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Drug release from lipid liquid crystalline phases: relation with phase behavior

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Abstract

Introduction: We studied the release of propranolol hydrochloride (PHCl), a water-soluble amphiphilic drug, from monoolein (MO)/water and phytantriol/water systems. *Methods:* We related the dissolution profiles with phase behavior and viscosity of the different liquid crystalline phases. Diolein has been added aiming to stabilize the cubic phases and thus preventing formation of less viscous (lamellar) phases. *Results:* Formulations display first-order release rates and diffusion release mechanism. Some formulations (mostly MO) were close to zero-order release in the first 120 minutes. *Discussion:* Release mechanism can be influenced by phase changes during dissolution. *Conclusions:* Both MO and phytantriol show good potential to be used for propranolol hydrochloride sustained drug release.

Key words: *Cubic phase; diolein; drug release; monoolein; phytantriol; propranolol hydrochloride*

Introduction

Monoolein (glyceryl monooleate, MO) and phytantriol (tetramethyl hexadecanetriol, PHY) (Figure 1) are both lipids with an extraordinary ability to form a large range of different liquid crystalline phases in contact with water, including a reverse bicontinuous cubic phases[1,2]. Because of its special structural properties, these phases have been extensively explored for different applications in drug delivery systems[3]. MO has also been widely used as emulsifier in the food and cosmetic industries. PHY is a well-known active ingredient for the cosmetics industry in hair and skin care. It improves the moisture retention properties of the skin and hair and acts as a penetration enhancer of panthenol, vitamins, and amino acids. For MO and PHY[2] the bicontinuous cubic phases are until now the most used in pharmaceutical applications. These phases have a transparent, stiff, gel-like appearance, and constitute a three-dimensional network of curved lipid bilayers separated by a network of congruent water channels[1]. This property has been used to sustain the delivery of various drugs. Sustained release formulations have the advantage of reducing the dosing frequency and producing controllable and sustained plasma levels that tend to minimize the risk of undesirable side effects. Both water-soluble and water-insoluble drugs have been incorporated into the MO bicontinuous cubic phase, including peptides[4], and it has been tested through different administration routes, for example, oral[5,6], periodontal, mucosal, and vaginal[3]. Transdermal delivery[7] and in vivo topical administration have also been reported[8,9]. Recently the performance of these two lipids both in topical applications[8,9] and in oral administration[10,11] have been compared. In oral administration, PHY may offer a benefit over MO as a drug delivery excipient because of its nondigestability, meaning that, unlike MO[5,6], the liquid crystalline structure can be retained in the presence of digestive enzymes[10].

The formation of a particular phase depends on the geometry of the lipid, water content, temperature, and other factors. Usually, molecules with a large hydrophilic moiety (headgroup) and a small hydrophobic tail associate into normal structures, that is, with positive

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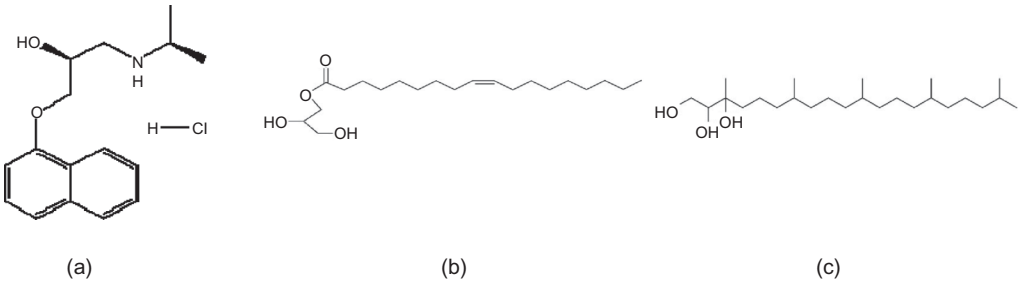

(a) (b) (c)

Figure 1. Chemical structure of (a) PHCl, propranolol hydrochloride; (b) MO, monoolein (glycerol monooleate); and (c) PHY, phytantriol (tetramethyl-hexadecanetriol).

curvatures, whereas molecules with a small headgroup and big hydrophobic moiety tend to form reverse structures, with negative curvatures[12]. Both MO and PHY can be found in this last group, forming reverse phases. Furthermore, both these systems exhibit unusual properties, in that its sequence upon hydration is reverse to what is generally expected[13], and, at room temperature, it evolves in the sequence micellar→lamellar→reverse cubic Ia3d phase→reverse cubic Pn3m, that is, curvature decreases with the hydration of the lamellar phase[14,2]. Between 20 and 40 wt% of water content, MO forms reverse bicontinuous cubic phases (space groups Ia3d and Pn3m). PHY needs a lower amount of water to form bicontinuous cubic phases, which might provide an alternative to MO in certain applications, particularly cubic liquid crystalline phases[2]. At higher water contents, the space group Pn3m reverse bicontinuous cubic phase coexists with excess water, keeping its physical stability.

For pharmaceutical applications, one should consider that the presence of a drug may modify the cubic phase structure and alter the drug release profile of the system[15]. There are some studies reporting that the type of drug as well as the drug loading affect the stability of the cubic phases that transform into lamellar or hexagonal liquid crystalline phases[16–18]. Some of these studies also relate phase changes with the drug release profiles from the systems[15,19,20].

In this work, we have used MO/water and PHY/water systems and aimed at stabilizing the cubic phases with different drug loadings. The MO and PHY formulations are doped with diolein (glyceryl dioleate, DO) to promote the formation of the cubic phases. It has previously been demonstrated that the addition of DO to MO induces an extensive reversed hexagonal region in the phase diagram at high water contents, which does not exist in the MO/water system[21]. The model drug used in this study is propranolol hydrochloride (PHCl), which is a nonselective β-adrenoreceptor blocker that is widely used in the treatment of many cardiovascular diseases. PHCl is a water-soluble cationic amphiphilic drug that forms lamellar phases with MO and water[22]. In agreement with the packing concept of amphiphilic molecules[12], the reverse cubic phase can be stable in the presence of both DO and PHCl as these molecules compensate for each other in that DO causes an increase of the hydrophobic region volume and PHCl increases the volume of the lipid polar region. The conditions where the cubic phase is formed in systems containing MO or PHY doped with DO and PHCl is therefore a promising route to be explored. An analysis of MO and PHY formulations for application in transdermal delivery, in which both dissolution and permeation studies through human epidermis are included, has been previously presented in another study[23].

