International Journal of Pharmaceutics 358 (2008) 23–26

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Rapid communication

Lyotropic liquid crystal preconcentrates for the treatment of periodontal disease

A. Fehér[a], E. Urbán[b], I. Erős[a], P. Szabó-Révész[a], E. Csányi[a,*]

[a] Department of Pharmaceutical Technology, University of Szeged, Eötvös Street 6, H-6720 Szeged, Hungary
[b] Institute of Clinical Microbiology, University of Szeged, Somogyi Square 1, H-6725 Szeged, Hungary

ARTICLE INFO

Article history:
Received 29 November 2007
Received in revised form 18 February 2008
Accepted 21 February 2008
Available online 29 February 2008

Keywords:
Periodontal disease
Lyotropic liquid crystals
Kirby–Bauer disk diffusion method
Drug release

ABSTRACT

The aim of our study was to develop water-free lyotropic liquid crystalline preconcentrates, which consist of oils and surfactants with good physiological tolerance and spontaneously form lyotropic liquid crystalline phase in aqueous environment. In this way these preconcentrates having low viscosity can be injected into the periodontal pocket, where they are transformed into highly viscous liquid crystalline phase, so that the preparation is prevented from flowing out of the pocket due to its great viscosity, while drug release is controlled by the liquid crystalline texture. In order to follow the structure alteration upon water absorption polarization microscopical and rheological examinations were performed. The water absorption mechanism of the samples was examined by the Enslin-method. Metronidazole-benzoate was used as active agent the release of which was characterized via in vitro investigations performed by means of modified Kirby–Bauer disk diffusion method. On the grounds of the results it can be stated that the 4:1 mixture of the investigated surfactants (Cremophor EL, Cremophor RH40) and oil (Miglyol 810) formed lyotopic liquid crystalline phases upon water addition. Polarization microscopic examinations showed that samples with 10–40% water content possessed anisotropic properties. On the basis of water absorption, rheological and drug release studies it can be concluded that the amount of absorbed water and stiffness of lyotropic structure influenced by the chemical entity of the surfactant exerted major effect on the drug release.

The term of periodontal disease describes certain pathological changes of the tissues surrounding the teeth. These pathological states can be characterized by the destruction of the periodontal connective and the alveolar bone tissue. The clinical symptoms of periodontitis include the morphological changes of the gingival tissue, gingival haemorrhage and the formation of periodontal pocket. This pocket creates ideal conditions for the colonisation and multiplication of pathogenic anaerobic bacteria. These bacteria are primarily responsible for the arising inflammation and for the destruction of tissues. Certain bacteria were shown to produce a great quantity of biologically active substances, which act directly on the surrounding tissues, thereby leading to their destruction. On the other hand, a large number of inflammatory and immune mediators also result in tissue damage. (Armitage, 2004; Pihlstrom et al., 2005; Tsai et al., 2005).

As pathogenic microorganisms play such a major role, either directly or indirectly, in the development and sustaining of periodontitis, it is evident to treat the patients with systemic or local antibiotics as a supplement to traditional mechanical therapy. However, systemic treatment has numerous side effects, the necessary great dose may cause hypersensitivity reactions and gastrointestinal intolerance, and it may also facilitate the development of bacterial resistance. Moreover, in spite of being administered in a great dose, the drug cannot provide the therapeutic concentration in the pocket for the necessary duration. These disadvantages can be decreased substantially with the local delivery of the antimicrobial material (Seymour and Heasman, 1995; Greenstein and Polson, 1998; Schwach-Abdellaoui et al., 2000). The local concentration of the drug can be increased if it is incorporated into a therapeutic system which can be injected directly into the site of application and the viscosity of which is increased drastically upon the effect of body temperature or water (saliva) (Wang and Johnston, 1995; Haglund et al., 1996; Edsman et al., 1998; Negishi et al., 1999; Scherlund, 2000; Chang et al., 2002; Kubo et al., 2003; Vernon et al., 2004; Bertram and Bodmeier, 2006).

