PHYSICOCHEMICAL CHARACTERIZATION AND DISSOLUTION STUDIES OF SOLID DISPERSIONS OF CLOTRIMAZOLE WITH CHITOSAN

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Abstract

The aim of the present study was to increase the solubility of clotrimazole. Among the methods to increase the solubility selected solid dispersions of the drug with the polymer. Chitosan was used as the polymer. Clotrimazole was incorporated into the chitosan type 652 with molar masse chitosan Mη = 429 kDa. Solid dispersions were prepared by using different ratios of clotrimazole and chitosan (1:9, 3:7, 5:5, 7:3, 9:1). Formulations were tested dissolution rate of the drug.

The highest dissolution of clotrimazole, amounting to 47.95%, was observed after 60 minutes from solid dispersion prepared by grinding method and 42.84% from physical mixtures with drug-polymer weight ratio 1:9 in the presence chitosan. The solubility of the drug improved more than 37-fold. XRPD analysis indicates the presence of the clotrimazole in crystalline form in the solid dispersion obtained by kneading method.

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

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1. Introduction

Clotrimazole is used to treatment fungal infections of skin and mucous membranes. Is an antifungal medicine characterized by a potent antifungal especially yeasts and dermatophytes It is also a promising agent in the pharmacotherapy of malaria and cancer [1-2]. This drug belongs to class II in The Biopharmaceutical Classification System, which means that despite the good permeability, this drug has poor solubility. This is a lipophilic drug with a low aqueous solubility. Thus the low bioavailability of clotrimazole is due to its solubility and dissolution limitations [3].

One way to improve the solubility of solid substances are solid dispersions. These are the systems containing a hardly water-soluble drug substance, which is dispersed in a carrier which improves the wettability of the particles. It is a result to reduce the size and increase the number of particles, which leads to increase a contact area between a substance's particle and a solvent.

Solid dispersions with the use of polymers, especially chitosan, as a carrier, play an exceptional role. Chitosan, dispersing in water environment, causes a significant increase in the contact area of the drug with solution, increases its hydrophilic properties and may affect its crystalline structure. All these factors lead to increased solubility of the drug. Chitosan in dispersions may prevent agglomeration of clotrimazole molecules and increase wettability of the drug molecules, thus intensifying the drug solubility. Chitosan gradually swells upon contact with the mucous membrane, which contributes to the controlled release of active substance. And by bioadhesion, prolonged residence time of application at the application site. The polymer also shows antimicrobial activity, which enhances the action of the drug 4-[6].

Thus a study was undertaken to investigate the effect of chitosan on the solubility of clotrimazole incorporated into this polymer carrier. Solid dispersions were prepared by using different ratios of clotrimazole and chitosan (1/9, 3/7, 5/5, 7/3, 9/1).

Demonstration of the effect of chitosan in various formulations or with various methods of preparation of the solid dispersions on the solubility of clotrimazole may enable development of new preparations of this drug with increased dissolution. Solid dispersions were prepared by using two methods: grinding and kneading methods.

2. Materials and methods

2.1. Materials

The study was performed with the use of clotrimazole (CLT) was kindly to use by P.P.F."Hasco-Lek" S.A. Poland incorporated into natural, highly purified chitosan B (CHIT) with 95% deacetylation and viscosity average molecular weight M_η=429, intrisic viscosity $\eta[dm^3\ g^{-1}]$ =0,3132 (Chitozan 652 p.a., Chitine, France). In the studies were also used sodium lauryl sulfate (SLS) 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 for 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 weighted on the analytical balance SARTORIUS and transferred quantitatively into a mortar in which the mixture was grinding for 10 minutes. The weight ratios of drug to polymer were: 1/9, 3/7, 5/5, 7/3, 9/1. Then the mixtures were sieved through a sieve with a mesh size of 315 μm and placed in the sealable glass vials. The samples were stored in a desiccator. Then, the prepared samples were

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weighed solid dispersions were prepared by using two methods: grinding and kneading methods. 100 mg. The sample were pressed under a pressure of 1 ton using a hydraulic press Specac. The resulting tablets were weighed. The tablets for a pure clotrimazole were made in the same way (Table1.)

