

CHITOSAN/HPMC-BASED HYDROGELS CONTAINING ESSENTIAL OILS FOR TOPICAL DELIVERY OF FLUCONAZOLE: PRELIMINARY STUDIES

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Abstract

Among the pharmaceutical excipients of natural origin, polysaccharides (i.e. chitosan and cellulose derivatives) and essential oils have attracted increasing interest as gelling agents and penetration enhancers respectively, for development of topical semisolid preparations, including hydrogels. Therefore, in this research an attempt has been made to design and prepare new biocompatible hydrogels, using fluconazole (FZ) as model drug, two mixed polysaccharides (chitosan and hydroxypropylmethylcellulose) as gelling agents, clove oil (CVO) and cinnamon oil (CNO) as potential penetration enhancers and synergistic antifungal components, cosolvents and a non-ionic synthetic surfactant as solubilizers. The prepared hydrogels were characterized for drug content, pH and rheological properties (flow behaviour, viscosity, consistency and spreadability). Also, *in vitro* fluconazole release through hydrophilic synthetic membrane was evaluated by vertical diffusion cell method. The obtained results revealed that all experimental formulations were homogeneous, with adequate rheological characteristics and possessed pH values very close to the lower limit of the compendial range. Based on the obtained results, it can be suggested that both essential oil composition and its concentration in the formulations have significant effects on the mechanical properties and pharmaceutical availability of the tested hydrogels. *In vitro* drug release results sustained that the most desirable topical vehicles for fluconazole delivery, the formulations FZ-CNO 3%, FZ-CNO 5% and FZ-CVO 5%, containing 3% or 5% cinnamon oil and respectively 5% clove oil incorporated into a chitosan-hydroxypropylmethylcellulose based hydrogel. In conclusion, these hydrogels should be further investigated by *in vitro* and *in vivo* studies as promising carriers for topical fluconazole delivery.

Rezumat

Dintre excipienții farmaceutici de origine naturală, polizaharidele (ex. chitosanul și derivații de celuloză) și uleiurile esențiale prezintă un interes crescând ca agenți de gelifiere și respectiv promotori de penetrare pentru dezvoltarea preparatelor topice semisolide, inclusiv hidrogeluri. Prin urmare, în cadrul acestui studiu s-a încercat formularea și prepararea unor noi hidrogeluri biocompatibile, utilizând ca substanță medicamentoasă fluconazolul (FZ), două polizaharide în amestec (chitosan și hidroxipropilmetilceluloză) ca agenți gelifianți, ulei de cuișoare (CVO) și ulei de scorțișoară (CNO) ca potențiali promotori de penetrare și componente antifungice sinergice cu FZ, cosolvenți și un surfactant sintetic neionic ca solubilizanti. Hidrogelurile preparate au fost caracterizate în ceea ce privește conținutul în substanță activă, pH-ul și proprietățile reologice (comportamentul de curgere, vâscozitatea, consistența și capacitatea de întindere). De asemenea, a fost evaluată eliberarea *in vitro* a fluconazolului prin membrana sintetică hidrofilă, utilizând metoda cu celulă de difuzie verticală. Rezultatele obținute au arătat că toate formulările experimentale au fost omogene, prezentând caracteristici reologice adecvate și pH foarte apropiat de limita inferioară a intervalului recomandat de farmacopee. Pe baza rezultatelor obținute, se poate sugera că atât compoziția uleiurilor esențiale, cât și concentrația lor în formulări au efecte semnificative asupra proprietăților mecanice și disponibilității farmaceutice a hidrogelurilor testate. Rezultatele testului de eliberare *in vitro* a substanței medicamentoase au susținut ca fiind cele mai bune vehicule topice pentru administrarea fluconazolului, formulările FZ-CNO 3%, FZ-CNO 5% și FZ-CVO 5%, conținând 3% sau 5% ulei de scorțișoară și respectiv 5% ulei de cuișoare, încorporate într-un hidrogel pe bază de chitosan-hidroxipropilmetil-celuloză. În concluzie, aceste hidrogeluri ar trebui investigate în continuare prin studii *in vitro* și *in vivo* ca transportori promițători pentru administrarea locală a fluconazolului.

