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Drug Delivery

Stimuli-Responsive Lipidic Cubic Phase: Triggered Release and Sequestration of Guest Molecules

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Abstract: New stimuli-responsive nanomaterials, made up of host–guest lipidic cubic phases (LCPs) are presented. These biocompatible, stable, transparent and water-insoluble LCPs are composed of monoolein (MO) as a neutral host, and small amounts of one of three judiciously designed and synthesized designer lipids as guest that preserve the structure and stability of LCPs, but render them specific functionalities. Efficient pH- and light-induced binding, release and sequestration of hydrophilic dyes are demonstrated. Significantly, these processes can be performed sequentially, thereby achieving both temporal and dosage control, opening up the possibility of using such LCPs as effective carriers to be used in drug delivery applications. Specifically, because of the inherent optical transparency and molecular isotropy of LCPs they can be envisaged as light-induced drug carriers in ophthalmology. The results presented here demonstrate the potential of molecular design in creating new functional materials with predicted operating mode.

Lipidic cubic phases (LCPs) are nano-compartmentalized biomaterials composed of specific lipids and water that form thermodynamically stable, nontoxic, and biocompatible gels.[1,2] The structure of LCPs is akin to an ordered molecular sponge consisting of a lipid bilayer that is curved in three dimensions,[3] surrounded by two identical, yet nonintersecting aqueous channels.[4] These materials are optically transparent and isotropic by molecular symmetry. Moreover, they are bicontinuous, and enable diffusion in both compartments. Compartmentalization of the LCPs can be utilized to introduce either hydrophilic, lipophilic, or amphiphilic molecules.[5–8] Hydrophilic compounds are embedded in proximity of the lipids' head groups or in the aqueous channels, whereas lipophilic molecules partition into the lipid bilayer, and amphiphilic ones to the interface between the bilayer and the aqueous compartments, thus rendering LCPs ideal molecular carriers.[9] Entrapment of guest molecules within the LCP protects them from chemical and physical degradation.[10] Based on their phase diagrams, they can coexist stably with any amount of excess water,[2] implying that they are water insoluble. Finally, LCPs are soft solids that can be manipulated to adopt any shape. The combination of these material properties is unique, and offers advantages in their utilization as biomaterials for various applications.

Stimuli-responsive chemical systems have been used in a number of areas of science and technology.[11] In the field of drug release, such systems enable control of drug distribution in response to exogenous (e.g., temperature, light) or endogenous (e.g., pH, redox) stimuli.[12] The materials used respond either to a specific physical incitement, or are made up of molecular building blocks that undergo chemical transformations such as protonation, cleavage or conformational change. They have potential therapeutic advantage when continuous release of active ingredients might be toxic,[13] and an "on/off" switching should provide control over effectiveness of the therapy. Responsive drug delivery systems used to date include liposomes, micelles, polymer nanoparticles, dendrimers, and inorganic nanoparticles. Despite great advances in material science and technology, a number of problems still persist, including toxicity, stability, biocompatibility and biodegradability, as well as efficient targeting to the diseased tissue. Synthetic polymeric systems often exhibit toxicity issues, which may limit the utility of these materials in vivo, since only nontoxic molecules and macromolecules can be used.[1] Biocompatibility and biodegradability are practical problems that are often encountered with inorganic nanoparticles such as Au–Ag and gold nanorods. Moreover, in most drug-targeting systems less than approximately 5% of the active compound reaches the diseased site.[11]

To overcome these problems, designer host–guest lipidic cubic phases are presented as alternative delivery and sequestration systems that can be triggered by external stimuli. Diffusion of embedded molecules within LCP depends, among others, on the size and polarity of the molecules. In the case of hydrophilic drugs, modification of lipids' head groups allows external control over drug release. For example, incorporation of a negatively charged lipid results in slower release of positively charged molecules from the cubic phase.[14] Stimuli-responsive drug delivery systems that use various external stimuli have been designed, including light,[15] pH and salt concen-

