ADSORPTION STUDIES OF KETOCONAZOLE WITH CHITOSAN

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Abstract
Combinations of polymers and biopolymers of biologically active compounds in the last period have been the subject of intensive research. The active substance linked to a polymer often has a modified function and sometimes no action. On the other hand, the use of unsuitable polymers may result in a type of mismatch of drug-polymer. The aim of the study was to examine whether the active substance ketoconazole causes incompatibility with dietary supplements containing chitosan. Antifungal drug adsorption phenomenon was investigated by the static method in the concentration range of the single dose using a pharmaceutical model of the gastrointestinal tract. The test results show that the antifungal agent is adsorbed onto the chitosan in the pH ranges used, and the binding ability of the chitosan depends on the variety, or indirectly from the reaction environment. It was observed that the average sorption, depending on the variety of chitosan, was located in the limit from 94% to 100%. The increase of the size of the adsorption of anti-inflammatory drugs on the polymer with increasing pH from 7.6 to 8.0 can be explained by the swelling properties of chitosan, which grow with increasing pH of the environment in the direction of alkaline pH. Thus, the specific surface area and polymer sorption capacity is increased. Based on the above considerations, it can be concluded that, between the test drug and the polymer, there is antagonistic interaction involving the adsorption of the drug of this group on a polymer which is chitosan.

Key words: *ketokonazol, absorption, chitosan.*

1. Introduction

Combinations of polymers and biopolymers of biologically active compounds in the last period are the subject of intensive research. The active substance linked to a polymer often has a modified function and sometimes no action at all. On the other hand, the use of unsuitable polymers may result in a type mismatch of drug-polymer.

The aim of the study was to examine whether the active substance ketoconazole causes incompatibility with dietary supplements containing chitosan. For this reason, the study attempted to investigate the *in vitro* effect of selected physicochemical factors on the adsorption capacity of different types of chitosan and evaluation of the assumption that the use of chitosan formulations is important for bioavailability of ingested and simultaneously orally administered drug substances with an explanation of the interaction mechanisms of the drug ketoconazole with dietary supplements containing chitosan.

2. Materials and methods

2.1. Materials

In the study, natural chitosans with a high deacetylation degree of 85% to 95%, treated with IR radiation doses from 5 to 30 kGy, from different producers were used (***Table 1***).

Dietary supplements Bio-active® (380 mg chitosan/capsule), Chromdiet® (300 mg chitosan/capsule) and Vitana® (300 mg chitosan /capsule) were used.

Ketoconazole (Piperazine,1-acetyl-4-[4-[[2-(2,4-dichlorophenyl)-2-(1H-imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]-,cis-.(±)-cis-1-Acetyl-4-[p-[[2-(2,4-dichlorophenyl)-2-(imidazol-1-ylmethyl)-1,3-dioxolan-4-yl]methoxy]phenyl]piperazine (Pharmacopoea Europaea, Ph. Eur.) [Cas. Nr. 65277-42-1].

2.2. Method

The phenomenon of adsorption of the drug was studied by static and dynamic pharmaceutical models (modified model of the Polish Pharmacopoeia IX) simulating *in vitro* conditions. The amount of the drug adsorbed by the chitosan concentration was calculated from the difference between the tested preparations before and after sorption. Tests were

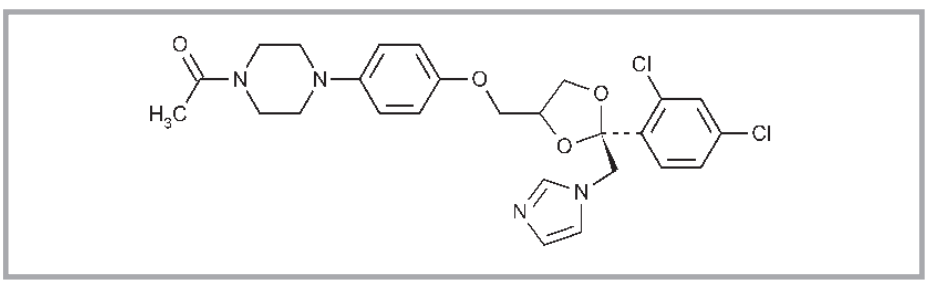

Figure 1. *Ketokonazole.*

carried out spectrophotometrically at 296 nm wavelength (determined by the regression line for ketoconazole was y = 0.017 + 33.21 x), using blank solution + MeOH as a reference.

