Analysis of Liquid Crystalline Nanoparticles by Small Angle X-Ray Diffraction: Evaluation of Drug and Pharmaceutical Additives Influence on the Internal Structure

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ABSTRACT: The goal of this work was to study the liquid crystalline structure of a nanodispersion delivery system intended to be used in photodynamic therapy after loading with photosensitizers (PSs) and additives such as preservatives and thickening polymers. Polarized light microscopy and light scattering were performed on a standard nanodispersion in order to determine the anisotropy of the liquid crystalline structure and the mean diameter of the nanoparticles, respectively. Small angle X-ray diffraction (SAXRD) was used to verify the influence of drug loading and additives on the liquid crystalline structure of the nanodispersions. The samples, before and after the addition of PSs and additives, were stable over 90 days, as verified by dynamic light scattering. SAXRD revealed that despite the alteration observed in some of the samples analyzed in the presence of photosensitizing drugs and additives, the hexagonal phase still remained in the crystalline phase. © 2011 Wiley-Liss, Inc. and the American Pharmacists Association J Pharm Sci 100:2849–2857, 2011

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INTRODUCTION

Liquid crystals belong to a state of matter with properties between those of an atomic ordered solid and an isotropic liquid. A liquid crystal phase may have structural similarities found in crystalline solids, as well as some disorder, evidenced by the ease of flow, as found in liquids. The transition between different phases corresponds to the breaking of some symmetry and can be described in terms of the so-called order parameter. This parameter represents the extent to which the configuration of the molecules in the less symmetric (more ordered) phase differs from that in the more symmetric (less ordered) one.[1]

The polar lipid monoolein (MO) can form several liquid crystalline mesophases in contact with water, a lamellar phase and a reverse bicontinuous cubic phase at room temperature, and a reverse hexagonal phase that is obtained at relatively high temperatures.[2–4]

The reverse hexagonal phase (referred to here as the hexagonal phase for simplicity) requires the addition of a third compound, such as oleic acid (OA), to be formed at room temperature.[5] Aqueous dispersions of the hexagonal phase can be obtained by adjusting the composition and by dispersing this liquid crystalline phase in an excess of water in the presence of a polymeric stabilizer (such as poloxamer 407).[6]

In the pharmaceutical field, the potential use of the reverse hexagonal phase to deliver compounds has been analyzed. These studies have included intravenous and skin administration[7–9] because this phase has important features, such as maintenance of

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chemical stability [9,10] controlled release,[11] and an increase of the penetration of drugs into skin. [7,8]

Phase diagrams formed by MO and water show that the structure of liquid crystalline phases may suffer phase transitions after the addition of lipids, drugs, polymers, and solvents.[5,8,12,13] In addition, a systematic study concerning dispersions composed of MO/OA verified that their structures are sensitive to alterations in pH, lipid composition, and concentration.[14] Therefore, it is important to study the inner structure of delivery systems composed of liquid crystals after the addition of pharmaceutical additives, as they can significantly alter their structure.[8,15]

Zinc and chloroaluminum phthalocyanines (ZnPc and ClAlPc, respectively), and protoporphyrin IX (PpIX) are highly hydrophobic and amphiphilic photosensitizers (PSs), respectively, used in photodynamic therapy (PDT) to treat cancers. PpIX, an endogenous PS, is mainly used to treat nonmelanoma skin cancers.[16,17] To date, a sulfonated aluminum phthalocyanine mixture has been approved in Russia,[18] and a silicon phthalocyanine is in phase I PDT trials to treat cutaneous T-cell lymphoma.[19] However, the administration of these PSs is difficult because they are insoluble in the solvents commonly used and, in addition, they present aggregation that reduces their photodynamic efficacy.[20] These features make them good candidates for the development of delivery systems that enable their solubilization and promote skin penetration.

In this context, hexagonal phase nanodispersions (HPNs) formed by MO/OA that contain phthalocyanines and PpIX were developed to improve their skin penetration in the treatment of nonmelanoma skin cancer by topical PDT. The mean average size (nm) and the internal structure of the HPNs were studied by dynamic light scattering and small angle X-ray diffraction (SAXRD) before and after the addition of PSs, a preservative (Merguard® 1200; Nalco, Naperville, Illinois) and a thickening polymer [hydroxyethyl cellulose (HEC)]. The lattice parameter and full width at half maximum (FWHM) were determined to observe the effect of the addition of additives and PSs on the phase structure.

MATERIALS AND METHODS

Materials

Monoolein (Myverol® 18–99K), with 94.5% minimum monoglyceride, and 1.2% maximum of free glycerine and fatty acid was supplied by Quest (Norwich, New York). OA, PpIX, ClAlPc, ZnPc, and *N*-methylpyrrolidone (NMP) were obtained from Sigma (St. Louis, Missouri). Poloxamer 407, a polyoxyethylene (101 units)–polyoxypropylene (56 units) copolymer, was obtained from BASF (Florham Park, New Jersey). Merguard® 1200 (~20% purified 1,2-dibromo-2,4-dicyanobutane in 2-phenoxyethanol) was obtained from Nalco) and the thickening HEC (Natrosol® 250 HHr) was obtained from Aqualon (Hopewell, Virginia).

Preparation of the HPN

Bulk hexagonal phases containing excess water were prepared by mixing MO (melted at 42°C) and OA at 8:2 (w/w), and by adding a 1.5% aqueous citrate buffer solution (pH 6.0) of poloxamer 407 to the lipid mixture to achieve an MO/OA/poloxamer 407/H_2O system (8:2:1.35:88.65, w/w/w/w). The selected proportion has been shown to allow the formation of a nanodispersion of the hexagonal phase, as described in an earlier publication.[7] The system was allowed to equilibrate at room temperature for 24 h. Then, the hexagonal phase with an excess of water was vortex mixed and sonicated (22.5 kHz) in an ice-bath for 3 min. The preservative Merguard® 1200 (0.1%, w/w), for topical (skin) intended use, was added to the aqueous phase of the nanodispersion and the PSs, predissolved in NMP, were added to the melted MO/OA mixture. To increase the viscosity of the liquid nanodispersion, the thickening polymer, HEC, was added (1.0%, w/w) to the final nanodispersion, followed by 24 h of rest. Finally, the nanodispersion was submitted to mechanical homogenization (1500 rpm) for 15 min at room temperature to disperse the polymer.

CHARACTERIZATION OF THE HPNS

Polarized Light Microscopy

The MO/OA/poloxamer 407/H_2O system (8:2:1.35:88.65, w/w/w/w), MO/OA/NMP/poloxamer 407/H_2O system (8:2:5.5:1.35:83.15, w/w/w/w), and the previous system containing PSs were characterized under a polarized light microscope (Axioplan 2 Image Pol microscope; Carl Zeiss, Oberkochen, Germany) before and after the sonication process used to disperse the bulk phase.

Dynamic Light Scattering

The unloaded and drug-loaded nanodispersions, with or without the thickening polymer, were analyzed by light scattering at 25°C using a Zetasizer Nano system ZS (Malvern Instruments, Worcestershire, UK). Samples ($n = 3$) were diluted in particle-free purified water and 12 measurements for each sample were carried out. The instrument contained a 4 mW He–Ne laser operating at a wavelength of 633 nm and incorporated noninvasive backscatter optics (NIBS; Malvern Instruments). The measurements were made at a detection angle of 173° and the measurement position within the cuvette was automatically determined by the software.