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Research Article

Liquid Crystalline Systems for Transdermal Delivery of Celecoxib: *In Vitro* Drug Release and Skin Permeation Studies

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Abstract. Liquid crystalline systems of monoolein/water could be a promising approach for the delivery of celecoxib (CXB) to the skin because these systems can sustain drug release, improve drug penetration into the skin layers and minimize side effects. This study evaluated the potential of these systems for the delivery of CXB into the skin based on *in vitro* drug release and skin permeation studies. The amount of CXB that permeated into and/or was retained in the skin was assayed using an HPLC method. Polarizing light microscopy studies showed that liquid crystalline systems of monoolein/water were formed in the presence of CXB, without any changes in the mesophases. The liquid crystalline systems decreased drug release when compared to control solution. Drug release was independent of the initial water content of the systems and CXB was released from cubic phase systems, irrespective of the initial water content. The systems released the CXB following zero-order release kinetics. *In vitro* drug permeation studies showed that cubic phase systems allowed drug permeation and retention in the skin layers. Cubic phase systems of monoolein/water may be promising vehicles for the delivery of CXB in/through the skin because it improved CXB skin permeation compared with the control solution.

KEY WORDS: celecoxib; drug delivery systems; liquid crystalline system; monoolein; skin permeation.

INTRODUCTION

Celecoxib (CXB) is a non-steroidal anti-inflammatory drug used in the treatment of various inflammatory conditions, including the symptomatic treatment of osteoarthritis, acute pain and rheumatoid arthritis. CXB also has chemopreventive activity against some types of cancer, such as ultraviolet B radiation-induced skin cancer and breast cancer. The activity of CXB is due to the selective inhibition of cyclooxygenase (COX-2) activity (1–3). Currently, CXB is marketed in a conventional capsule form for oral administration. However, toxic systemic side effects limit its long-term oral administration. Nevertheless, other routes of administration, such as skin delivery, have been explored (3–8). The application of CXB to the skin could be an interesting strategy for CXB delivery because skin delivery could increase the local drug concentration and reduce the risk of systemic toxicity. Skin delivery of a drug in a sustained release system also provides constant drug levels at a specific location for prolonged periods of time. In addition, this route of administration avoids first-pass metabolism and minimizes side effects because the drug is applied directly at the site of inflammation (2,9–11). However, skin drug permeation can be a limiting step for skin delivery for many drugs because the organized structure of the stratum corneum is a highly effective barrier (9,10). Some strategies to decrease the effectiveness of the skin barrier function and improve drug delivery to the skin require the use of penetration enhancers applied on the skin or the use of proper carriers (2,8,12,13). The skin delivery of CXB has been studied using several drug carriers, such as micro- and nanoemulsions, nanostructured lipid carriers, and cyclodextrins (2,4–7). However, a promising drug delivery system that could be useful for this purpose is lyotropic liquid crystals, which increase the diffusion coefficients of drugs through the skin (9,10,12).

Liquid crystals are systems that can be formed by polar lipids that, when in contact with water, spontaneously reorganize into three-dimensional structures (named liquid crystalline phases) that can be used for drug delivery (14).

Monoolein is a natural polar lipid that swells in water and forms various phases of lyotropic liquid crystals. It is also non-toxic, biodegradable and biocompatible. Liquid crystalline systems of monoolein and water can incorporate both hydrophilic and lipophilic substances, and these systems have been studied as sustained delivery systems for several drugs (9,14–24).

Several liquid crystalline phases of monoolein and water with different properties may be formed depending on their water content, temperature and the presence of a drug or additive. The incorporation of molecules with different polarities may lead to phase transitions due to changes in the packing

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parameters of the liquid crystalline systems (18,21,23,25). The effect of a molecule on the phase behavior of the systems should be evaluated in the development of a drug delivery system because the structure of the system can influence the drug release profile (15,21,26).

