ORIGINAL ARTICLE

NOVEL TOPICAL CHITOSAN/HYDROXYPROPYLMETHYL-CELLULOSE – BASED HYDROGELS CONTAINING FLUCONAZOLE AND SUCROSE ESTERS. FORMULATION, PHYSICOCHEMICAL CHARACTERIZATION, *IN VITRO* DRUG RELEASE AND PERMEATION

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Abstract

The purpose of this work was to formulate, prepare and evaluate novel topical hydrogels for the fluconazole delivery, containing biocompatible, biodegradable, non-toxic, non-irritating excipients which have inherent antifungal activity, considering that a synergistic therapeutic effect can be obtained. Fluconazole (FZ) and two non-ionic natural surfactants of sucrose esters group (sucrose laurate, sucrose palmitate) as potential penetration enhancers were incorporated into a hydrogel vehicle prepared using a mixture of two biopolymers as gelling agents (chitosan and hydroxypropylmethylcellulose, HPMC). The experimental hydrogels were evaluated for physicochemical properties (drug content, pH and viscosity) and *in vitro* drug release through hydrophilic synthetic membrane. Also, *ex vivo* FZ permeation test using excised pig ear skin was performed in order to investigate the penetration enhancement effect of the two sucrose esters. Franz diffusion cells method was used in the drug release and permeation experiments. The resulted data indicated that all tested hydrogels fulfilled the official requirements regarding drug content, pH and viscosity. High flux and release rate values obtained for all FZ chitosan/HPMC-based hydrogels in the *in vitro* drug release study indicate the formulations performance. Based on the *ex vivo* drug permeation data, sucrose laurate can be considered an effective penetration enhancer for fluconazole, encouraging additional *in vitro* and *in vivo* investigations.

Rezumat

Scopul acestei lucrări a fost formularea, prepararea și evaluarea unor noi hidrogeluri topice pentru administrarea fluconazolului, cu excipienți biocompatibili, biodegradabili, netoxici, neiritanți şi cu activitate antifungică intrinsecă, considerând că se poate obţine un efect terapeutic sinergic. Fluconazolul (FZ) şi doi surfactanţi neionici din grupul esterilor de zaharoză (laurat de sucroză, palmitat de sucroză) au fost încorporați într-un vehicul de tip hidrogel preparat folosind un amestec de doi biopolimeri ca agenți gelifianți (chitosan și hidroxipropilmetilceluloză, HPMC). Hidrogelurile experimentale au fost evaluate în ceea ce priveşte proprietățile fizico-chimice (conținutul în substanță activă, pH-ul şi vâscozitatea) și eliberarea *in vitro* a substanţei medicamentoase prin membrană sintetică hidrofilă. De asemenea, s-a efectuat testul de permeație *ex vivo* a FZ, utilizând piele excizată de pe urechea de porc, pentru a investiga efectul de promotori de penetrare al celor doi esteri de zaharoză. În experimentele de eliberare și permeație a substanţei medicamentoase s-a utilizat metoda cu celule de difuzie Franz. Datele obţinute au arătat că toate hidrogelurile testate au îndeplinit cerințele oficiale privind conținutul în substanță activă, pH-ul și vâscozitatea. Valorile mari ale fluxului și vitezei de eliberare obținute pentru toate hidrogelurile cu FZ pe bază de chitosan/HPMC în studiul de eliberare *in vitro* a substanţei medicamentoase, indică performanța formulărilor. Pe baza datelor testelor pe permeabilitate *ex vivo* a substanţei medicamentoase, lauratul de sucroză poate fi considerat un promotor de penetrare eficient pentru fluconazol, încurajând investigații *in vitro* și *in vivo* suplimentare.

Keywords: fluconazole, chitosan, sucrose esters, hydrogel

Introduction

Due to the emergence of new mycotic agents and to the re-emergence of several mycotic infections, nowadays fungal diseases are regarded as a global public healthcare problem, that pose a critical threat on immune-deficient individuals [2, 9, 30]. To improve the prognosis for the patients with both systemic and superficial fungal infections, different classes of antifungal drugs have been approved for oral and parenteral therapy [18]. Among these drugs, fluconazole (FZ), a synthetic fluorinated bis-triazole derivative, is widely used due to its highly effective antifungal activity and its wide anti-mycotic spectrum [15, 33]. FZ systemic administration offer several advantages

determined by its specific physicochemical properties. Compared to other antifungal agents with azole rings, like itraconazole, ketoconazole or miconazole, FZ is less lipophilic (with a logP = 0.5), has a better water solubility (of 8 mg/mL, at 37°C), and a higher bioavailability and antifungal activity, all explained by the presence of the halogenated phenyl ring and the two triazole rings [8]. However, systemic therapy with FZ presents two major inconveniences: the well-known adverse effects (nausea, vomiting, bloating, diarrhoea and abdominal pain) that reduce the patient's compliance in case of a long-term treatment, and various drug interactions [9, 29]. On the other hand, after systemic administration, FZ is rapidly and highly accumulated in the *stratum corneum*, acquiring the minimal inhibitory concentration for most dermatophytes [12, 22, 38]. Due to this characteristic, FZ is considered a good candidate for topical delivery, which could be an interesting alternative route in the treatment of superficial fungal infections, because the above-mentioned inconveniences are avoided.