This work addresses the phase behavior, viscosity, and drug release from liquid crystalline structures prepared with MO and PHY (Figure 1) doped with DO, aiming to achieve sustained release dosage forms. The different phases were analyzed by small-angle X-ray scattering (SAXS), and the dissolution profiles were evaluated to establish a possible relation with phase behavior and viscosity. Mean dissolution times (MDT) and fit factors were used to compare the dissolution profiles. Also, dissolution mechanisms were evaluated by the application of several models.

Materials and methods

Materials

We have used propranolol hydrochloride (Batch No. PRP 190; Becpharma, Lisbon, Portugal) as a water-soluble model drug. MO (Rylo MG 19 Pharma ID98-027, a technical grade constituted by 90% MO, 5% of other monoglycerides, and 5% of mainly diglycerides, fatty acids, and free glycerol; Danisco Ingredients, Copenhagen, Denmark), diolein (DO, Danisco Ingredients), and PHY (BASF, Ludvigshafen, Germany, Lot: 40356188QO) were used without further purification. The water used

Table 1. Formulation codes and composition. Liquid crystalline phase, birefringence, and pH results are also shown. The nonhomogenous formulations were rejected from further studies.

	Composition (wt.%)						
Formulation code	PHCl	lip[a]:w	lip[a]:DO	Homogenous	Birefringence	pH (±0.05)	L.C. phase
MO (blank)	0	65:35	—	Yes	No	5.5	Pn3m
MO2	2	65:35	—	Yes	No	5.5	Ia3d
MO5	5	65:35	—	No	NA	NA	Ia3d + Lα
MO10	10	65:35	—	Yes	Yes	5.7	Lα
MO15	15	65:35	—	Yes	Yes	5.8	Lα
MO20	20	65:35	—	No	NA	NA	Lα + solid
MOD1 (blank)	0	65:35	92:8	Yes	Yes	5.2	H_{II}
MO2D1	2	65:35	92:8	Yes	No	5.3	Ia3d
MO5D1	5	65:35	92:8	No	NA	NA	Ia3d + Lα
MO10D1	10	65:35	92:8	Yes	Yes	5.5	Lα
MO15D1	15	65:35	92:8	Yes	Yes	5.6	Lα
MO20D1	20	65:35	92:8	No	NA	NA	Lα + solid
MOD2 (blank)	0	65:35	88:12	Yes	Yes	5.1	H_{II}
MO2D2	2	65:35	88:12	Yes	No	5.2	Ia3d
MO5D2	5	65:35	88:12	Yes	No	5.3	Ia3d
MO10D2	10	65:35	88:12	Yes	Yes	5.5	Lα
MO15D2	15	65:35	88:12	Yes	Yes	5.6	Lα
MO20D2	20	65:35	88:12	No	NA	NA	Lα + solid
PHY (blank)	0	73:27	—	Yes	No	5.6	Pn3m
PHY2	2	73:27	—	Yes	No	5.6	Ia3d
PHY5	5	73:27	—	Yes	No	5.7	Ia3d
PHY10	10	73:27	—	No	NA	NA	Ia3d + Lα
PHY15	15	73:27	—	No	NA	NA	Ia3d + Lα
PHY20	20	73:27	—	No	NA	NA	Lα + solid
PHYD1 (blank)	0	73:27	92:8	Yes	Yes	5.4	H_{II}
PHY2D1	2	73:27	92:8	Yes	No	5.5	Ia3d
PHY5D1	5	73:27	92:8	Yes	No	5.6	Ia3d
PHY10D1	10	73:27	92:8	No	NA	NA	Ia3d + Lα
PHY15D1	15	73:27	92:8	No	NA	NA	Ia3d + Lα
PHY20D1	20	73:27	92:8	No	NA	NA	Lα + solid
PHYD2 (blank)	0	73:27	88:12	Yes	Yes	5.2	H_{II}
PHY2D2	2	73:27	88:12	Yes	No	5.4	Ia3d
PHY5D2	5	73:27	88:12	Yes	No	5.5	Ia3d
PHY10D2	10	73:27	88:12	No	NA	NA	Ia3d + Lα
PHY15D2	15	73:27	88:12	No	NA	NA	Ia3d + Lα
PHY20D2	20	73:27	88:12	No	NA	NA	Lα + solid

[a]Lipid: MO or PHY, depending on whether the formulation code starts by MO or PHY, respectively; NA, not applicable or not determined.

was deionized and purified (Milli-Q water system, Billerica, MA, USA).

Preparation of formulations

MO was melted at 45°C and PHY at 50°C. Appropriate amounts of these lipids were weighted into different glass vials. Water, DO, and drug were also weighted into the vials as appropriate (Table 1). The vials were then sealed and centrifuged back and forth several times at 45°C or 50°C for MO- and PHY-containing formulations, respectively. They were then left to equilibrate for 7 days at 23 ± 2°C.

Visual inspection

Crossed polarizers were used to examine the formulation homogeneity. Only the homogenous one-phase samples, isotropic or anisotopic, were considered for further studies.

Formulations pH

Determinations were carried out with a pH meter (pH 526, WTW, Weilheim, Germany), and pH was measured directly with the electrode inside the sample vials, after calibration with pH 4 and pH 7 buffer solutions.

Small-angle X-ray scattering

The measurements were performed on a Kratky compact small-angle system equipped with a position-sensitive detector (OED 50 M from M. Braun, Graz, Austria) containing 1024 channels with 53.0 μm width. Cu Kα radiation of wavelength 1.542Å was provided by a Seifert ID300 X-ray generator operating at 55 kV and 40 mA. A 10-μm thick nickel filter was used to remove the Kβ radiation, and a 1.55-mm tungsten filter was used to protect the detector from the primary beam. The sample-to-detector distance was 277 mm. The volume between the sample and the detector was kept under vacuum during data collection to minimize the background scattering. Samples were measured in a sample holder with mica windows at 32°C. Temperature was kept constant at each value (±0.1°C) with a Peltier element.