Monoolein is also a skin permeation enhancer because it improves drug partition on the skin *via* the promotion of ceramide extraction and the enhancement of lipid fluidity in the stratum corneum. Liquid crystalline systems of monoolein and water have been proposed as drug delivery systems for the skin delivery of drugs because they can control drug delivery and increase skin permeation due to the permeation enhancer properties of monoolein. Liquid crystalline systems of monoolein and water are reported to increase the skin permeation of lipophilic compounds, such as CXB, and improve the bio-distribution of drugs in the skin (9,10,12,27–29).

Liquid crystalline systems of monoolein and water may be a promising approach for the delivery of CXB to the skin because these systems can sustain drug release, improve drug penetration into the skin layers and minimize the side effects associated with the oral administration of this drug. The present study evaluated the potential of liquid crystalline phases of monoolein and water for the delivery of CXB into the skin based on *in vitro* drug release and permeation studies.

METHODS

Myverol 18-99, a commercial grade of monoolein, was kindly offered by Kerry of Brazil (Campinas, São Paulo, Brazil). CXB (99.4%) was purchased from Exim Pharm International (India), Polysorbate 20 (Tween 20) from Merck and Synth. All other chemicals were analytical grade.

Preparation of Liquid Crystalline Systems

Liquid crystalline systems of cubic phase (monoolein/water ratio of 7:3) were prepared containing CXB at increasing drug loads (0.5, 1.0, 2.0, and 5.0% (*w*/*w*)). The saturation solubility of CXB in the systems can be estimated by an analysis of increasing drug loads, until the drug crystals can be observed microscopically in the systems (30).

The systems were prepared by melting monoolein at 40°C followed by the addition of the required amount of water at the same temperature. The CXB was dissolved in the molten monoolein prior to the addition of water. The resulting formulation was lowered to room temperature to equilibrate for 24 h.

In order to evaluate systems with different initial water contents which presents different liquid crystalline phases, lamellar and cubic phase containing 1.0 and 2.0 (*w*/*w*) of CXB were prepared at monoolein/water ratios of 9:1 and 7:3, respectively. These ratios were selected to obtain lamellar and cubic phases based on phase diagrams and phase behavior reviews of monoolein/water systems reported previously in the literature (14–16,23,24,31).

The identification of the obtained liquid crystalline phases was performed by the macroscopic aspect of the systems and polarizing light microscopy at 37°C using an Axioplan 2 Image microscope (Carl Zeiss, Oberkochen, Germany) fitted with a hot stage plate and an Axiocam HRc digital camera using automatic image acquisition.

In Vitro Drug Release Studies

In vitro drug release studies were performed in quintuplicate on a vertical diffusion cell with a surface area of 3.8 cm^2. The donor compartment was separated from the acceptor compartment by a FH fluoropore membrane (polytetrafluoroethylene, PTFE, 0.54 μm pore size, 47 mm diameter). The acceptor compartment was maintained at constant agitation (50 rpm) and temperature (37±0.5°C). Liquid crystalline systems with different initial water content (monoolein/water ratios of 9:1 and 7:3, lamellar and cubic phase, respectively) containing 2.0% *w*/*w* CXB were evaluated. A cubic phase system containing 1.0% (*w*/*w*) CXB was also evaluated. The same amount of CXB was dissolved in propylene glycol and used as a control solution. Samples of the liquid crystalline systems (0.5 g) were placed on the membrane surface in the donor compartment. The acceptor phase was 200.0 mL of 0.01 M phosphate buffer pH 7.4±0.2 containing 2.0% (*w*/*w*) Tween 20® to achieve *sink* conditions. Samples of the acceptor phase (3.0 mL) were taken periodically (0.5, 1, 2, 3, 4, 5, 6, and 24 h) and the amount of CXB released was assessed spectrophotometrically at 251 nm in a UV-VIS Femto 800 XI spectrophotometer. The determination of the release kinetics was conducted by linear regression analysis of the *xy* scatter chart, applying the zero-order kinetic model (amount of drug released *versus* time) and the Higuchi model (amount of drug released *versus* square root of time).

