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SEMISOLIDS SYSTEMS ON A LIQUID-CRYSTALINE STRUCTURE BASE FOR THE PROLONGED RELEASE OF LIPOSOLUBLE ACTIVE INGREDIENTS.

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ABSTRACT

In the present work we set as objective to investigate the diffusion in vitro over 48 h using Franz cells of a liposoluble active principle, Econazole (ECZ), incorporated in emulsion prepared by conventional techniques and in emulsions with liquid crystalline structure. The concentration release of ECZ was determined by high performance liquid chromatography (HPLC), using a mobile phase of methanol water (50:50) on a gradient elution and a column of phase reverse C18 was used for the chromatographic separation. The conventional emulsions exhibits a kinetic liberation that adjusts to the Higuchi model during the 48 h of study, while the emulsions with liquid crystalline characteristics adjusts to a kinetic liberation of order zero in the firsts 8 h of study, such release is associated with the ECZ dissolved outside of the liquid crystalline structure, and then with the disintegration of the multilayer from the liquid crystalline, the ECZ incorporated in their structure is released, allowing a controlled release.

KEYWORDS: Liquid crystals, econazole, prolonged release, emulsions.

INTRODUCTION

It is of utmost important in the drug industry and research and development the study of active principle through prolonged release whose two most important aspect are the action site and rhythm with which the active ingredient is supplied. In actuality there are many products of prolonged release to be administered via oral and injectable but not for topical application. In the pharmaceutical forms of sustained release the limiting step for the absorption is the release of the principle active from the pharmaceutical form. To achieved a therapeutic level and sustain for a period of time, the formulation must supply an initial loading dosis that release the drug immediately of being applied and a maintenance dose or prolonged delivery.[1]

Sustained release medications share the objective of improving in the supply of pharmaceutical active regarding conventional medication. The most important reason for these drugs is to improve treatment efficiency and reduce the frequency of administration.

Skin is a stratified organ composed of epidermis, dermis and the subcutaneous adipose layer. In the skin surface there are keratinized cells forming the stratum corneum, which is covered by a layer of emulsified lipids. The skin is continually submitted to aggression of physical and chemical agent from the external environment. It is a physiological barrier against the penetration of gases, liquid or solids that are part of the environment and the loss of the constituents of our body. In spite of it, the skin could be slightly permeable to chemical substances so it is possible to use a topical way for the administration of medication and to achieve local or systemic therapeutic effects, especially when carriers and vehicles suitable are used to facilitate drug penetration. The determination of the pharmacological activity of preparations for application on the skin highlights the importance of the excipients in the process of release and absorption of the active principles. Through an adequate choice of excipients, the bioavailability of drugs could be modulated and thus achieve a conventional medication or sustained release.[2-3]

The conditions for the percutaneos penetration and absorption of drugs depends mainly on their physicochemical characteristics. Penetration can occur by diffusion through the stratum corneum, either via intercellular or intracellular, or by passage through the ducts of the sweat glands or the pilosebáseas structures. Most chemical molecules are absorbed through the skin by passive diffusion.

Lyotropic liquid crystals are suitable as sustained release of active ingredients, due to the high viscosity and the ability to dissolve both liposoluble and hidrosoluble drugs. In the case of our formulation, liposoluble drugs are accommodated between the hydrocarbon chains of stearic acid and soluble in the polar zone stearate triethanolamine.[4]

The objective of this work was to investigate the liberation, in vitro, at different times an liposoluble principle active incorporated into a conventional emulsion and into a emulsion with liquid crystals structure. A formulation consisting of stearic acid, liquid petrolatum, triethanolamine, methyl paraben, propyl paraben and water was used, and Econazole (ECZ) was incorporated as an active ingredient model, which is a drug with antifungal activity for topical use, imidazole derivative, its chemical formula is $C_{18}H_{15}Cl_3N_2O.HNO_3$, its molecular weight is 318.68 g/mol, besides is slightly soluble in water and is soluble in oils.[5]

Like other imidazole derivatives (bifonazole, ketoconazole), ECZ inhibits ergosterol biosynthesis and other esters of the fungal cell membrane disrupting its structure and function. When applied topically, much of the drug remains on the skin surface in the stratum corneum concentrated, which reaches a high inhibitory concentration to destroy dermatophytes, tricófitos and ringworm.

