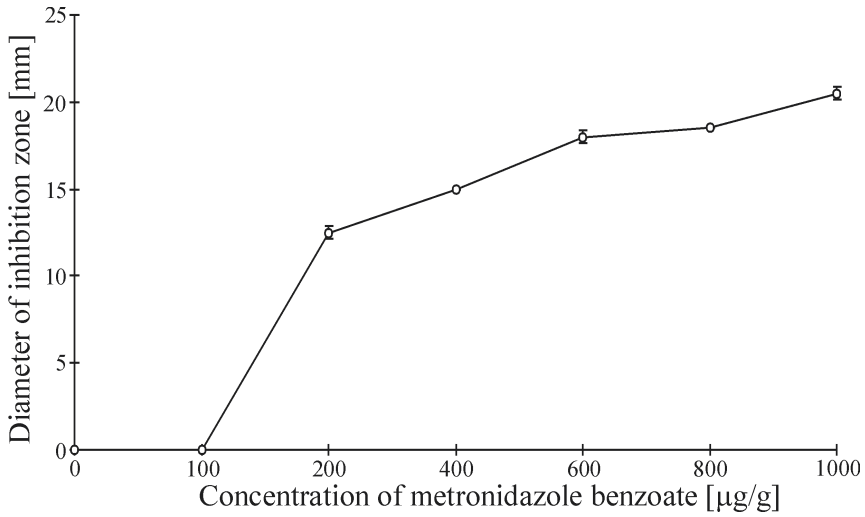


Figure 27. Alteration of the inhibition zones as a function of metronidazole benzoate concentration in case of sample I/4.

On the basis of these data 200 μg/g drug content was chosen, because it prognosticated measurable inhibition zones in case of all investigated samples. In Figure 28 inhibition zones after 72 hour of incubation at 37 ^{o}C and under anaerobic condition can be seen.

The dependence of the inhibition zone on the water concentration was also investigated. In Figure 29 it can be seen that linear relationship was found between the water concentration of samples and the diameters of the inhibition zones. This phenomenon is presumably associated with the water absorption, the degree of which varies with the initial water content of the investigated compositions. Furthermore the results are in good agreement with data obtained by the in vitro drug release studies: inhibition zones measured in case of Cremophor RH40 containing systems are significantly greater ($p<0.05$) than in inhibition zones observed in case of Cremophor EL containing samples.

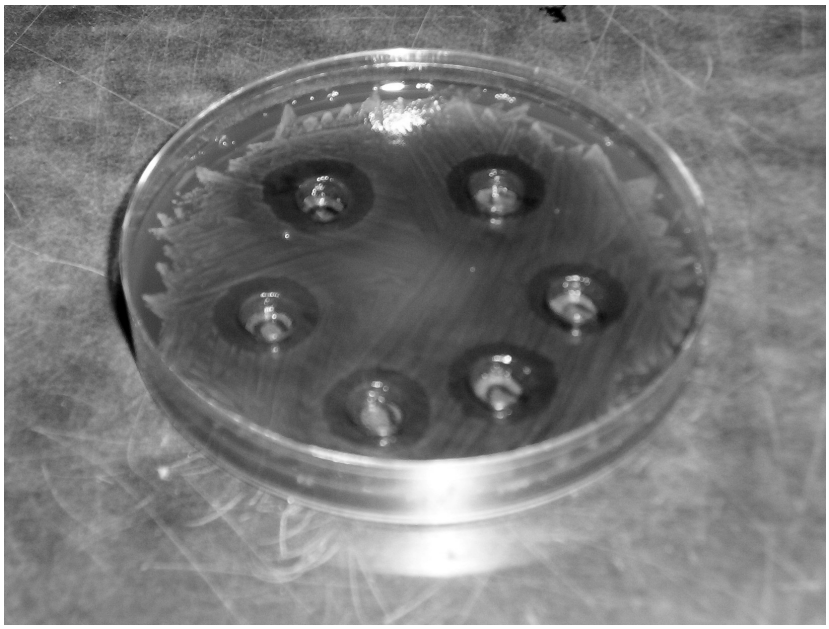


Figure 28. Inhibition zones developed after incubation under anaerobic condition at 37 ^{o}C for 72 hours.