Keywords: fluconazole, chitosan, essential oil, hydrogel

Introduction

For the treatment of various dermatological diseases, such as superficial fungal infections, semisolid dosage forms including ointments, creams and gels are preferred by both physicians and patients. Within the group of these preparations, the use of hydrogels as vehicles for numerous drugs has been extended in the last decades [4, 9, 11, 12, 19]. It is well known that the hydrogel excipients, especially the gelling agents, cosolvents and penetration enhancers, play an essential role in the drug release from the vehicle and in the drug ability to overcome the *stratum corneum*, considered the main barrier for drug penetration into and through the skin after dermal administration [8, 16]. Therefore, during the development of the hydrogel vehicles, the selection of appropriate excipients is an essential phase to provide drug delivery systems with improved properties, such as: enhanced skin penetration, therapeutic efficacy, safety and enhanced patient compliance. Nowadays, the developers and formulators of semisolid dosage forms commonly use excipients of synthetic and semi-synthetic origin, presenting unique properties and offering several benefits over natural excipients (i.e. no inherent variability, low sensitivity to moisture and different formulation components). However, in recent decades there has been increasing interest in the use of pharmaceutical excipients of natural origin which are biocompatible, biodegradable and toxicologically harmless [17].

Natural polysaccharides represent a group of biopolymers widely used as excipients in the formulation of semisolid dosage forms, especially hydrogels, in which they act as gelling agents, emulsifiers, stabilizers and mucoadhesives [1, 2, 10]. Chitosan, also named deacetylchitin, poly-D-glucosamine and poliglusam, is an abundant natural polysaccharide resulted from alkaline deacetylation of chitin, with interesting unique properties for biomedical applications, namely biocompatibility, biodegradability, non-toxicity, low allergenicity and mucoadhesivity. Besides, chitosan is a renewable polymer and exhibits inherent antimicrobial properties, including antifungal activity. Chitosan is the only polysaccharide positively charged and soluble in diluted aqueous acidic solutions [2, 15]. This basic polysaccharide has a rigid crystalline structure, determined by inter and intra-molecular hydrogen bonding, but due to the polymer chain relaxation commonly observed during hydration, the chitosan hydrogels show a relatively low mechanical strength [7, 15] which is not favourable when used as topical preparations. Therefore, the association of chitosan with hydrophilic polymers forming gels with higher mechanical strength is beneficial in the case of topical hydrogel formulations. Among the hydrophilic polymers tested in this purpose, cellulose derivatives, such as methylcellulose and hydroxylpropylmethylcellulose (HPMC) are of great interest,

being excipients of semi-synthetic origin, nontoxic and non-irritating, with a “Generally Regarded As Safe” (GRAS) status attributed by the Food and Drug Administration of the United States of America [3, 18].

Within the group of natural penetration enhancers, essential oils and their constituents have gained increasing interest in the recent years, being preferred over the commonly used synthetic compounds, as they are safe (non-irritating, nontoxic, non-allergenic) with GRAS status, compatible with drugs and other excipients and suitable skin penetration promoters for both hydrophilic and lipophilic drugs. Furthermore, essential oils and their constituents possess numerous biological activities, including antibacterial, antifungal and antiviral effects [6]. Thus, the antimycotic activity of clove oil and cinnamon oil against various pathogenic fungi species in humans was recently reported and attributed to the high level of eugenol in their composition [5, 13]. Therefore, these essential oils considered non antibiotic agents, could be associated with common antifungal drugs to enhance their efficacy after topical administration, by synergistic effect and enhancing the skin penetration of drugs. On the other hand, it is necessary to mention that the incorporation of essential oils into hydrophilic dosage forms, like hydrogels, presents some technological limitations, because of the hydrophobic, reactive and volatile nature of the bioactive components of these oils.

In this study, fluconazole (FZ) was selected as model drug for two reasons: 1) it is still one of the most used antifungal drug in medical practice for the treatment of both superficial and systemic fungal infections; 2) after oral administration, it accumulates in the *stratum corneum* of the skin for which it has high affinity and therefore it may be a good candidate for the transport through the skin, which could be an attractive alternative to enteral transport especially in the case of the treatment of superficial fungal infections. The purpose of this study was to explore the possibility of formulating some hydrogels for topical delivery of fluconazole, using two mixed biopolymers (chitosan and hydroxypropylmethylcellulose) as gelling agents, clove oil and cinnamon oil as potential penetration enhancers and synergistic antifungal components with FZ, cosolvents and a non-ionic synthetic surfactant as solubilizers for drug and essential oils. In addition, the influence of essential oils on the physical properties, rheological behaviour and *in vitro* drug release of the experimental hydrogels was investigated.