The aim of our research was to develop preconcentrates consisting of oil and surfactant, which contain components with good physiological tolerance and spontaneously form lyotropic liquid crystalline phase of high viscosity in an aqueous medium. This mixture can be injected into the periodontal pocket, where it is

* Corresponding author. Tel.: +36 62 545 571; fax: +36 62 545 571.
E-mail address: csanyi@pharm.u-szeged.hu (E. Csányi).

0378-5173/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.ijpharm.2008.02.025

Table 1
Compositions of the studied systems

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

transformed into a liquid crystalline phase through water absorption, and the preparation is prevented from flowing out of the pocket by its great viscosity, while drug release is controlled by the liquid crystalline structure.

Metronidazole-benzoate (Ph. Eur. 5) was chosen as the active agent. 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 Miglyol 810 as oil phase. The composition of these samples is shown in Table 1.

The structure of the samples was examined with a polarization microscope (LEICA Q500 MC) at room temperature. The magnification was 40 fold.

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 water-free sample was placed on the G1 glass filter of the instrument filled with bubble-free water, and then the quantity of the absorbed water was measured as the function of time. The duration of the measurement was 2 h (List, 1985).

Rheological measurements were carried out with a Rheostress 1 Haake instrument. A cone-plate measuring device was used in which the cone angle was 1°. The flow and viscosity curves of the samples were determined by changing the shear rate between 0.01 and 100 s^{-1} (Schram, 2000).

The in vitro drug release was characterized via modified Kirby–Bauer disk diffusion method (Bauer et al., 1996; Peng et al., 2005). The tests were carried out using cultures of *Fusobacterium varium* ATCC 27725. In each petri-dish filled with blood agar 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 °C for 72 h. 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.

The obtained results can be summarized as follows. In the course of polarization microscopic examinations samples with different water content were investigated, and their liquid crystalline textures were identified. The phase diagrams of the investigated systems can be seen in Fig. 1. According to the results, samples with water content of 10–40% showed anisotropic behavior. Below this concentration 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 immiscible 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 10–20% (w/w) – samples possessed lamellar structures. Above this water concentration a phase transformation occurred: the lamellar phase turned into hexagonal one. In Fig. 2 polarizations micrograph of samples II/3 and II/4 can be seen with 20 and 30% water content, respectively. In case of 20% water content Maltese crosses – typical pattern of lamellar 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 (Fig. 3).

The water absorption of water-free compositions was investigated using different surfactants (system I/1 and II/1). Fig. 3 shows the amount of absorbed water when Cremophor EL and Cremophor RH40 were applied as surfactants and Miglyol 810 as oily component. It can be seen that the system I/1, which has Cremophor

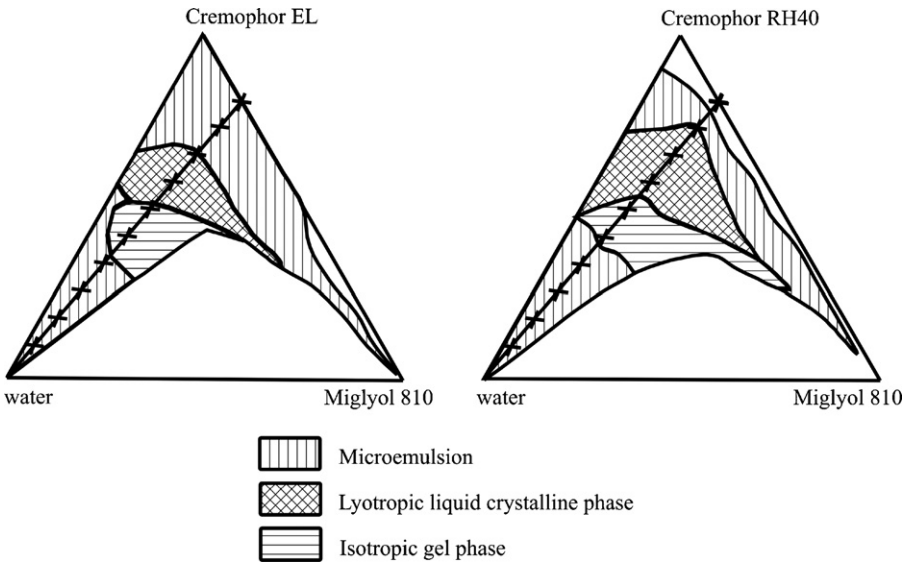

Fig. 1. The phase diagrams of the investigated systems.