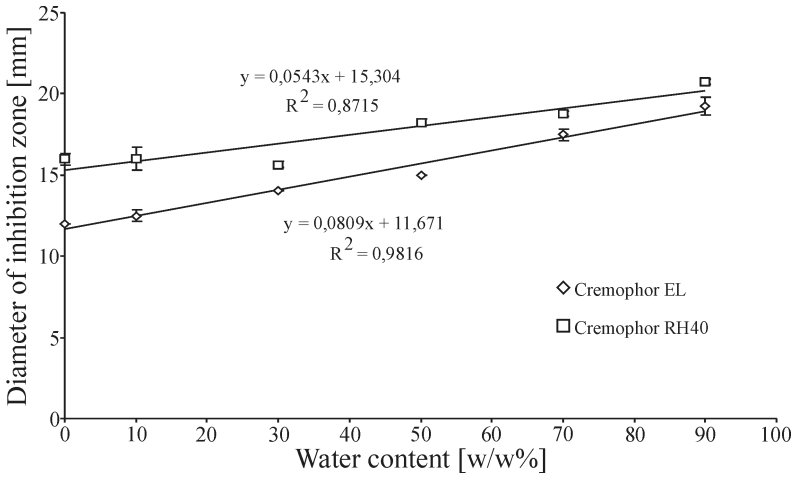


Figure 29. Alteration of the inhibition zones as a function of water content.

6 SUMMARY

The aim of this researh was the formulation and ivestigation of in situ gelling drug delivery system based on low-viscous isotropic phase which turns into high-viscous lyotropic liquid crystalline phase in aqueous environment. Summarizing my experimental work it can be concluded that:

- The 4:1 mixture of the investigated surfactants (Cremophor EL, Cremophor RH40) and oils (Miglyol 810, isopropyl myristate) – selected on the basis of preliminary experiments – forms lyotopic liquid crystalline phases upon water addition.
- On the basis water absorption investigations it can be seen that the amount of absorbed water and the speed of water absorpion was influenced by the chemical entity of the surfactant.
- Polarization microscopic examinations showed that samples with 10 % - 40 % water content possessed anisotropic properties. Below this conentration limit optically isotropic samples were described as reversed micellar solutions – and in case of water contaninig systems as w/o microemulsions. However, in case of Cremophor RH40 containing water-free samples the surfactant and the oil were inmiscible in each other thus they formed a biphasic mixture. On the addition of small amount of water these systems turned into w/o microemulsion as well. On the effect of further water addition lamellar, hexagonal and an optically isotropic gel phase and finally o/w microemulsion area were identified.
- Phase transformations were affirmed by rheological and thermoanalitical investigations as well. The alterations in the elastic – plastic properties associated with phase transformations were pointed out by oscillation measurements. The free and bound water fraction was examined by means of SZT-DSC measurements. The appearance of free water at about 30 w/w% water content was in good agreement with the lamellar-hexagonal phase transformation. After the saturation of the surfactant and below 80 % water concentration the amount of bound water forming the hydration layer around the surfactant molecules was practically independent of the total water content of samples. Above 80 % water content the decrease of the amount of the bound water related to the surfactant concentration can be explained by the fact that

these systems are situated at the border region of colloid systems and can be characterized by lower stability.

- Based on in vitro drug release and microbiological investigations it can be concluded that a considerable amount of the drug was liberated in the course of the examination and the chemical entity of the surfactant exerted major influence on drug release. Linear relationship was found between the viscosty values and the amount of released drug.

In conclusion, the investigated water-free systems characterized by low viscosity values and pharmaceutically acceptable components are promising vehicles for the formulation of injectable in situ gelling drug delivery systems.

7 REFERENCES

[1] A. N. Martin, Physical Pharmacy, Lea & Febiger, Philadelphia, (1962).

[2] X.-G. Wen, Ann. Phys. 316 (2005) 1.

[3] J. S. Lange, Nucl. Phys. A 718 (2003) 367.

[4] Z. Nazario, D. I. Santiago, Phys. Lett. A 328 (2004) 207.

[5] T. Z. Rizvi, J. Mol. Liq. 106/1 (2003) 43.

[6] H. Stegemeyer, Lyotrope Flüssigkristalle, Steinkopff Verlag, Darmstadt (1999).

[7] P. Davidson, J.-C. P. Gabriel, Curr. Opin. Colloid Interf. Sci. 9 (2005) 377.

[8] Z. Dogic, S. Fraden, Curr. Opin. Colloid Interf. Sci. Article in Press.

[9] J. M. Seddon, Curr. Opin. Colloid Interf. Sci. 8 (2004) 424.