Materials and Methods

Materials

Fluconazole (FZ) and chitosan (CTS) (Chitopharm[®]M) have been generously donated by S.C. Vim Spectrum SRL (Romania) and respectively S.C. Antibiotice SA Iași (Romania). Essential Cinnamon Oil (CNO) and

Clove Oil (CVO) were purchased from Elemental, Romania. The surfactant Cremophor A25 (polyethylene glycol 25 cetostearyl ether) was generously donated by BASF Chem Trade GmbH (Germany). Tetraglycol (TG) was purchased from Merck Schuchardt OHG (Germany). Acetic acid and sodium chloride were purchased from S.C. Chimopar (Romania), and ethanol (96%) was supplied by S.C. Chemical Company S.A. (Romania). Hydroxypropylmethylcellulose (HPMC) (Methocel K4M) was generously donated by Colorcon L.t.d. (England). Copper (II) chloride ($\text{CuCl}_2 \cdot \text{H}_2\text{O}$) was supplied by Sigma Aldrich (Germany). Distilled water was used to prepare the hydrogels, and bidistilled water was used to prepare the isotonic sodium chloride solution (0.9%). Synthetic hydrophilic polysulfone membranes, 25 mm in 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

Preparation of chitosan/HPMC-based hydrogels containing fluconazole and essential oils. Experimental hydrogels were prepared in two steps. In the first step, a hydrogel base was prepared using two hydrophilic biopolymers, chitosan and HPMC. In the second step, the medicated liquid phase containing FZ, cosolvents, non-ionic surfactant and essential oil was incorporated into the hydrogel base to obtain the final chitosan/HPMC-based hydrogel formulations. The codes and the composition of the five experimental formulations are given in Table I.

Table I

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

Hydrogel components	Weight (%) and formulation codes				
	FZ 2%	FZ-CNO 3%	FZ-CNO 5%	FZ-CVO 3%	FZ-CVO 5%
Fluconazole	2.0	2.0	2.0	2.0	2.0
Cinnamon oil	-	3.0	5.0	-	-
Clove oil	-	-	-	3.0	5.0
Tetraglycol	10.0	10.0	10.0	10.0	10.0
Ethanol	20.0	20.0	20.0	20.0	20.0
Cremophor A25	0.5	0.5	0.5	0.5	0.5
Hydrogel CTS 3%	22.5	21.5	20.83	21.5	20.83
Hydrogel HPMC 4%	45.0	43.0	41.67	43.0	41.67

Hydrogel base preparation was carried out by mixing a 3% chitosan hydrogel with a 4% HPMC hydrogel in a ratio of 1:2. The hydrogel with 3% chitosan was prepared by dissolving the polymer by gently stirring in 1% acetic acid aqueous solution. The hydrogel based on 4% HPMC was prepared by the "hot/cold" method: the polymer was dispersed in distilled water heated to 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 (2-8°C) for 30 minutes.

Preparation of medicated chitosan/HPMC-based hydrogels containing essential oils. Pre-weighed amounts of fluconazole and Cremophor A25 were dissolved in the co-solvent mixture (tetraglycol-ethanol 1:2). In the obtained solution, the essential oil (CNO or CVO) in 3% or 5% concentration (w/w) was added under stirring, until complete dissolution. Into the hydrogel base, the solution of fluconazole, essential oil and Cremophor A25 in the cosolvent mixture was added by moderate agitation (300 rpm). Stirring was continued under the same conditions, avoiding as much as possible air incorporation, until a homogeneous hydrophilic gel was obtained.

Preparation of 2% fluconazole hydrogel without essential oil (control formulation) was performed similarly to the preparation of hydrogels with

fluconazole and essential oils, but without the use of the essential oil.

In the final preparations, the fluconazole concentration was 2% (w/w), and the volatile oil (cinnamon or clove) content was 3% or 5% (w/w) (Table I).

After preparation, all experimental hydrogels were sonicated for de-aeration and stored cold for 24 hours before further evaluation.

Determination of drug content and pH

The FZ content was determinate using an UV-VIS spectrophotometric method (T70+UV-VIS spectrophotometer, UVWIN5 soft, PG Instruments, England). Approximately 0.4 g of the hydrogel was weighed, and transferred to a 25 mL volumetric flask with 96% ethanol. The obtained dispersion was filtered through a membrane filter (0.45 μm , 25 mm, Teknokroma, Germany) and the concentration of fluconazole in the filtrate was spectrophotometrically analysed at 253 nm.

The pH value of the experimental hydrogels was carried out by the potentiometric method, using a portable digital pH meter (Sension™1, Hach Company, U.S.A.). The procedure was as follows: about 5 g of hydrogel and 20 mL of distilled water previously heated to $37 \pm 2^\circ\text{C}$ were added and stirred vigorously for 1 minute. After cooling, the obtained dispersion was filtered and the pH was determined in the filtrate.

