EFFECTS OF GUEST MOLECULES ON GMO-BASED LYOTROPIC LIQUID CRYSTALS

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Lipid-based lyotropic liquid crystals, such as lamellar (LP) and cubic (CP) phases made up of glyceryl monooleate (GMO) and water, are well known for their potential as sustained drug release matrices, thanks to highly ordered and thermodynamically stable internal nanostructure [1]. The amphiphilic nature of these systems allows to encapsulate drugs characterized by different physical-chemical features [2]. In this study fluorescein disodium salt, methylene blue, and nile red (0.5 mg/g) were used as hydrophilic, amphiphilic, and lipophilic drug prototypes to evaluate their effects on physic characteristics of LP and CP. In detail, we prepared LP by using specific ratio of GMO: PBS pH 7.4 (80:20 w/w), and subsequently LP was transformed into CP, adding an excess of hydration medium. Kinexus Rotational Rheometer was used to perform frequency sweep test under constant shear stress and complex viscosity measurements of LP and CP. Additionally, CP were subjected to some cycles of large amplitude oscillatory strain and recovery at low strain. The results provide no differences between rheological profiles of empty and loaded systems, even after exposing the samples to sterilization process; along with in vitro water uptake values, which were not affected by the presence of drug models. On the contrary, the addition of the guest compounds influenced in vitro CP spontaneous degradation, leading to strengthened matrices. Release studies showed three different kinetic profiles for each drug model loaded as function of their solubility. Overall, these results open new possibilities for a differentiated multi-drug delivery through in situ gelling lyotropic systems.

RHEOLOGICAL CHARACTERIZATION

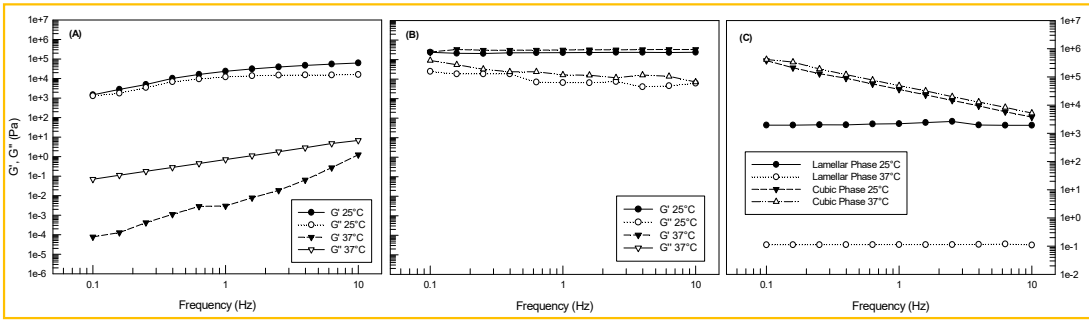


Figure 1. Rheological properties of lamellar and cubic phase obtained in oscillatory mode. Dynamic moduli change of lamellar (A) and cubic phase (B) and viscosity flow (C) of samples were represented as a function of temperature (25 and 37 ± 1°C) and within a frequency range of 0.1-10 Hz and at constant shear stress (1 Pa). Lamellar phase shown a strong dependence of temperature, indicating a "liquid-like" behavior. On the contrary the panel B confirmed the successful gelation of the sample in the presence of an excess of medium and the formation of a strong three-dimensional network of cubic phase. As shown in panel C the temperature variation induced a marked reduction of lamellar phase viscosity when analyzed at body temperature; but the sample preserved a resistance to the imposed stress. No-temperature influence was recorded analyzing complex viscosity profile of CP: this in another confirmation of the transition to lyotropic cubic phases.