[10] M. Makai, E. Csányi, Zs. Németh, J. Pálinkás, I. Erős, Int. J. Pharm. 256 (2003) 95.

[11] T. Suzuki, H. Takei, S. Yamazaki, J. Colloid Interf. Sci. 129 (1989) 491.

[12] G. Burducea, Romanian Reports in Physics 56 (2004) 66.

[13] K. L. Mittal, Micellization, Solubilization, and Microemulsions, Plenum Press, New York (1977).

[14] E. Wolfram, Proceedings of the International Conference on Colloid and Surface Science Volume 1, Publishing House of the Hungarian Academy of Sciences, Budapest (1975).

[15] T. Cosgrove, Colloid Science: theory, methods and applications, Blackwell Science Publ. Ltd, Ames (2005).

[16] W. M. Gelbart, A. Ben-Shaul, D. Roux, Micelles, Membranes, Microemulsions and Monolayers, Springer-Verlag Inc., New York (1994).

[17] B. Widom, P. Bhimalapuram, K. Koga, Phys. Chem. Chem. Phys. 5 (2003) 3085.

[18] P. C. Hiemenz, Principles of Colloid and Surface Chemistry, Marcel Dekker Inc., New York (1986).

[19] C. Tanford, The hydrophobic effect, John Wiley and Sons, New York (1973).

[20] J. B. F. N. Engberts, J.-J. H. Nusselder, Pure & Appl. Chem. 62 (1990) 47.

[21] P. S. Goyal, V. K. Aswal, Curr. Sci. 80 (2001) 972.

[22] D. J. Mitchell, B. W. J. Ninham, J. Chem. Soc. Faraday Trans. 77 (1981) 601.

[23] J. N. Israelachvili, Intermolecular and Surface Forces, Academic Press, London (1985).

[24] C. C. Müller-Goymann, Eur. J. Pharm. Biopharm. 58 (2004) 343.

[25] E. Farkas, R. Zelkó, Zs. Németh, J. Pálinkás, S. Marton, I. Rácz, Int. J. Pharm. 193 (2000) 239.

[26] J. H. Clint, Surfactant Aggregation, Blackie, London (1992).

[27] J. Charvolin, Liq. Crist. 13 (1993) 829.

[28] H. Binder, A. Anikin, G. Lantzsch, G. Klose, J. Phys. Chem. B 103 (1999) 461.

[29] M. Holmes, M. Leaver, A. Smith, Langmuir 11 (1995) 356.

[30] K. Berger, K. Hiltrop, Progr. Colloid Polym. Sci. 100 (1996) 9.

[31] K. Fontell, K. K. Kox, E. Hansson, Mol. Cryst Liquid Cryst. Letters 1 (1985) 9.

[32] K. Fontell, Coll. Polymer Sci. 268 (1990) 264.

[33] V. Luzatti, P. A. Spegt, Nature 215 (1976) 707.

[34] A. Tardieu, V Luzatti, Biochim. Biophys. Acta 11 (1970) 219.

[35] M. Scherlund, Thesis, Acta Universitatis Upsaliensis 237 (2000) 1.

[36] P. Chen-Chow, S. G. Frank, Int. J. Pharm. 8 (1981) 89.

[37] S. C. Miller, M. D. Donovan, Int. J. Pharm. 12 (1982) 147.

[38] S. Miyazaki, C. Yokouchi, T. Nakamura, N. Hashiguchi, W. M. Hou, M. Takada, Chem. Pharm. Bull. 34 (1986) 1801.

[39] A. Abd Elbary, N. Foda, S. Tayel, Pharm. Ind. 53 (1991) 1151.

[40] P. L. Wang, T. P. Johnston, Int. J. Pharm. 113 (1995) 73.

[41] K. Edsman, J. Carlfors, R. Petersson, Eur. J. Pharm. Sci. 6 (1998) 105.

[42] B. O. Haglund, R. Joshi, K. J. Himmelstein, J. Control. Release 41 (1996) 229.

[43] S. Kumar, B. O. Haglund, K. J. Himmelstein, J. Ocul. Pharmacol. 10 (1994) 47.

[44] K. Schwach-Abdellaoui, N. Vivien-Castioni, R. Gurny, Eur. J. Pharm. Biopharm. 50 (2000) 83.