The release data were also modeled mathematically to describe the release mechanism by the method proposed by Rigter-Peppas (32):

$$\frac{M^t}{M_\infty} = Kt^n$$

where M^t/M_∞ is the fractional drug release, K is a kinetic constant dependent on the system, t is the release time, and n is a release exponent, which is indicative of the release mechanism from matrices of varying shapes and swelling or non-swelling systems. For moderately swelling systems (*i.e.*, equilibrium swelling ratio not greater than 1.33, equivalent to an increase in the volume of 25.0%), a value of 0.5 indicates Fickian diffusion in which the drug is released *via* usual molecular diffusion through the system. A value between 0.5 and 1.0 indicates anomalous transport in which some influence of swelling and/or erosion occurs. A value of 1.0 indicates a Case II relaxational mechanism, which is associated with the stresses and state-transitions that occur during the swelling (32,33).

In Vitro Skin Permeation Studies

In vitro skin permeation studies were performed in the same vertical diffusion cell described before using skin samples from the dorsal region of porcine ears as the biological membranes. Fresh porcine ears were obtained from a local slaughterhouse (Frigorífico Olhos d'água, Ipuã, SP, Brazil) and immediately cleaned with running water. The skin samples were carefully dissected for the maximum removal of the subcutaneous tissue and fat, dermatomized to obtain a uniform tissue thickness of approximately 500 μm (Dermaton Nouvag, Switzerland) and stored at −18 to −20°C for a

maximum period of 30 days. Before the experiment, the skin samples were thawed and mounted on diffusion cells (surface area of 3.8 cm) with the stratum corneum facing the donor compartment and the dermis facing the acceptor phase. Liquid crystalline systems of cubic phase containing 1.0% (*w*/*w*) CXB (0.5 g) and a solution of CXB (0.5 g) in propylene glycol at the same concentration (used as control) were applied to the surface of the skin in the donor compartment. The acceptor phase was 200.0 mL of 0.01 M phosphate buffer pH 7.4±0.2 containing 2.0% *v*/*v* Tween 20®, which was stirred at 50 rpm and maintained at 37°C to keep the skin surface at 32°C. Samples from the acceptor phase (3.0 mL) were taken after 24 h, and the amount of CXB permeated through skin was quantified using HPLC (11).

In Vitro Skin Retention Studies

At the end of the *in vitro* skin permeation study, the skin samples were taken from the donor compartment, rinsed to remove the excess formulation and dried with filter paper. The permeation corresponding area was used to evaluate CXB retention in the skin layers. The stratum corneum (SC) was separated from the remaining skin layers epidermis and dermis (EP+D) by the tape-stripping technique, using 15 adhesive tapes (3M, Scotch Book Tape no. 845; 3 M, St. Paul, MN, USA) with the first tape being rejected. Both stripping tapes (SC) and the remaining tissue EP+D were used for CXB extraction and quantification by HPLC according to a validated method (11). For the CXB extraction from the SC and EP+D samples, aliquots of 5.0 and 3.0 mL, respectively, of the extractor solution (methanol/water 72:28 *v*/*v*) were added, followed by incubation in an ultrasound bath for 15 min and centrifugation at 2,500 rpm for 3 min. The supernatant was filtered, and the amount of CXB was assayed using HPLC. The *in vitro* permeation/retention studies were performed in four replicates.

Analytical Methodology

The CXB permeated/retained in the skin was assayed using an HPLC method proposed by Praça *et al.* (11). The HPLC equipment used was a Shimadzu system (Kyoto, Japan) equipped with 2 LC-10AD pumps and a SPD-10A/10AV UV detector operating at 251 nm. The mobile phase was methanol–water (72:28 *v*/*v*) previously degassed with helium gas. The chromatographic column was an RP-C18 (10 cm length, 5 μm, 4 mm id LiChrospher; Merck, Darmstadt, Germany), and the flow rate was 0.8 mL/min. The running time was 5 min, and the injection volume was 20 μL.

Results are reported as mean±S.D. Data were statistically analyzed by *T* test. Values were considered significant for CXB retention from the control solution between the SC and EP+D (***$p<0.001$) and for CXB retention in the SC from the control solution and the cubic phase gel (*$p<0.05$).