Over the last two decades different semisolid formulations have been investigated as topical delivery systems of FZ. Numerous studies have evaluated the potential of various gel systems (surfactant-based gels, organogels, hydrogels, emulgels, micro-emulsion-loaded hydrogels and liposomal gels) as topical vehicles for FZ incorporation [1, 6, 11, 13, 14, 17, 23, 31, 39] because of their well-known advantageous properties. Hydrogels are conventional semisolid hydrophilic vehicles widely used to incorporate different drugs [23, 26]. Among hydrogels, the biopolymers-based hydrogels, in particular polysaccharide-based hydrogels, currently attracted great interest as topical drug delivery systems, due to their advantageous specific properties such as: hydrophilic nature, three-dimensional network structure, biocompatibility, biodegradability, non-toxicity, mucoadhesivity and ease of formulation and preparation [3, 28]. Chitosan and cellulose derivatives are two of the most frequently used biopolymers as gelling agents in the pharmaceutical domain for hydrogels preparation, because of their improved processability and unique characteristics.

Chitosan is a natural poly-cationic biopolymer obtained by alkaline N-deacetylation of chitin, consisting of units of β-(1,4)-2-amino-2-D-glucose and β-(1,4)-2-acetamido-2-D-glucose. Chitosan is a weak base (pKa = 6.2 - 6.8), soluble in diluted organic acids (acetic acid, lactic acid, formic acid or succinic acid) and insoluble in water and most organic solvents. In addition, cationic character gives chitosan significant antimicrobial (antibacterial, antifungal and antiviral) activity, which underlies most of its biomedical applications [5, 19, 21, 25, 27, 32]. Therefore, it would be expected that by using chitosan-based hydrogels which have inherent anti-mycotic activity, as topical vehicles for an antifungal agent (i.e. fluconazole), a synergistic therapeutic effect would be obtained, thus leading to a more effective management of superficial fungal infections.

Chitosan hydrogels possess relatively low mechanical strength, which is a drawback that limits their applications as pharmaceutical semisolid topical preparations. In order to improve mechanical property of the chitosan hydrogels, the addition of another hydrophilic polymer, namely a cellulose derivative, which forms gels with higher mechanical strength, has been proposed and investigated [4, 34-36].

Considering the fact that among the skin layers, *stratum corneum* possess the highest resistance to the drugs cutaneous transport, the use of penetration enhancers (i.e. non-ionic surfactants) is the most frequently applied approach in the formulation stage of a topical preparation in order to overcome the skin barrier, because of their ability to alter temporarily the *stratum corneum* structure, thus facilitating the drug penetration and permeation through the skin [10].

Furthermore, the selection of appropriate and relatively innocuous non-ionic surfactants, such as those from the group of natural surfactants, is currently considered a particularly important objective of the formulation development stage of topical drugs [16]. Sucrose esters are non-ionic, biocompatible and biodegradable natural surfactants, possessing also favourable dermatological characteristics (i.e. non-toxic and non-irritating to the skin). Due to these valuable properties, lately, sucrose esters are increasingly used in the formulation of dermatological preparations as solubilizers, penetration enhancers, emulsifiers and stabilizers [7, 24, 37]. They also possess important pharmacological properties, namely antimicrobial and anti-mycotic activities [20].

This experimental study aimed to formulate and evaluate new hydrogels for the topical application of fluconazole using biocompatible, biodegradable, non-toxic, non-irritating auxiliary substances with antifungal activity. For this purpose, FZ was incorporated into an innovative hydrogel vehicle, containing two biopolymers as gelling agents (chitosan and hydroxypropylmethylcellulose) and a sucrose ester (sucrose laurate or sucrose palmitate, in different proportions) as potential penetration enhancer. The chitosan/hydroxypropylmethylcellulose hydrogels containing FZ and sucrose esters were characterized regarding the drug content, pH, viscosity and the *in vitro* drug release. Diffusion studies were performed in vertical diffusion cells using both hydrophilic synthetic membranes and excised pig ear skin, in order to evaluate the release of FZ from the hydrogel vehicle, and the drug skin penetration and permeation respectively. The influence of the two penetration, enhancers, sucrose laurate (SL) and sucrose palmitate (SP) was also investigated.

Materials and Methods

Materials

Fluconazole and chitosan (Chitopharm®M) have been generously donated by S.C. Vim Spectrum S.R.L (Romania) and S.C. Antibiotice S.A. Iaşi (Romania). Hydroxypropylmethylcellulose (HPMC) (Methocel K4M) was generously donated by Colorcon Ltd. (England). Sucrose laurate (SL) and sucrose palmitate (SP) were received as free samples from Mitsubishi Chemical Foods Corporation (Japan). Glycerol and tetraglycol (TG) were purchased from S.C. Silal Trading S.R.L. (Romania) and Merck Schuchardt OHG (Germany), respectively. Ethanol, isopropyl alcohol (ISP) and acetic acid were purchased from Chimopar (Romania). Polyethyleneglycol 400 (PEG 400) and polyethyleneglycol 600 (PEG 600) were received as gift samples from BASF ChemTrade GmbH, Germany. Distilled water was used to prepare the studied hydrogels, and bidistilled water was used to prepare phosphate buffered saline, pH 7.4. Synthetic hydrophilic polysulfone membranes, with 25 mm diameter and 0.45 μm pores (Tuffryn HT membranes) were purchased from Pall Corporation, USA. All materials used in the study were of pharmaceutical or analytical purity, and were used as such.

Methods

Solubility studies

Solubility behaviour of FZ in water and different co-solvents, currently used in hydrogel formulations, namely glycerol, tetraglycol, PEG 400 and PEG 600, was measured by saturation shake-flask method. The two sucrose esters (SL and SP) solubility in water and three co-solvents (ethanol, isopropyl alcohol and tetraglycol) was also determinated using the same method.