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tration.[16] Fong et al. used spiropyran laurate to form light-responsive LCPs as drug-delivery systems that photoisomerize reversibly following irradiation, thereby switching from the inverted bicontinuous cubic (fast release) to the inverted hexagonal liquid crystal structures (slow release).[15] Negrini et al. developed a pH-sensitive LCP that can be used for release of molecules in the gastrointestinal tract, while preventing the release in the stomach environment.[16]

Herein, we present a host–guest system composed of monoolein (MO) as the host lipid, and one of three designer synthetic lipids as the guest. These complex systems form stimuli-responsive, functional LCPs that are either light or pH sensitive. Lipids **1** and **2** were designed in order to obtain light-responsive biomaterials, whereas lipid **3** (Figure 1) is utilized in pH-sensitive formulations.[10] MO's glycerol head group is localized at the interface between the lipid bilayer and the aqueous compartment.[17] Addition of small amounts of the designer guest lipids results in modified LCPs with functionalized head groups that exhibit electrostatic interaction with hydrophilic molecules residing in the aqueous compartments. Conditions are established for the triggered release and sequestration of two hydrophilic dyes, the anionic crocein orange G (CO), and the cationic methylene green zinc chloride double salt (MG), upon interaction with LCPs doped with the guest lipids.

The current work explores two regimes: 1) triggered release from the LCPs using pH-sensitive lipid **3** and light-sensitive lipid **2**; 2) sequestration into the LCPs using light-sensitive lipid **1**. In the release experiment, specially designed stainless steel holders were filled with 15 mg of LCP containing the solubilized dye.[18] The surface area of the LCP was flattened with a spatula and the holder was placed in a spectroscopic cuvette. LCPs were overlaid with 1 mL of various aqueous solutions and the time-dependent concentration of the dye released into the overlay was evaluated (by using Eq. (1), see the Supporting Information) at different pH values (MQ water at pH≅7, 20 mM NaCl, and 20 mM citric acid at pH values of 2.5, 3, and 5) for LCPs containing the pH-sensitive lipid **3**, and following exposure to UV light for LCPs with the light-sensitive lipid **2**. Release of the positively charged dye MG (0.24 mM in LCPs) from the aqueous compartments of pure MO LCP, and from MO-based LCPs doped with lipid **3** (denoted herewith as MO/**3**, composed of 99/1 %, w/w, in which the molar ratio between lipid **3** and MG is 84:1) was investigated at various pH conditions. The *p*-nitro benzoic acid head group (pK_a≅3.6) renders lipid **3**, and the ensuing LCPs pH sensitive. As shown in Figure 2, deprotonation of the carboxylate head group at pH 7 results in binding of the positively charged MG, in contrast to the pure MO LCP, the head groups of which are neutral. Upon successive decrease of the pH from 7 to 5, 3 and 2.5, the carboxylate head group is progressively protonated, resulting in decreased binding between lipid **3** and MG, and leading to an increase of MG release. At pH 5 approximately 95 % of lipid **3** is ionized, whereas at pH 3 and 2.5, that is, below the pK_a value of the *p*-nitro benzoic acid head group, MG release is faster than with pure MO-based LCPs at pH 7 (Figure 2), and MO-based LCPs at pH 2.5 (Figure S3 in the Supporting Information).