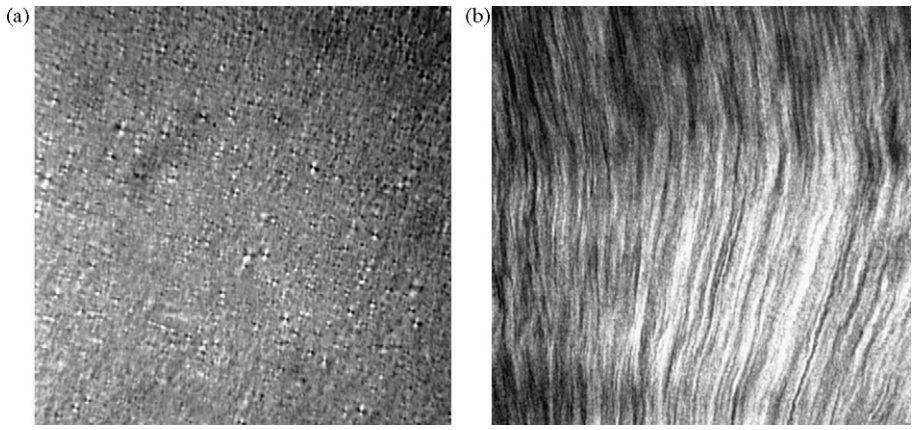

Fig. 2. Polarizations micrograph of sample II/3 (a) with 20% water content, and II/4 (b) with 30% water content at magnification of 40×.

Table 2
Kinetic parameters of the water absorption

Sample	Surfactant	Equation of the straight line	R^2
I/1	Cremophor EL	$y = 0.0739x - 0.0771$	0.9993
II/1	Cremophor RH40	$y = 0.057x - 0.0779$	0.9994

EL as surfactant absorbed significantly more water (0.725 ml/g) during the 2 h of examination than the system II/1 (0.555 ml/g), which contains Cremophor RH40. Plotting the amount of absorbed water as the function of square root of time resulted straight lines (Table 2). On the bases of the equations the time, which is needed to reach the given water content was calculated (in case of 50% water content by extrapolation). In the same graph the viscosity value of the samples with corresponding water content measured at $\dot{\gamma} = 90\,s^{-1}$ was plotted as well. In this way the viscosity change as the function of time can be evaluated. On the bases of the figure it can be concluded that system with Cremophor EL absorbed more water, in this way less time was needed to reach the same water content than in case of Cremophor RH40 containing system. Furthermore Cremophor EL formed more rigid system with considerably higher viscosity values (Fig. 3). The water absorption and the stiffness of the structure are presumably important factors in point of view of drug release. The results of drug release studies supported this presumption. In Fig. 4 the diameter of the inhibition zone as the function of water content can be seen. It can be observed that application of Cremophor RH40 as surfactant resulted higher inhibition zones, which can be explained by the lower rigidity of this system. However, considering the effect of water content on the diameter of inhibition zones in case of both systems, it can be concluded, that the higher water content coinciding to less water absorption led to the formation of wider inhibition zones. On the grounds of these results it can be concluded, that beside the rheological features describing the lyotropic structure,