Each reported solubility result was obtained by performing in parallel three independent experiments. For each experiment, an excess amount of solid was dispersed in 5 mL of each of the mentioned solvents in 10 mL stoppered glass vials and stirred for 10 min in order to facilitate a proper mixing of the solid with the liquid.

The mixture vials were kept and shaken for 98 h at 25 ± 1°C in an isothermal shaker bath (Memmert, Germany) to get to equilibrium and then the saturated solution and precipitate were separated by centrifugation (at 12,000 rpm for 15 min), followed by filtration (membrane filter 0.45 μm, 25 mm, Teknokroma, Germany). The concentration of the FZ and sucrose esters in the filtrated saturated solution and diluted appropriately (with ethanol or methanol), was determined by UV spectrophotometry (T70+ spectrophotometer, PG Instruments, UK) at the wavelength of 253 nm and 213 nm respectively.

Selection of the 2% fluconazole hydrogels components

The co-solvent for FZ was selected based on the results of drug solubility study, so as to ensure that the active substance is maintained in a dissolved state, at a concentration of 2%.

Based on their specific and unique properties, a mixture of two biopolymers (chitosan and HPMC) as gelling agents, and two non-ionic natural surfactants (SL and SP) as potential penetration enhancers were selected.

Also, a hydrogel with 2% fluconazole without sucrose ester (control formulation) was formulated to assess the potential effects of the sucrose esters on both physico-chemical and rheological properties of the experimental hydrogels, as well as on the *in vitro* fluconazole release and permeation.

The composition of the experimental chitosan/HPMC-based hydrogels containing 2% fluconazole is presented in Table I.

Table I

The composition of the chitosan/HPMC-based hydrogels with 2% fluconazole

Hydrogel components	Weight (%) and formulation codes						
	FZ	FZ-SL 0.5%	FZ-SL 1%	FZ-SL 2.5%	FZ-SL 5%	FZ-SL 0.5%-10% ISP	FZ-SP 0.5%-10% ISP
Fluconazole	2.0	2.0	2.0	2.0	2.0	2.0	2.0
Sucrose laurate	-	0.5	1.0	2.5	5.0	0.5	-
Sucrose palmitate	-	-	-	-	-	-	0.5
Tetraglycol	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Isopropyl alcohol	-	-	-	-	-	10.0	10.0
Distilled water	10.0	10.0	10.0	10.0	10.0	-	-
Hydrogel CTS 3%	22.66	22.5	22.33	21.83	21.0	22.50	22.5
Hydrogel HPMC 4%	45.34	45.0	44.67	43.67	42.0	45.0	45.0

Preparation of the chitosan/HPMC-based hydrogels containing 2% fluconazole: the preparation of the hydrogel base was carried out by mixing the 3% chitosan hydrogel with the 4% HPMC hydrogel, in a ratio of 1:2. The hydrogel with 3% chitosan was prepared by dissolving the polymer under gentle stirring in a 1% acetic acid aqueous solution. The 4% HPMC hydrogel was prepared by the "hot/cold" method: the polymer was dispersed in distilled water heated at about 70 - 80°C, with continuous stirring at 2000 rpm, using a laboratory shaker (Eurostar Digital, IKA Werke, Germany), then the

obtained dispersion was kept cold (at 2 - 8°C) for 30 minutes.

Preparation of the hydrogels containing 2% fluconazole and sucrose esters: fluconazole was dissolved in tetraglycol, while the sucrose ester (SL or SP) was dissolved in 10 g of distilled water or isopropyl alcohol. The solution of fluconazole in tetraglycol was added to the hydrogel base under moderate stirring, and then the aqueous/alcoholic solution of the sucrose ester was added to the obtained hydrogel. The resulted hydrogel was easily mixed for homogenization, avoiding as much as possible air incorporation and foaming.

Preparation of the 2% fluconazole hydrogel: was performed similarly to the preparation of the hydrogels with fluconazole and sucrose esters, but using distilled water instead of aqueous sucrose esters' solution.

After preparation, all experimental hydrogels were left for 24 hours at room temperature before testing.

Quantitative drug analysis

The determination of FZ content was performed according to the procedure described in our previous study [6]. In brief, approximately 0.4 g of FZ hydrogel was weighed and quantitatively brought with 96% ethanol in a 25 mL volumetric flask, filling with the same solvent. The obtained solution was spectrophotometricaly analysed using a UV-VIS spectrophotometer (T70+, UVWIN5 soft, PG Instruments, UK), at 253 nm. Each determination was made in triplicate.

pH and viscosity measurements

The pH of the experimental hydrogels was determined at 25 ± 2°C by potentiometric method (Sension™ 1 portable digital pH meter, Hach Company, U.S.A.), using aqueous solutions containing 5% (w/w) hydrogel. A stress-controlled rheometer (RheoStress 1, HAAKE, France) equipped with a cone-plate geometry (1/60) was used to perform the viscosity measurements. Each experiment was carried out in triplicate.

Preparation of the skin membrane

Ex vivo permeation studies were conducted using as model membrane dermatomed pig ear skin excised from 4-month-old domestic pig (female or male) ears, obtained from a local slaughterhouse. Immediately after excision, the pig ears were cleaned up with tap water, then the outer region of the ears was clipped of bristles and then the skin was dermatomed to a thickness of around 500 µm. The dermatomed skin samples were immediately used for the permeation experiments or stored at -20°C for a maximum period of 2 months. Before use, the dermatomed pig skin was removed from the freezer and allowed to thaw at room temperature. The integrity of the skin was examined, the thickness of each sheet was measured with a micrometer and then squares of 2 cm^2 were cut from the skin sheet.

