THE INFLUENCE OF A DENTAL FORMULATION PREPARED WITH CHITOSAN ON THE PHARMACEUTICAL AVAILABILITY OF CLOTRIMAZOLE

Bożena Grimling[1*], Jan Meler[1], Maria Szcześniak[1], Martyna Kocoń[1], Bożena Karolewicz[1], Adrianna Złocińska[2], Agata Górniak[2]

1 - Faculty of Pharmacy, Department of Pharmaceutical Technology, The „Silesian Piasts" Memorial Medical University of Wroclaw 50-556 Wroclaw St. Borowska 211a, Poland
e-mail:bozena.grimling@umed.wroc.pl

2 - Faculty of Pharmacy, Laboratory of Elemental Analysis and Structural Research The „Silesian Piasts" Memorial Medical University of Wroclaw 50-556 Wroclaw St. Borowska 211a, Poland

Abstract

The present work involves the development of a dental gel composition obtained on the basis of clotrimazole incorporated into chitosan in order to improve drug solubility. Solid dispersions were prepared by using two methods: grinding and kneading. The solid dispersion varied the ratio of chitosan to drug to increase the volume of the drug; the ratios were 5:5, 3:7, 2:8, 1:9. The mixtures were subjected to the dissolution rate of clotrimazole. The presence of chitosan improved the drug solubility; a better solubility from the solid dispersion prepared by the grinding method was obtained from the ratio of drug to polymer of 1+9. The rate of dissolution of clotrimazole was improved 17 times compared to the pure drug. Fourier transform infrared spectroscopy (both infrared and X-ray diffraction) revealed no new chemical structure of the tested connections and concluded that there was no interaction between the drug and the polymer in the test diffractions.

Solid dispersions with the best parameters were used to prepare hydrogels, and the pharmaceutical availability of clotrimazole was analysed. The best properties were characterized by a hydrogel that was composed of the ratio of the amount of drug to polymer 5:5. The study demonstrated the availability of a pharmaceutical drug release at a therapeutic concentration in the first hour of the study.

The use of the appropriate balance between clotrimazole and chitosan and the development of the hydrogel composition may affect the improvement of the drug solubility and may create the possibility of obtaining sustained or controlled release of the drug substance.

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Key words*: clotrimazole, chitosan, solid dispersion, hydrogel, carrier XRPD, FTIR*

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1. Introduction

The physical and chemical properties of a drug substance affect its rate of dissolution and permeation through biological membranes, which in effect determines whether the therapeutic concentration is reached at the site of action. This dependence plays an important role especially in the case of poorly soluble active substances. An example of such a drug is clotrimazole (1-[(2-chlorophenyl)(diphenyl)methyl]-1H-imidazole), which, due to low water solubility (0.49 mg/mL at 25°C) [1] and it being highly lipophilic. This drug belongs to class II in The Biopharmaceutical Classification System. Clotrimazole is used to treat fungal infections of the skin and mucous membranes. The spectrum of its activities includes i.a. dermatophytes and yeasts [1,2]. It is also a promising agent in the pharmacotherapy of malaria and cancer [1,2].

Currently, drug formulations containing clotrimazole are being developed in combination with polymers that prolong its release time. Singh et al. developed a buccal bioadhesive film containing clotrimazole, with carboxymethylcellulose and carbopol 974P [3]. Harish et al. prepared based gels on carbopol 934P and hydroxypropyl methylcellulose containing clotrimazole, which have demonstrated prolonged release of the drug [4].

Chitosan is a natural polymer. It has many properties, including adequate mucoadhesiveness, biocompatibility, biodegradability and desirable physicochemical characteristics: appropriate viscosity, wetting and high swelling, fungicidal and bactericidal. It is often used in drug form technology as a film-forming agent, drug carrier, or tablet disintegrant. There are few reports of the effect of chitosan on improving the solubility of BCS class II substances.

