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Note

Small particles of a heparin/chitosan complex prepared from a pharmaceutically acceptable microemulsion

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Abstract

A water-in-oil microemulsion based on ingredients acceptable for oral administration of substances to human was investigated. The microemulsion was studied with and without biologically active ingredients by dynamic light scattering, turbidity, diffusion-NMR, and conductivity. Also, a technology based on mixing of appropriate microemulsions was investigated as a means to produce particles of nanometer size of a heparin/chitosan complex.

It was found that the microemulsion existed up to about 15% (w/w) of water with or without active ingredients. Formation of nanosized heparin/chitosan particles inside the water droplets was confirmed. It was concluded that the investigated microemulsion could be an interesting system for the oral administration of a heparin/chitosan complex.

Keywords: Microemulsions; Heparin; Chitosan; Pharmaceutical; Drug delivery

Conventional drug therapy has relied on the use of small molecules based on reversibility at the site of action. However, biotechnology has provided, and will do so even more in the future, new types of medical agents like peptides, proteins and DNA for different types of new therapies, e.g. as vaccines and in gene therapy. Such substances call for new types of delivery systems, e.g. for inhalation, for injections, and perhaps also for oral administration.

One type of delivery makes use of the drug in the form of small particles, either as such, or in combination with carrier substances. Popular methods have been based on the use of colloidal systems such as emulsions and liposomes (Couvreur et al., 1996; Sjöström et al., 1993), to produce small particles. In this work we present preliminary studies of an interesting water-in-oil microemulsion (Gattefossé Formulation Guide) due to its composition of ingredients that are pharmaceutically acceptable for oral delivery of water soluble substances. Also, the microemulsion was utilized to form a complex between heparin and chitosan. The surfactants (S) used were a mixture of 85.4% (w/w) Transcutol®P (purified (>99.7%) di(ethylene glycol)monoethyl ether) and 14.6% (w/w) Labrafil®M 1944 CS (an oleoyl macrogol-6 glyceride) and the oil used was Plurol®Oleique (a polyglyceryl-6 dioleate). The microemulsion ingredients were obtained as gifts from BioNord AB, Kungälv, Sweden (a Gattefossé agent). The use of heparin in an oral

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hygiene composition has been described before and it has been shown that the molecule can complex with oppositely charged macromolecules, e.g. chitosan, to give formulations suitable for treatment of periodontitis (Larm, 1995/30403). In other reports the use of heparin in the treatment or prophylax of infections caused by or associated with the bacterium *Helicobacter pylori* is described (Larm and Wadström, 1994/16714). A microemulsion-based formulation could be an interesting alternative in such applications.

A phase diagram for ternary system oil–water-surfactant without active ingredients was constructed by visual inspection (Fig. 1), knowing that microemulsions are transparent and clear (Jönsson et al., 1998). The phase diagram was then used as guidance to produce microemulsions containing heparin and chitosan (added together with the water). Small particles of heparin and chitosan were prepared by the route described by Holmberg (1994). Thus, two water-in-oil microemulsions, one containing heparin and one containing chitosan in the water pools, were mixed under vortexing, and the mixture obtained was characterized by different techniques. Visual inspection of the systems was used to monitor the appearance of turbidity. Dynamic light scattering (Brown, 1993) experiments were carried out at an observation angle of 90° with vertical polarization of the analyzing polarizer using a Brookhaven BI-200SM goniometer equipped with a Lexel 95, 2 W argon-ion laser, and with 25 ns as the shortest delay time of the correlator. The sample temperature was 25 °C. Self-diffusion of water in the microemulsions were followed by the pulsed gradient spin echo NMR technique (Stilbs, 1987; Callahan and Aust, 1984) on a Bruker DMX 200 NMR spectrometer by recording the ^{1}H water signals after addition of $^{2}H_2O$ to the samples.