In vitro fluconazole release and permeation studies

The study was performed with a Franz diffusion system (Microette-Hanson system, model 57-6AS9, USA), with an effective diffusion area of 1.767 cm^2 of the diffusion cell and a volume of 6.5 mL of the receptor compartment. Approximately 0.300 g of sample was weighed into the donor compartment and placed on a polysulfone synthetic membrane, with a 25 mm diameter and 0.45 µm pores (HT Tuffryn membranes, Pall Corporation, USA) for the *in vitro* test, and on porcine ear skin for the *ex vivo* study. The synthetic and biological membranes fixed between the donor and the receptor compartment, were maintained in contact with the receiving medium for 30 minutes prior to the sample application. To ensure sink conditions throughout the *in vitro* tests, the receptor compartment of each diffusion cell contained a phosphate buffered saline (pH 7.4) with 60% ethanol solution, freshly prepared, heated and de-aerated. For the *ex vivo* study, as receptor medium a phosphate buffered saline (pH 7.4) solution was selected. The receptor medium was maintained at 32 ± 1°C throughout the experiments. The magnetic stirrer was set at 600 rpm to stir the receptor medium and avoid the influence of the diffusion layer. The experiments were performed for 8 h (*in vitro*) or 24 h (*ex vivo*). 0.5 mL of the receptor fluid were automatically taken and replaced with a fresh receptor medium to maintain the constant volume (of 6.5 mL). For the *in vitro* tests the samples withdraw occurred at 30 min, 1, 2, 3, 4, 5, 6, 7, and 8 hours, while for the *ex vivo* studies samples from the receptor fluid were taken at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 22, and 24 hours.

The quantitative measurement of FZ from the samples was carried out with an UV-VIS T70 + spectrophotometer, with UVWIN5 soft (PG Instruments, UK) at the wavelength of 266 nm, corresponding to the maximum absorption of FZ in phosphate buffered saline (pH 7.4) with 60 % ethanol, and at 261 nm for the absorption of FZ in the phosphate buffered saline (pH 7.4) solution. The determinations were made in triplicate.

Data analysis of in vitro drug release and permeability studies

Permeation parameters, namely steady-state flux (J_{ss}, µg/cm^2/h) and lag time (t_L, h), were calculated from the slope and respectively the *x*-axis intercept of the plots of the cumulative amount of permeated FZ (µg/cm^2) *vs*. time. To obtain the permeability coefficient (Kp, cm/h) value, steady-state flux was divided by the initial concentration of the drug in the donor compartment. The release rate (k) values were calculated using the steady-state slopes from the plots of the

cumulative amount of FZ that permeated through the membrane (μg/cm^2) *vs.* square root of time. The values of the diffusion coefficient (D) were calculated from the release rate values.

Statistical analysis

The experimental data were statistically analysed using Statistica software (version 7.0). The data showed as mean ± standard deviation (SD). The differences were considered statistically significant for $p < 0.05$. For graph clarity reason, standard deviation is not presented in the figures.

Results and Discussion

Solubility studies

As shown in Table II, fluconazole exhibited the lowest solubility in water (0.555 ± 0.034 mg/mL). A slightly higher FZ dissolution capacity than water was observed for glycerol, property indicated by the FZ solubility value of 8.75 folds higher (Table II). Unlike water, the two tested polyethyleneglycols exhibited a much higher FZ dissolution capacity, their calculated enhancement factor was 162.71 for PEG 600, and 187.48 for PEG 400. The highest FZ solubility value was determined in tetraglycol, with an enhancement factor of about 358. Based on the highest solubility value of FZ in these water-miscible solvents, tetraglycol was selected as co-solvent for the hydrogel formulations.

Table II

The solubility of fluconazole in the tested solvents at 25 ± 2°C

Solvent	Solubility (mg/mL)
Water	0.555 ± 0.034
Glycerol	4.859 ± 0.425
Polyethylene glycol 400	104.049 ± 15.281
Polyethylene glycol 600	90.306 ± 7.084
Tetraglycol	198.773 ± 10.589

Table III presents the solubility values of the sucrose esters in the tested solvents (water, tetraglycol, ethanol, isopropyl alcohol). In the case of SP, it can be observed that the lower solubility value was determined in water. In comparison, the SP solubility in tetraglycol and ethanol was 178 fold and respectively 139 fold higher. Isopropyl alcohol presented the highest solubility capacity of SP, with a calculated enhancement factor of 367, as compared to water.

Table III

The solubility of sucrose palmitate and sucrose laurate in tested solvents at 25 ± 2°C

Solvent	Sucrose palmitate solubility (mg/mL)	Sucrose laurate solubility (mg/mL)
Water	0.725 ± 0.034	424.032 ± 8.115
Ethanol	100.758 ± 4.281	1040.356 ± 6.581
Isopropyl alcohol	265.992 ± 6.357	665.465 ± 5.019
Tetraglycol	129.092 ± 3.874	540.656 ± 7.214

For SL the lowest solubility value was obtained in water, and the highest value in ethanol (2.45 fold higher than in water). As for tetraglycol and isopropyl alcohol, the solubility values of SL in these solvents were 1.28 fold higher, and respectively 1.57 fold higher compared to water. Comparing the solubility data obtained for the two tested sucrose esters, SL was more soluble than SP in all the tested solvents.