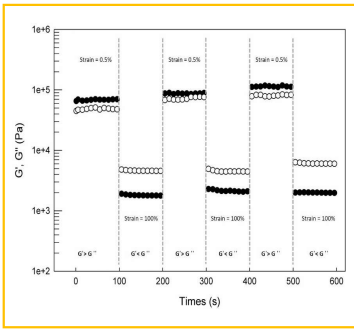


Figure 2. Self-recovery properties of cubic phase, analyzed at 37 ± 1°C. Three cycles of 100% and 0.5% strain (frequency=1Hz) were applied on the same sample, evaluating G' (filled circles) and G'' (empty circles) values. The increase of strain (maximum deformation) induced a reverse of G' and G'' values, highlighting a liquid-like behavior, but at low strain cubic phase showed a quick recovery of its inner network and confirmed the self-recovery ability of CP.

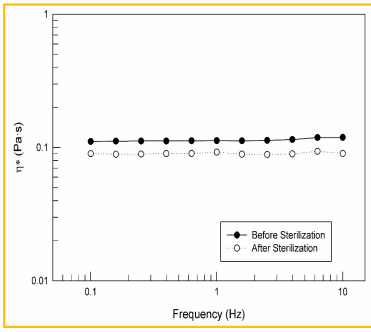
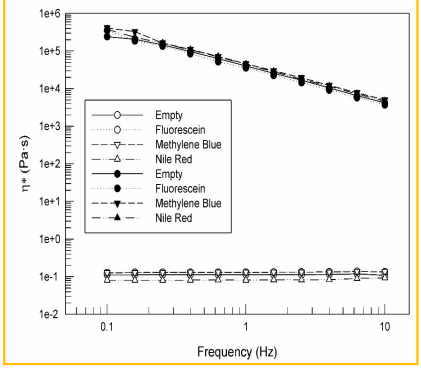


Figure 3. Complex viscosity curves vs frequency of lamellar phase analyzed at 37 ± 1°C before and after sterilization process, carried out by using an autoclave (120°C for 15 min).

The complex viscosity profile recorded during frequency sweep test maintained its trend also after sterilization process, with a slight reduction of η* that could be considered an advantage for an easier *in vivo* administration of LP.

Figure 4. Complex viscosity (η* - Pa·s) curves vs frequency (Hz) analyzed at 37 ± 1° for lamellar (empty symbols) and cubic (filled symbols) prepared with (0.5 mg/g) and without model drugs. The result was a representative experiment of five independent experiments. The addition of chosen concentration of each model drug did not influence or altered the transition from lamellar to cubic phase. The complex viscosity of LP and CP was maintained almost constant during all range of frequency analysis. Rheological profiles were not changed by the incorporation of three chosen model drugs, in fact the curves of η* vs frequency were overlapped for all four tested samples. These results encourage future studies because this in situ cubic phase system could be suitable to deliver a wide range of active molecules with pharmacological activity, without loss the specific feature of GMO-based systems.



WATER UPTAKE

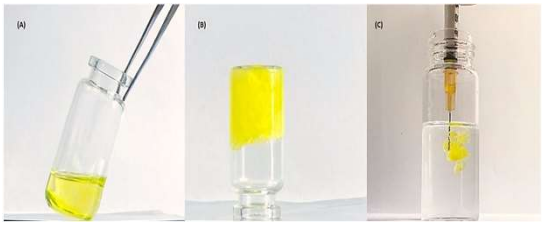


Figure 5. Lamellar (A) ---> Cubic phase (B) transition after exposure of lamellar phase to an excess of PBS (in this case fluorescein disodium salt was added to stain sample); and representative figure of instantaneous transition (C).

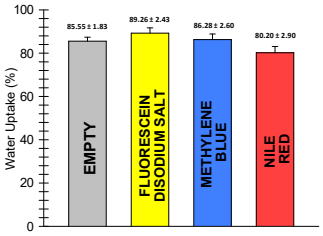


Figure 6. Water Uptake (WU %) values, obtained for empty and drug-loaded lamellar phase during its cubic phase transition. The absorption of PBS and the formation of CP were not modified when the model drugs were present into LP; the differences in weight gain between empty and fluorescein-, methylene blue- and nile red-loaded cubic phase were not statistically significant.