The forming technique of liquid crystalline structures involves adding the oil phase to a part of the aqueous phase forming a concentrated of liquid crystals and then dilute with the remaining water. In a previous work by our group, the incorporation of ECZ in the emulsion with liquid crystals did not change the presence of extinction crosses that identifies. The proposed formula was found to be a plastic system with thixotropy, and showed good stability, even when the active ingredient was incorporated and a slightly decreased of the viscosity was detected in both the conventional method and the method prepared with liquid crystals. Rheological profiles proves to have adequate values of viscosity and thixotropy for the formulation of a dermatological emulsion.[6]

METHODOLOGY

The raw materials Stearic acid, Liquid Petrolatum, Propylparaben and Methylparaben quality Pharmacopeia Argentina were acquired in commercial drug stores with manufacturer test protocols. The Econazole nitrate used, manufactured by Sharon Bio-Medicine Ltd, was USP grade. To prepare the samples freshly distilled water was used. Analytical reagents used to perform release studies and quantitation by HPLC were: methanol HPLC grade, distilled

water and purified with Milli-Q systems, Propylene Glycol pro analysis, Disodium Phosphate and Monosodium Phosphate HPLC grade.

Composition systems: Four systems were prepared (Table 1): two were prepared by conventional systems (Standard 1 and Sample 1) and the remaining two with liquid crystals formation technique (Standard 2 and Sample 2).

Table 1: Composition of the 4 systems.

	Standard 1	Sample 1	Standard 2	Sample 2
Stearic acid	15,00 g	15,00 g	15,00 g	15,00 g
Liquid Petrolatum	20,00 g	20,00 g	20,00 g	20,00 g
Propylparaben	0,03 g	0,03 g	0,03 g	0,03 g
Triethanolamine	4,14 g	4,14 g	4,14 g	4,14 g
Methylparaben	0,07 g	0,07 g	0,07 g	0,07 g
Econazole Nitrate	–––	1,00 g	–––	1,00 g
Water	60,76 g	59,76 g	60,76 g	59,76 g

Preparation technique: Standard 1 and Sample 1 were prepared by conventional technique, the aqueous phase consisting of methyl paraben, triethanolamine and distilled water was added to the oil phase, consisting of liquid petrolatum, stearic acid and propylparaben (both at a temperature of 70ºC) with stirring until the formation of the emulsion.[6]

Standard 2 and Sample 2 were prepared by a technique of liquid crystals formation [7], the working temperature was 70ºC. The stearic acid, the liquid petrolatum and the propylparaben were placed into a stainless steel capsule (oil phase), after been melted they were added to the aqueous phase formed by triethanolamine, methylparaben and part of the water (20ml), stirring the system mechanically. Four-bladed agitator was employed at a speed of 50 rpm. Once homogenised the system, it was diluted with the remaining water and increasing gradually the agitation speed from 50 rpm up to 500 rpm and this speed was maintained for 10 minutes.[6]

Econazole nitrate were incorporated to the Sample 1 and 2 as an principle active by dissolving it in part of the aqueous phase of the initial preparation formed by the mixed of triethanolamine and methylparaben before the emulsification. With the alkaline pH of the solution the Econazole nitrate release the base and when mixed the aqueous phase with the oily phase, the ECZ, by diffusion coefficient and affinity, pass to the oil phase of both Sample (Sample 1 and Sample 2) and into the liquid crystals structure of the sample 2.[6]

Release: Diffusion studies were made in vitro, simulating in vivo conditions. Assay of release for both formulations were carried out in diffusion cells Franz [8], this consist of two compartment: the top for the release system and the lower for receiving medium (volume 15cm3, in our case), both separated by a semipermeable membrane. Artificial cellulose nitrate membrane (Sartorius) were used with pore size of 0.65µm and a contact surface or diffusion area of 3,14 cm^2.

For the study, 0,5 g samples were homogeneously distributed over the membrane in the donor compartment. As receiving medium, propilenglicol (70%) and buffer phosphate (30%, pH 7,4) were used, to ensure the sink condition during the assay. This solution was kept in constant stirring to avoid concentration gradients. The temperature of the compartments was maintained with a thermostatic bath at 32 ° C (± 0.5 ° C), which is the average temperature of the skin surface.[9]

We worked with Franz cells in parallel for: white, samples of conventional emulsion and emulsion samples with liquid crystalline characteristics, in triplicate.The test duration was 48 hours. In the pre-established times (0.5, 1, 2, 3, 4, 5, 6, 7, 8, 24 and 48 h) a 3ml aliquot was taken with subsequent refill of the medium. Drug was quantitated by HPLC.

Kinetic models used to characterize diffusion behavior: The active ingredient (ECZ) release from the systems studied can follow among others, any of the release kinetics detailed below.

- **Zero Order** (Ec.1) the release rate of the active ingredient from the releasing system to the receiving medium system is independent of the initial concentration of the active ingredient. This kinetic fits a straight line by plotting concentration vs. time.[10]

Equation 1: $-dc / dt = k$, where k is the constant for the order release zero.