One way to improve the solubility of solid substances is solid dispersions. Thus, the combination of clotrimazole and chitosan has been identified here to improve drug solubility, prolong drug release time and obtain antifungal synergism. The polymer also shows antimicrobial activity, which enhances the action of the drug [5,6]. This work involves the development of dental gel composition obtained by incorporating clotrimazole onto chitosan in order to improve drug solubility.

2. Materials and methods

2.1. Materials

This study was performed with the use of clotrimazole (CLT), which was kindly given to us by P.P.F. “Hasco-Lek” S.A. Poland. This was incorporated into natural, highly purified chitosan of high molecular weight (CHIT) >75%, deacetylation of 95% and viscosity of 800–2000 cP was kindly given to us by “Sigma-Aldrich”. In the studies sodium lauryl sulphate (SLS) was also used, which was purchased from PPH “Stanlab” in Poland, Aqua purification and Ethanol 760 g/l acc. to FP IX. Other materials used in the study were of analytical grade.

2.2. Methods

2.2.1. Technology of the preparation of investigated formulations

Solid dispersions were prepared by using two methods: grinding and kneading methods.

2.2.1.1. Grinding method

An appropriate amount of polymer and clotrimazole were weighed on a SARTORIUS analytical balance and transferred quantitatively into a mortar in which the mixture was ground for 10 minutes. The weight ratios of drug to polymer were 5:5, 3:7, 2:8 and 1:9. The mixtures were then sieved using a mesh size of 315 μm and placed in

sealable glass vials. The samples were stored in a desiccator. Then, the prepared samples were weighed and solid dispersions were prepared by using two methods: grinding and kneading methods. A quantity of 100 mg of sample was pressed under a pressure of 1 ton using a hydraulic press (Specac). The resulting tablets were weighed. Tablets of pure clotrimazole were made in the same way.

2.2.1.2. Kneading method

The sample weight of clotrimazole was quantitatively transferred to a mortar. The drug was then dissolved in the appropriate amount of ethanol. The sample weight of chitosan of a suitable molecular weight was added to the solution. The weight ratios of drug to polymer were 5+5, 3+7 and 1+9. The mixture was ground for 10 minutes and the solvent was allowed to completely evaporate at room temperature. The dry solid dispersion was sieved using a mesh of 315 μm and placed in sealed vials. The samples were stored in a desiccator. Next, the tablets were prepared as described above (table 1). Additionally, a dispersion was made as a MF 2:8 formulation. It was made because of the inability to obtain a hydrogel with the use of a MF 1:9 dispersion due to a high chitosan concentration and a too-high viscosity.

Table 1. The quantitative composition of solid dispersions prepared by the kneading and grinding methods of clotrimazole onto chitosan

Solid dispersion	Drug/polymer ratio	Quantity of drug CLO [mg]	Quantity of polymer CHIT [mg]
MF 1:9	1:9	100	900
MF 2:8	2:8	200	800
MF 3:7	3:7	300	700
MF 5:5	5:5	500	500
SR 1:9	1:9	100	900
SR 3:7	3:7	300	700
SR 5:5	5:5	500	500

MF – the physical mixture (grinding method), SR – the solid dispersion (kneading method), CLO – clotrimazole, CHIT – chitosan

2.2.2. Examination of pure clotrimazole and its solid dispersion dissolution rate

The examination of solubility was carried out in a tablet dissolution apparatus according to FP X, which determines the active substance dissolution rate from solid drug forms. This was performed using a VanKel VK 7025 dissolution apparatus, which was connected to a fraction collector Varian Inc. A volume of 500 mL of a 1% solution of SLS was used as releasing medium.

Dissolution was evaluated after compressing 100 mg of samples, which were placed in each of the six chambers of the apparatus at 37 ± 0.5°C, with a velocity of 100 rpm. The trial was continued for 1 hour, and 5 mL samples were collected at 10 time intervals, i.e. after 5, 10, 15, 20, 25, 30, 35, 40, 50 and 60 minutes. Collected samples were filtered on filters with 10 μm pore size.