This study also attempted to examine the impact of the chosen solution of chitosan (Chitosan type 352, 20 kGy) on the solubility of ketoconazole [1].

The study was conducted in the environment of the stomach (pH 2) using two samples, A and B. Sample A contained only therapeutic substances, while sample B contained a medicinal substance in the presence of the polymer.

Sample A: weighed quantities of medicinal substances were added to 20.0 mg of ketoconazole (this quantity is present in commercially available drug formulations) and brought to pH 2 with 0.05 N HCl.

Sample B: 300 mg of the chitosan was added and shaken until dissolved and then adjusted to pH 2 with 0.05 N HCl and Ketoconazole was added in an amount of 20.0 mg. Received layouts were shaken (300 r.p.m.) for 2 hours at 37 °C, simulating the conditions of the stomach. Then, samples were brought to room temperature, spun in a centrifuge (2100×g) for 20 minutes and allowed to stabilise for 0.5 hours. The supernatant was obtained from 1.5 ml of the sample, transferred to Eppendorf tubes and spun in a centrifuge again (15000×g) for 10 minutes. Then, the tubes were collected into a clean specific volume of sample added to the supernatant and the specific volume of the solvent (MeOH) for the determination of the sample: Ketoconazole 1.5 ml sample was transferred to a test tube and added to 10 ml of the reference solution;

After stirring the contents in the tubes, spectrophotometric measurements were performed.

2.3. Study of Ketoconazole adsorption

Ketoconazole adsorption phenomenon was investigated by static and dynamic concentrations, generally in the range of the dose of a single pharmaceutical composition, using a model of the gastrointestinal tract based on a modification of Polish Pharmacopoeia test for the formulations [1 - 3].

The study was conducted in a water bath shaker, maintaining conditions as close as possible to the conditions seen in the gastrointestinal tract. The amplitude of vibration (300 r.p.m.), and the process temperature (37 °C) were established.

Vials containing 5 ml were centrifuged and 2 ml was measured of the respective chitosan solutions; solutions were adjusted to pH 2, which corresponds to the fasting stomach environment. The volume of solution used corresponded to 0.03 g of chitosan. Then, a corresponding amount of medicinal substance 20.0 mg (dose in therapeutic treatments) was added and shaken (300 r.p.m.) for 2 hours. The contents of the tubes were adjusted with 0.2 N Na_2CO_3 to pH 7.0 - 7.6, which corresponds to the allergic reaction and colonic intestinal juice. Samples were incubated at 37 °C with shaking (300 r.p.m.) for 2.5 hours. [4] The test layout was brought to room temperature and spun in a centrifuge (2100×g) for

20 minutes and then allowed to stabilise at 0.5 hours. Then, aliquots of the supernatant solution were collected into clean 1.5 ml tubes and the specific volume of the solution was added to determine the reference sample.

2.4. Research and determining the viscosity average molecular weight

Measurements were made at a constant temperature of 25 °C. The Ubbelohde viscometer [Polish Pharmacopoeia IX] was used. An aqueous solution of 0.1 M acetic acid and 0.2 M sodium chloride solution was then filtered to separate the insoluble fraction. Five measurements were made for each concentration. Mark-Houwink parameters were used to convert the intrinsic viscosity to an average viscosity molecular weight, which are known for the chitosan composition of the solvent - $K = 1.81 \times 10^{-6}$ dm^3 h^{-1}, $\alpha = 0.93$ (***Table 1***)[5]

Table 1. *The values of intrinsic viscosity [η] and the viscosity average molecular weight M [η] chitosan tested; * - degree of deacetylation.*