Triggered release of MG was demonstrated in a dynamic experiment shown in Figure 3. Initially MG is bound within the LCP at pH 7. After four hours the pH of the overlay was de-

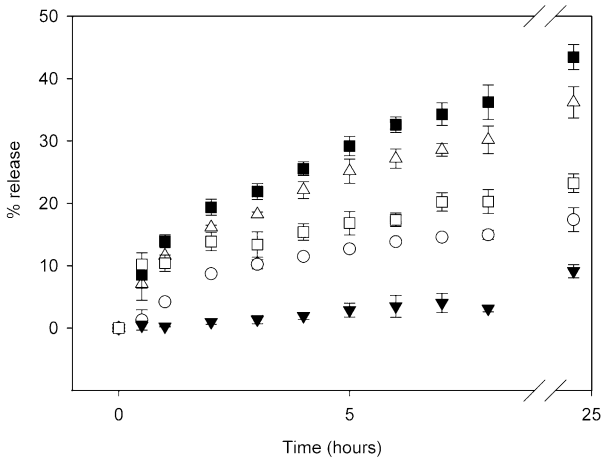

Figure 2. Percentages of MG release as a function of time from the aqueous compartments of the following LCPs into the overlay: MO, pH 7 (white squares); MO/**3**, pH 7 (black triangles); MO/**3**, pH 5 (white circles); MO/**3**, pH 3 (white triangles); MO/**3** pH 2.5 (black squares).

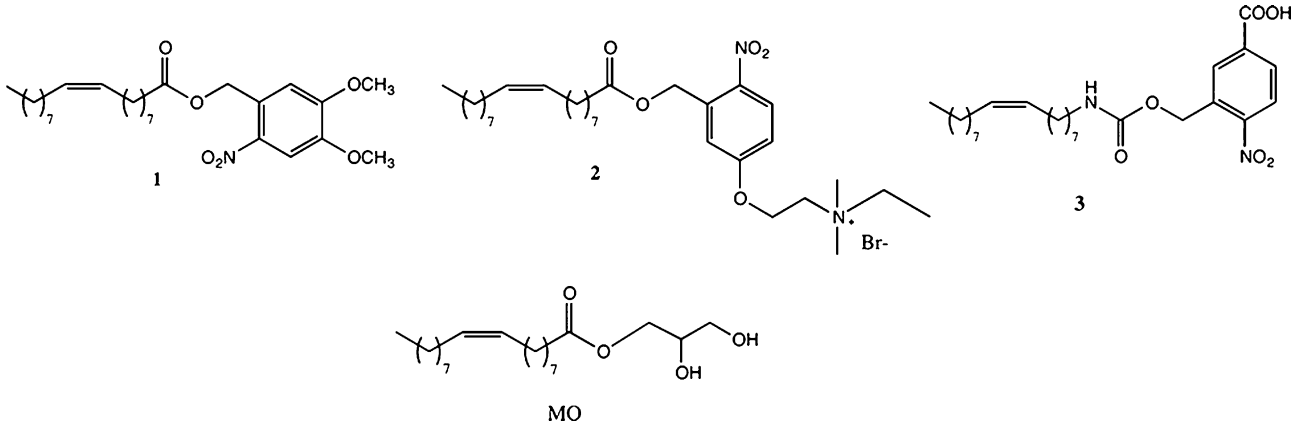

Figure 1. Structures of the synthetic guest lipids **1**, **2**, and **3** (additives), and of the host lipid MO.

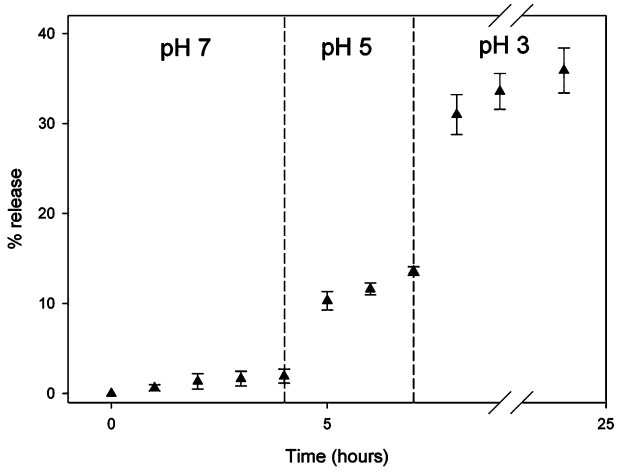

Figure 3. Percentages of MG release as a function of time from the aqueous compartment of MO/**3** LCP into overlay.