The drug content and pH of each hydrogel formulation was determinate in triplicate.

Rheological measurements

Viscosity and rheological profiles of the hydrogels were assessed by the steady state flow test, using a stress-controlled rheometer (RheoStress 1, HAAKE, France) equipped with a cone-plate geometry (1/60); the analysis of the experimental data was carried out using HAAKE RheoWin 4 version 4.30 software. To evaluate the consistency of the experimental hydrogels, the penetration values and spreadability were measured by penetrometry and parallel-plate method respectively. The penetrometric measurements were performed using a penetrometer (PNR 12, Petrolab, Germany) equipped with a micro-cone and suitable container and according to the compendial procedure. The spreadability determination was carried out using the Pozo Ojeda-Sune Arbussa extensometer, following the technique described in the literature [14]. The values of the areas occupied by the sample, obtained as an average of three determinations, were plotted vs. to the corresponding standardized weight, resulting the extensometric profiles. All rheological tests were performed in triplicate at 25°C.

In vitro drug release experiment through synthetic membrane

The study of the *in vitro* cumulative release of FZ from the chitosan/HPMC-based hydrogels containing volatile oils was performed to evaluate the effect of the formulation variables on the performance of the preparations.

The experiments were carried out in freshly prepared, heated and de-aerated 0.9% sodium chloride with 60% ethanol solution as receptor medium, using a Franz diffusion cells system (Microette-Hanson system, model 57-6AS9, USA) with a polysulfone synthetic membrane (25 mm diameter, 0.45 µm pores, HT Tuffryn membranes, Pall Corporation, USA) under sink conditions. The diffusion cells presented a diffusion area of 1.767 cm² and a volume of 6.5 mL for the

receptor compartment. Synthetic membranes were mounted between the donor and receptor compartment of the vertical diffusion cells and were kept in contact with the receptor medium for 30 minutes before the sample application. Approximately 0.300 g of sample was weighed into the dosing capsule of each diffusion cell. Throughout the test, the system was maintained at 32 ± 1°C and the receptor medium was stirred continuously (at 600 rpm) by means of a magnetic stirrer to avoid the effects of the diffusion layer. At pre-determined time intervals, 0.5 mL of the receptor solution were automatically withdrawn and replaced with equal volume of fresh receptor medium. In the collected samples, 0.5 mL of 0.0514% (w/V) cupric chloride solution was added to form the fluconazole-Cu (II) complex.

The cumulative amounts of FZ were determined by UV spectrophotometric method (UV-VIS T70+ spectrophotometer with UVWIN5 soft, PG Instruments, England) at the wavelength of 330 nm, corresponding to the maximum absorption of the FZ-Cu (II) in physiological saline with 60% ethanol solution. The determinations were made in triplicate.

The *in vitro* release data were analysed by calculating the permeation and release parameters: the steady state flux (J_{ss} , µg/cm²/h), the permeability coefficient (Kp , cm/h) and the lag time (t_L , h), and respectively the release rate (k , µg/cm²/h^{1/2}) and diffusion coefficient (D , cm²/h).

Statistical analysis

All the results were expressed as mean ± standard deviation (SD) using Statistica software (version 7.0).

Results and Discussion

Determination of drug content and pH

The fluconazole content and pH of the experimental chitosan-HPMC-based hydrogels with or without essential oils is shown in Table II.

Table II

Drug content, pH, viscosity and penetration values of the experimental FZ hydrogels

Formulation code	Drug content (%)	pH at preparation	pH at 30 days after preparation
FZ 2%	102.36 ± 2.57	4.06 ± 0.004	4.1 ± 0.003
FZ-CNO 3%	99.47 ± 1.82	4.08 ± 0.005	4.04 ± 0.001
FZ-CNO 5%	98.23 ± 3.17	4.07 ± 0.002	4.09 ± 0.009
FZ-CVO 3%	97.61 ± 1.33	4.07 ± 0.006	4.12 ± 0.002
FZ-CVO 5%	98.95 ± 0.86	4.06 ± 0.005	4.09 ± 0.008

When evaluating the drug content of the experimental semisolid formulations, consideration was given to the official monograph, which permits a ± 3% deviation from the declared value for products containing 0.5% and more than 0.5% of the active drug [20]. The FZ content of experimental hydrogels found to be in the range of 97.61 - 102.36% (Table II), was within the admitted official limits

and indicates the uniform distribution of the active drug in the preparations.