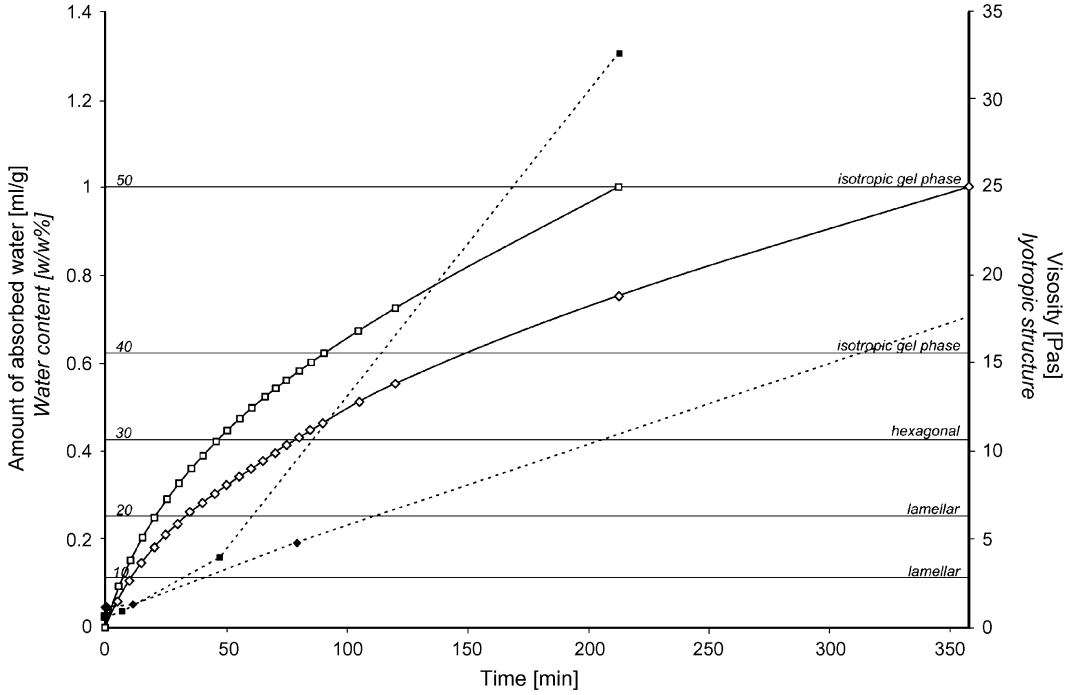

Fig. 3. Amount of absorbed water and alteration of lyotropic structure and viscosity as the function of time. (□), water absorption of system I/1; (◊) water absorption of system II/1; (■) viscosity of system I; (♦) viscosity of system II.

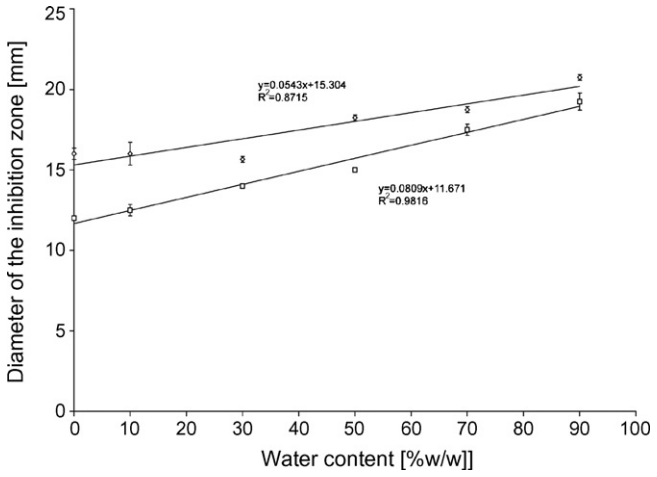

Fig. 4. Diameter of the inhibition zone as the function of water content. (□), system I/1; (◊), system II/1.

the water absorption capacity has an effect on the drug release as well.