The findings from the visual inspections were supported by the conductivity and turbidity results

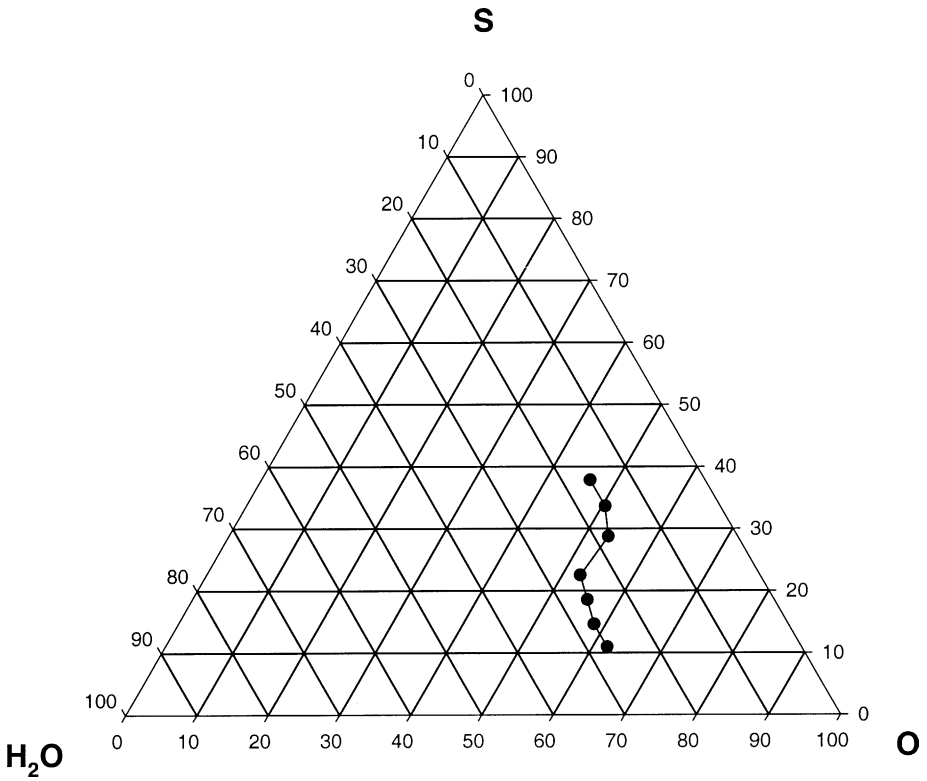

Fig. 1. Phase diagram of the of the "empty" microemulsion showing the phase boundary for where it exist. The microemulsion consisted of S (85.4%, w/w Transcutol P and 14.6%, w/w), O (Plurol Oleique) and water.

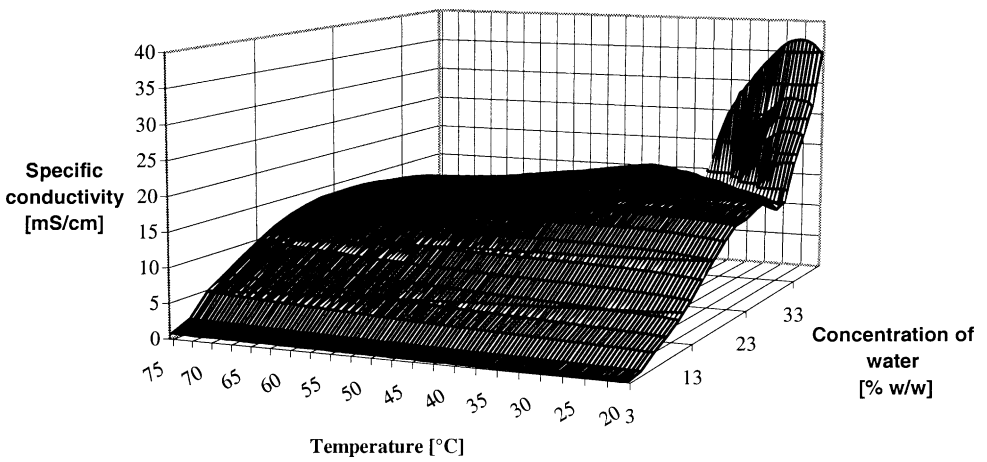

Fig. 2. Conductivity of the "empty" microemulsion as a function of temperature and added water.

presented in Figs. 2 and 3, respectively, as obtained with a Scanalys instrument (Sun and Sköld, 2001). It is seen that both the conductivity (Fig. 2) and the turbidity (Fig. 3) increased when the water concentration was increased. The changes were smooth up to a concentration of 25–30% water, where abrupt changes in conductivity and turbidity indicated phase changes (Joubran et al., 1993; Meier, 1995; Liu et al., 1999; Komives et al., 1994; Binks, 1993; Usacheva et al., 1997). It can also be seen that the systems were quite insensitive to changes in temperature. The results of the measurements were in accordance with the expected structure of small water droplets in an oil-continuous medium for water contents below 25–30%.