RESULTS

Identification of Liquid Crystalline Systems

The identification of the liquid crystalline phases by polarizing light microscopy showed that the addition of CXB at the loads studied did not disrupt the liquid crystalline structure of the monoolein/water systems because cubic phases were obtained in the systems. The microscopic results showed that the cubic phase systems containing 0.5 and 1.0% (*w*/*w*) CXB solubilized the drug regardless of the observation that, at higher loads, the presence of drug crystals indicated that saturating concentrations of the drug had already been reached. Drug solubility and the presence of suspended drug in the systems can influence the drug release properties (30).

The evaluation of systems with different initial water contents showed that lamellar and cubic phases were obtained in the presence of CXB. Typical textures of the lamellar phase systems are shown in Fig. 1. The cubic phase is isotropic under polarized light microscopy, and may be identified by the isotropy and its particular macroscopic aspect. The systems were also evaluated using polarizing microscopy after 7 and 14 days, and no changes in the mesophases were observed in the cubic phase. However, the lamellar systems showed minor liquid crystalline structures at 14 days (data not shown). These results suggest that the cubic phases containing CXB were more stable than the lamellar phase.

In Vitro Drug Release Studies

In vitro drug release from the monoolein/water systems with different initial water contents was studied. Figure 2 presents the cumulative amount of CXB released as a function of time from the monoolein/water systems with different initial water contents: monoolein/water ratios of 9:1 and 7:3 containing 2.0% (*w*/*w*) of CXB, which represented the lamellar and cubic phases, respectively. The amount of CXB released was very similar for both systems studied, which indicates that the initial water content did not affect the drug release profile. After drug release studies, the systems were submitted to phase identification (polarizing light microscopy and macroscopic aspect) and both systems presented cubic phase. Figure 2 also shows that both liquid crystalline systems showed a lower release compared with the control solution. The fraction of CXB released from the lamellar and cubic phases after 24 h were 30.5 and 30.4% of the amount of the drug applied, respectively, while the control solution released 80.8% of the total drug amount. These results show that the studied liquid crystalline systems decreased drug release, as expected.

In vitro drug release studies were also performed using monoolein/water cubic phases containing 1.0 and 2.0% (*w*/*w*) CXB, which represented the drug dissolved and suspended, respectively. The cubic phase was chosen because it is almost completely hydrated, and stable in the presence of CXB at these concentrations. Figure 3 presents the drug release data expressed as a percentage of the CXB released as a function of time. The release profiles of the control solutions containing 1.0 and 2.0% CXB were similar, and the fractions of CXB released after 24 h were 81.12 and 80.8%, respectively. The fractions of CXB released at 6 h were similar for the liquid crystalline systems that contained both drug dissolved (1.0% *w*/*w* CXB) ou suspended (2.0% *w*/*w* CXB). However, the fractions of CXB released from the cubic phase systems after 24 h revealed a reduced release with the higher drug load.

The *in vitro* release data were analyzed to describe the release mechanism and the mathematical models used

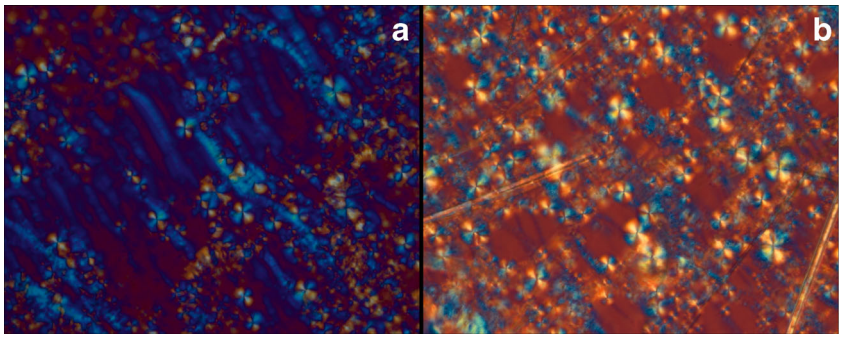

Fig. 1. Photomicrographs of the monoolein/water lamellar systems containing 1.0 and 2.0% (*w*/*w*) CXB (**a** and **b**, respectively)