2.2.1.2. Kneading method

The sample weight of clotrimazole quantitatively was transferred to the mortar. Then the drug was dissolved in the appropriate amount of ethanol. The sample weight of chitosan having a suitable molecular weight was added to the solution. The weight ratios of drug to polymer were: 1/9, 3/7, 5/5, 7/3, 9/1. The mixture was grinding for 10 minutes and allowed to completely evaporate the solvent at room temperature. The dry solid dispersion was sieved through a sieve with 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).

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

Solid dispersion	Drug/polimer ratio	Quantity of drug [mg]	Quantity of polymer [mg]
MF**B** - CLO**1** : CHIT**9**	1:9	100	900
MF**B** - CLO**3** : CHIT**7**	3:7	300	700
MF**B** - CLO**5** : CHIT**5**	5:5	500	500
MF**B** - CLO**7** : CHIT**3**	7:3	700	300
MF**B** - CLO**9** : CHIT**1**	9:1	900	100
SD**B** - CLO**1** : CHIT**9**	1:9	100	900
SD**B** - CLO**3** : CHIT**7**	3:7	300	700
SD**B** - CLO**5** : CHIT**5**	5:5	500	500
SD**B** - CLO**7** : CHIT**3**	7:3	700	300
SD**B** - CLO**9** : CHIT**1**	9:1	900	100

SD - the solid dispersion (kneading method), B- Chitozan Mη=429 kDa,
MF - the physical mixture(grinding method), CLO-clotrimazole, CHIT-chitosan

2.2.2. Examination of pure clotrimazole and its solid dispersions 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. The studies were performed in a VanKel VK 7025 dissolution apparatus, which was connected to a fraction collector Varian Inc. 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°C +/- 0.5°C, with velocity of 100 rotations per minute. The trial was continued for 1 hour, 5 ml samples were collected in 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 10μm pore size.

The collected samples were diluted in methanol and next their content was evaluated with the use of JASCO V650 spectrophotometer with the use of 1 cm cuvette at wavelength λ=261 nm.

The drug concentration in samples and an average percentage of dissolved clotrimazole were calculated using linear regression equation for clotrimazole y=2.0126x + 0,0015. 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 means of 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 source operating at 30 mA and 40 kV. Samples were scanned from 7 to 50° 2θ at a scanning rate of 0.02° 2θ s-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 4000 to 450 cm^{-1}.

2.2.6. Statistical analysis

Statistical analysis was performed for the average dissolution rate of clotrimazole expressed in percentages after 60 minutes of dissolution in 1% SLS for the pure substance and the solid dispersions of chitosan. The tests were done the Shapiro-Wilk normality for all formulations. In order to verify the homogeneity of variance, Levene's test was used. The analysis of variance was performed using ANOVA. Statistically significant differences between the average data rates for the dispersions were tested by the test NIR. All tests were performed at a significance level of $p < 0.05$.

3. Results and Discussion

3.1. Dissolution of clotrimazole in presence of chitosan

Table 2 presents the solubility of pure clotrimazole without chitosan. The dissolution findings of pure clotrimazole in1% solution SLS were used as reference to compare solubility of the drug incorporated into chitosan. The drug dissolution was found to increase gradually with time and it was from 0.8% to 1.3 % of the investigated dose.

Table 2 Dissolution of clotrimazole pure in 1% solution SLS

time intervals collected samples [min]	the average of dissolubility [%]	RSD [%]
5	0.806	1.307
10	0.971	4.108
15	1.085	5.037
20	1.172	3.378
25	1.195	5.148
30	1.209	4.205
35	1.224	3.127
40	1.251	0.570
50	1.301	1.055
60	1.313	3.017

RSD-relative standard deviation

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Analysis of data from Tables 3-4 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 of the demonstrated that all the investigated solid dispersions of clotrimazole with chitosan the solubility of clotrimazole. The presence of chitosan improved markedly the dissolution of clotrimazole, which increased with time with amount of the chitosan in formulations.