Type of chitosan	Radiation dose-degrading	Intrinsic viscosity [η] [dm^{3}g^{-1}]	Viscosity average molecular weight M [η] [kDa]
PRIMEX (85)*	Sample	0.2852	348
	5	0.2545	343
	10	0.2282	293
	15	0.2057	270
	20	0.1872	242
	30	0.1576	205
CHITO CLEAR TM 1015 (95)*	Sample	0.5100	725
	5	0.4172	584
	10	0.3440	453
	15	0.2910	396
	20	0.2580	348
	30	0.2550	344
Chitosan HUASU (92)*	Sample	0.7437	1087
	5	0.5843	839
	10	0.5185	738
	15	0.3717	612
	20	0.3303	454
	30	0.2986	407
CHITOZAN 352 (95)*	Sample	0.2117	282
	5	0.1949	258
	10	0.1696	222
	15	0.1639	214
	20	0.1575	177
	30	0.1497	194
Chromdiet®	Sample	0.1872	242
Bio – active®	Sample	0.1576	205
Vitana®	Sample	0.1774	229

3. Results and discussion

3.1. Effect of chitosan on the solubility of Ketoconazole

The amount of chitosan used in tests corresponds to the values commonly used in the supplement formulations. Tests were carried out in the acidic environment of the stomach, and a drug under such conditions as weak acid is poorly soluble and hardly dissociated (***Figure 2***). In the study of chitosan, simulating a gastric environment in the gel cannot prevent the effect of chitosan on increasing the solubility of ketoconazole (about 8%), since this possible feature of the polymer may be masked by the more pronounced adsorption process. It can be assumed that, in the tested concentrations, chitosan does not impact on the solubility of ketoconazole, and occurring in the gastric environment, the ketoconazole adsorption process helps to reduce the damaging effect of a drug to the gastric mucosa.

3.2. Impact of intrinsic viscosities and viscosity average molecular weights on the Ketoconazole adsorption process by chitosans

Analysis of the impact of radiation dose on the adsorption capacity of degrading Ketoconazole by chitosans shows a pattern where the decrease in the intrinsic viscosity of chitosan indicates an increase in the amount of bound drug (see ***Figure 3***).

Analysis of the viscosity determinations of average molecular weights that result from the intrinsic viscosity determinations showed that the values for chitosan vary depending on the degree of degradation of the radiation of the polymer. The test results show that ketoconazole is adsorbed onto the chitosan in pH ranges used, and the binding ability of the chitosan depends on the variety and its degradation.

The measurement results of adsorption by chitosan Ketoconazole contained in the concomitant preparations generally available for purchase have confirmed the raised hypothesis

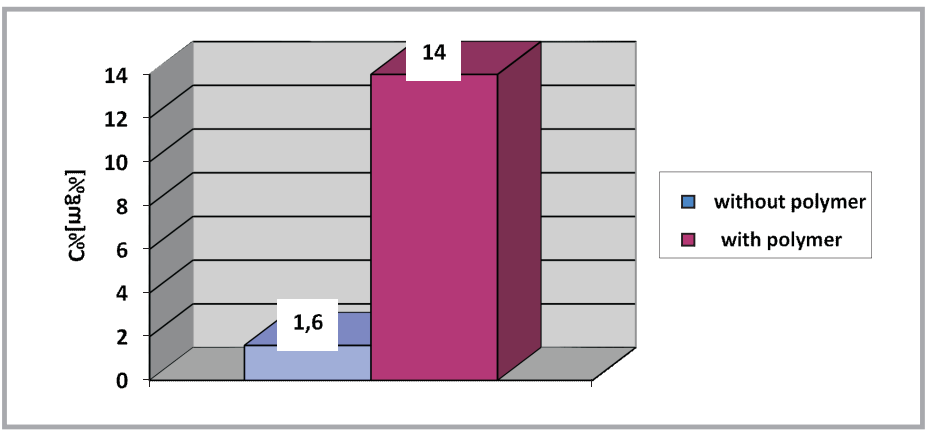

Figure 2. *Change the solubility of Ketoconazole without the addition of polymer (1) and in its presence (2) on the basis of changes in the concentration (C% [mg%]).*