Selection of the 2% fluconazole hydrogels components

Based on the solubility studies, tetraglycol was used as a co-solvent for fluconazole, and also as a potential penetration enhancer, in a concentration of 20%.

The final formulation concentrations of the gelling agents were approximately of 0.65% for chitosan and of 1.7% for HPMC. These hydrophilic polymers were used in the form of hydrogels, which were blended in a ratio of 1:2, and the polymer concentration in the hydrogel was 3% for chitosan and 4% for HPMC.

As potential penetration enhancers, two non-ionic natural surfactants, sucrose laurate and sucrose palmitate were selected. The sucrose laurate surfactant was used in concentrations of 0.5%, 1%, 2.5% and 5% in the formulations containing water, while in the formulation with ISP it was dissolved only at a 0.5% concentration. Due to its very low water solubility (Table III), sucrose palmitate was used only at 0.5% concentration; even at this reduced concentration, the presence in the hydrogel of ISP as co-solvent was absolutely necessary. According to the solubility studies results, ISP was selected at a concentration of 10%.

Quantitative drug analysis

The FZ content of the studied hydrogels (Table IV) ranged between 98.87 ± 1.35 and 103.26 ± 0.78% of the theoretical value (2%, w/w), which corresponds to the compendial specifications for drug content. The obtained data show the uniform distribution of the active substance in the experimental hydrogels.

Table IV

Drug content, pH, and viscosity of the experimental chitosan/HPMC-based hydrogels, containing 2% fluconazole

Formulation code	Drug content (%)	pH	Viscosity (Pa · s)
FZ	103.26 ± 0.78	4.59 ± 0.02	1.008
FZ-SL 0.5%	101.16 ± 1.47	4.69 ± 0.07	1.243
FZ-SL 1%	99.57 ± 2.41	4.55 ± 0.02	0.637

Formulation code	Drug content (%)	pH	Viscosity (Pa · s)
FZ-SL 2.5%	101.42 ± 1.57	4.62 ± 0.01	1.036
FZ-SL 5%	98.87 ± 1.35	4.65 ± 0.01	0.780
FZ-SL 0.5% - 10% ISP	100.31 ± 1.82	4.52 ± 0.01	0.785
FZ-SP 0.5% - 10% ISP	99.64 ± 3.58	4.57 ± 0.01	0.827

pH and viscosity measurements

The pH values of the chitosan/HPMC-based hydrogels containing 2% FZ varied in a very narrow range, between 4.52 ± 0.01 and 4.69 ± 0.07 (Table IV), being mainly determined by the presence of acetic acid at a concentration of 1%, the other variable components of the formulation (type and concentration of the sucrose ester) having an insignificant effect on this parameter. Also, it can be observed that the measured pH values were within the recommended range (4.5 - 8.5) for semisolid preparations by the national pharmacopoeia [40], so it can be suggested that they will be well tolerated by the skin.

The viscosity values obtained for the tested fluconazole hydrogels (Table IV) varied within a relatively wide range of values, from 0.637 to 1.243 Pa · s, highlighting the influence of the system's composition on this property. The highest viscosity was observed for the FZ-SL 0.5% formulation, while the lowest was obtained for formulation FZ-SL 1% (1.95 fold lower). In the case of the two hydrogels containing 0.5% SL, the presence of 10% ISP reduced by 1.58 fold its viscosity. However, ISP in concentration of 10% did not influenced in great measure the viscosity of the hydrogels, regardless of the type of sucrose ester used in the preparation, SL or SP at a concentration of 0.5% (0.785 Pa · s, and 0.827 Pa · s respectively). Comparing the apparent viscosity values of the hydrogels containing sucrose esters with that of the control hydrogel (without the sucrose esters), it can be seen that, except for the formulations FZ-SL 0.5% and FZ-SL 2.5%, all the other formulations showed a lower viscosity.

Changes in viscosity of the experimental chitosan/HPMC-based hydrogels containing 2% FZ and sucrose esters can be attributed to the formation of spherical or vermicular micelles, with a hexagonal or lamellar liquid crystal structure. Also, the concentration of the sucrose ester surfactant can influence the rheological properties of the hydrogels, by altering the micelle hydration, which leads to the growth or shape modification of the micelles (from spherical to vermicular). Thus, increasing the size of the micelles or the transition from spherical to vermicular form, the viscosity of the systems can be increased. In addition, sucrose esters may influence the gel properties of the gelling agents (chitosan and HPMC). Consequently, these results can be attributed to the combined effects of the type and concentration of the sucrose ester on micelle formation, their shape and degree of hydration, and on the gelling ability of the hydrophilic polymer. For all formulations, the viscosity was found within the range value specific for semisolid preparations.

In vitro fluconazole release

The values of the specific release and permeation parameters of fluconazole from the experimental hydrogels, through hydrophilic synthetic membrane, calculated from the *in vitro* release data, are illustrated in Figure 1 and listed in Table V.