A quantitative determination of CLT was performed using an HPLC Ultimate 3000 z system with autosampler (Thermo Scientific) with a column of Zorbax ODS C18 (5 μm, 4.6 × 150 mm, Agilent). Analyses were performed using isocratic elution with a mixture of

solvents, with a composition ratio of acetonitrile to water of 70:30 with a steady flow rate of 1.5 mL/min.

Substances eluted from the column were identified by a spectrophotometric detector at 230 nm. External standards of CLT were used to obtain calibration curves. Evaluation methods were linear in the range of 0.00025–0.025 mg/mL for both compounds (linearity r^2 = 0.9994) [7].

Quantitative drug to polymer ratios in which the solid dispersion had the most beneficial properties improving the drug dissolution were determined.

2.2.3. Examination of samples by X-ray diffraction (XRD)

Powder X-ray diffraction patterns for solid dispersions containing clotrimazole and chitosan and pure substances were recorded on an X-diffractometer (Bruker D2 Phaser, detector LynxEye, USA), employing CuK∞ radiation at room temperature. Samples were scanned from 7 to 50° (2θ) at a scanning rate of 0.05° through s^t the measurement range at 0 5 s $step^{-1}$.

2.2.4. Fourier transform infrared spectroscopy (FTIR)

The FTIR spectra of clotrimazole and chitosan and its solid dispersions were obtained by using a spectrometer (Scientic Thermo Nicolet IS50 FT-IR). In order to measure this, a suitable amount of the sample was applied on the crystal plate surface of the device so that it covered the entire surface of the prism. The sample was then pressed against the head to the point of transition of the radiation beam. Spectra were recorded in the range of 4000 to 450 cm^{-1}.

2.2.5. Preparation of hydrogels

Hydrogels were prepared using a mechanical stirrer model Eprus® U500. Weighed on a Carbopol 974P, solid dispersions, prepared using different ratios of clotrimazole and chitosan, were transferred to a container. Glycerol and degassed purified water were weighed on the surface of the powders. Ingredients were mixed at 1630 rpm for 2 minutes. Triethanolamine was added dropwise to the gel to achieve a pH of 6 to 6.5. The hydrogels thus prepared were degassed on an ultrasonic cleaner for 15 minutes and left for 72 hours in a refrigerator. The composition of the investigated hydrogels is presented in Table 2.

Table 2. Quantitative and qualitative composition of hydrogels

Ingredients	Formulation code			
	H0	H 5+5	H 3+7	H 2+8
Carbopol 974 P	0.25	0.25	0.25	0.25
Glicerolum 86%	2.5	2.5	2.5	2.5
MF – drug/polymer clotrimazole	0.5	0.5	0.5	0.5
chitosan	-	0.5	1.17	2.0
Triethanoloamine	q.s.	q.s.	q.s.	q.s.
Deionized water	to 50	to 50g	to 50g	to 50g

MF – the physical mixture (grinding method),

2.2.6. Examination of the pharmaceutical availability of clotrimazole from hydrogels

The study was carried out with a Vankel VK 7025 autosampler Varian and an overlap for the release of semi-fluid formulations at fixed parameters over 5 hours. Hydrogel packs for pharmaceutical availability were prepared as described in Section 2.4.5. The samples were assayed by HPLC Ultimate 3000 with a KNAUER column with a diameter of 4.6 mm, a length of 15 cm and a bed size of 5 μm. The clotrimazole assay was carried out under isocratic conditions, eluting with a mixture of acetonitrile and water at a ratio of 70:30 (v/v), the phase flow rate through the column was 1.5 mL/min. Substances eluted from the column were determined spectrophotometrically at 230 nm. The resulting chromatogram was analyzed by the CHROMELEON 7 program from Dionex.

Based on the data obtained, the release rate constant and the half-release time of the active substance were calculated from the drug form. The results are shown in Table 6 and in Figure 4.

3. Results and Discussion

3.1. Dissolution of clotrimazole in presence of chitosan

Table 3 presents the solubility of pure clotrimazole without chitosan. The dissolution findings of pure clotrimazole in 1% SLS solution were used as a reference to compare solubility of the drug incorporated into chitosan. The drug dissolution was found to increase gradually with time and was 3.34–5.08% of the investigated dose.