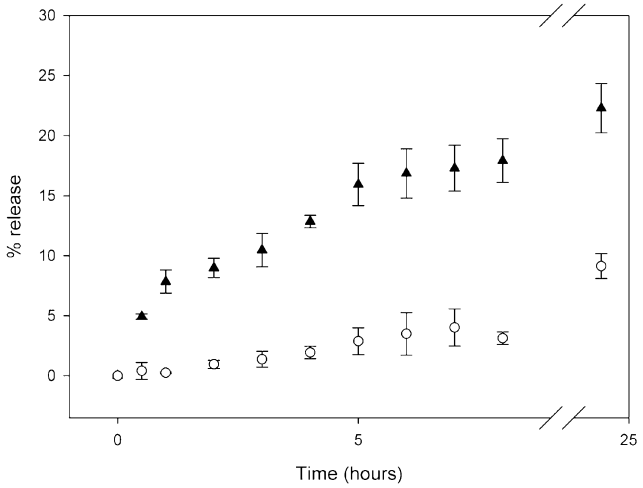

Figure 4. Percentages of MG release as a function of time from the LCP's aqueous compartments into the overlay: MO/**3**, pH 7 (white circles); MO/**3**, pH 7, 20 mM NaCl (black triangles).

creased to 5, causing partial release, and after an additional two hours the pH was decreased to 3, resulting in an additional step-like release profile.

The effect of ionic strength on dye binding and release is shown in Figure 4. MG, bound to a LCP doped with lipid **3** at pH 7, is effectively released upon addition of 20 mM of NaCl, as the carboxylate moieties are shielded by the salt, thereby reducing the electrostatic attraction to MG.[19]

These results are in excellent agreement with the control of release by host–guest electrostatic interactions in cubic phases reported recently by Negrini et al.[20] Small angle X-ray scattering (SAXS) experiments (Figure 5) performed on LCPs doped with guest lipid **3** under identical conditions to those implemented in the triggered-release experiments demonstrate that addition of the synthetic guest lipid does not affect the phase identity of the MO/H_2O system, which is *Pn*3*m* throughout.[2] The symmetry of the LCP is unchanged after 24 h, at both pH 7 and 3. The unit-cell parameters are 101.0, 102.3 and 102.2 Å, respectively (Table S1 in the Supporting Information).

Photochemical triggering of drug release represents a powerful alternative to pH, provided that the medium is transparent. LCPs are ideal for these purposes, as they are optically transparent and isotropic gels.[8] Incorporation of designer light-responsive lipids[21,22] into LCPs was therefore envisaged. Lipids containing *o*-nitrobenzyl ester moieties are particularly well suited to modulate chemical reactivity because they undergo rapid cleavage reactions when exposed to UV (≅360 nm) light.[23] Towards this goal, photochromic lipid additive **2** was incorporated into LCPs (0.1%, w/w). Photoinduced cleavage of lipid **2** with UV light was characterized spectroscopically (Figure S2 in the Supporting Information). Lipid **2** (0.1%, w/w of total lipid content) was incorporated into an MO-based LCP, and the release characteristics of CO were assessed. As shown in Figure 6 (black triangles), efficient binding of the negatively charged CO to the positively charged ammonium head group of **2** is affected at pH 7 (the molar ratio between **2** and CO is 1:1). Exposure of the lipid **2**-doped LCP to UV light for 15 min initiates a photochemical cleavage of the of nitrobenzyl group,[24] yielding a negatively charged carboxylate head group located at the lipid–water interface of the LCP. The resulting release of CO, which is due to electrostatic repulsion, is shown in Figure 6 (white circles). Further exposure to UV radiation for 15 min after 4 and 6 h results in additional release of CO. In order to ascertain that CO release is due to the UV-triggered photocleavage of the lipid's head group, and is not induced by temperature change or any other accompanying reaction that may take place, a control experiment was carried out using the positively charged oleyl amine (0.1%, w/w) as additive. Lacking light-responsive groups, no release was ob-