Viscosity

Viscosity measurements were performed on a controlled stress Physica UDS 200 rheometer using a 1° cosine-and-plate geometry (25 mm diameter). All measurements were performed within the linear viscoelastic region, which was checked for each sample. The obtained shear stress values were in the range of 2–7 Pa. Each sample (1 g) was placed on the plate held at 32°C. Oscillatory tests were made in the angular frequency range of 0.05–5 Hz. The above experimental procedure allowed the recording of the complex viscosity (η*) as function of the angular frequency of oscillation.

In vitro dissolution studies

Dissolution was conducted using teflon supports vt(surface area of 63.6 mm^2 and 2 mm height) in a USP rotating paddle apparatus, at a speed of 50 rpm, in 6 × 900 mL of deionized water, maintained at 32 ± 0.5°C, using an automated assembly that consisted of a water bath fitted with a variable-speed stirrer (Dissolutest Prolabo, Fontenay-sous-Bois, France), a peristaltic pump (Ismatec IPN, Zurich, Switzerland), a UV/visible spectrophotometer (Perkin Elmer Lambda 2, Uberlingen, Germany) with an automatic multicell programer (Perkin Elmer B012-7391). The released PHCl absorbance was recorded automatically at 290 nm every 12 minutes for 48 hours. A total of five dissolution curves were obtained for each formulation, those were back-correlated with the respective blank (Table 1). MDT at different time points were determined from mean profiles obtained from these five curves as done by Costa et al.[24] The dissolution profiles were also compared using the fit factors f_1 and f_2 recommended by FDA and EMEA guidance documents[25,26].

Results and discussion

Phase behavior and viscosity

Tables 1 and 2 show the formulation composition and summarize the results obtained.

After visual inspection with crossed polarizers, some of the samples were eliminated from further consideration (Table 1), as they presented phase separation. Samples with 20% of drug load were also eliminated because of incomplete solubilization of PHCl. Figure 2 shows five representative SAXS spectra of formulations at different liquid crystalline phases. Our blank samples (MO/water 65:35 wt% and PHY/water 73:27 wt%) form a Pn3m cubic phase (Figure 2e). This phase is unstable to the addition of an amphiphilic drug-like PHCl, and a transition to the Ia3d cubic phase (Figure 2c and d) and to the Lα lamellar phase (Figure 2a) occurs with successive higher drug loads. When we add DO to MO and PHY, a reverse hexagonal H_{II} phase (Figure 2b) is formed. The lattice spacing values of both MO and PHY Pn3m cubic phases (blanks), respectively, of 97.2Å and 67.1Å (Table 2), are in close agreement with values previously determined by other authors[2,27].

The viscosity of different formulations is summarized in Table 2 and Figures 3 and 4. A general observation is that the viscosity is mainly determined by the phase behavior (Figure 3). PHY formulations present higher viscosity than MO formulations in a similar phase and a lower lattice spacing (Table 2, Figure 3), probably because they need less water to form the cubic phase. In both systems we can observe that the drug load induces the decrease of viscosity, related with phase changes (Table 2, Figure 4). The PHY cubic phases are more resistant to a phase transition upon drug load compared to the MO cubic phases ($PHY5_{Ia3d}$ is still a homogenous cubic phase whereas $MO5_{2\Phi}$ is not). A possible explanation is that PHY forms the cubic phases at lower water content than MO. The decrease of the viscosity with PHCl was also shown by other authors[28]. The formulations were designed so that the lipid to water ratio is kept constant and the drug was added to this formulations at different concentrations. This implies that the formulations with the higher drug load also have lower total water content, which should also be taken into account when evaluating the data. This clearly affects the lattice spacing and also the viscosity.

While PHCl has the effect of decreasing the viscosity, the addition of DO has the opposite effect. DO increases the relative volume of the hydrophobic region, which favors the transition to the reverse hexagonal phase instead of the lamellar phase, which gives a higher viscosity compared to the formulations without DO. However, when a small amount of PHCl is added together with DO, an amphiphile that promotes normal structures, the

Table 2. Formulation codes, liquid crystalline phase, lattice spacing (SAXS results), viscosity, and MDT (drug dissolution parameter) results are also shown.

Formulation code	L.C. phase	L. space (Å)	Viscosity (Pa. s)	$MDT_{48\ hours}$ (hours)
MO (blank)	Pn3m	97.2	1.13E + 06 ± 3.21E + 04	NA
MO2	Ia3d	129.3	3.09E + 05 ± 2.00E + 03	5.75
MO10	Lα	45.1	3.02E + 03 ± 4.00E + 01	0.89
MO15	Lα	39.1	2.66E + 03 ± 7.07E + 01	1.05
MOD1 (blank)	H_{II}	57.2	6.29E + 04 ± 2.12E + 02	NA
MO2D1	Ia3d	128.0	5.49E + 05 ± 2.00E + 03	11.85
MO10D1	Lα	56.1	1.12E + 03 ± 2.00E + 01	2.13
MO15D1	Lα	50.5	2.23E + 03 ± 6.35E + 01	2.43
MOD2 (blank)	H_{II}	55.5	1.35E + 04 ± 3.54E + 02	NA
MO2D2	Ia3d	140.0	5.80E + 05 ± 9.71E + 03	12.49
MO5D2	Ia3d	171.0	4.01E + 05 ± 1.20E + 03	9.18
MO10D2	Lα	50.8	2.46E + 03 ± 2.00E + 01	3.20
MO15D2	Lα	46.1	2.30E + 03 ± 3.51E + 01	2.96
PHY (blank)	Pn3m	67.1	6.02E + 06 ± 7.07E + 04	NA
PHY2	Ia3d	105.0	2.59E + 06 ± 3.51E + 04	13.45
PHY5	Ia3d	109.1	8.16E + 05 ± 2.45E + 03	11.60
PHYD1 (blank)	H_{II}	49.24	4.25E + 05 ± 4.25E + 02	NA
PHY2D1	Ia3d	103.0	2.04E + 06 ± 4.93E + 04	14.42
PHY5D1	Ia3d	97.1	8.45E + 05 ± 3.25E + 03	15.37
PHYD2 (blank)	H_{II}	48.46	6.54E + 04 ± 8.49E + 02	NA
PHY2D2	Ia3d	103.8	3.47E + 06 ± 4.16E + 04	13.69
PHY5D2	Ia3d	103.0	1.83E + 06 ± 2.08E + 04	11.40

