University of Szeged, Faculty of Pharmacy
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Summary of Ph.D. Thesis

Formulation and Investigation of in Situ Gelling Lyotropic Liquid Crystalline Systems

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INTRODUCTION

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. Factors inducing gelation include alterations in the pH, ionic strength, solvent concentration and temperature. In situ gelling systems possess several advantageous properties. In certain cases the systematically applied active agent cannot achieve the required concentration and/or retention time at the site of action. 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 the site of application where they undergo a sol-gel transformation by meeting physiological conditions. The increased viscosity ensures the desired retention time while the local release of the drug the adequate concentration. In this way site-specific action, 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.

Lyotropic liquid crystals are usually formed from water and one or two surfactants and possibly oils within a definite concentration and temperature range. Depending on the solvent concentration different liquid crystalline structures – called mesophases – occur. Figure 1 shows the arising liquid crystalline phases as the function of concentration and polarity of the 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.

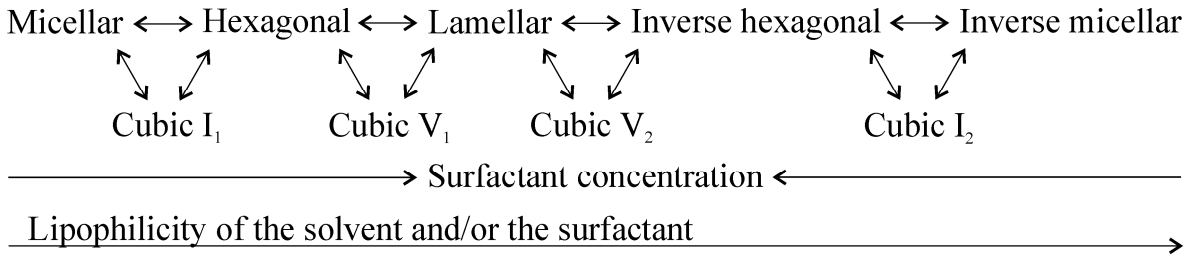

Figure 1. Transformations of lyotropic liquid crystalline mesophases.

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.

EXPERIMENTAL AIMS

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

- On the bases of preliminary experiments the 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 triangular phase diagrams;
- investigation of the 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.

MATERIALS AND METHODS

Materials

Metronidazole benzoate (Ph. Eur. 4) was chosen as the active agent and 3.5% of the drug was dissolved in the samples. The carrier was the 4:1 mixture of a non-ionic surfactant: Cremophor EL (polyoxyl 35 castor oil USP/NF) or Cremophor RH40 (polyoxyl 40

hydrogenated castor oil USP/NF) and neutral oil (Ph. Eur. 4) or isopropylmyristate (Ph. Eur. 4). The applied surfactants and oil and their ratio were determined on the basis of triangular phase diagrams.

Methods

Preparation of samples

Formulations were prepared with and 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.

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.

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.

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.

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 for 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.

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.

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.

RESULTS

Ternary phase diagrams of the ivestigated systems

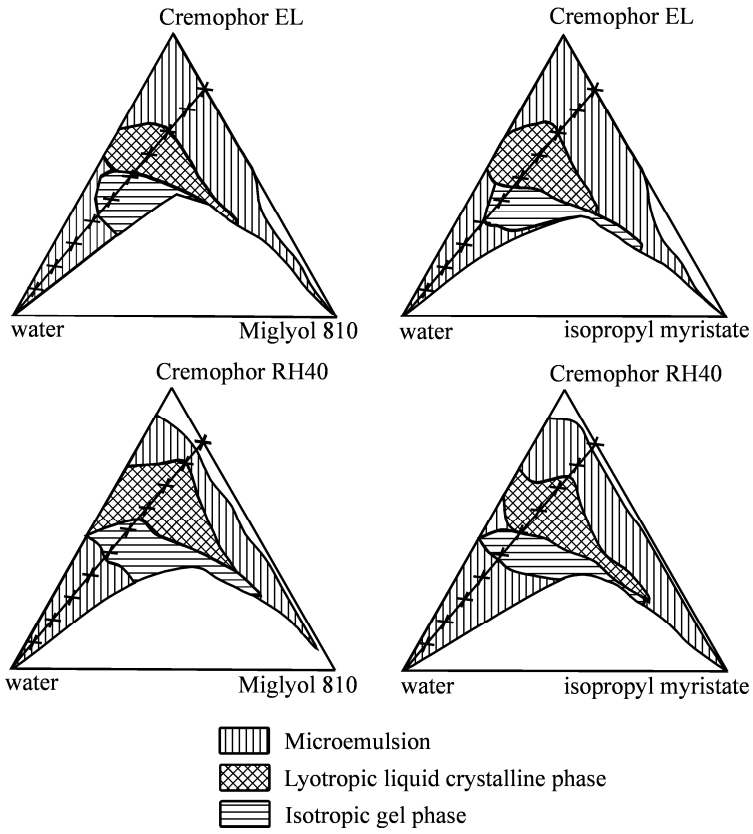

Figure 2. Ternary phase diagrams of the investigated systems.