indicated a zero-order release. Although the monooelin/water systems had shown a diffusion control release (Fickian release) for several drugs (30) the correlation coefficients obtained for both studied systems fit better with zero-order model kinetics (0.9907 for cubic phase system with 1.0% *w*/*w* of CXB and 0.9625 for cubic phase system with 2.0% *w*/*w* of CXB). No evidence of matrix erosion was observed during the *in vitro* drug release studies. The release mechanism of the systems was also evaluated using the method proposed by Rigter–Peppas (32) in which the value of the release exponent (n) is calculated, and it is indicative of the release mechanism for matrices of varying shapes and swelling or non-swelling systems. The release exponents (n) obtained in this study were higher than 1.0 (1.7374 for cubic phase system with 1.0% of CXB and 1.3649 for cubic phase system with 2.0% *w*/*w* of CXB), which suggests that Fickian diffusion did not control drug release. A value of $n=1$ means that the drug release is independent of time, and therefore, a zero-order release can be achieved (32).

In Vitro Skin Permeation/Retention Studies

The *in vitro* skin permeation/retention results are presented in Fig. 4. The amount of CXB retained in the SC and EP+D from the cubic phase systems after 24 h were 39.00 and 60.62 μg/cm^2, respectively. The cubic phase systems also promoted CXB permeation through the skin. No drug permeation from the control solution was observed within the lower detection limit of the analytical HPLC method used. These results are interesting because they show that CXB released from the cubic phase system reached deeper skin layers (EP+D) instead of being retained exclusively within the SC. The control solution showed a similar retention rate in the EP+D (60 μg/cm^2) compared with the cubic phase, and a significant difference (***$p<0.001$) in the amount of CXB retained in the SC was observed (22.07 μg/cm^2). However, as previously indicated, no drug was found in the permeate solution. In this case, the whole amount of the drug that entered into the skin was retained in the SC and EP+D. However, drug retention in the SC and the EP+D as well as skin permeation was observed using the cubic phase system.

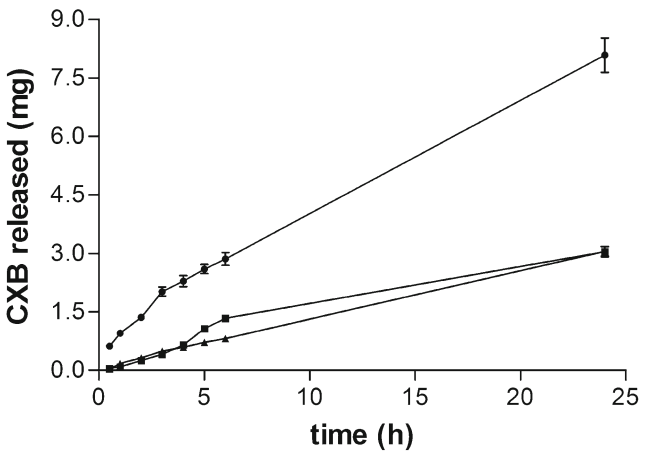

Fig. 2. Cumulative amount of CXB released as a function of time for monoolein/water systems with different initial water contents: monoolein/water ratio of 9:1 (*black triangle*) and monoolein/water 7:3 (*black square*) *w*/*w* and a solution of CXB in propylene glycol (*black circle*). All the systems contained 2.0% *w*/*w* CXB ($n=5$)

