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Formulation, physicochemical characterization and stability study of lithium-loaded microemulsion system

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ABSTRACT

Lithium biocompatible microemulsion based on Peceol®, lecithin, ethanol and water was studied in attempt to identify the optimal compositions in term of drug content, physicochemical properties and stability. Lithium solubilization in microemulsion was found to be compatible with a drug-surfactant binding model. Lithium ions were predominantly solubilized within lecithin head group altering significantly the interfacial properties of the system. Pseudo-ternary phase diagrams of drug free and drug loaded microemulsions were built at constant ethanol/lecithin weight ratio (40/60). Lithium loaded microemulsion has totally disappeared in the Peceol® rich part of phase diagram; critical fractions of lecithin and ethanol were required for the formation of stable microemulsion. The effect of lithium concentration on the properties and physical stability of microemulsions were studied using microscopy, Karl Fischer titrations, rheology analyses, conductivity measurements and centrifugation tests. The investigated microemulsions were found to be stable under accelerated storage conditions. The systems exhibited low viscosity and behaved as Newtonian fluid and no structural transition was shown.

1. Introduction

Lithium is a metallic element discovered in 1818 and introduced into medical practice in 1850 for the treatment of gout and then for other uses as stimulant and sedative (Gershon et al., 1973; Ehrlich and Diamond, 1980). Currently, lithium is used as a mood stabilizer and it was the first drug approved by the FDA (Food and Drug Administration) in 1974 in the treatment of bipolar disorder (Sachs et al., 2003; Schou, 1999). It has antimanic and antidepressant action in acute and prophylactic treatment (Marmol, 2008; Schou, 2001).

In recent years, there has been a renewed interest for lithium following the discovery of a neuroprotective effect (Chuang et al., 2002; Chen and Chuang, 1999; Hashimoto et al., 2002; Nonaka and Chuang, 1998; Chiu et al., 2013; Rowe and Chuang, 2004). These results pave the way for the use of lithium as therapeutic agent in a number of neurodegenerative diseases such as Huntington's disease (HD) (Wada, 2009), Alzheimer (Diniz et al., 2013), amyotrophic lateral sclerosis or Parkinson's disease (Chiu and Chuang, 2010). However, many side effects such as: urinary disorders, renal dysfunction, hypotension, hand tremors, muscle weakness, blurred thyroid, etc remain an obstacle to its widespread clinical use (Oakley et al., 2001; Hestbech et al., 1977; McKnight et al., 2012; Sheard, 1975; Christodoulou et al., 1977; Vestergaard et al., 1980; Johnson and Mannisto, 1980; Kane et al., 1978). Indeed, lithium has a narrow therapeutic window (lithium efficacy appears at blood concentrations between 0.5 and 1.5 mEq/L, while the first toxicological effect occurs above 1.5 mEq/L, increasing in severity with lithium blood concentration) requiring regular monitoring of patients with blood test. The development of lithium in its current dosage form for the long term is therefore impossible for the indication of neuroprotection (Kane et al., 1978; Sheean, 1991).

Amongst the diverse routes of drug delivery, oral transmucosal route has acquired considerable attention in recent years because of it distinct advantages for systemic drug delivery such as increased absorption, faster onset of actions and reduced intensity of local or systemic side effects (Abhang et al., 2014; Zhang et al., 2002; Hearnden et al., 2012). Indeed, oral mucosa is well supplied with vascular and lymphatic drainage and first-pass metabolism in the liver and presystemic elimination in the gastro intestinal tract are avoided (Shojaei, 1998).

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New low-dose lithium microemulsion based on Peceol®, lecithin and ethanol administrated by transmucosal route (Maurel, 2013a, 2013b) was reported to improve motor and neuropathological phenotypes in the YAC128 mouse model of HD, without toxic side effects observed with conventional therapeutic doses (Pouladi et al., 2012). The dose of lithium used in this study resulted in a blood concentration that is 500-fold lower (blood concentration below 0.002 mEq/L in mice) compared to the therapeutic blood levels already observed (around 1.0 mEq/L) (Moscovich, 1993). These positive findings support development of lithium microemulsion as agent therapeutic in HD for human clinical trials and open the way for tests on other hydrophilic drugs.

The water solubilization capacity of the four-component microemulsion containing Peceol®, lecithin, ethanol and water has been investigated in detail in our previous publications (Mouri et al., 2014a, 2014b). The microstructure of the low viscous microemulsion studied using Small Angle X-ray Scattering (SAXS) and electronic microscopy consists on nano-sized polar domains (stacks of small smooth lamellae) randomly oriented in apolar medium. Obviously, the incorporation of drug in microemulsion can change its microstructure and may even influence the stability of the system. The detailed information available on drugs must be supplemented with more information on the interaction between the different components and structural entities. The characteristic properties of drug loaded colloidal systems are mostly dictated by the interactions between its components.