Table 3. Dissolution of pure clotrimazole in 1% SLS solution

Time intervals of collected samples [min]	Average dissolubility [%]	RSD [%]
5	3.34	0.40
10	3.90	0.39
15	4.20	0.34
20	4.44	0.32
25	4.60	0.23
30	4.75	0.22
35	4.85	0.19
40	4.98	0.16
50	5.04	0.11
60	5.08	0.16

RSD – relative standard deviation

Analysis of data from Tables 4-5 and Fig.1 revealed that the addition of chitosan has a considerable effect on clotrimazole dissolution in the range of investigated solid dispersions.

The results showed that all tested clotrimazole dispersions with chitosan tested improved clotrimazole solubility. The presence of chitosan improved markedly the dissolution of clotrimazole, which increased with time with amount of the chitosan in formulations.

The highest dissolution of clotrimazole, amounting to 90.48%, was observed after 60 minutes from physical mixtures prepared by the grinding method and 79.8% from solid dispersion prepared by the kneading method with a drug to polymer weight ratio of 1:9 in the presence of chitosan. In dispersions containing 30% of the drug and 70%of the

polymer the solubility of clotrimazole from solid dispersion prepared by the kneading method was at the level of 66.27%, and from physical mixtures, it was 76.36%.

Table 4. Influence of chitosan on the dissolution of clotrimazole from physical mixtures prepared by the grinding method in a 1% solution of SLS

Clotrimazole to chitosan weight ratio of solid dispersions prepared by grinding method								
Time intervals of collected samples	**1:9**		**2:8**		**3:7**		**5:5**	
	Dissolution [%]	**RSD**	**Dissolution [%]**	**RSD**	**Dissolution [%]**	**RSD**	**Dissolution [%]**	**RSD**
5	73.53	0.12	68.25	0.03	63.81	0.12	37.30	0.07
10	76.33	0.12	71.01	0.02	67.88	0.06	46.85	0.09
15	76.75	0.12	73.62	0.03	70.01	0.03	53.58	0.08
20	77.10	0.12	74.72	0.03	70.97	0.04	57.22	0.07
25	77.13	0.11	75.40	0.02	71.51	0.04	60.11	0.07
30	78.08	0.11	75.97	0.02	72.14	0.03	61.04	0.07
35	80.14	0.09	76. 89	0.02	72.86	0.03	62.31	0.07
40	83.41	0.11	77.74	0.03	73.68	0.03	63.37	0.07
50	85.93	0.11	78.69	0.03	74.31	0.03	65.39	0.08
60	90.48	0.04	79.07	0.03	76.39	0.02	67.74	0.05

RSD – relative standard deviation

Table 5. Influence of chitosan on the dissolution of clotrimazole from solid dispersion prepared by kneading method in a 1% solution of SLS

Clotrimazole to chitosan weight ratio of solid dispersions prepared by kneading method						
Time intervals of collected samples	**1:9**		**3:7**		**5:5**	
	Dissolution [%]	**RSD**	**Dissolution [%]**	**RSD**	**Dissolution [%]**	**RSD**
5	57.68	0.04	45.22	0.12	35.70	0.05
10	59.22	0.04	48.58	0.06	46.48	0.01
15	59.50	0.04	50.05	0.03	50.61	0.01
20	60.48	0.02	50.82	0.04	54.39	0.01
25	64.05	0.08	51.17	0.04	57.19	0.01
30	65.10	0.09	51.87	0.03	58.52	0.01
35	66.15	0.09	52.36	0.03	58.38	0.01
40	68.60	0.08	55.86	0.03	58.73	0.01
50	75.81	0.09	59.15	0.03	59.08	0.02
60	79.80	0.05	66.27	0.02	59.57	0.02

RSD – relative standard deviation

In the ratios of drug to polymer 1:9, 3:7 and 5:5, the rate of dissolution of the drug decreased with higher doses of the drug and lower amounts of polymer. The lowest solubility was observed in dispersions in which the drug to polymer weight ratio was 5:5, in which case the drug solubility was slightly above 67.74% and 59.57% depending on the prepared dispersion.