On the basis of the preliminary experiments it was concluded that neutral oil and isopropyl myristate were the most suitable oils, in the case of applying Cremophor EL and Cremophor RH40 as surfactants. Figure 2 shows the phase diagrams of the studied ternary systems. 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 could be observed. 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 %

Water absorption of the water-free liquid crystal preconcentrates

The knowledge of the mechanism of the 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). On the basis of the results it can be concluded that the amount of absorbed water increased as the function of Cremophor EL content.

Polarization microscopy

In the course of polarization microscopic examinations samples with different water content were investigated, and their liquid crystalline textures were identified. On the grounds of the results it could 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 a small amount of water these systems turned into a 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 occurred: the lamellar phase turned into a hexagonal one. 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.

Rheological investigations

Results obtained from the rheological examinations were in good agreement with the data of polarization microscopy. The alteration in the flow behaviour on the effect of water addition was similar in case of all the four systems. 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. In case of samples with 10 % water content an ideal viscous flow behaviour was observed which characteristic is, among others, of real and micellar solutions and microemulsions. With increasing water concentration due to the appearance of coherent structure a non-Newtonian – thixotropic – flow behaviour occured. The values of thixotropic area showed maximum value as the function of water content, which refers to the appearrance of lamellar, hexagonal, isotropic gel and o/w microemulsion phase.

Besides the determination of flow curves, the viscoelastic properties of samples were also examined by means of oscillatory tests. Németh et al. reported an oscillatory rheological method for the identification of pharmaceutically important lamellar phases. In the case of 10 % water content the loss modulus (G”) value was higher than the storage modulus (G’) value, that is the system showed plastic behaviour. At 20 % water content the value of G’ exceeded that of G” with about one order of magnitude, and the G” curve showed a minimum. This behaviour is in good correlation with Németh’s observations for the lamellar phase. When the water concentration was 30 %, higher G’ values could be observed, which indicated the presence of the more elastic hexagonal structure.

Subzero temperature DSC measurements

In the course of SZT-DSC experiments samples with different water content were investigated. The sharp exothermic and the wider endothermic peak caused by freezing and melting of water were observed just above a definite water concentration. 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 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. A linear relationship was found 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). On the bases of the results the amount of free and bound water was calculated. Knowing the amount of bound water and the composition of samples, it was examined how the surfactant : bound water ratio varied with the increasing water content. On the basis of results 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 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 were situated at the border region of colloid systems and can be characterized by lower stability.

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. In order to study the applicability of my compositions for such purpose, metronidazole benzoate was dissolved in 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. 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 (systems V-VII). The drug release of these compositions was also examined. Metronidazole benzoate release from the systems of different compositions could be described with good correlation by the following semi-empirical equation:

$$M_t/M_\infty = kt^n \qquad (1)$$

The value of release exponent *n* gives useful information of the kinetic of drug release mechanism. 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 an 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 was approximately 1.0, consequently the drug release rate was independent of time. This behaviour corresponds to zero-order release kinetics. In the case of system VII non-Fickian type of drug release could be observed as a result of the interaction of Fickian diffusion and dynamic swelling.

Considering the results 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 was liberated depending on the oil used. It can be concluded that unlike the oils, the type of the surfactant has a 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. When the amount of the released drug was plotted according to structural viscosity, a linear relationship could be observed. Drug release was

markedly influenced by the chemical entity of the surfactant applied, as they formed compositions with different rheological behaviour.

Modified Kirby-Bauer disk diffusion method

In the course of modified disk diffusion experiment American Type Culture Cell line of *Fusobacterium varium* (ATCC 27725) was used. Fusobacterium species play an important role in the development of different forms of periodontal diseases, such as gingivitis, adult periodontitis, HIV-periodontitis and periimplantitis. The dependence of the inhibition zone on the water concentration was investigated. A linear relationship was found between the water concentration of samples and the diameters of the inhibition zones. This phenomenon was presumably associated with water absorption, the degree of which varies with the initial water content of the investigated compositions. Furthermore, the results were in good agreement with data obtained by the in vitro drug release studies: inhibition zones measured in case of Cremophor RH40 containing systems were significantly greater ($p<0.05$) than in inhibition zones observed in case of Cremophor EL containing samples.

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 – formed lyotopic liquid crystalline phases upon water addition.
- On the basis of 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. With increasing water content lamellar, hexagonal and an optically isotropic gel phase 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 could be explained by the fact that these systems were situated at the border region of colloid systems and could 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 a major influence on drug release. A 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.

ANNEX

Publications related to the Ph.D. thesis

Publications

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)