The pH of all tested hydrogels fell into the weak acid range, and varied very little around 4. It was noticed that no significant changes in the pH values occurred over the entire test period, which suggests the chemical stability of the formulation components under storage conditions.

Rheological measurements

The results of the steady state flow test are shown in Figure 1, in form of rheograms (the shear stress, τ , as a function of shear rate, $\dot{\gamma}$) and viscosity curves (the apparent viscosity, η , as a function of shear

rate). Also, Table III lists the apparent viscosity and thixotropy values obtained for the experimental preparations.

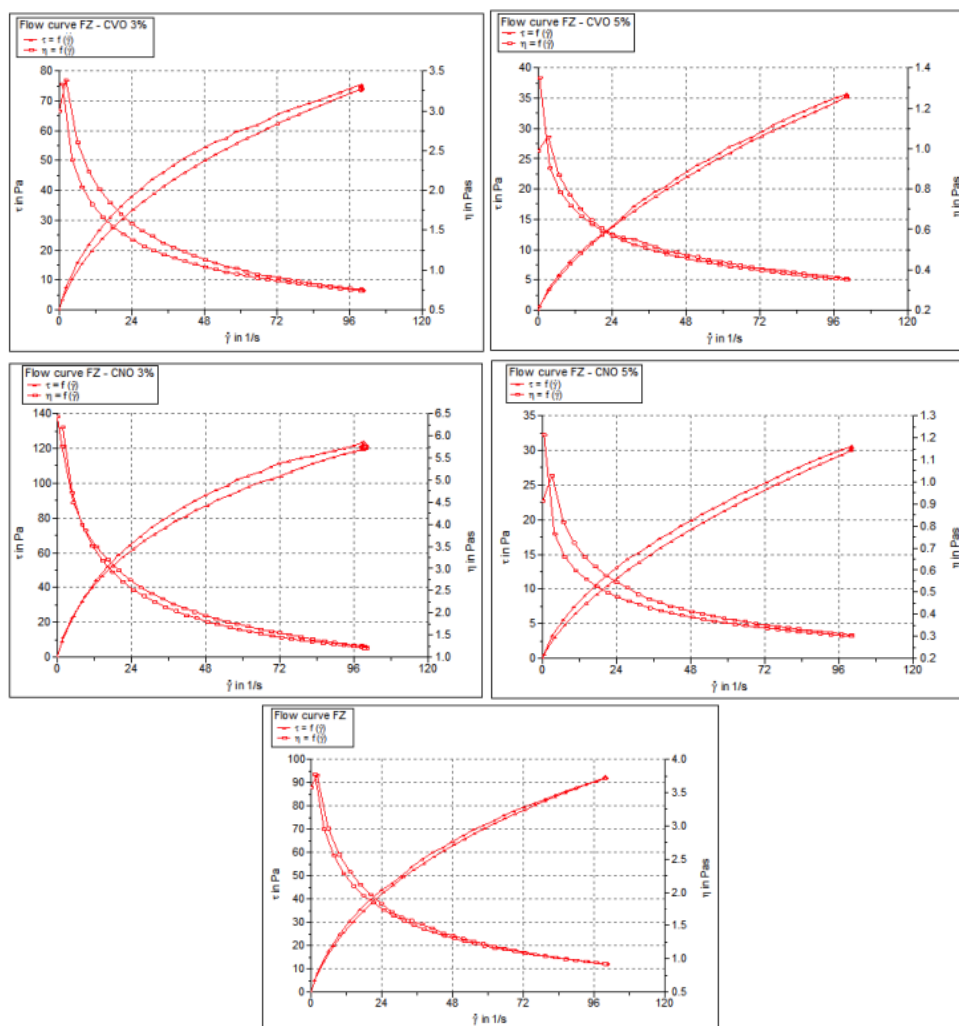


Figure 1.

Rheograms and viscosity curves of the experimental hydrogels

Table III

Viscosity, thixotropy and penetration values of the experimental FZ hydrogels

Formulation code	Viscosity (Pa·s)	Thixotropy (Pa/s)	Penetration value (mm)
FZ 2%	0.924 ± 0.56	1220	212.00 ± 1.63
FZ-CNO 3%	1.219 ± 0.87	4040	272.67 ± 3.86
FZ-CNO 5%	0.303 ± 0.22	182.5	488.00 ± 8.04
FZ-CVO 3%	0.745 ± 0.61	1254	281.67 ± 1.70
FZ-CVO 5%	0.354 ± 0.48	305.7	358.00 ± 5.89