Comparing data from Tables 3–5, a significant increase in drug solubility can be observed, which increased 17 times, 15 times and almost 13 times in the presence of chitosan in relation to the amount of added polymer in comparison to the solubility of the pure drug

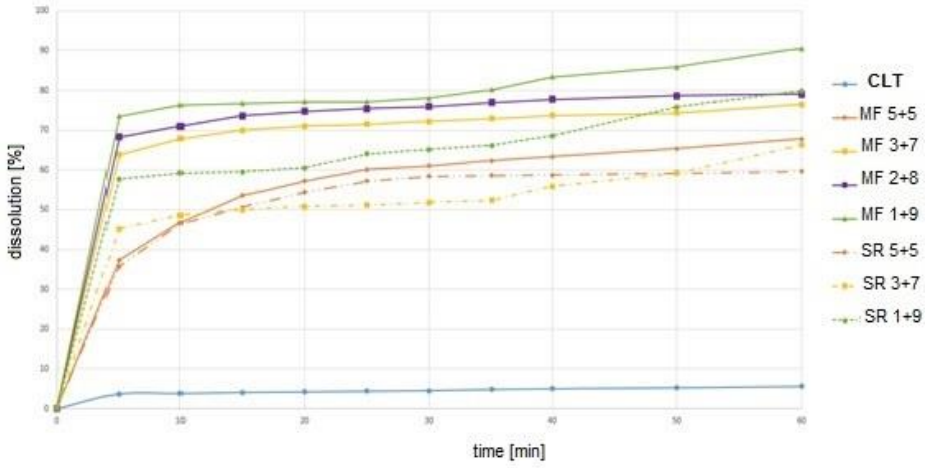

Figure 1. Dissolution profiles of clotrimazole from solid dispersion in 1% solution of SLS. MF – the physical mixture (grinding method), SR – the solid dispersion (kneading method), CLO – clotrimazole, CHIT – chitosan.

Analysis of clotrimazole release profiles in the presence of chitosan indicated a significant increase in drug release rate compared to the release of pure clotrimazole. Out of the analyzed curves, the highest rate of time-shedding and the highest percentage of released clotrimazole are observed for a physical mixture containing a drug-to-polymer ratio of 1: 9

The solubility line of pure clotrimazole is characterized by a low inclination angle to the time axis, and the drug solubility in time increased slightly from 3.34% to 5.08%.

Increased solubility of clotrimazole in solid dispersion with chitosan may be explained by numerous factors. Chitosan, when dispersing in water, may cause molecular dispersion of the drug by increasing the surface of the drug solubility [4–6]. Chitosan in dispersions may prevent agglomeration of clotrimazole molecules and increase wettability of the drug molecules, thus intensifying drug solubility.

3.2. Analysis of X-ray diffractograms (XRD) of clotrimazole, chitosan and their solid dispersions

The X-ray diffractograms of pure components and solid dispersions of clotrimazole with chitosan are shown in Figure 2. The positions of diffraction peaks for the chitosan pure were revealed successively to be 2θ 9–10° and 19–20° [8,9]. The positions of the diffraction peaks for clotrimazole pure were successively at 2θ 9.2°, 12.4°, 18.5°, 19.5°,

20.7°. These positions coincide with the literature [10], which demonstrates the presence of the drug in crystalline form.