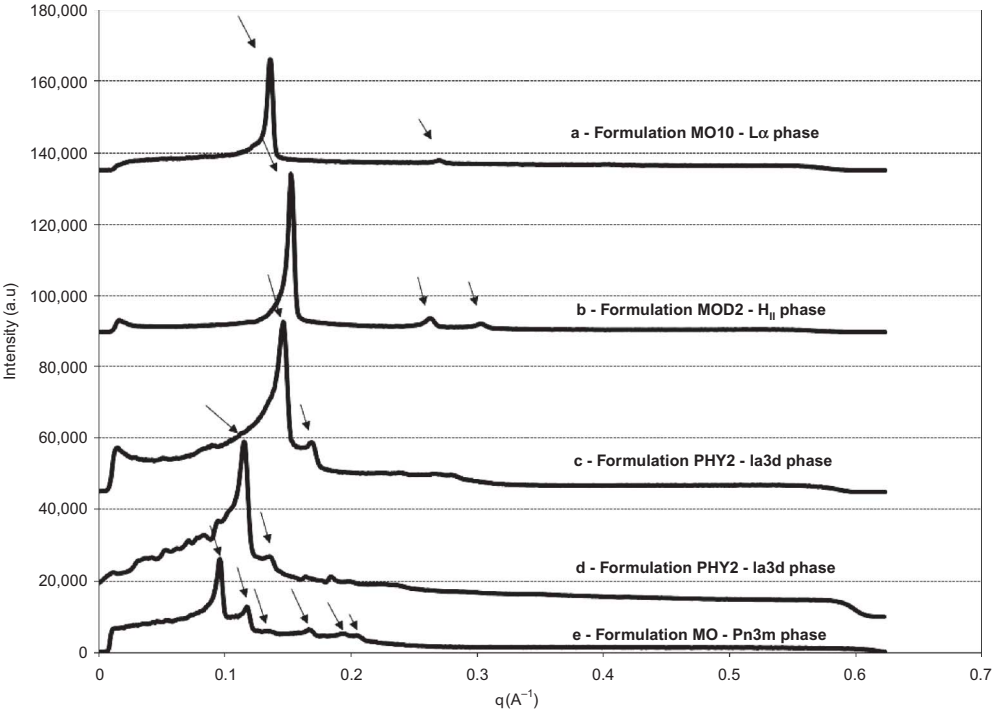

Figure 2. Representative SAXS spectra of formulations showing different liquid crystalline phases. The intensity is plotted versus the wave vector, $q = 2\pi/D$, where D is the spacing between the lattice planes. The arrows signal the first Bragg's reflection.

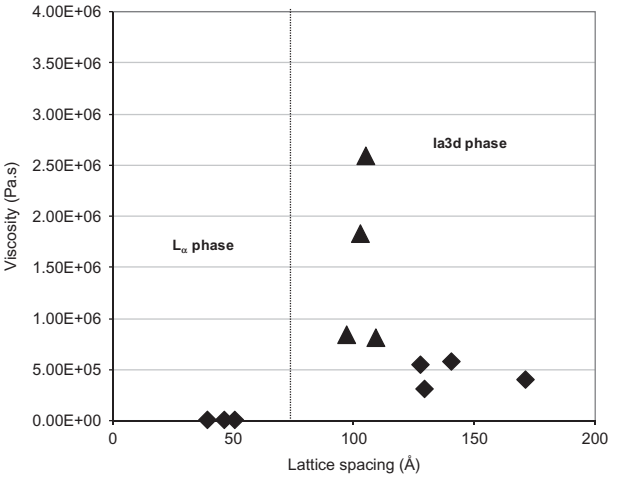

Figure 3. Formulation viscosity, η, versus lattice spacing, *D*, obtained with SAXS measurements. The lamellar, Lα, phases present much lower viscosity. ◆, MO formulations; ▲, PHY formulations.

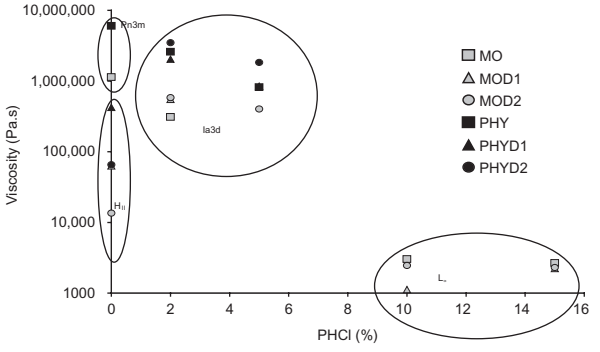

Figure 4. Relation between formulation viscosity, η, and composition (drug load) for the indicated liquid crystalline phases.

combined effect is the stabilization of the cubic phase. In formulations $MO10D1_{L\alpha}$, $MO10D2_{L\alpha}$, $MO15D1_{L\alpha}$, and $MO15D2_{L\alpha}$ we observe mainly the effect of the drug PHCl in changing the structure and affecting the curvature of the interface inducing a lamellar phase (with lower viscosity). However, at higher DO contents and lower concentrations of PHCl, the stabilizing effect of DO on the cubic phase is present. This is exemplified by the formulation $MO5D2_{Ia3d}$ that is in the Ia3d cubic phase while $MO5_{2\Phi}$ contains two coexisting liquid crystalline phases (Table 2). Within the PHY formulations, we do not observe the corresponding effect. For drug loads above 5%, the PHY-containing formulations are not homogenous and the Lα lamellar phase is present together with the Ia3d cubic phase; the phase boundaries to these two-phase regions were not further investigated.

Figure 4 shows the relation between formulation viscosities and their composition in PHCl. It is observed that PHY formulations (cubic phases) present higher viscosity than MO formulations in the same phase. The increase in drug load makes the viscosity of the Ia3d cubic phase to slightly decrease, whereas DO causes a general increase in viscosity of Ia3D at the same drug level. The cubic phases with the lower water contents (Pn3m) have the highest viscosity, followed by the Ia3d cubic phases and the hexagonal phases. The lamellar phases have the lowest viscosity.