[45] B.N.A. Vandekerckhove, M. Quirynen, D. VanSteenberghe, J. Periodontol. 68 (1997) 353.

[46] A. Hatefi, B. Amsden, J. Control. Release 80 (2002) 9.

[47] L. E. Hollister, Neurobiol. Aging. 10 (1989) 628.

[48] R. J. Levy, L. Vinod, S. A. Strickberger, T. Underwood, J. Davis, Drug Deliv. 3 (1996) 137.

[49] A. K. Dash, Z. Gong, D. W. Miller, H. Huai-Yan, J.-P. Laforet, Int. J. Pharm. 190 (1999) 21.

[50] B. Balakrishnan, A. Jayakrishnan, Biomaterials 26 (2005) 3941.

[51] K. S. Mallapragada, B. Narasimhan, Biomaterials 23 (2002) 4305.

[52] J. Carlfors, K. Edsman, R. Petersson, K. Jornving, Eur. J. Pharm. Sci. 6 (1998) 113.

[53] C. C. Mueller-Goymann, H.-J. Hamann, J. Control. Release 23 (1993) 165–174.

[54] S. Wahlgren, A. L. Lindstrom, S. Friberg, J. Pharm. Sci. 73 (1984) 1484.

[55] C. C. Mueller-Goymann, S. G. Frank, Int. J. Pharm. 29 (1986) 147.

[56] A. Schneeweis, C. C. Mueller-Goymann, Pharm. Res. 14 (1997) 1726.

[57] A. Schneeweis, I. Papantoniou, C. C. Mueller-Goymann, Pharm. Pharmacol. Lett. 7 (1997) 42.

[58] S. C. Sweetman, Martindale 33rd edition, Pharmaceutical Press, London, (2002).

[59] The United States Pharmacopeia 23rd edition, The United States Pharmacopeial Convention Inc., Rockwille, (1994).

[60] European Pharmacopoeia 4th edition, Directorate for the Quality of Medicines of the Council of Europe, Strasbourg (2002).

[61] J. E. F. Reynolds, Martindale 28th edition, Pharmaceutical Press, London, (1982)

[62] British Pharmacopoeia 1998, Stationary Office, London, (1998).

[63] U. Noyan, S. Yilmaz, B. Kuru, T. Kadir, O. Acar, E. Buget, J. Clin. Periodontol. 24 (1997) 158.

[64] L. Bata, Folyadékkristályok. Liquid crystals. Műszaki Könyvkiadó, Budapest, (1986).

[65] A. Saupe, J. Colloid Interf. Sci. 58 (1977) 549.

[66] C. Ho, R. Goetz, M. El-Asser, J. Vandrehoff, F. Fowkes, Langmuir 7 (1991) 56.

[67] K. Holmberg, Handbook of Applied Surface and Colloid Chemistry, John Wiley & Sons Ltd, New York, (2001).

[68] G. Persson, Thesis, Department of Chemistry Biophysical Chemistry Umeå University, (2003).

[69] S. Mackeben, M. Müller, C. C. Müller-Goymann, Colloid. Surface. A 183–185 (2001) 699.

[70] G. A. Kossena, W. N. Charman, B. J. Boyd, C. J.H. Porter, J. Control. Release 99 (2004) 217.

[71] A.-S. Sallam, E. Khalil, H. Ibrahim, I. Freij, Eur. J. Pharm. Biopharm. 53 (2002) 343.

[72] B. Demé, M. Dubois, T. Zemb, Biophys. J. 82 (2002) 215.

[73] M. Harms, S. Mackeben, C. C. Müller-Goymann, Colloid. Surface. A 259 (2005) 81.

[74] J. Hao, H. Hoffmann, Curr. Opin. Colloid Interf. Sci. 9 (2004) 279.

[75] V. M. Sadtler, M. Guely, P. Marchal, L. Choplin, J. Colloid Interf. Sci. 270 (2004) 270.

[76] D. Laba, Rheological Properties of Cosmetics and Toiletries, Marcel Dekker Inc., New York, (1993).

[77] A. Hasse, S. Keipert, Eur. J. Pharm. Biopharm. 43 (1997) 179.

[78] C. Rodriguez-Abreu, K. Aramaki, Y. Tanaka, M. A. Lopez-Quintela, M. Ishitobi, H. Kunieda, J. Colloid Interf. Sci. 291 (2005) 560.

[79] H. Gochman-Hecht, H. Bianco-Peled, J. Colloid Interf. Sci. 288 (2005) 230.