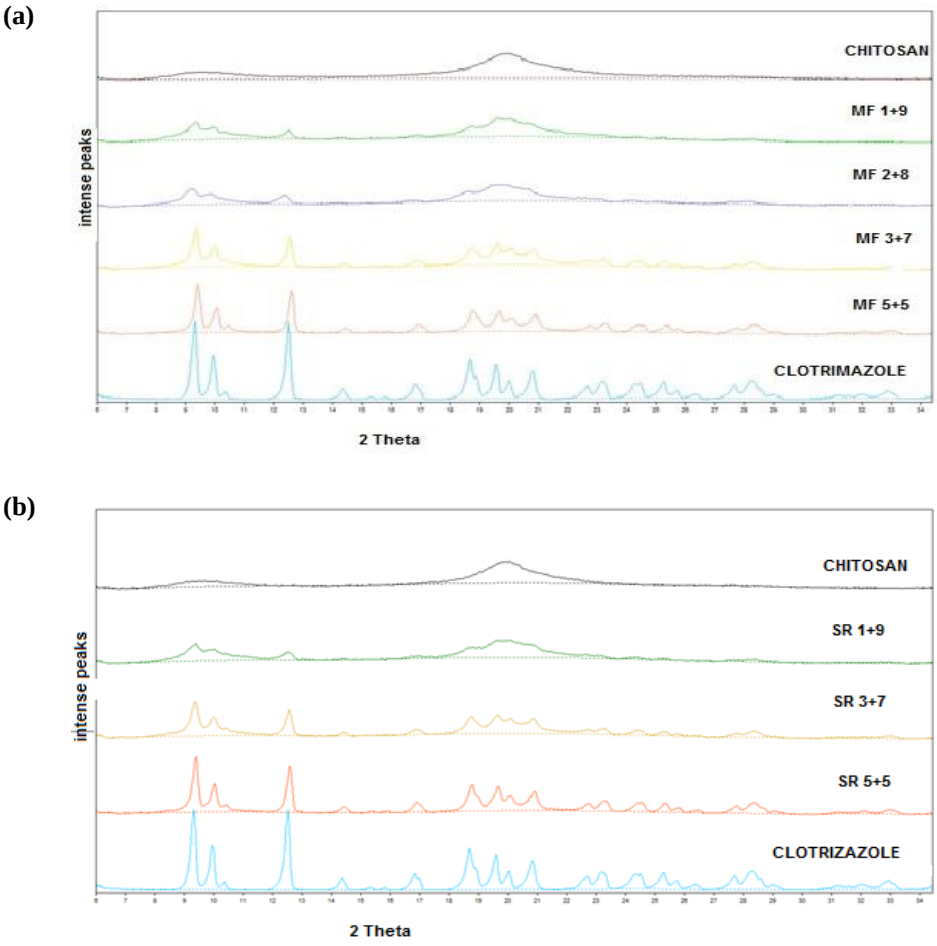

Figure 2. XRPD spectra of clotrimazole, chitosan and their solid dispersions obtained by the grinding method (MF) **(a)** and the kneading method (SR) **(b)**

From comparative analysis of diffractions of clotrimazole, chitosan and their solid dispersions, it was found that the spectra of each mixture revealed characteristic reflection angles for clotrimazole of varying intensities. The low intensity of the read files in comparison to the pure substance was possibly due to the lower content of the drug in formulations. On the basis of the spectra, it was concluded that clotrimazole was present in each case in the crystalline form. There was no observed peaks that could suggested the creation of an amorphous form of the drug.

3.3. Analysis of Fourier transform infrared spectroscopy spectra of clotrimazole, chitosan and their solid dispersions

The FTIR spectra of clotrimazole and solid dispersions of clotrimazole with chitosan are shown in Figure 3