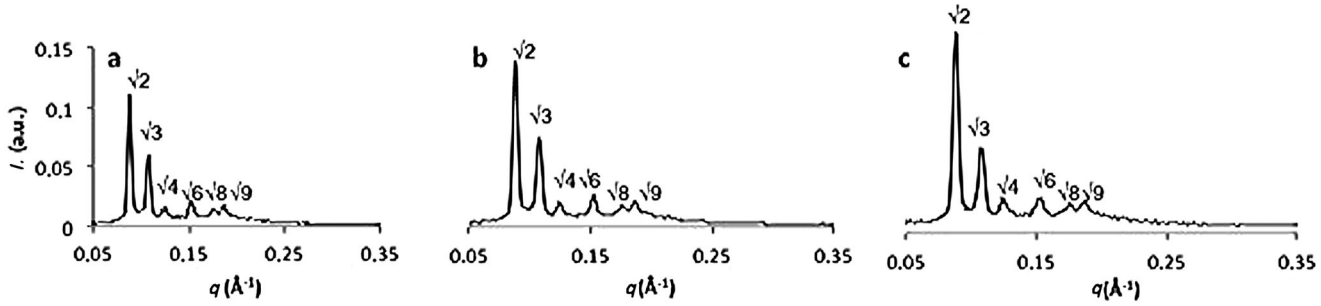

Figure 5. SAXS data on MO/**3** LCPs under different conditions: a) immediately after preparation; b) after 24 h at pH 7; c) after 24 h at pH 3.

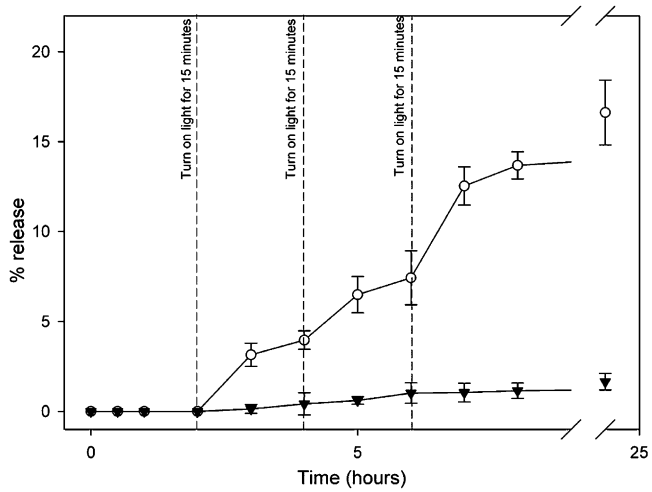

Figure 6. Percentages of CO release as a function of time from the aqueous compartments of MO/**2** LCP into the overlay: without irradiation (black triangles); with UV irradiation (white circles). The UV light was turned on for 15 min after 2, 4 and 6 h, respectively.

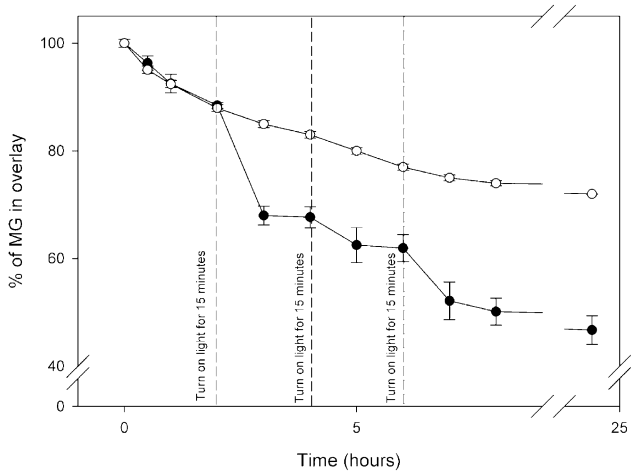

Figure 7. Percentages of MG sequestrated as a function of time from the overlay to the aqueous compartments of LCPs: MO (white circles); MO/**1** (black circles). The UV light was turned on for 15 min after 2, 4 and 6 h, respectively.

served even after 24 h (Figure S1 in the Supporting Information).