DISCUSSION

Liquid crystals are interesting systems to be used as vehicles for lipophilic drugs, such as CXB, because these drugs can be easily dissolved in the lipid domain of the system and further released through the system. The identification of the liquid crystalline phases formed using monoolein/water systems in the presence of drugs is fundamental to the development of drug delivery systems based on liquid crystals because the structure of the mesophase presented by the system can influence drug release. The addition of drugs or additives to liquid crystalline systems can modify the phase structures and system properties, which may influence drug release profiles (20,21,26,34,35). Several authors have reported altered properties in the liquid crystalline phase of monoolein/water systems due to the addition of drugs and solvents (18,20,25,34,36–39). The presence of a drug can affect the curvature of the lipid bilayer and change the average area of the lipid head group, which changes monoglyceride molecular packing and promotes a phase transition (18,23). The influence of a drug on the liquid crystalline structure depends on its location in the liquid crystalline systems (*i.e.*, the lipidic or aqueous domain of the system). Generally, hydrophilic drugs favor lamellar phases, and non-polar solutes, which partition strongly into the lipid phase, tend to favor the formation of inverted non-lamellar phases (16,23,24). No changes in the liquid crystalline phases were found in this study due to the presence of CXB, which indicates that these systems can be used as a vehicle for CXB administration with no damage to the system properties.

As mentioned above, drug release from liquid crystalline systems can be altered by the mesophase structure in the

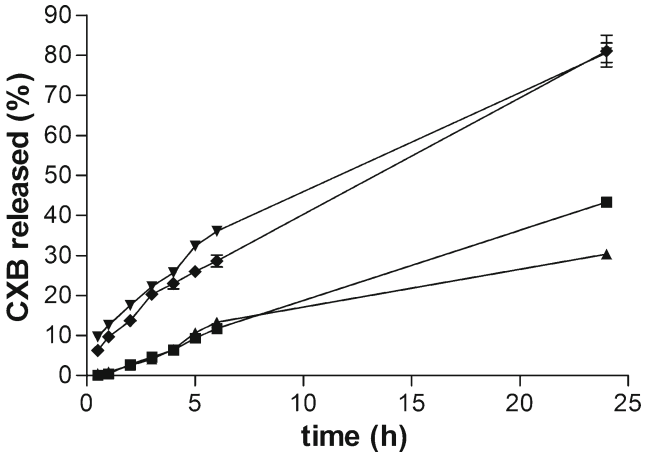

Fig. 3. Drug release profiles of monoolein/water cubic phase systems containing CXB at drug loads of 1% *w/w* (*black square*), 2.0% *w/w* (*upward-pointing triangle*) and solutions of CXB in propylene glycol at drug loads of 1% *w/w* (*downward-pointing triangle*) and 2.0% *w/w* (*black diamond*). (n=5)

systems, which, in turn, is dependent on the initial water content. The amount of CXB released was very similar for the two systems studied (lamellar and cubic phase systems with different initial water contents), which indicates that the initial water content did not affect the drug release profile. Monoolein absorbs water in the presence of excess water until its maximum water content is reached (approximately 40.0% *w/w*), after which it adopts a cubic phase. It is suggested that this water uptake is rapid and that drug release occurs primarily from the cubic phase formed (40). The liquid crystalline systems evaluated in this study showed rapid water uptake and the very quick transition to the cubic phase. Therefore, CXB was released from the cubic phase system, irrespective of the initial water content. Similar results were obtained by Burrows *et al.* (30), Geraghty *et al.* (41), and Rizwan *et al.* (42). However, some authors have reported differences in drug release from liquid crystalline systems with different initial water contents (43,44). These differences may be attributed to an increase in the hydrophilic channels available for the release of the drug because the water content increases, and therefore, more swollen matrices provide a more rapid diffusion of the drug (42,43). Systems with higher water content have an increased water layer thickness, which increases the diffusivity and release of water-soluble drugs. These contrasting findings on the effects of the initial water content on the release properties of the systems may be related to the drug partitioning between the monoolein and aqueous phases (29), and the initial water content of the systems can increase or decrease the apparent diffusion coefficient, depending on the drug partitioning in the system.

The studied liquid crystalline systems decreased drug release, as expected. Cubic phases are reported to provide a slow release matrix for the incorporated drugs, and therefore, they are largely used as drug delivery systems for several routes of administration (19,20,23,40). This liquid crystalline phase is thermodynamically stable and macroscopically stiff, and it consists of one congruent lipid bilayer that extends in three dimensions, surrounded by water channels (9,14). These water channels are formed because the lipids, in contact with water, form curved non-intersecting bilayers organized to form a continuous system of water channels (14,15,20). This structure consists of a complex matrix containing lipidic and aqueous domains (curved lipid bilayers and interconnected water channels), which can modulate the release of drugs due to its complexity (19).