[80] L. Djordjevic, M. Primorac, M. Stupar, Int. J. Pharm. 296 (2005) 73.

[81] R. K. Mitra, B. K. Paul, J. Colloid Interf. Sci. 283 (2005) 565.

[82] P. Špiclin, M. Homara, A. Zupančič-Valant, M. Gašperlin, Int. J. Pharm. 256 (2003) 65.

[83] Th. F. Vandamme, Prog. Retin. Eye Res. 21 (2002) 15.

[84] F. E. Eirich, Rheology, Academic Press, New York, (1967)

[85] Gy. Mózes, E. Vámos, Reológia és Reometria, Műszaki Könyvkiadó, Budapest, (1968).

[86] R. Houwink, H. K. de Decker, Elasticity, plasticity and structure of matter, Univerity Press, Cambridge, (1971).

[87] G. M. Ravanagh, S.B. Ross-Murphy, Prog. Polym. Sci. 23 (1998) 533.

[88] G. Schramm, A Practical Approach to Rheology and Rheometry, Gebrueder HAAKE GmbH, Karlsruhe (1994).

[89] M. G. Bernia, C. J. Lawrence, D. Machin, Adv. Colloid Interfac. Sci. 98 (2002) 217.

[90] J. Flanagan, K. Kortegaard, D. N. Pinder, T. Rades, H. Singh, Food Hydrocolloid. 20 (2006) 253.

[91] K. Aramaki, Md. H. Kabir, N. Nakamurab, H. Kunieda, M. Ishitobi, Colloid. Surface. A 183–185 (2001) 371.

[92] C. Sinn, J. Non-Cryst. Solids 347 (2004) 11.

[93] C. Valenta, K. Schultz, J. Control. Release 95 (2004) 257.

[94] G. G. Chernik, Curr. Opin. Colloid Interf. Sci. 4 (2000) 381.

[95] M. Tansho, T. Imae, S. Tanaka, H. Ohki, R. Ikeda, S. Suzuki, Colloid. Surface. B 7 (1996) 281.

[96] M. Gradzielski, H. Hoffmann, J.-P. Panitz, A. Wokaun, J. Colloid Interf. Sci. 169 (1995) 103.

[97] P. Sherman, Industrial Rheology, Academic Press, New York, (1970).

[98] J. W. Goodwin, R. W. Hughes, Rheology for Chemists, Royal Society of Chemistry (2000).

[99] E. Csányi, Thesis, Department of Pharmaceutical Technology University of Szeged, (2004).

[100] Zs. Németh, L. Halász, J. Pálinkás, A. Bóta, T. Horányi, Colloid. Surface. A 145 (1998) 107.

[101] N. Garti, A. Aserin, I. Tiunova, M. Fanun, Colloid. Surface. A 170 (2000) 1.

[102] M. Fanun, E. Wachtel, B. Antalek, A. Aserin, N. Garti, Colloid. Surface A 180 (2001) 173.

[103] N. Garti, Thermal Behavior of Dispersed Systems, Marcel Dekker Inc., New York, (2000).

[104] P. Meisel, T. Kocher, J. Photoch. and Photobio. B 79 (2005) 159.

[105] B. L. Pihlstrom, B. S. Michalowicz, N. W. Johnson, Lancet 366 (2005) 1809.

[106] D. O. Klugh, Clinical Techniques in Equine Practice, 4 (2005) 135.

[107] S. Sridevi, B. R. Jayachandra, N. Mittal., D. S. Kumar, K. J. Pandit, Pharmazie, 50 (1995) 153.

[108] G. Greenstein, A. Polson, J. Periodontol. 69 (1998) 507.

[109] D. S. Jones, A. D. Woolfson, A. F. Brown, W. A. Coulter, C. McClelland, C. R. Irwin, J. Control. Release 67 (2000) 357.

[110] G. Bonacucina, M. Cespi, M. Misici-Falzi, G. F. Palmieri, Int. J. Pharm. 307 (2006) 129.

[111] C. R. Young, C. Dietzsch, M. Cerea, T. Farrell, K. A. Fegely, A. Rajabi-Siahboom, J. W. McGinity, Int. J. Pharm. 301 (2005) 112.

[112] J. Slots, M. A. Taubman, Contemporary Oral Microbiology and Immunology, Mosby-Year Book, St. Louis, (1991).

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ANNEX

I.

II.

III.