Addition of lipid **2** (0.1 %, w/w) to MO induces a cubic *Pn*3*m* (characteristic for the MO/H_2O system under these conditions) to cubic *Ia*3*d* phase transition, as shown by SAXS (Figure S4 and Table S1 in the Supporting Information). This transition occurs before and after UV irradiation, with unit-cell parameters of 157.0 and 157.3 Å, respectively. Based on the critical packing parameter (CPP),[1] this transition may be due to the larger polar head group area of lipid **2**, which leads to a smaller CPP and to the observed *Ia*3*d* cubic symmetry. Importantly, upon hydration of the *Ia*3*d* LCP for 24 h the phase reverts to the *Pn*3*m* symmetry ($a = 105.7$ Å), as expected for an MO cubic phase in excess water.

UV-induced control of the head-group structure and charge was shown to be effective in sequestration of charged molecules from the environment into LCPs. Sequestration experiments were conducted using the neutral lipid **1** embedded as additive in LCP (0.01 %, w/w). In this experiment the stainless steel holders were filled with 15 mg of MO/**1** LCP, the surface area of the LCPs was flattened with a spatula and the holder was placed in a spectroscopic cuvette overlaid with an aqueous solution containing 9.6 μM MG. The molar ratio between the light-sensitive lipid **1** embedded in the LCP and MG in the overlay was 1:1. Sequestration of MG from the overlay into the doped and pure LCPs was identical prior to UV illumination, as both LCPs are composed of neutral lipids. Following exposure to UV light, the head group of lipid **1** is converted to a negatively charged carboxylate, which is shown to sequester the positively charged dye (Figure 7). This effect can be repeated, resulting in sequential sequestration of the dye. As shown by SAXS, addition of the synthetic guest lipid **1** does not affect the phase identity of LCPs, which is *Pn*3*m* throughout. The symmetry of the LCP is unchanged after UV treatment or after incubation for 24 h, with unit-cell parameters of 103.1, 103.3 and 106.0 Å, respectively (Figure S5 and Table S1 in the Supporting Information).

The results presented here demonstrate the potential of molecular design in achieving new, stimuli-responsive nanomaterials. Small amounts of judiciously designed and synthesized lipid additives can be incorporated into MO-based LCPs without destabilizing them, while rendering them functional in binding, release and sequestration. In this investigation we present pH and light as external stimuli. In the case of pH, stable LCPs enable the controlled release of hydrophilic drugs from the aqueous channels of the mesophases into the surrounding environment. In the case of light, these systems can specifically release from, or sequester into LCPs. Significantly, these processes can be performed sequentially, thereby achieving both temporal and dosage control. Due to the thermodynamic stability of fully hydrated LCPs with any amount of excess water, that is, their absolute insolubility in water, and to their soft-gel consistency, we envisage the possibility of applying such nanostructured biomaterials to specific locations in vivo, thereby achieving exquisite spatial control of drug delivery. Moreover, the use of light as an external stimulus[24] has many advantages in comparison with other stimuli: it is milder than acids or bases, and variation of intensity, wavelength and duration can provide a high level of pharmacological control.[11] LCPs are ideal in this respect, as they are optically transparent and isotropic by molecular symmetry, and can thus be envisaged as light-induced drug carriers in ophthalmology. Finally, because of the ability of LCPs to incorporate molecules of virtually any polarity or charge, the strategy presented here is general, that is, molecule independent, and shows great promise for biomedical applications.

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