Table 3. 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 dispersion prepared method by grinding											
time intervals collected samples		CLO**1** : CHIT**9**		CLO**3** : CHIT**7**		CLO**5** : CHIT**5**		CLO**7** : CHIT**3**		CLO**9** : CHIT**1**	
		dissolution %	**RSD**	**dissolution %**	**RSD**	**dissolution %**	**RSD**	**dissolution %**	**RSD**	**dissolution %**	**RSD**
Chitosan Mη=429 kDa	**5**	38,65	0,83	28,86	2,20	13,42	2,76	6,78	9,26	3,21	7,44
	10	42,00	0,38	33,35	4,54	18,95	1,89	9,69	3,30	4,48	10,87
	15	43,43	0,02	34,65	1,00	22,65	0,27	12,32	3,13	5,84	10,05
	20	44,30	0,35	35,30	0,59	25,82	0,33	14,75	3,65	6,98	10,47
	25	44,83	0,46	36,73	0,19	27,21	0,26	16,82	2,48	7,99	9,84
	30	45,18	1,57	37,59	0,05	29,79	0,41	18,47	2,54	8,93	9,06
	35	46,03	0,44	37,90	1,27	31,23	0,43	19,95	3,08	9,83	9,12
	40	46,38	0,54	38,02	1,50	32,39	1,05	21,48	3,63	10,79	9,41
	50	47,30	0,15	38,51	1,06	34,16	0,90	23,92	2,99	12,31	7,25
	60	47,95	0,99	38,52	0,75	37,37	1,17	25,98	3,11	13,64	6,82

RSD-relative standard deviation

The highest dissolution of clotrimazole, amounting to 47.95%, was observed after 60 minutes from physical mixtures prepared by the grinding method and 42.84% from solid dispersion prepared by the kneading method with drug-polymer weight ratio 1:9 in the presence chitosan. In dispersions containing 30% of the drug and of the polymer, the solubility of clotrimazole from solid dispersion prepared by the kneading method was at the level of 29.6% and from physical mixtures was 38,5%.

The ratios of drug to polymer 5/5, 7/3 and 9/1 the rate of dissolution of the drug decreases as the higher doses of the drug and polymer to be added decrease.The lowest solubility was observed in dispersions in which the drug-to-polymer weight ratio was 9:1, in which case the drug solubility was slightly above 13.6% and 10.0% depending on the prepared dispersion.

Comparing data from Tables 1 and 2-3, we can notice a significant increase in the drug solubility, which in the presence of chitosan increased 37 times, 30 times and almost 10 times in relation to the amount of added polymer in comparison to the solubility of pure drug.

When the course of clotrimazole solubility curves in the presence of chitosan is observed, it becomes apparent that they are situated in the field above the clotrimazole solubility curve without polymer. The solubility curve for clotrimazole mixed with chitosan at 1:9 ratio assumes the highest position in the field, also the inclination angle of the straight line to time axis is significant, and the drug solubility in relation to time is from 33,58% to 42,84% from solid dispersion and 38.65% to 47.95%, i.e. it increases with time.

Table 4. 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 dispersion prepared method by kneading										
time intervals collected samples	CLO**1** : CHIT**9**		CLO**3** : CHIT**7**		CLO**5** : CHIT**5**		CLO**7** : CHIT**3**		CLO**9** : CHIT**1**	
	dissolution %	**RDS**	**dissolution %**	**RDS**	**dissolution %**	**RDS**	**dissolution %**	**RDS**	**dissolution %**	**RDS**
5	33,58	5,73	15,71	5,54	12,40	0,73	7,69	1,73	1,47	6,69
10	36,66	2,29	19,42	5,20	16,67	0,44	12,46	3,42	2,70	0,27
15	39,02	2,75	22,70	4,61	19,74	0,23	14,79	2,65	3,55	6,41
20	39,48	0,48	23,71	1,07	21,62	0,73	16,10	1,44	4,10	4,31
25	40,30	0,07	25,12	0,90	22,97	0,25	17,45	1,00	4,84	3,77
30	40,60	1,09	25,81	2,31	24,07	1,25	18,17	0,24	5,63	4,85
35	41,21	0,93	26,45	1,59	24,89	0,34	18,89	0,52	6,66	9,91
40	41,44	0,68	27,17	2,00	25,83	0,45	19,38	0,47	6,88	5,68
50	42,54	1,53	29,07	2,56	27,21	0,58	20,28	1,47	8,20	6,87
60	42,84	0,83	29,62	2,87	27,87	1,17	20,96	2,05	10,00	6,70

RSD-relative standard deviation

The solubility line of pure clotrimazole is characterized by a low inclination angle to the time axis, and the drug solubility in time increases slightly and is from 0.8% to 1.3%.