Drug release

Figure 5 shows the 48-hour dissolution profiles of PHCl from MO (a) and PHY (b) formulations. A general observation is that the release rate is lower for the formulations that include DO, and these formulations most often also exhibit higher viscosity. Furthermore, the release rate is slower for the formulations with lower drug content. Only six of the formulations investigated reach a plateau in the release profile within 8 hours, and these are the MO lamellar phase formulations with high drug load. Concerning drug release, we have observed the following trends for the same drug load: in the case of the lamellar phases with 15% PHCl, we see that the release rate increases with the amount of DO, in the order $MO15_{L\alpha}$ > $MO15D1_{L\alpha}$ > $MO15D2_{L\alpha}$. This variation in the release rate cannot be related to variations in the viscosities (Table 1). We note that the formulations containing DO do not release the drug completely. A similar dependence of the release rate on the DO content is observed for the lamellar formulations with lower dug content (10%), $MO10_{L\alpha}$ > $MO10D1_{L\alpha}$ > $MO10D2_{L\alpha}$.

For the formulations in the cubic phase, we can conclude that for a certain lipid and drug load, the order of viscosities correlates inversely with release, except for a few exceptions. Furthermore, the introduction of DO retards the release for both the cubic phase and the lamellar formulations. This is probably because of the higher viscosity in these formulations, which helps to keep the shape and tortuosity of the aqueous channels and lowers the diffusion of both drug and water.

The MO formulations with lower drug load $MO2_{Ia3d}$, $MO2D1_{Ia3d}$, $MO2D2_{Ia3d}$, and $MO5D2_{Ia3d}$ present a slower and incomplete release. They do not reach 80% of drug release in 48 hours. The same applies to $MO10D1_{L\alpha}$ and $MO10D2_{L\alpha}$. This indicates that the drug partitions into the lipid bilayers. The value of log *P* for PHCl has previously been determined as –0.45[29], and it is therefore considered water soluble. Still, when present in a formulation consisting of amphiphilic molecules in lipid bilayers, it is also of uttermost importance to consider the surface activity of the drug molecule

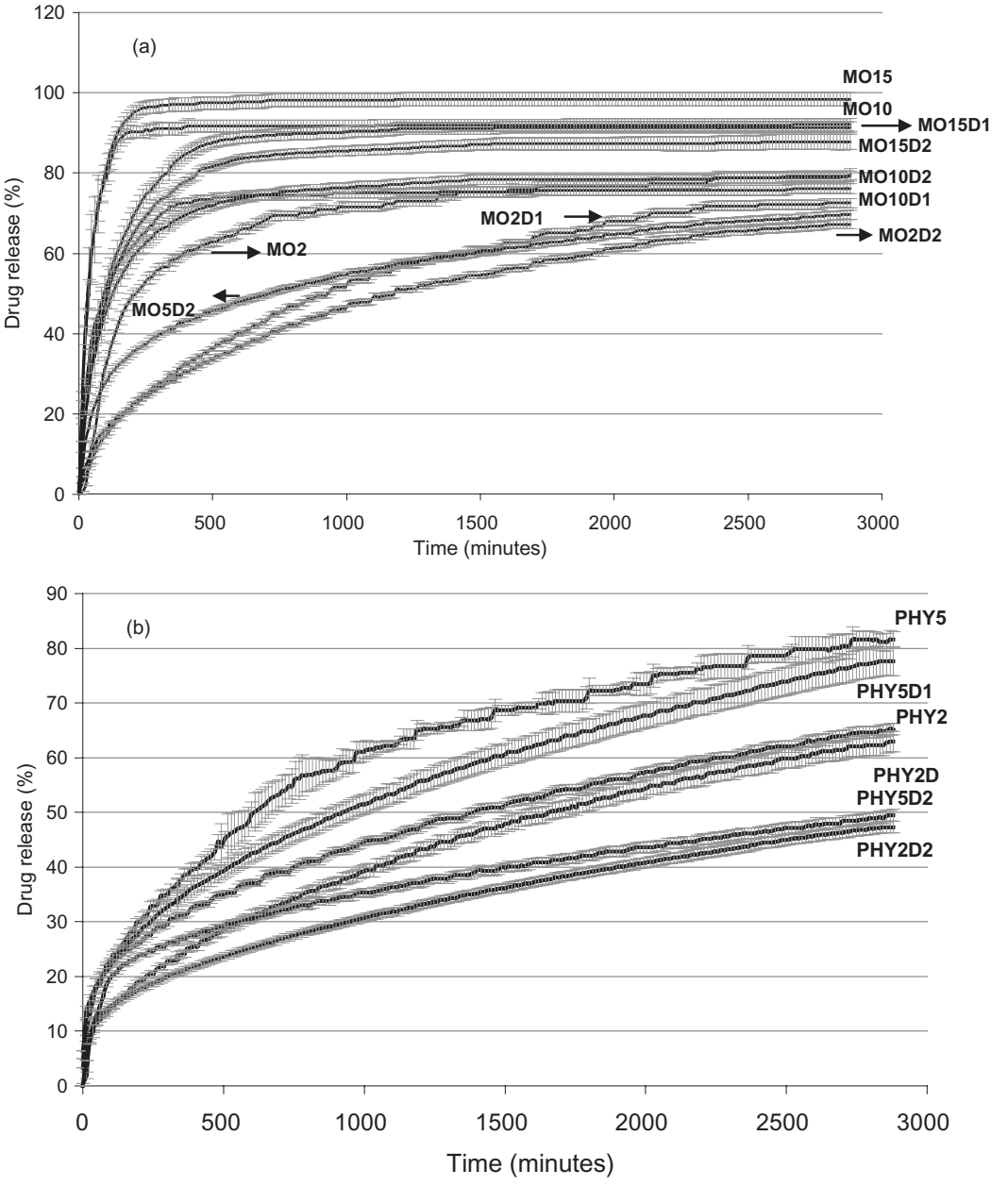

Figure 5. Dose percentage of PHCl released in 48 hours from MO (a) and PHY formulations (b).

to get a molecular understanding of the experimental observations. PHCl contains a hydrophobic moiety and hence has an amphiphilic nature, and it has also been reported to self-associate in aqueous solutions to form aggregates with an aggregation number of 10[30]. Bearing this in mind, the partitioning of the drug into the lipid bilayers is expected, which can explain the observed incomplete release within the time of the experiment. The release of drugs from lipid-based liquid crystalline systems is diffusion controlled[11,31], and when the formulation is intact a very slow release is expected[5,6]. This can also explain why we observed an incomplete release within the time of the experience in spite of the sink conditions used. Some authors[22] refer that the complexation of the cationic drug with oppositely charged fatty acids that might be present in the lipid samples (see 'Materials') can be responsible for the sustained and incomplete release from MO formulations. However, if this was the explanation for the sustained release, it should also be applicable to the formulations in the lamellar phase, which does not appear to be the case. As shown in Figure 5, the

formulations that are in the lamellar phase, without DO (e.g., MO15$_{L\alpha}$), show almost complete drug release over 48 hours.