- **First order** (Eq. 2): the release rate of the active ingredient from the releasing system to the receiving medium is proportional to its concentration, decreasing exponentially over time. This kinetic fits a straight line by plotting the logarithm of the concentration vs. time.[10]

Equation 2: $ln\ c = kt + cte$, where k is the constant release

- **Higuchi equation** (Eq. 3): Higuchi proposed a mathematical model widely used to describe the empirical process of drug delivery. This approach fits a straight line by plotting concentration of active ingredient released into the receiving medium vs. square root of time.[11]

Equation 3: $Qt / Q\infty = k.\ t^{1/2}$, where Qt is the amount of active substance released at time t, Q∞ is the amount released at infinite time, which is equal to the initial amount of drug and k is the Higuchi constant speed.

Quantitation by high performance liquid chromatography (HPLC)

The content of ECZ from the sample was analyzed by HPLC, using a chromatograph Hewlett Packard Series 1100 model equipped with a G1315A DAD detector. Chromatographic separation was performed on a C18 reverse phase column (Luna®). A calibration curve was performed with Econazole Nitrate standards for the range 0.050 mg / ml - 0.003 mg / ml, in methanol-water (50:50), adjusting to a line with r^2 of 0.9827. The measuring samples were prepared with a aliquot of the solution from the Franz cell diluted to the 50% with a mix of metanol-water (50:50). The movil phase was metanol-water, worked with a gradient elution with 57% methanol at zero minutes and was gradually increased to 72% at 6.5 minutes, then it increased to 98% at 10 minutes and remained in 98% methanol to 15 minutes, using a flow rate of 1.0 ml / min.[12] The detection was performed at 220 nm. Injected volume: 20 µl.

RESULTS AND DISCUSSION

Table 2: Release profile of conventional emulsion ECZ

Tpo (h)	Q (mg)	SD	Q/Sup	SD	%Q	SD
0.5	0.207	0.062	0.066	0.020	4.472	1.533
1.0	0.277	0.075	0.088	0.024	5.967	1.840
2.0	0.354	0.081	0.113	0.026	7.620	1.978
3.0	0.444	0.096	0.141	0.031	9.557	2.414
4.0	0.507	0.090	0.162	0.029	10.901	2.301
5.0	0.568	0.100	0.181	0.032	12.204	2.574
6.0	0.630	0.111	0.201	0.035	13.535	2.823
7.0	0.681	0.116	0.217	0.037	14.639	3.014
8.0	0.734	0.123	0.234	0.039	15.771	3.225

Q = amount of ECZ released at different times. Q / Sup = amount of ECZ released per unit area (mg / cm2). % Q = percentage of ECZ released to the receiving medium. SD = standard deviation of the data.

Table 2 details the profile liberation of the principal active (ECZ) from the conventional emulsion during the 48 h of the assay. Figure 1 represents the percentage of ECZ released, where the system proved to have a kinetic liberation that adjusts to the Higuchi model.[11-13] through the entire assay and with a square coefficient correlation (r^2) of 0.9969 (0 - 8 h) and 0.9411 (8 - 48 h), according to the table 4.

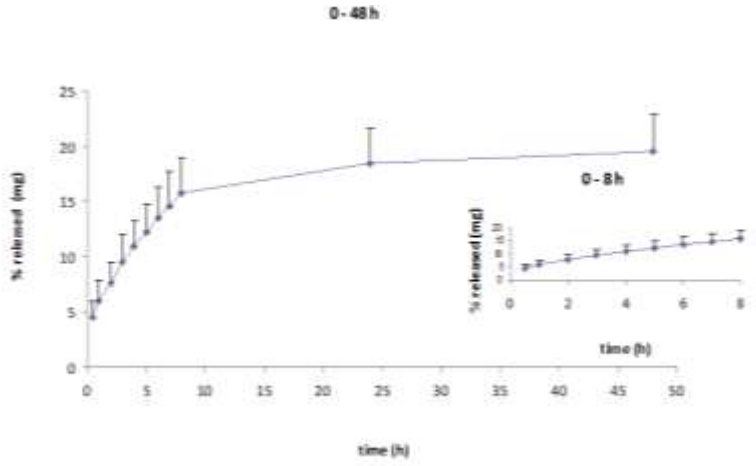

Figure 1: Release of the conventional emulsion ECZ.

Table 3 details the profile liberation of ECZ from the emulsion with liquid crystals during the 48 h of the assay. Figure 2 represents the percentage of ECZ released, where the system shows a kinetic liberation of order zero in the first 8 h of assay with a square coefficient correlation (r^2) of 0.9968 and from 8 to 48 h the release curve fits with the Higuchi model with a coefficient correlation of 0.9542 (table 4).

Table 3: Release profile of ECZ emulsion with liquid crystals.