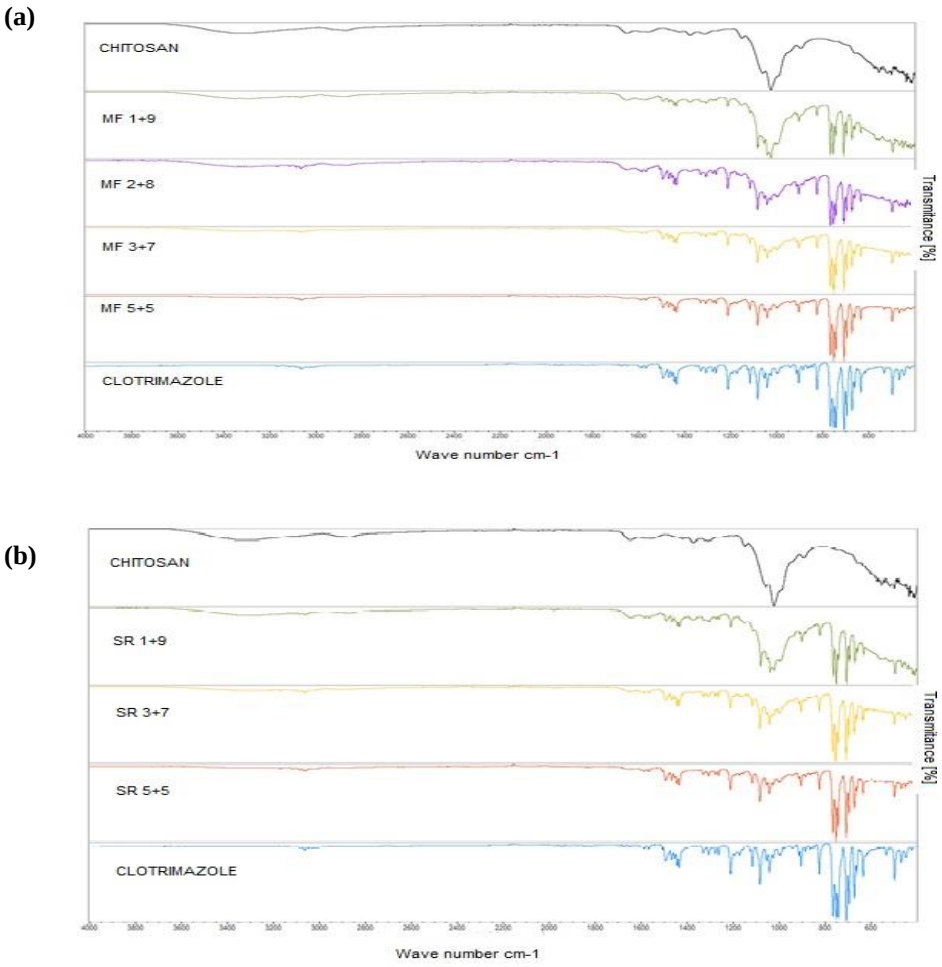

Figure 3. FTIR spectra of clotrimazole, chitosan and their solid dispersions obtained by the grinding method (MF) (a) and the kneading method (SR) (b)

The observed characteristic bands for clotrimazole are consistent with the literature [2]. The vibration bonds in an aromatic ring are in the form of a bond at 3050 cm^{-1}, which is characteristic of bonds C-H, a peak at 1580 cm^{-1}, which conforms to the C=N group, and the bands at 1480 cm^{-1} and 1440 cm^{-1} are specific for the C=C group. Moreover, visible peaks were found at 700–900 cm^{-1} and at 1300 cm^{-1} and 1200 cm^{-1}, which correspond to the vibration of C-H bonds.

The spectra for chitosan are characteristic for this compound [9–11]. The broad band visible at 3400 cm^{-1} is due to stretching vibrations of O-H and N-H bonds. The stretching vibration derived from the CH bonds of groups CH_3 and CH_2 are present in the range of 2800–3000 cm^{-1}. For these groups, vibrations are also evident at 1300–1500 cm^{-1}. In the range of 1500–1800 cm^{-1}, bands corresponding to C=O and N-H bonds from the amide group are noticeable. The range 900–1200 cm^{-1} shows the band of peaks for the following bonds: C-C, C-O-C, C-O-H. In this range, at 1150 cm^{-1}, the peak is characteristic for glycoside linkages.

On the basis of spectra analysis for clotrimazole, chitosan and their solid dispersions, there is no indication of changes in the course bands. The spectra of physical mixtures are the resulting alignment of the spectra of clotrimazole and polymer. There is reduction in the intensity of a characteristic peak for the drug, and this may be due to the decreasing amount in the solid dispersion.