The slow and incomplete release is more obvious for PHY formulations compared to the MO formulations (Figure 5b). All the PHY-containing formulations investigated are highly viscous reverse bicontinuous cubic phases of space group Ia3d. As was also observed for the MO formulations, the addition of DO presents a strong effect in both sustaining the release and retaining the drug. This strong effect might be related with the fact that these formulations have lower water content. The decrease in water content decreases the thickness of the aqueous channels in the bicontinuous structure and therefore decreases the release rate of the drug.

Figure 6 presents MDT values for MO (a) and PHY (b) formulations at different cumulative time points (2, 10, 24, 36, and 48 hours). MDT 48 hours, which represents the whole dissolution curve of each formulation, is also shown in Table 1.

The increase in the MDT values with time is much more evident when the formulation has a prolonged release, as we can see with the cubic phases, especially with PHY formulations and MO2D1$_{Ia3d}$ and MO2D2$_{Ia3d}$, but also with MO5D2$_{Ia3d}$ and MO2$_{Ia3d}$. Here, it is important to note that the phase (Table 1) refers to that

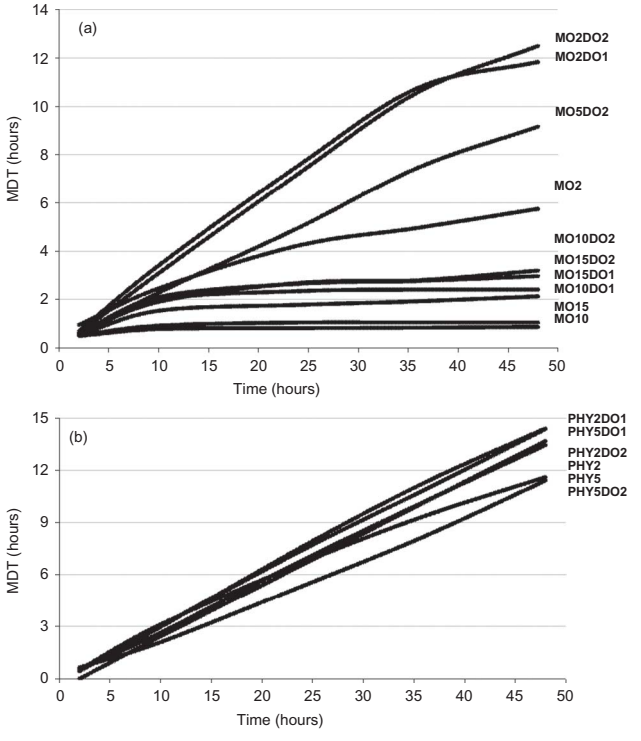

Figure 6. MDT values for MO (a) and PHY (b) formulations at different time points: 2, 10, 24, 36, and 48 hours.

of the formulation when the dissolution experiment starts. Because of uptake of water and release of drug, the composition of the formulation changes during the experiment, and this may also lead to phase transitions. This will be further discussed later in this section. Among the MO formulations we clearly see the sustained release effect of DO in the dissolution data in Figure 6. We also notice the fast release from MO lamellar phases (MO10$_{L\alpha}$, MO15$_{L\alpha}$, and the corresponding DO formulations) when compared to bicontinuous cubic phases. The lamellar formulations reach a plateau (dissolution end) after 10 hours, or even before, for those without DO (MO10$_{L\alpha}$ and MO15$_{L\alpha}$).

The data in Figure 6 can be used to compare the release from the different cubic phase formulations at different stages of the release process. After 10 hours, the MDT values of the PHY formulations are all higher than the corresponding values of MO formulations. Up to this point, we observe that, for example, PHY2D1$_{Ia3d}$ and PHY2D2$_{Ia3d}$ (Figure 6b) have lower MDT than MO2D1$_{Ia3d}$ and MO2D2$_{Ia3d}$ (Figure 6a), which reflects a higher dissolution rate. However, when the experiment is conducted for longer times, this effect is no longer present. The dissolution of these formulations, PHY2D1$_{Ia3d}$ and PHY2D2$_{Ia3d}$, reaches the highest MDT values, showing an alteration in release rate or dissolution mechanism. The opposite behavior is observed for the formulation PHY5D2$_{Ia3d}$ that starts with the slower release rate (higher MDT) and ends with the highest (lowest MDT). One possible explanation could be a change in the phase behavior of the formulation, when the respective composition changes, as also supported from the phase studies of formulations with different drug loadings (Table 1). The decrease in PHCl concentration upon dissolution could induce phase transformations between the Ia3d cubic phase and the Pn3m cubic phase or a hexagonal phase (Table 1).

A general observation is that the PHY formulations show a slower release (higher MDT) than the MO formulations. In what concerns release, we have observed that MO5D2$_{Ia3d}$ > PHY5D2$_{Ia3d}$ and MO2 >> PHY2$_{Ia3d}$ release is faster from MO formulations than from corresponding PHY formulations. However, the effect of DO may supersede the change of lipid, for example, PHY5$_{Ia3d}$ > PHYD1 > MO5D2$_{Ia3d}$.

Most of the release control seems to be determined by the lipid phase behavior, where the lamellar phase gives rise to faster release of the drug than the reverse cubic phase. The lamellar phase is also less viscous than the cubic phase. However, in the lamellar phases, the drug load is higher, and thus the gradient in the drug as driving force for diffusion is also higher. Despite the latter remark, note that MO15D2$_{L\alpha}$ >> MO5D2$_{Ia3d}$ ~ MO2D2$_{Ia3d}$, which seems to indicate that the change to

a lamellar phase is more relevant than the drug content. It has also been pointed out by other authors that changes in liquid crystalline phase structure, and hence in the dimensions of the internal aqueous domains, have an impact on the diffusivity of molecules through the matrix altering the drug release rate from liquid crystalline systems[5,6].