The aims of the current study were to investigate lithium microemulsion properties in a wide composition range, to optimize the system in order to adapt lithium dose for clinical trials and finally to evaluate the physical stability of the system. Different techniques were used to characterize the drug loaded microemulsions: optical microscopy, Karl Fischer titration, Bowcott and Schulman titration, rheology and conductivity.

2. Materials and methods

2.1. Materials

Glycerol monooleate 40 (Peceol®) that consists of 45.3 wt% of monoglycerides, 44.5 wt% of diglycerides and 8.6 wt% of triglycerides (acid value 1.2, water 0.05 wt% and free glycerol 0.5 wt%) was supplied by Gattefossé (France). The fatty acid composition of Peceol® was oleic acid 81 wt%, linoleic acid 12 wt% and 7 wt% of saturated fatty acid. Soybean lecithin (Epikuron® 200) containing 94.5 wt% of phosphatidylcholine was purchased from Cargill, France. Anhydrous ethanol was purchased from Carlo Erba, France and high purity water, from SDS. Phosphate buffer solution (1.0 M) and NaOH were purchased from Sigma Aldrich and lithium citrate tetrahydrate (purity 99,4 wt%) from Chemcon. All chemicals were used as received, without further purification.

2.2. Phase diagrams

The Peceol®/lecithin/ethanol/water system was explored by the construction of pseudo-ternary phase diagrams at constant weight ratio of ethanol to lecithin (40/60) in the presence of the hydrophilic drug lithium citrate. To prepare samples, Peceol® was stirred gently at 37 °C in a mixture of lecithin and ethanol (prepared separately by weighing the surfactant and alcohol into a glass vial containing a magnetic stirrer until a transparent and homogenous yellow solution was obtained). To avoid evaporation of ethanol during stirring, all glass vials used were tightly stopped with a screw-cap. After cooling down of the oily phases to about 25 °C, the lipid mixtures were diluted with the aqueous phase (either pure water or lithium citrate solution). The single-phase microemulsion boundary was pre-determined by visual observation with the appearance of turbidity and phase separation.

It should be noted that no buffer solution was used and pH value of the lithium citrate solution was around 10.

2.3. Polarizing optical microscopy

Samples were analyzed under a polarized light microscope (Axiolab, Zeiss) at 10× magnification. A drop of each sample was placed between a coverslip and a glass slide, and then examined at ambient temperature (25 °C) under cross-polarized light.

2.4. Karl fischer titration

The water fraction in the isotropic liquid was determined by Karl Fischer titration (Karl Fischer Mettler Toledo). The HYDRANAL® Composit 5 KF reagent and Methanol as alcohol solvent were used. The sample was introduced directly into the measuring cell, and the amount of water in the test sample was calculated according to the volume of consumed reagent. To minimize experimental error, all samples were measured three times.

2.5. Rheology

Rheology measurements were performed using an AR 2000 EX Rheometer (TA instruments). Cone plates with 4 cm and 6 cm in diameter and an angle of 2° and 1.1°, respectively, were used. Temperature was maintained at 25 ± 0.1 °C. Shear rate measurements were performed between 0.01 and 1000 s^{-1}. A sample volume of 1 ml was used.

2.6. Conductivity

The conductivity meter Crison model used is the MultiMeter MM41 and conductivity measurements were performed at room temperature (25 ± 1 °C). All measurements were autocorrected for the temperature variation. The conductivity cell 5071 used was a platinium cell for very low conductivities measurements. The measuring range was 0.05 μS/cm to 30 mS/cm and the constant of cell was 0.1 cm^{-1}. All measurements were repeated three times to ensure repeatability and accuracy of the results. Microemulsions without drug incorporation were prepared with a 0.01 M phosphate buffer solution at pH 7.4 instead of water in order to increase the conductance of the aqueous phase. It was verified that the addition of this small amount of electrolyte to water had no impact on the microemulsion phase boundary before performing conductivity measurements.

2.7. Centrifugation

The stability of the microemulsions in the presence of lithium citrate as hydrophilic drug was checked using centrifugation. Centrifugation was carried out at 8000 rpm for 15, 30 and 45 min using Eppendorf 5804R centrifuge. If the system is not stable, the high effective gravitational force on a test tube caused a phase separation with precipitation of water in the bottom of the tube.

2.8. Potentiometric titration

Aliquots of stock lecithin, stored under nitrogen at −20 °C, were solubilized with ethanol in order to give three concentrations: 0.1, 0.4 and 20 mM/L. The acid-base titrations were performed by adding amounts of 0.1 M HC1 or NaOH to 25 ml of stirred and thermostated suspensions of lecithin the bulk pH being monitored