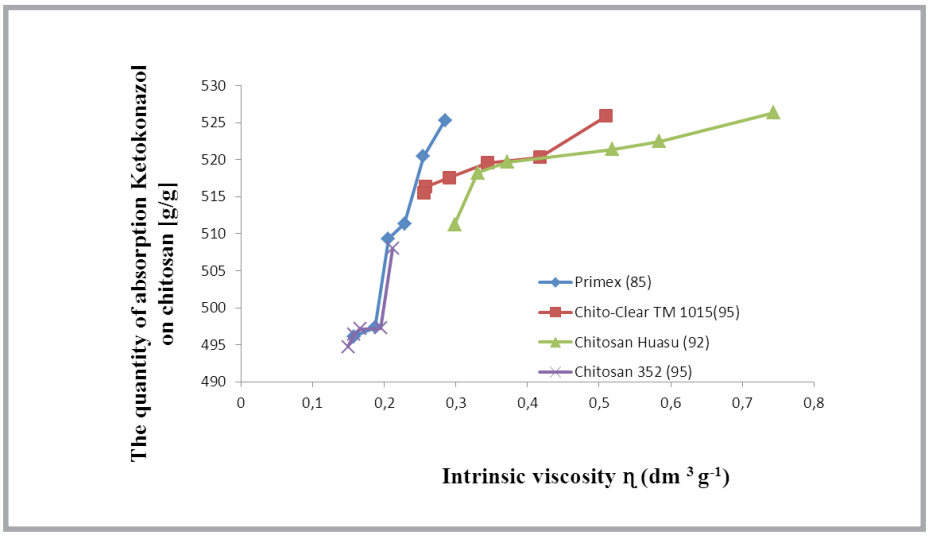

Figure 3. *Ketoconazole binding by various types of chitosan according to the intrinsic viscosity [η].*

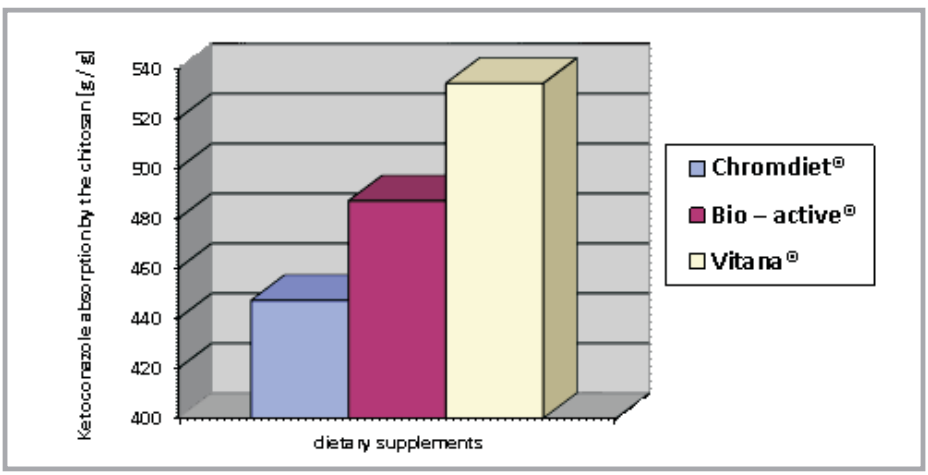

Figure 4. *Binding of Ketoconazole for dietary supplements containing chitosan [g/g]*

that adsorption is highly variable for different preparations. The strongest is bound by the preparation Vitana®, and the weakest by the preparation Chromdiet®.

The binding of Ketoconazole by individual slimming preparations showed similar values, but were much larger in comparison with adsorption of the drug by various manufacturer's chitosans. Chitosan drug formulations contained in the binding capacity have almost 100% of the dose, and thus significantly effect the bioavailability of ketoconazole when

used simultaneously (***Figure 4***). It was observed that the average sorption, depending on the variety of chitosan, was in the range from 94% to 100%.

The fact that the lowest value of adsorption at pH 6.4 can be explained by the chemical properties of chitosan, which shows the load until pH < 6.7 and the electrostatic adsorption may have a relation to the nature of therapeutic substances of weak acids [6 - 8].

At a pH above 7.6, corresponding to the environment of the filled gut, the average size at the highest dose of the drug on chitosan was in the range from 94% to 100%.

4. Conclusion

Increased size of adsorption of ketoconazole on the polymer with increasing pH from 7.6 to 8.0 can be explained by swelling properties of chitosan, forms of which are present in the form of a conglomerate of the emulsion system.

On the basis on the above considerations, it can be stated that, between the test drug and the polymer, there is antagonism of an interaction consisting of adsorption of the drug on the polymer, which is chitosan.

5. References

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