After 48-hour dissolution tests, the lipid liquid crystalline phases were characterized again with SAXS. This showed that, after dissolution, the formulations form the same phases as the blank drug-free formulations. In other words, the MO and PHY formulations that contain DO form a hexagonal phase after the dissolution test and the MO and PHY formulations without DO form the Pn3m cubic phases, which is the fully swollen cubic phase again after the dissolution test. The formation of these phases in excess of water, which is the final state of this experiment, is also consistent with the phase diagrams of MO and PHY[14,2]. This means that during this process the lipid/water systems suffer phase changes that may influence the rate and mechanism of drug release. Lamellar phases change into cubic phases, and the ones with DO also change to hexagonal phases (except MO10D1, MO10D2$_{L\alpha}$ that after 48 hours is still a mixture of phases, Ia3D and hexagonal) and there must be intermediate stages where the different phases coexist. The change in phase behavior during the release process can be predicted from the phases shown in Table 1, although the water content is lower for these cases. Also, the reverse Ia3d cubic phases change to the more swollen Pn3m cubic phase or to hexagonal phases (except PHY5D2$_{Ia3d}$ that after 48 hours is still a mixture of phases, Ia3d and hexagonal). These phase transition cannot be resolved from our PHCl release curves. We note that these exceptions to form after dissolution, the same phases as the blank drug-free formulations, are mainly related to incomplete release.

Fit factors

Table 3 shows fit factor values for the considered formulations compared with formulation MO2$_{Ia3d}$ taken as reference. Here we have considered the dissolution points up to 120 minutes plus the last point at 48 hours to follow the FDA and EMEA Guidelines concerning the inclusion of only one measurement after 85% dissolution for both products under comparison.

Generally, f_1 values up to 15 and f_2 greater than 50, which means an average difference of no more than 10% at the sample time points, ensure equivalence of the two curves under comparison. For our formulations these two factors together do not ensure equivalence of any of the dissolution curves. However, the difference factor f_1 was more sensitive in finding dissimilarity between the curves than the similarity factor f_2. In fact, if we only consider f_2, we have a performance equivalence between formulation MO2$_{Ia3d}$ and PHY5$_{Ia3d}$, PHY5D1$_{Ia3d}$, PHY5D2$_{Ia3d}$, and MO5D2$_{Ia3d}$. The effect of increasing drug load from 2% to 5% seems to have been compensated by changing MO for PHY despite a much higher viscosity of the PHY formulations (Table 1 and Figure 7). It is likely that other parameters like size and number of aqueous channels of the different formulations may also affect the release. This can also explain the burst effect in the initial release from PHY formulations (Figures 5 and 6). Considering the similarity between the MO2$_{Ia3d}$ and the MO5D2$_{Ia3d}$ formulations, we find that the effect that the addition of DO compensates for is the increasing drug load and thereby stabilizes the cubic phase by increasing the relative volume of the hydrophobic region. In this case both formulations present similar viscosity values.

When other formulations are compared (data not shown) we have equivalence, considering both f_1 and f_2

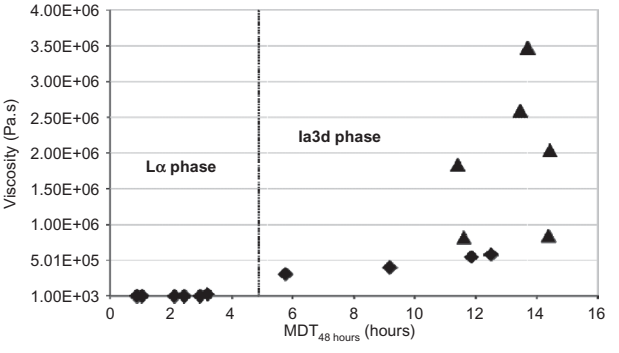

Figure 7. Formulation viscosity, η, versus MDT values at 48 hours for MO and PHY formulations. ◆, MO formulations; ▲, PHY formulations.

Table 3. Fit factors values for the considered formulations compared with formulation MO2$_{Ia3d}$ taken as reference.

Fit factors	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV
f_1	52.12	43.14	54.55	49.35	49.31	36.33	171.28	173.66	37.35	36.56	26.44	83.32	57.99	84.80	67.80
f_2	37.17	54.01	46.37	47.41	51.61	54.47	18.94	19.01	48.24	49.06	58.28	34.61	42.07	34.91	39.63

I = MO2/PHY2; II = MO2/PHY5; III = MO2/PHY2D1; IV = MO2/PHY2D2; V = MO2/PHY5D1; VI = MO2/PHY5D2; VII = MO2/MO10; VIII = MO2/MO15; IX = MO2/MO2D; X = MO2/MO2D2; XI = MO2/MO5D2; XII = MO2/MOD1; XIII = MO2/M10D2; XIV = MO2/M15D1; XV = M2/M15D2.

factors. This happens with the pairs $PHY2_{Ia3d}$ and $PHY5D1_{Ia3d}$, and $PHY2D1_{Ia3d}$ and PH5D2, where we can observe the effect of DO mentioned above; $PHY5_{Ia3d}$ and $PHY5D1_{Ia3d}$, $PHY2D1_{Ia3d}$ and $PHY2D2_{Ia3d}$, and MO2D1 and $MO2D2_{Ia3d}$, where the increasing of DO concentration does not alter the release. All these pairs of formulations also show similar values of viscosity.

Figure 7 presents formulation viscosity versus MDT values at 48 hours. Here we can see a clear relation between viscosity and MDT and therefore with the drug release rate. In general, with the exceptions for the cases discussed above, the formulations with highest MDT also present the highest viscosity values. PHY formulations are all bicontinuous cubic phases with space group Ia3d and so are the cubic phases in the MO formulations. However concerning viscosity and release rate they behave differently. In general, PHY formulations show a slower release (higher MDT) than MO formulations. The formulations that form a lamellar phase show the fastest release, and advantage was taken of this characteristic in a previous study on skin permeation from MO and PHY formulations[23]. While slow release is usually desired for drug sustained delivery, faster release is generally required for transdermal drug delivery.