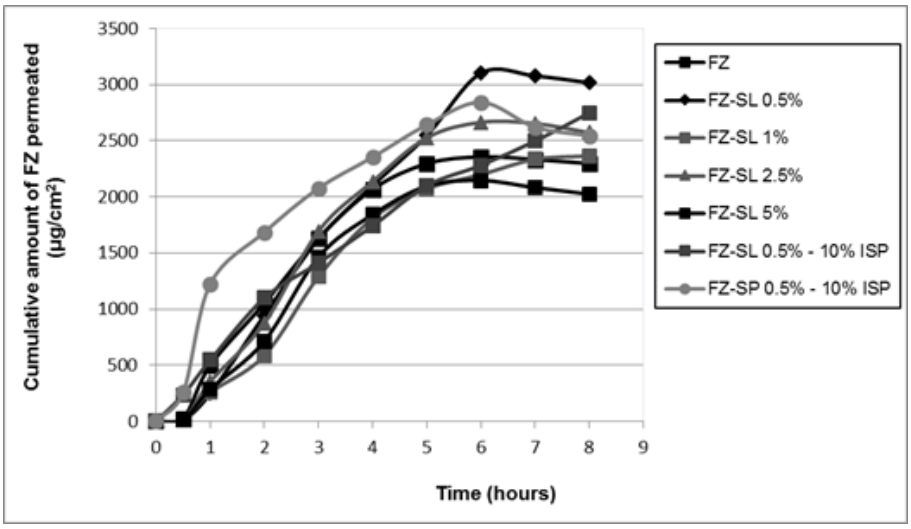

Figure 1.

In vitro fluconazole permeation profiles through synthetic membrane from experimental hydrogel formulations

Table V

The specific parameters for the *in vitro* release of fluconazole from the studied formulations, through hydrophilic synthetic membrane

Formulation code	Permeation parameters			Release parameters	
	J_{ss} (μg/cm^2/h)	K_P x 10^{-6} (cm/h)	t_L (h)	k (μg/cm^2/h$^{1/2}$)	D x 10^{-3} (cm^2/h)
FZ	528.6 ± 3.31	264.30	-	1584.0 ± 9.15	4.92
FZ-SL 0.5%	566.0 ± 3.81	283.01	0.40 ± 5.71	1799.7 ± 15.34	6.36
FZ-SL 1%	481.6 ± 3.99	240.81	0.49 ± 3.58	1414.5 ± 12.45	3.93
FZ-SL 2.5%	620.4 ± 8.15	310.20	0.46 ± 6.12	1677.0 ± 7.45	5.52
FZ-SL 5%	537.8 ± 6.25	268.90	0.49 ± 5.09	1450.0 ± 5.28	4.13
FZ-SL 0.5%-10% ISP	302.1 ± 3.43	151.07	-	1190.7 ± 16.8	2.78
FZ-SP 0.5%-10% ISP	321.2 ± 2.81	160.61	-	1128.4 ± 9.75	2.49

J_{ss}: steady-state flux; K_P: permeability coefficient; t_L: lag time; k: release rate; D: diffusion coefficient

It can be observed that, except for the formulations FZ-SL 1% and FZ-SL 0.5%-10% ISP, the fluconazole diffusion from the tested hydrogels (with and without sucrose ester) was most intense and increased progressively over the first 6 hours of testing, when 61.85 ± 1.23% - 85.91 ± 2.76% of the total amount of fluconazole was released (Figure 1). Afterwards, the release and diffusion process were slower, and the steady state was reached. The highest cumulative amount of fluconazole (85.91 ± 2.76%) was released after 6 h of testing from the FZ-SL 0.5% formulation, followed by the FZ-SL 2.5% and FZ-SP 0.5%-10% ISP formulations, from which 76.92 ± 0.95% and respectively 75.99 ± 1.78% of fluconazole was released. The formulations FZ-SL 1% and FZ-SL 0.5%-10% ISP released, after 8 hours of testing, a slightly lower cumulative amount of fluconazole (64.38 ± 1.26% and respectively 73.33 ± 0.48%), while the control hydrogel released a cumulative amount of 68.04 ± 2.86% FZ, after 6 hours of testing (Figure 1). The high percentages of the released fluconazole from the experimental hydrogels indicate the following: the used release medium was adequate, the reduced affinity of the active substance for the vehicle in which it was dissolved, the ability of the hydrogel base to release the drug substance, thereby assuring the performance of the formulation.

By comparing the values of the specific parameters for permeation and *in vitro* release of the fluconazole from the studied hydrogels through the synthetic membrane (Table V), it can be observed that the hydrogel containing 2.5% sucrose laurate produced the highest transfer rate of FZ (620.4 ± 8.15 μg/cm^2/h), followed by FZ-SL 0.5% hydrogel with 0.5% sucrose laurate (566.0 ± 3.81 μg/cm^2/h). Relatively close values of the FZ flux (537.8 ± 6.25 μg/cm^2/h and 528.6 ± 3.31 μg/cm^2/h) were calculated for the FZ-SL 5% formulation and the control hydrogel respectively. In contrast, the hydrogels FZ-SL 0.5%-10% ISP and FZ-SP 0.5%-10% ISP produced steady state flux values of 2.05 and respectively 1.93 times lower than that obtained for the formulation containing 2.5% sucrose laurate. Table V shows that from all experimental hydrogels, except the control formulation and those containing 10% ISP, the fluconazole transfer through synthetic membrane began after a very short lag time, approximately 30 minutes, which, in the case of synthetic membrane testing is not significant because the membrane resistance to diffusion is negligible, and the sink conditions favouring the diffusion of the drug in the receiving medium are provided.

The fluconazole release rate values from the studied hydrogels through the synthetic membrane varied similarly to the steady state flux. It is noteworthy that the fluconazole release rate values were much higher than the values of the steady state flux, suggesting that the permeation of the drug through the membrane was independent of its release from the preparations.