DRUGS RELEASE

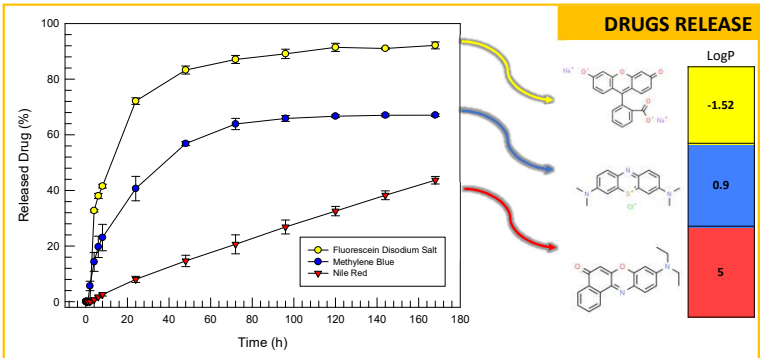


Figure 7. In vitro release of fluorescein disodium salt, methylene blue and nile red from GMO-cubic phase in pH 7.4 buffer solution at 37°C. The results are expressed as mean values ± standard deviation. The affinity between the tested model drugs and the inner structure of formed cubic phase was confirmed by these results. Cubic phase can be considered as a sustained drug delivery system, that induced a slow and a controlled release over time of hydrophilic, amphiphilic and lipophilic drugs, in a manner dependent of physico-chemical characteristics of chosen molecules. As show, thanks to its high hydrophilicity, fluorescein disodium salt was able to easy diffuse through the aqueous channels, obtaining an amount of released drug equal to 92.15 ± 1.25% after 7 days; at the same time point, only the 43.68 ± 1.35% the lipophilic model (nile red) was released, while amphiphilic methylene blue release profile was interposed between those of the other two dyes (with a value of 67.04 ± 0.45%).

SPONTANEOUS DEGRADATION

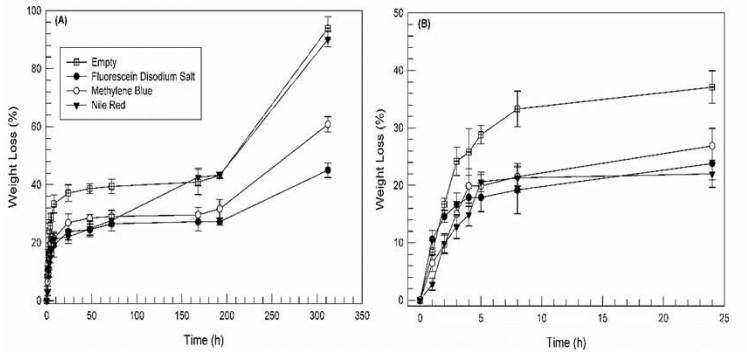


Figure 8. In vitro degradation profiles was shown as weight loss (%) curves vs time (h), for empty and drug-loaded cubic phase. The experiments were carried out in PBS pH 7.4 buffer solution at 37°C. Panel B is representative for the first 24 hours of entire experiments (panel A). The results are expressed as mean values ± standard deviation. Different LogP dyes incorporation led to a greater resistance of the cubic phase in the first 24 hours of experiment (panel B), recording a weight loss for empty CP equal to 37.10 ± 2.84%, in comparison with the values obtained for fluorescein-, methylene blue- and nile red-loaded CP equal to 23.80 ± 2.84%, 26.88 ± 3.04% and 22.00 ± 2.36%, respectively.

References: [1] Chen, Y., Ma, P., & Gui, S. (2014). Cubic and Hexagonal Liquid Crystals as Drug Delivery Systems. BioMed research international, 2014, 815-981. [2] Shah, J. C., Sadhale, Y., & Chilukuri, D. M. (2001). Cubic phase gels as drug delivery systems. Advanced drug delivery reviews, 47(2-3), 229-250.