Drug release was also studied with cubic phases systems containing CXB dissolved (1.0% *w/w*) and suspended (2.0% *w/w*). It is reported that drug solubility and the presence of suspended drug in the system can influence drug release properties. Drug release from systems containing suspended drugs involves the dissolution of suspended drug in the systems as dissolved drug is released in addition to the processes of diffusion of dissolved drug through the channels of the system and finally the transfer of drug to the release medium (30). The release profiles obtained until 6 h for both systems were similar, but at 24 h CXB release decreased in the higher loaded system. Kumar *et al.* 2004 (16) reported that drug release increases with an increasing drug load for a polar drug

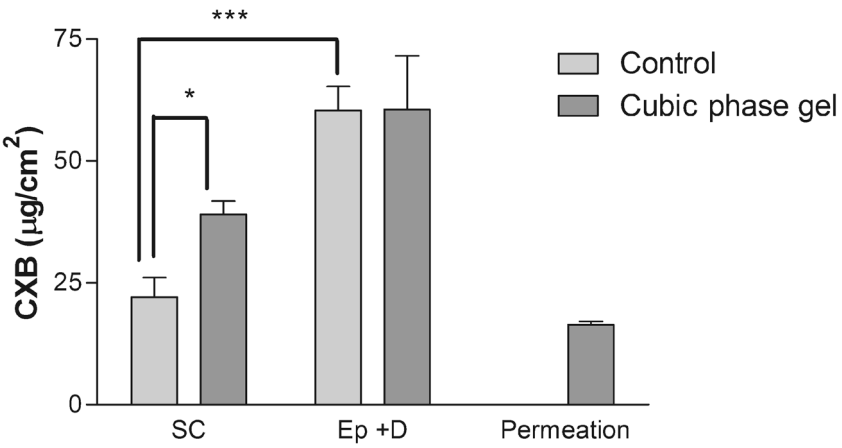

Fig. 4. Amounts of CXB retained or permeated (μg/cm^2) in the SC and [EP+D] from the control formulation or the cubic phase system after 24 h. Statistical analysis: *T* test. Values are considered significant for CXB retention from the control solution between the SC and EP+D (***p<0.001) and for CXB retention in the SC from the control solution and the cubic phase gel (*p<0.05)

but decreases for a non-polar drug. This concept is consistent with our results (Fig. 3), considering that CXB is a highly lipophilic molecule. Bertram and Bodmeier (45) studied drug release from polymeric inserts and also found decreased release with increasing drug loading for some drugs. This result was attributed to the interaction of the drug with the charged polymer formation of a poorly soluble drug-polymer salt, and therefore, the release was determined by the local solubility of the drug. This interaction led to a zero-order release. The same study observed that drugs that do not interact with the polymer and form a poorly soluble complex showed a drug release independent of the drug load.

The release profiles of several drugs from monoolein/water systems were reported as a linear function with the square root of time, which indicates a diffusion-controlled release (25,31,39–43). However, when the dissolution of a drug is the rate-limiting step for drug release, the release data can follow zero-order kinetics (30). Dissolution in the penetrating release solution is very slow for poorly soluble drugs, and only the dissolved drug diffuses out of the matrix through the matrix channels. This result has been described for poorly soluble drugs with zero-order release, but the release followed the square root of time kinetics for more soluble drugs (*i.e.*, diffusional release) (46). The release data obtained in this study showed that the drug release which followed zero-order kinetic and this result can be related to poor CXB solubility.

In vitro skin permeation/retention studies were performed to evaluate the potential of liquid crystalline systems of monoolein and water for the delivery of CXB into the skin. CXB is highly lipophilic (log*P* value of 4.21), and could be retained in superficial skin layers (1). Very lipophilic drugs enter into the stratum corneum, but these drugs remain in this superficial layer and do not reach the deep layers of the skin.