Diffusion models

Several common models were applied using nonlinear least squares, based on the Marquardt algorithm. (i) Zero-order release model for constant release rates, $M_t/M_0 = c_1 t$; (ii) Korsmeyer–Peppas model for diffusion-based mechanism, $M_t/M_0 = c_2 t^{c_1}$; (iii) Hixson–Crowell model for erosion-based mechanism, $M_t/M_0 = c_2\,[1 - (1 - c_1 t)]^3$; (iv) first-order model for mass balance, $M_t/M_0 = c_2\,[1 - \exp(-c_1 t)]$. We have fitted these various functions for dissolution times up to 120 minutes and also for the curves attaining 70% or 80% of drug release. In the first case we consider only the ascending part of the curves; in the second case we remove the curves plateau for MO formulations and consider the full curves for PHY formulations, with the exception of $PHY5_{Ia3d}$ that exceeded 80% drug release. We also consider the full curves of MO2D1, $MO2D2_{Ia3d}$, and $MO5D2_{Ia3d}$ for the same reason. Note that the $MO10_{L\alpha}$ and $MO15_{L\alpha}$ curves considered up to 80% drug release become shorter than when we consider 120 minutes of release.

Both functions, first-order release and Korsmeyer–Peppas function, describe the profiles well. For 120 minutes, the first-order function presents the best fit to both lamellar and cubic phase formulation dissolution curves: $MOD1_{HII}$, $MO5D2_{Ia3d}$, $MO10_{L\alpha}$, $MO10D2_{L\alpha}$, $MO15_{L\alpha}$, $PHY2_{Ia3d}$, $PHY2D2_{Ia3d}$, and $PHY5D2_{Ia3d}$. For the other formulations, the best fit is given by the Korsmeyer–Peppas function.

The 70% and 80% drug release curves indicate, for MO formulations, a best fit to the first-order model, while for PHY formulations this is achieved with the Korsmeyer–Peppas function. The differences found between the two fittings (120 minutes and 70% and 80% curves) are probably because of phase changes that occur during drug release.

The first-order model has been associated with particular characteristics of the delivery system, which reflect to some extent a reservoir-type delivery system. Moreover, it was suggested that first-order kinetics could describe the release profile of water-soluble drugs, where they would be released at rates proportional to the amounts of drug remaining inside the dosage form[32].

The Korsmeyer–Peppas model function can be related to the drug diffusion mechanism. This is consistent with the behavior of our formulations, which are not destroyed; they do not suffer erosion or disintegration during the drug release, although they suffer phase transitions. The water-soluble drug diffuses through them into the surrounding liquid (water), and there is also a concomitant uptake of water and swelling of the liquid crystalline phases originating in the phase changes that we have discussed above. Drug diffusion through liquid crystalline phases was also found by other authors[6].

The parameter c_1 in the Korsmeyer–Peppas function is used to characterize different release mechanisms. If the c_1 value is 0.5 or less, the release mechanism follows Fickian diffusion, and higher values, $0.5 < c_1 < 1$, for mass transfer correspond to a non-Fickian model (anomalous transport)[33]. All PHY formulations present c_1 values between 0 and 0.5, indicating a Fickian diffusion mechanism, except the $PHY5D2_{Ia3d}$ that, with a value of 0.62, indicates anomalous transport.

The Hixson–Crowell function was not adequate to fit our profiles. The zero-order fit, although not producing the best least-squares fit, was close in some cases. These include the 120-minute curves of $MO2_{Ia3d}$, $MO2D1_{Ia3d}$, $MO2D2_{Ia3d}$, $MO15D2_{L}\alpha$, and $PHY5D2_{Ia3d}$. Zero-order release rate is considered ideal for sustained drug delivery as it allows constant drug plasma levels.

Figure 8 shows some of the results of the fitting procedure. Figure 8b presents a zero-order fit to the $MO2_{Ia3d}$ profile, which, despite the obvious lack of quality, helps to point an overall trend following that model. This would especially be the case if the first part of the curve, which represents some delay, would be discarded. This delay can be explained by the uptake of water in the initial phase, leading to the swelling of the formulation.

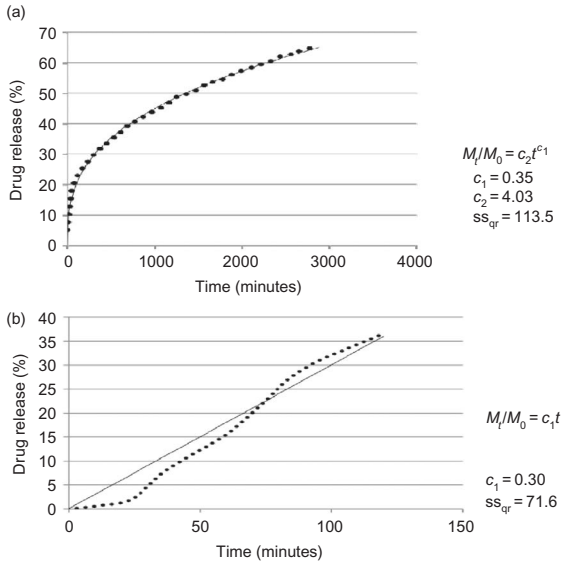

Figure 8. (a) Release curve of formulation PHY2 fitted with the Korsmeyer–Peppas function (solid line). (b) Release curve (120 minutes) of formulation $MO2_{Ia3d}$ fitted with the zero-order function (solid line); ss_{qr}, sum of squares.

Conclusions

The release of PHCl, a water-soluble drug, from formulations prepared with MO/water and PHY/water was studied and the dissolution profiles were related with the phase behavior and viscosity of the different crystalline phases. DO was added to these systems aiming to stabilize the cubic phases. The main findings are that DO can prevent the formation of less viscous phases, and for lower drug loads this substance stabilizes the MO cubic phases and retards release. In general, cubic phase formulations with higher viscosity present a slower drug release. It was also found that PHY is more resistant to a phase change upon drug load than MO and that PHY formulations present a slower drug release than MO formulations. Despite the sink conditions used, PHCl was not completely released from the formulations in particular from those that contain DO. From SAXS experiments, it was shown that the formulations exhibit transitions between different lyotropic phases with clearly different diffusion characteristics during drug release. This may also lead to deviations from the release models as these do not generally include such changes in formulation diffusion characteristics. The drug was partitioned into the lipid bilayers because of its amphiphilic nature. Regarding the release order and mechanism, the formulations showed first-order release rate and a diffusion release mechanism. Some formulations, mostly MO formulations, were close to zero-order release in the first 120 minutes of release. In some cases, the function that best describes the curve depends on the number of points considered in the fitting, which is probably because of phase changes that occur during drug release.

The final conclusion is that MO and PHY formulations show a good potential to be used in PHCl-sustained drug release.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this paper.

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