Although the viscosity of the sucrose esters containing hydrogels, except the FZ-SL 1% formulation, was comparable to that of the control hydrogel (without sucrose ester), the total amount of the released and membrane permeated fluconazole from these hydrogels was notably higher. This is most likely due to the presence of sucrose laurate, and only secondary to the viscosity of the vehicle. The significant influence of the sucrose laurate in the hydrogels formulation is also evidenced by the fact that, for the 5% concentration of sucrose laurate, the flux increase was reduced, most probably due to the inclusion of a larger fraction of fluconazole in the micelles and also due to the increase of its affinity towards the gel base. Consequently, the FZ solubility increase in the presence of the surfactant, which also acted as solubilizer [24, 37]. Instead, sucrose palmitate and isopropyl alcohol determined the slowest diffusion of fluconazole from the FZ-SP 0.5%-10% ISP and FZ-SL 0.5%-10% ISP hydrogels, that can be attributed to the higher solubilizing potential of the surfactant and co-solvent for FZ, increasing the solubility of the drug in the gel base and consequently, increasing the affinity of FZ for the vehicle and lowering the thermodynamic activity of the substance in the vehicle.

The results of the *in vitro* release study showed differences in the formulations, that may be attributed to the combined effects of the following factors: the type and concentration of the sucrose ester in the formulation, the interaction of FZ with surfactants (sucrose esters) micelles, the solubility of the drug substance in the gel vehicle, thus its affinity for the active compound, the viscosity of the respective systems.

Study of ex vivo fluconazole permeation through excised pig ear skin

This study investigated the influence of two natural penetration enhancers, sucrose laurate and sucrose palmitate, on the *ex vivo* permeation of FZ from chitosan/HPMC-based hydrogels containing the sucrose ester in different concentrations. Figure 2 presents the cumulative amount of FZ permeated through excised pig ear skin.

The results of *ex vivo* drug permeation experiments were different than those of *in vitro* drug release test through synthetic membrane. The FZ permeation profile from chitosan/HPMC-based hydrogels, with and without sucrose esters, through pig ear skin presented two linear portions: prior the steady state (the first 5 or 7 h) and during the steady state (from 5 - 9 to 21 - 22 h).

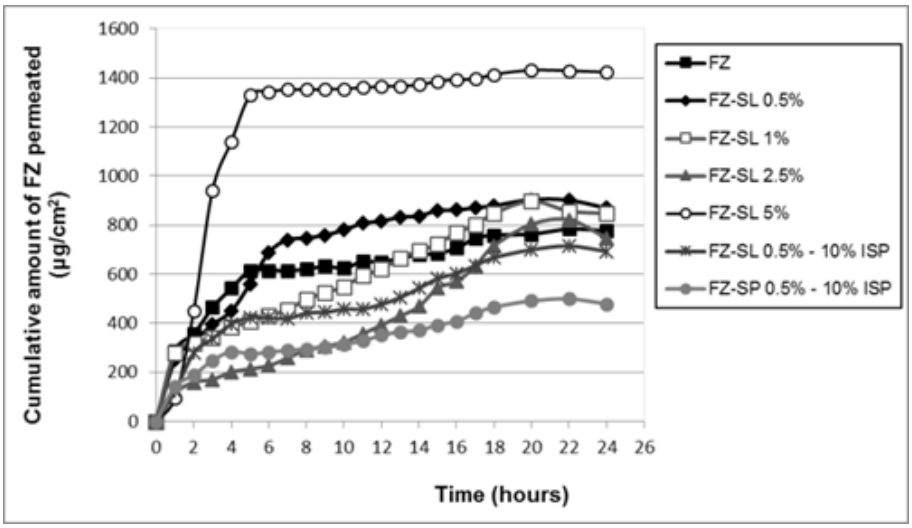

Figure 2.

Ex vivo FZ permeation profiles through pig ear skin from chitosan/HPMC-based hydrogels, with and without sucrose esters

Fluconazole from chitosan/HPMC-based hydrogel containing the highest SL concentration (5%) permeated through the pig ear skin faster and in higher amounts than FZ from all other studied hydrogels, including the sucrose ester-free gel. Although the concentration of sucrose laurate in the formulation FZ-SL 0.5% was one tenth of that in FZ-SL 5% formulation, fluconazole from FZ-SL 0.5% hydrogel permeated also in high amount, which was approximatively 1.7 fold lower than that produced by FZ-SL 5% formulation, but higher than that measured for all other hydrogel vehicles. However, a transdermal fluconazole permeation decrease could be observed in case of FZ-SL 1% formulation followed by FZ-SL 2.5% formulation, even though the SL content was higher than that in the FZ-SL 0.5% hydrogel. These findings suggest that the percutaneous permeation enhancing effect of SL depends in high extent of its concentration in the FZ chitosan/HPMC-based hydrogels. Moreover, the addition of 10% isopropyl alcohol in the 0.5% SL and 0.5% SP containing hydrogels significantly decreased the FZ permeation through pig ear skin, which was lower than that from control formulation (sucrose ester-free).

The abovementioned results were confirmed by the calculated steady state flux, permeability coefficient, release rate and diffusion coefficient values, listed in Table VI.

The highest *ex vivo* permeation parameters values were produced by the FZ-SL 5% hydrogel, followed by the FZ-SL 0.5%-10% ISP formulation (about 3 times lower). A slightly lower and slower drug transfer can be observed for FZ-SL 0.5%, FZ and FZ-SP 0.5%-10% ISP formulations compared to that of FZ-SL 5% and FZ-SL 0.5%-10% ISP hydrogels, while the FZ-SL 1% and FZ-SL 2.5% formulations produced the lowest values of drug permeation through pig ear skin.