It can be observed that all of the tested FZ hydrogels showed a pseudoplastic-thixotropic behaviour, but the degree of thixotropy was different. Compared to the control formulation (FZ 2%), the gels with 3% cinnamon or clove oil exhibited a thixotropy of 3.3 times, respectively 1.03 times higher, whereas the degree of thixotropy of the gels with 5% cinnamon or clove oil was 6.7 times, respectively 4 times lower. It was found that the essential oil,

especially the cinnamon oil, in a concentration of 3% caused an increase in the thixotropic character of the tested hydrogels, and at a higher concentration (5%) produced a drastic decrease of the thixotropy degree. Thus, it can be suggested that the type and the concentration of the essential oil considerably influenced only the degree of thixotropy, the rheological behaviour being the same.

The profile of the viscosity curves of the experimental hydrogels showed that their viscosity decreases with the increase in shear stress and, respectively shear rate that is specific to pseudoplastic non-Newtonian systems. As can be observed from Table III, the presence of the essential oil in the formulation affected the viscosity of the studied hydrogels, the extent of modification depending on its concentration and composition. Thus, it was found that at a concentration of 5% both essential oils produced about a 3-fold decrease in the viscosity of the experimental hydrogels, while at a concentration of 3% the cinnamon oil increased the viscosity of the control gel 1.32 times, and the clove oil decreased 1.24 times the viscosity of the control gel.

Both the viscosity and thixotropy differences can be attributed to the changes in the chitosan/HPMC binary three-dimensional gel structure, because of the different polarity and chemical composition of the essential oil (mainly eugenol, a phenylpropanoid derivative for the clove oil, and cinnamaldehyde for the cinnamon oil), as well as to possible interactions between the molecules of different components (water, cosolvents, terpenes and macromolecular compounds). For all formulations, the viscosity values ranged in the specific field of semisolid preparations.

The results of the consistency measurements through penetrometry are listed as penetration values in Table III. It can be noticed that for the formulation *FZ-CNO 5%* the highest penetration value was measured, indicating the lowest consistency. Also, the hydrogel containing 5% clove oil (*FZ-CVO 5%* formulation) led to a high penetration value (only 1.36 times lower than that produced by the formulation *FZ-CNO 5%*) resulting in low consistency. On the other hand, the consistency of hydrogels containing 3% essential oil was 1.73 to 1.78 times higher than that of the hydrogel with 5% cinnamon oil, as indicated by lower penetration values (Table III). The hydrogel with 2% fluconazole but without essential oil was the most consistent, showing the lowest penetration value. Similar to the steady state flow test, the results of the penetrometry measurements suggested the influence of the essential oil composition and concentration on the consistency of the chitosan/HPMC-based hydrogels containing 2% FZ. In addition, these results are consistent with those obtained by the viscosimetric test.

Along with viscosity, thixotropy and flowability, spreadability of the semisolid dosage forms, is an important rheological property related to consistency, as it contributes both to the application or delivery of a suitable drug dose on the skin and to the ease of semisolid application on the substrate. Besides, the spreadability considerably influences the patient's preference for the respective semisolid formulation. The results of the spreadability test are shown in Figure 2 as extensometric profiles.

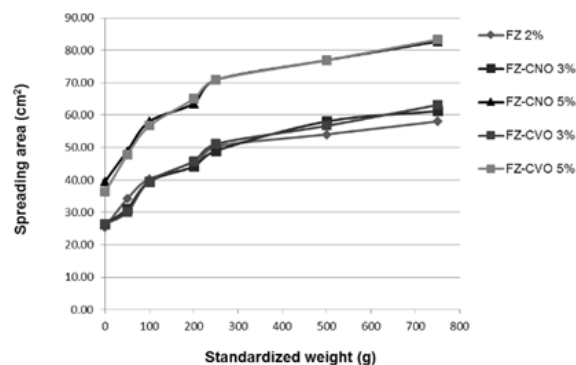


Figure 2.