Increased solubility of clotriamazole 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 the drug solubility.

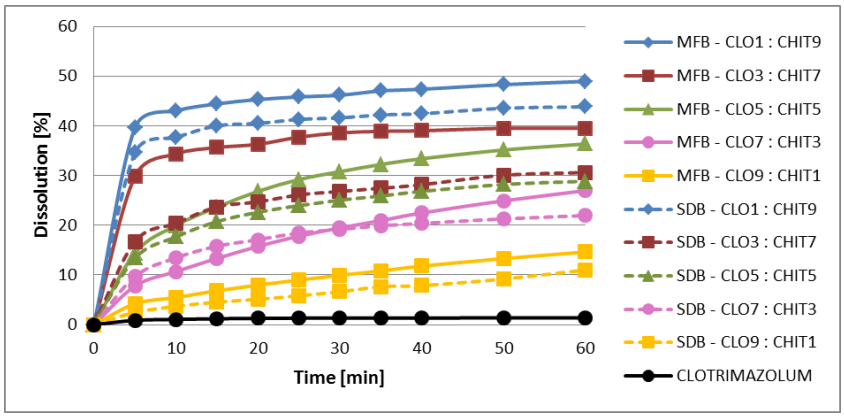

Figure 1. Dissolution profiles of clotrimazole from solid dispersion in 1% solution of SLS

3.2. Analysis 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 Fig 2. The position of diffraction peaks for the chitosan revealed successively to 2θ 9°-10° and 19°-20° [7-8]. The position of the diffraction peaks for clotrimazole shown up successively to 2θ 9,24° 12,44° 18,58°, 19,50°, 20,72°. These positions coincide with the literature [9], which proves the presence of the drug in crystalline form.

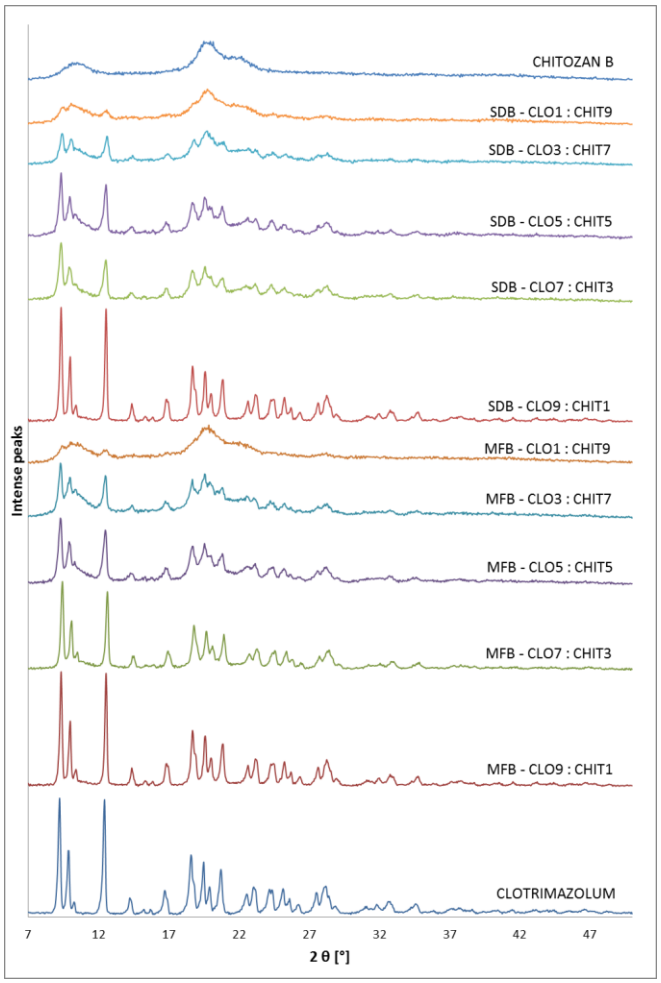

Figure 2. XRPD spectra of clotrimazole, chitosan B (Chitosan Mη=429 kDa**)** and their solid dispersions and their solid dispersion were obtained by grinding (MF) and kneading (SD) method.