Table VI

The specific parameters for the *ex vivo* release of fluconazole from the studied formulations, through excised pig ear skin

Formulation code	Permeation parameters			Release parameters	
	J_{ss} (µg/cm^2/h)	K_P x 10^{-6} (cm/h)	t_L (h)	k (µg/cm^2/h$^{1/2}$)	D x 10^{-5} (cm^2/h)
FZ	83.8 ± 3.25 (1-5 h)	41.87	-	270.7 ± 21.68	14.37
	12.5 ± 3.41 (7-22 h)	6.23	-	90.4 ± 18.66	1.61
FZ-SL 0.5%	84.2 ± 3.12 (1-7 h)	42.08	-	305.3 ± 10.08	18.3
	13.0 ± 0.36 (7-21 h)	6.51	-	93.19 ± 11.05	1.7
FZ-SL 1%	31.1 ± 2.27	15.53	-	180.9 ± 5.36	6.42
FZ-SL 2.5%	25.2 ± 1.73 (1-14 h)	12.59	-	121.5 ± 6.43	2.9
	46.5 ± 2.81 (14-22 h)	23.27	-	394.7 ± 8.05	30.6
FZ-SL 5%	315.5 ± 4.28 (1-5 h)	157.75	0.49 ± 3.78	1036.0 ± 12.63	210.6
	7.1 ± 0.52 (9-20 h)	3.53	-	51.92 ± 3.47	0.53
FZ-SL 0.5%-10% ISP	100.7 ± 3.38 (0-4 h)	50.34	-	203.9 ± 4.37	8.16
	21.2 ± 1.19 (6-22 h)	10.62	-	149.3 ± 7.01	4.37
FZ-SP 0.5%-10% ISP	67.5 ± 0.49 (0-4 h)	33.76	-	141.7 ± 2.63	3.94
	14.7 ± 1.06 (5-22 h)	7.35	-	100.2 ± 5.87	1.97

J_{ss}: steady-state flux; K_P: permeability coefficient; t_L: lag time; k: release rate; D: diffusion coefficient

These differences could be explained based on the biophysical mechanism of the penetration enhancing activity of sucrose esters and isopropyl alcohol, as well as the influence of sucrose ester chemical structure on its interaction with the *stratum corneum* lipids. Recent studies suggested that sucrose esters may temporarily alter the *stratum corneum* integrity and its barrier properties by fluidizing the lipids of this layer, as a result of the intercalation of the sucrose esters hydrocarbon chains between the lipophilic tails of the bilayer, and consequently the interaction of the sucrose ring with the lipids polar heads. The above mentioned sucrose esters-*stratum corneum* lipids interaction is notably influenced by the surfactant structure, namely the length of the hydrocarbon chains [24, 37]. Similarly, the percutaneous enhancement activity of isopropyl alcohol was attributed to its interaction with the polar groups of the *stratum corneum* lipids. Further, both types of penetration enhancers, sucrose esters and isopropyl alcohol, promote the drug skin permeation by increasing the drug solubility in the vehicle, acting as micellar solubilizers and respectively as co-solvents. However, the drug entrapment into surfactant micelles can be considered a permeation back-draw by hindering the drug transfer from the vehicle into the skin.

This inconvenient can be suggested as the main reason for which the formulations FZ-SL 1% and FZ-SL 2.5% conducted to the lowest FZ skin transfer, among all the studied hydrogels, although their concentration in sucrose laurate was 2-5 fold higher than that of the FZ-SL 0.5% gel, ranked the second considering the FZ permeation values. In the case of FZ-SL 5% formulation, which seems to be the best in terms of FZ skin permeation, it can be suggested that in concentration of 5% sucrose laurate has a more pronounced penetration enhancement effect than as that of drug solubilizer. Also, this study revealed the synergistic effect of isopropyl alcohol and sucrose laurate as penetration enhancers for fluconazole in case of the formulation FZ-SL 0.5%-10% ISP, with FZ permeation values slightly higher than that obtained for the hydrogel containing the same SL concentration, but without ISP.

However, at the same sucrose ester concentration, SL (with 12 C-atoms in the fatty acid chain) was a much effective enhancer than SP (with 16 C-atoms in the fatty acid chain), increasing the FZ skin permeation by 1.5 fold.

Conclusions

In this study, several innovative hydrogels for topical delivery of fluconazole were successfully formulated and prepared using a mixture of two biocompatible, natural biodegradable, non-toxic, polyhydric biopolymers as gelling agents (chitosan and hydroxypropylmethylcellulose) and two non-ionic natural surfactants of the sucrose ester group, well tolerated by the skin, as potentially penetration enhancers. The results of the characterization tests proved that all experimental 2% fluconazole chitosan/HPMC-based hydrogels containing 0.5-5% sucrose laurate or 0.5% sucrose palmitate corresponded to official recommendations in terms of drug content, pH and viscosity. According to the results of the *in vitro* drug release and *ex vivo* drug permeation study, it can be concluded that the *in vitro* drug release measurement using synthetic membranes is a valuable quality control test revealing the performance of the new developed formulations, but it is necessary to be completed with *ex vivo* skin permeation studies in order to evaluate the effect of penetration enhancers. Our results showed that sucrose laurate alone in 5% concentration or mixed in 0.5% concentration with 10% isopropyl alcohol is an effective penetration enhancer for fluconazole.

However, further investigations on experimental hydrogels stability, safety and therapeutic efficacy are needed in order to develop commercially viable topical formulation of fluconazole.

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