Influence of the type and concentration of essential oils on the spreadability of the tested hydrogels (mean $\pm$ SD, $n = 3$)

From the comparative analysis of the extensometric profiles of the experimental chitosan/HPMC-based hydrogels containing fluconazole and essential oils (Figure 2), it can be observed that for all tested hydrogels the spreadability increased progressively with the increase in the applied weight. The largest spreading areas were measured, for all weights applied, for the fluconazole hydrogels containing 5% essential oil (*FZ-CNO 5%* and *FZ-CVO 5%* formulations); moreover, the extensometric profiles of these systems were super-posable. Similar extensometric profiles, but with smaller spreading areas, showed the experimental hydrogels containing 3% volatile oil. The spreading areas produced by the medicated hydrogel without volatile oil were similar to that of the hydrogels containing 3% essential oils when 0 - 250 g were applied, and smaller when more than 250 g were applied, showing the lowest spreadability. However, all tested systems showed good spreadability, demonstrated by the obtained high extensometric values. The results of the extensometric measurements were consistent with those of consistency and viscosity, indicating that the experimental hydrogels with lower consistency and viscosity had a higher spreadability. Also, the influence of essential oil, by its chemical composition and its concentration in the formulation, on the spreadability of the experimental gels is noted.

In vitro fluconazole release from the experimental chitosan/HPMC-based hydrogels through synthetic membrane

The *in vitro* cumulative release of FZ from chitosan/HPMC-based hydrogels containing volatile oils was investigated in order to evaluate both the performance of the formulations (the ability of the vehicle to release the drug) and the effect of the volatile oils (formulation variables) and of the mechanical properties of the gel base (viscosity, consistency) on the *in vitro* availability of fluconazole. Based on the results of this test, it is possible to select the best formulation or to create premises for the

optimization of the evaluated formulations. The results of this study are illustrated in Figure 3 and listed in Table IV.

In vitro fluconazole permeation profiles through hydrophilic synthetic membrane from chitosan/HPMC-based hydrogels (mean $\pm$ SD, n = 3)

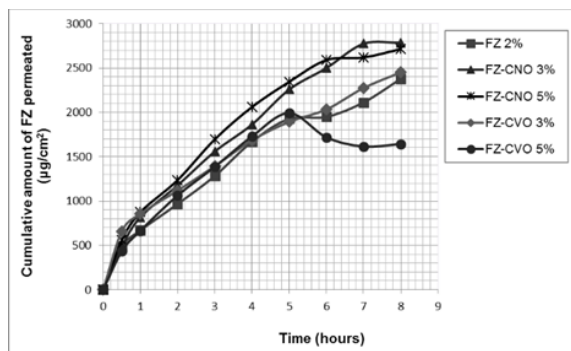


Figure 3.

Table IV

Specific permeation and release parameters of the hydrogels with 2% fluconazole, through synthetic membrane

Formulation code	Permeation parameters		Release parameters	
	J ($\mu\text{g}/\text{cm}^2/\text{h}$)	$K_p \times 10^{-6}$ (cm/h)	k ($\mu\text{g}/\text{cm}^2/\text{h}^{1/2}$)	D $\times 10^{-3}$ (cm^2/h)
FZ 2%	323.75 $\pm$ 1.02	161.88	950.64 $\pm$ 0.98	1.77
FZ-CNO 3%	328.90 $\pm$ 0.62	164.45	1213.30 $\pm$ 2.05	2.89
FZ-CNO 5%	395.95 $\pm$ 1.28	197.98	1174.70 $\pm$ 2.26	2.71
FZ-CVO 3%	227.03 $\pm$ 7.95	113.52	886.59 $\pm$ 9.57	1.54
FZ-CVO 5%	346.15 $\pm$ 2.41	173.08	1026.60 $\pm$ 2.85	2.07

Results indicated that after 8 hours of testing, the highest cumulative amounts of fluconazole (85.14% and 81.43%) were released from FZ-CNO 3% and FZ-CNO 5%. The FZ release for hydrogels containing clove oil was lower, namely 71.11% at the end of the test period (for FZ-CVO 3% formulation) respectively 60.05% after 5 hours of testing (for FZ-CVO 5% formulation). The control formulation (without essential oil) released the lowest cumulative amount of FZ until reaching the steady state (within 5 hours of testing), but also at the end of testing, when the released amount of FZ increased to 68.66%. The high percentages of fluconazole released from the experimental hydrogels, especially from those containing cinnamon oil, indicated the following: the used receptor medium was adequate; the relative low affinity of the active substance for the vehicle in which it was dissolved; the ability of the hydrogel base to release the drug, thereby assuring the performance of the formulation. In addition, the time interval after which almost all fluconazole was released from the samples suggests the influence of the viscosity and formulation consistency on this process.