The comparative analysis of diffraction clotrimazole, chitosan and their solid dispersions, it was found that the spectra of each mixture revealed characteristic the reflection angle for clotrimazole of varying intensity. The low intensity of the read files in comparison to the pure substance due to the lower content of the drug in formulations. On the basis of the

spectra, it was concluded that clotrimazole present in each case in the crystalline form. There was not observed any peaks that could provide for the creation the amorphous form of the drug.

3.3. Analysis 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 Fig.3.

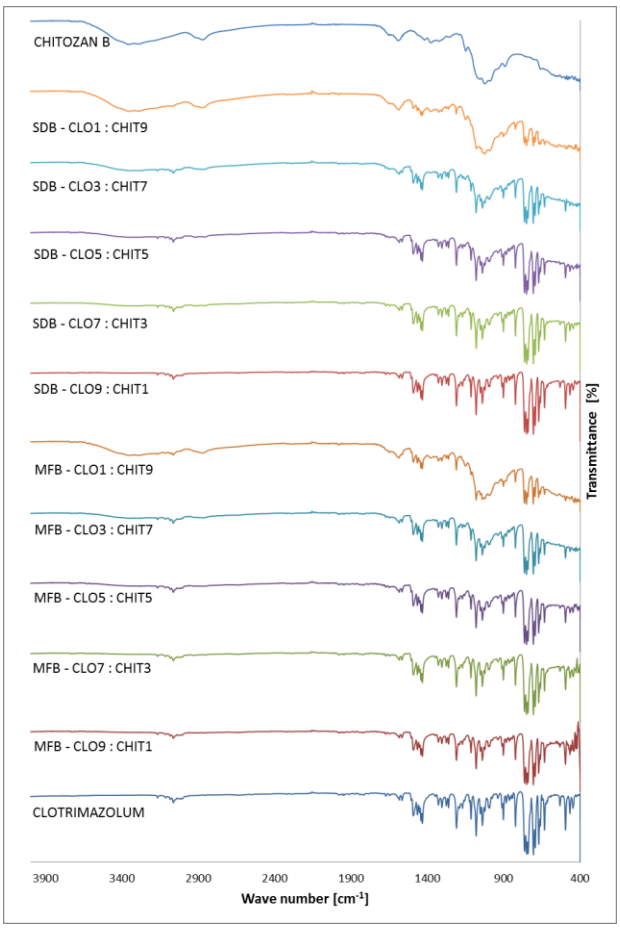

Figure 3. FTIR spectra of clotrimazole and solid dispersions of clotrimazole with chitosan

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

The spectra for the chitosan are characteristic for this compound [9-11]. The broad band visible at 3400 cm^{-1} is due 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 are also evident vibrations at 1300-1500 cm^{-1}. In the range

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of 1500-1800 cm^{-1} are noticeable bands corresponding bindings C=O and N-H from the amide group. 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 does not indicate changes in the course bands. The spectra of physical mixtures are the result alignment of the spectra of clotrimazole and polymer. The reduction the intensity of characteristic peak for the drug. It follows from the decreasing amount in the system.
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.

4. Conclusions

1. Solid dispersions prepared kneading and grinding method with chitosan increased the dissolution of clotrimazole. The effect depends on the drug/polymer weight ratio.
2. Highest dissolution of clotrimazole was achieved at drug/polymer ratio 1:9 in the presence solid dispersion prepared grinding method.
3. The results of FTIR spectroscopy reveal that there was no chemical interaction between drug and the polymer. X-ray analysis of solid dispersions studies showed the crystalline character of the drug.
4. Chitosan has been proposed as a useful excipient for enhancing the bioavailability of poorly water-soluble compounds.

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