No additional peaks attests to the fact that there was no creation of a new chemical structure in mixtures. At the same time, it is concluded that there was no chemical interaction between the drug and the polymer.

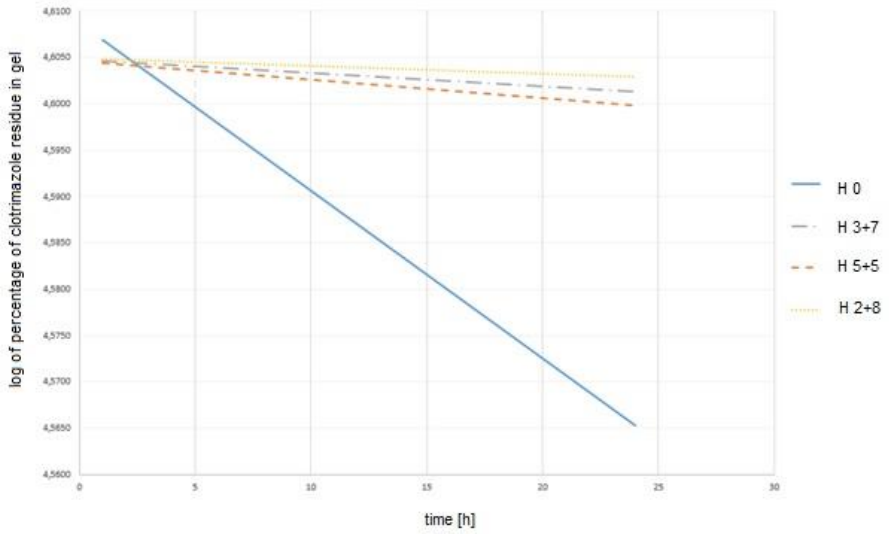

Figure 4. Influence of chitosan on clotrimazole release from hydrogels

The release process of all the formulations follows first order kinetics. The logarithm of the percentage of clotrimazole as a function of time is presented in Figure 4. The investigated gels with chitosan have prolonged half release period of clotrimazole, with the release process lasting from 3438 h to 8250 h (Table 6).

Table 6. Release rate constant [K], semiliberation rates $T_{0.5}$ of drug from hydrogels on the basis of solid dispersions of clotrimazole and chitosan

Formulation code	Regression equation	Correlation coefficient	Release rate constant K [h^{-1}]	Semiliberation rates $T_{0.5}$ [h]
H 0	Y = -0.0018x + 4.6088	0.9911	0.0018105	382
H 5+5	Y = -0.0002x + 4.6044	0.9257	0.0002016	3438
H 3+7	Y = -0.0001x + 4.6048	0.9374	0.0001477	4691
H 2+8	Y = -0.00008x + 4.6049	0.9791	0.0000840	8250

H 0 – hydrogel without chitosan; H 5+5, H 3+7, H 2+8 – Hydrogels containing solid dispersions in different ratios of the drug to the polymer

Hydrogel H 5+5, with 1% chitosan content, released clotrimazole the fastest compared to other chitosan systems. Based on these results, it was found that the highest amount of clotrimazole was released from the hydrogel with 1% chitosan content. The amount of released hydrogel drug corresponded to concentrations equal to or higher than the minimum inhibitory concentration of antifungal clotrimazole.

4. Conclusions

1. Solid dispersions prepared by kneading and grinding methods with chitosan increased the dissolution of clotrimazole. The effect depended on the drug/polymer weight ratio.

2. The highest dissolution of clotrimazole was achieved at a drug/polymer ratio of 1+9 in the presence of solid dispersion prepared by the grinding method.

3. The results of FTIR spectroscopy revealed that there was no chemical interaction between the drug and the polymer. X-ray analysis of solid dispersions showed the crystalline character of the drug.

4. The presence of chitosan in the hydrogel prolonged the drug release time. The best pharmaceutical availability was obtained from a hydrogel containing a solid dispersion, in which the drug to polymer ratio was 5 + 5.

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