From Figure 3 it can be observed that all permeation profiles of fluconazole for the analysed hydrogels, except for that corresponding to the FZ-CVO 5% formulation, showed a linear portion with an increasing slope over 1 - 7 hours or 1 - 8 hours, suggesting an intense and progressive transfer of FZ through the hydrophilic synthetic membrane. The FZ permeation

from the FZ-CUI 5% formulation was similar, but only for 5 hours, after which the steady state was installed. By comparing the values of the specific *in vitro* permeation and release parameters of fluconazole from the studied hydrogels through synthetic membrane (Table IV), it was found that the systems containing 5% cinnamon or clove oil produced the highest FZ transfer rate values (395.95 $\pm$ 1.28 $\mu\text{g}/\text{cm}^2/\text{h}$, respectively 346.15 $\pm$ 2.41 $\mu\text{g}/\text{cm}^2/\text{h}$), followed by the FZ-CNO 3% and FZ 2% hydrogels, which led to close values of the steady state flow (328.90 $\pm$ 0.62 $\mu\text{g}/\text{cm}^2/\text{h}$, and 323.75 $\pm$ 1.02 $\mu\text{g}/\text{cm}^2/\text{h}$ respectively). For the formulation FZ-CVO 3% a steady state flow value of 1.7 times lower than that produced by the FZ-CNO 5% formulation was obtained.

The values of the FZ release rate from the studied hydrogels through synthetic membrane varied in a similar manner to the steady state flux, with the following hierarchy in descending order: FZ-CNO 3% > FZ-CNO 5% > FZ-CVO 5% > FZ 2% > FZ-CVO 3%. It is also worth noting that the FZ release rate values were 3 - 4 times higher than the steady state flux values, suggesting that the drug permeation through the membrane was independent of its release from the preparations.

The results of the *in vitro* release study demonstrate the differences between the analysed formulations, which can most likely be attributed to the combined effects of the following factors: the different chemical composition and the concentration of the essential oil present in the gel base; the solubility

and thermodynamic activity of the drug in the hydrogel base, due to the presence of the two cosolvents (tetraglycol and ethyl alcohol) and of the essential oil, components in which fluconazole is more soluble than in water because it has a much lower polarity; the affinity of the gel base for the active compound, correlated with its solubility in the base; the viscosity/consistency of this systems. Thus, higher values of the steady flux and release rate were obtained for the systems containing essential oil (Table IV), although the solubility and affinity of fluconazole for these systems was most likely higher than those for the gel without the volatile oil. This can be attributed to the viscosity and lower consistency of the respective gels. It is generally accepted, that the lower viscosity of a topical formulation allows an easier diffusion of the active drug through the vehicle, due to the reverse relationship between viscosity and the capacity of the formulation to yield the drug. These results indicate the dominant effect of the thermodynamic activity (solubility) of the drug, and the viscosity of the hydrogels on the FZ release from these vehicles.

Conclusions

In the present study, fluconazole, a poorly water-soluble antifungal drug, and two essential oils having intrinsic antimycotic activity (cinnamon oil and clove oil), were successfully formulated and incorporated into chitosan/HPMC-based hydrogels containing cosolvents (tetraglycol and ethanol) and a non-ionic surfactant. The results of the rheological tests demonstrated that all studied hydrogels exhibited non-Newtonian pseudoplastic behaviour with more or less pronounced thixotropic character, and adequate viscosity, consistency and spreadability. The differences between formulations in terms of these properties could be attributed to chemical composition and concentration of the incorporated essential oil. Also, the pharmaceutical availability of the studied systems was different, depending on the composition and mechanical properties (viscosity, consistency) of the hydrogel base. The addition of essential oil in increasing concentration has increased the release and permeation rate of fluconazole from the vehicle through the synthetic hydrophilic membrane. Based on the results of the *in vitro* release study, the most desirable hydrogel vehicles for topical delivery of FZ were considered the formulations *FZ-CNO* 3%, *FZ-CNO* 5% and *FZ-CVO* 5%, containing 3% or 5% cinnamon oil and respectively 5% clove oil as potential penetration enhancers, 30% mixture of tetraglycol-ethanol (1:2) as cosolvents, 0.5% Cremophor A25 as solubilizer, 0.64% or 0.62% chitosan and 1.72% or 1.66% HPMC as gelling agents and water; these produced the highest FZ transfer rate through the hydrophilic synthetic membrane (328.90 - 395.95

$\mu\text{g}/\text{cm}^2/\text{h}$), thus demonstrating their *in vitro* performance. From this preliminary study it can be concluded that incorporation of cinnamon oil and clove oil in polysaccharides-based hydrogels can be explored for effective and safe topical delivery of fluconazole to improve the treatment of superficial fungal infections. Therefore, thorough *in vitro* and *in vivo* studies regarding their stability, safety and therapeutic efficacy need to be conducted for developing a commercially viable topical fluconazole hydrogel.

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