Sodium heparin (molecular weight 20 kD, gift from Astra Tech, Sweden) and chitosan (degree of deacetylation approximately 70%, molecular weight 200 kD, gift from Pronova Biopolymers, Norway) could be incorporated in amounts of slightly more than 1%

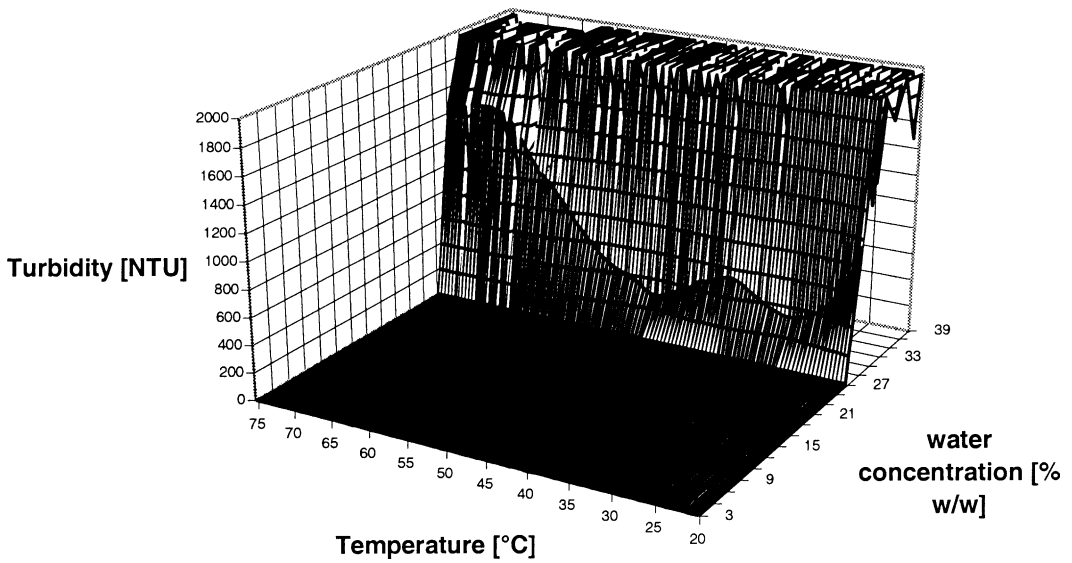

Fig. 3. Turbidity of the "empty" microemulsion as a function of temperature and water concentration.

(w/w) in the water component, which amounts to about 16% (w/w) of the microemulsion. Also, a mixture of the two microemulsions was clear and transparent, indicating that the heparin/chitosan complex that formed on mixing the two microemulsions gave particles in the nanometer range. It is known that surfactant-stabilized suspensions of nanoparticles are stable against sedimentation and clear to the eye (Petit et al., 1993). In the mixing experiments described below the chitosan concentration was kept at 0.05% (w/w) due to a slightly increased viscosity at higher concentrations.

Results from dynamic light scattering measurements gave a mean diameter of 1.7 nm (standard deviation was 0) for the water droplets without any added salt and 2.2 nm (standard deviation was 1.1) for the droplets containing heparin/chitosan complex as reported by the CONTIN analysis (Brown, 1993). In the analysis the values of the water viscosity and the refractive index were utilized. The analysis was carried out to give volume weighted diameters. This procedure emphasizes smaller diameters if these are dominating the systems (Brown, 1993). The results from the diffusion-NMR measurements were in the accordance with the dynamic light scattering results. The diffusion coefficients obtained are presented in Table 1 for the empty system, the microemulsion with chitosan, the microemulsion with heparin, and the mixture containing the heparin/chitosan complex. As can be seen, there was no essential difference between the diffusion coefficients of water in the different systems indicating that the internal structure is the same. The diffusion coefficients were in the range expected for water molecules in typical water-in-oil microemulsions (Jönsson et al., 1998).

Table 1
Diffusion coefficients for empty, chitosan, heparin and heparin/chitosan containing microemulsions gathered from the diffusion-NMR experiments

	Diffusion coefficients ($m^2\ s^{-1}$)
Empty	5.8×10^{-11}
Chitosan	5.6×10^{-11}
Heparin	5.7×10^{-11}
Heparin and chitosan	5.8×10^{-11}

The results obtained show that a pharmaceutically acceptable water-in-oil microemulsion can be used for the formation of heparin/chitosan nanoparticles.

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