The use of a drug delivery system based on monoolein should be more promising for drug delivery to the skin due to the penetration-enhancer properties of this polar lipid. Monoolein/solvent systems are effective penetration enhancers for lipophilic drugs and highly polar compounds, most likely acting through a reversible disruption of the ordered lamellar structure of the stratum corneum bilayers, as well as increasing the lipid fluidity of the stratum corneum. It is also reported that monoolein enhances the skin penetration of several compounds, such as vitamin K, cyclosporine A, and aminolevulinic acid (9,10,12,17,28) . We selected the cubic phase of monoolein and water containing 1.0% (*w*/*w*) CXB for the *in vitro* permeation studies because this system appeared to dissolve the drug and sustain CXB release compared with the control. The cubic phases of monoolein and water improve the topical/transdermal delivery of several drugs. It is also reported that monoolein cubic phases are superior for the delivery of drugs to the skin compared with commercial ointments and water solutions, and they promote a different distribution pattern of the drug in skin layers (9).

The *in vitro* skin permeation/retention results are presented in Fig. 4. Cubic phase systems showed skin CXB retention in the SC and EP+D after 24 h and some CXB permeation through the skin, but no drug permeation was observed from the control solution. These results are interesting because they show that the CXB reached deeper skin layers (EP+D) instead of exclusive retention in the SC. Moreover, CXB permeated through the skin. These results suggest that the cubic phase favored the transdermal delivery of CXB. Both formulations had higher retention in the EP+D compared with the amount of CXB retained in the SC. However, the whole amount of the drug that entered into the skin was retained in the SC and EP+D in the control solution. In contrast, drug retention was observed in both the SC and the EP+D using a cubic phase system, as was skin permeation.

More CXB was retained in the SC from the cubic phase compared with the control solution ($p<0.05$). Otherwise, similar amounts of the drug were found in the EP+D layers from both the cubic phase system and the control solution ($p>0.05$). The results suggest that some of the drug that had reached the EP+D from the cubic phase systems permeated the skin, while the whole amount of the drug that entered into the skin was retained in the skin layers in the control solution. It should also be considered that the amount of drug released from the cubic phase system evaluated in skin permeation/retention studies (containing 1.0% *w*/*w* CXB) at 24 h was 40.0% (Fig. 3) of the total amount of the drug present in the system, once this system was able to sustain drug release. Therefore, the amount of the drug available for penetration into the skin from the cubic phase system at 24 h was small compared with the control solution, which suggests that cubic phase systems can provide drug to the skin for a longer period of time. These results suggest that the cubic phase system favored the transport of CXB through the skin layers considering that the amount of the drug released from the cubic phase was limited by the system (and could be further released) and that this formulation provided greater CXB retention in the SC, a similar retention in the EP+D compared with the control solution and some drug permeation.

This result corroborates the effects of the cubic phase system in affecting the skin barrier to increase CXB permeation. The effect of monoolein on disrupting the SC structure may decrease its barrier properties because CXB reached the deep layers of the skin. Regardless, the amounts of CXB that permeated after 24 h in the cubic phase system were smaller than the amounts of the drug retained in the skin layers. However, the SC and EP+D can act as a depot for the continuous and sustained transdermal delivery of CXB. Regardless, this study evaluated the cubic phase systems containing CXB in direct contact with skin, and the performance of this liquid crystalline system for drug delivery into skin was described. However, this system, which may improve CXB skin delivery, can be also incorporated into suitable transdermal systems and evaluated. Further studies will be performed to examine transdermal drug delivery systems containing the liquid crystalline system described in this study.

CONCLUSIONS

Liquid crystalline phases of monoolein and water can be obtained in the presence of CXB at the loads studied, and the drug had no effect on the phase behavior of the systems.

Our results confirm the use of the cubic phase of monoolein/water as a drug release system for CXB. The drug release was independent of the initial water content and followed zero-order kinetics.

Cubic phase systems of monoolein and water are promising vehicles for the delivery of CXB in/through the skin because our results show a facilitation of skin permeation compared with the control solution.

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Conflict of interest The authors report no declarations of interest.

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