In conclusion, it can be stated that the 4:1 mixture of the investigated surfactants (Cremophor EL, Cremophor RH40) and oil (Miglyol 810) formed lyotopic liquid crystalline phases upon water addition. Polarization microscopic examinations showed that samples with 10–40% water content possessed anisotropic properties. On the addition of water lamellar, hexagonal and an optically isotropic gel phase were identified. In order to characterize the structure of the optically isotropic gel phase more precisely and to obtain results about the interaction of the drug and the lyotropic structure we are planning to perform X-ray diffraction examinations in the future. On the basis of water absorption, rheological and drug release studies it can be concluded that the amount of absorbed water and stiffness of lyotropic structure influenced by the chemical entity of the surfactant exerted major effect on the drug release.

References

Armitage, G.C., 2004. Periodontal diagnoses and classification of periodontal diseases. Periodontol. 2000 34, 9–21.
Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., 1996. Antibiotic susceptibility testing by a standardized single disk method. Tech. Bull. Regist. Med. Technol. 36, 49–52.
Bertram, U., Bodmeier, R., 2006. Parameters affecting the drug release from in situ gelling nasal inserts. Eur. J. Pharm. Biopharm. 63, 310–319.
Chang, J.Y., Oh, Y.K., Choi, H.G., Kim, Y.B., Kim, C.K., 2002. Rheological evaluation of thermosensitive and mucoadhesive vaginal gels in physiological conditions. Int. J. Pharm. 241, 155–163.
Edsman, K., Carlfors, J., Petersson, R., 1998. Rheological evaluation of poloxamer as an in situ gel for ophthalmic use. Eur. J. Pharm. Sci. 6, 105–112.
Greenstein, G., Polson, A., 1998. The role of local drug delivery in the management of periodontal diseases: a comprehensive review. J. Periodontol. 69, 507–520.
Haglund, B.O., Joshi, R., Himmelstein, K.J., 1996. An in situ gelling system for parenteral delivery. J. Control. Release 41, 229–235.
Kubo, W., Miyazaki, S., Attwood, D., 2003. Oral sustained delivery of paracetamol from in situ-gelling gellan and sodium alginate formulations. Int. J. Pharm. 258, 55–64.
List, P.H., 1985. Arzneiformenlehre. Ein Lehrbuch für Pharmazeuten. 4 Auflage. Wissenschaftlicher Verlag GmbH, Stuttgart.
Negishi, M., Hiroki, A., Horikoshi, Y., Miyajima, M., Asano, M., Katakai, R., Yoshida, M., 1999. Swelling and ketoprofen release characteristics of thermo- and pH-responsive copolymer gels. Drug Dev. Ind. Pharm. 4, 437–444.
Peng, Y., Han, B., Liu, W., Xu, X., 2005. Preparation and antimicrobial activity of hydoxypropyl chitosan. Carbohydr. Res. 340, 1846–1851.
Pihlstrom, B.L., Michalowicz, B.S., Johnson, N.W., 2005. Periodontal diseases. Lancet 366, 1809–1820.
Scherlund, M., 2000. In situ gelling drug delivery systems for periodontal anaesthesia. Ph.D. Thesis, Uppsala University, Sweden.
Schram, G., 2000. A Practical Approach to Rheology and Rheometry, second ed. Gebrueder HAAKE GmbH, Karlsruhe.
Schwach-Abdellaoui, K., Vivien-Castioni, N., Gurny, R., 2000. Local delivery of antimicrobial agents for the treatment of periodontal diseases. Eur. J. Pharm. Biopharm. 50, 83–99.
Seymour, R.A., Heasman, P.A., 1995. Pharmacological control of periodontal disease II. Antimicrobial agents. J. Dent. 23, 5–14.
Tsai, I.S., Tsai, C.C., Ho, Y.P., Ho, K.Y., Wu, Y.M., Hung, C.C., 2005. Interleukin-12 and interleukin-16 in periodontal disease. Cytokine 31, 34–40.
Vernon, B.L., Fusaro, F., Borden, B., Roy, K.H., 2004. Partition-controlled progesterone release from waterborne, in situ-gelling materials. Int. J. Pharm. 274, 191–200.
Wang, P.L., Johnston, T.P., 1995. Sustained-release interleukin-2 following intramuscular injection in rats. Int. J. Pharm. 113, 73–81.