Tpo (h)	Q (mg)	SD	Q/ Sup	SD	%Q	SD
0,5	0,181	0,024	0,058	0,008	3,655	0,510
1,0	0,231	0,010	0,074	0,003	4,670	0,323
2,0	0,310	0,002	0,099	0,001	6,265	0,315
3,0	0,388	0,001	0,124	0,000	7,844	0,385
4,0	0,452	0,001	0,144	0,000	9,139	0,441
5,0	0,519	0,014	0,165	0,004	10,494	0,580
6,0	0,582	0,011	0,185	0,004	11,775	0,782
7,0	0,648	0,025	0,206	0,008	13,097	0,656
8,0	0,710	0,018	0,226	0,006	14,369	1,069
24,0	0,871	0,017	0,277	0,005	17,619	1,176
48,0	0,942	0,021	0,300	0,007	19,046	1,284

Q = amount of ECZ released at different times. Q / Sup = Amount of ECZ released per unit area (mg / cm^2). % Q = Percentage of ECZ released to the receiving medium. SD = standard deviation of the data.

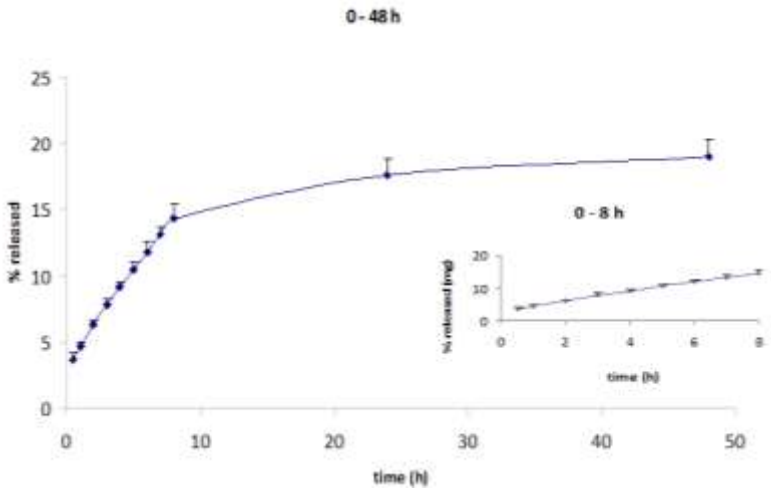

Figure 2: Release of ECZ emulsion with liquid crystals.

Table 4: Correlation coefficients of each system for the different kinetics release.

Formulation	Order zero r^2	First order r^2	Higuchi equation r^2
Conventional emulsion 0-8 h	0,9861	0,9194	0,9969
Conventional emulsion 8-48 h	0,8719	0,8566	0,9411
Emulsion with LC 0-8 h	0,9968	0,9424	0,9878
Emulsion with LC 8 - 48 h	0,891	0,8707	0,9542

Can be observed that in the case of conventional emulsion where the ECZ is dissolved into the globules of the internal phase of the emulsion, the kinetics follows the Higuchi model during the whole study, which would be explained considering the emulsion as homogeneous matrix and assuming that there is always a reservoir of drug to diffuse through the membrane.[14]

Contrary to the conventional emulsion, in emulsions with liquid crystals characteristics, the ECZ is found into the liposoluble structure of liquid crystals and in the oil from the globules of the internal phase, which in this case is not a homogeneous matrix.

These emulsions presents two release kinetics of the active principle, a kinetic liberation of order zero in the first 8 h of the assay and then a kinetic that adjust to the Higuchi model for the rest 48 h of the study. We could think that the kinetic of zero order is associated to the liberation of ECZ that was in the internal phase due to the destruction of some globules, either by defects in the membrane formed by liquid crystals or by contact with the receiving medium, the ECZ solubilized in the oil phase of the internal phase release. Another possibility is the breakdown of the liquid crystals outermost allowing the release of the ECZ

trapped in the lipid fraction. Or the combination of both factors. A lower release was also observed in the early hours of the assay compared to the conventional emulsion. After the first hour of the study, the multilayer of liquid crystals were continuously destroyed and releasing the active ingredient incorporated in its structure and allowing the extended release.
A t-test was carried out, which shows that although there is no significant difference ($p>0,05$) on the first liberation hours (0 - 8 h) and the last hour of the assay (48 h), there is a significant difference ($p<0,05$) when the drug release is analyzed in times ranging from 8 to 24 and 24 to 48 h, being higher for the system with liquid crystals characteristics. Furthermore, although there is no significant difference in the first 3 hours of release, it was observed that conventional emulsion showed higher standard deviations dispersion (SD) above those of the formula with liquid crystals.

CONCLUSIONS

We can conclude that although the amount of ECZ released in vitro at the end of the study is similar for both systems, the formula with liquid crystalline characteristic had a lower initial release with a profile release sustained over time.

It stands the importance in the choice of the excipient and the structure characteristics to modify the release time of the principle active from the pharmaceutical system.

Therefore it is assumed that after the topical application of the cream, the supply of active ingredient would be optimized from the conveyor system to the skin over time, which would imply a decrease in the frequency of application of the pharmaceutical form and improving the treatment efficacy.

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