

## IN VITRO STUDIES OF ADSORPTION OF ERYTHROMYCIN ON CHITOSANS FROM DIETARY SUPPLEMENTS

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### Abstract

*In clinical practice, in the treatment of obesity, a plurality of natural high-molecular compounds are used, the activity of which supports weight loss. During the use of dietary supplements containing chitosan, illness sometimes develops and other therapeutic agents are applied as antibiotics. The aim of our study was to determine the binding capacity of the antibiotic erythromycin depending on variable physicochemical factors, in the model of the gastrointestinal tract, by chitosans found in slimming dietary supplements. Erythromycin adsorption phenomenon was studied by static and dynamic pharmaceutical model (according to the modified method of the Polish Pharmacopoeia) simulating in vitro conditions. The amount of bound drug was used to calculate the average percentage of the adsorbed dose. The results obtained show that erythromycin is adsorbed by chitosans at various pH ranges, and the binding capacity of the environment depends on the pH, viscosity and concentration of the antibiotic, as well as the chitosan and type and additional substances present in the gastrointestinal tract. The average level of the adsorption of erythromycin in the chitosan-nutrients system depends on the pH of the medium. The highest amount of adsorption was noted above pH 7 (chitosan precipitated polymer forming the emulsion-gel system).*

**Key words:** Erythromycin, adsorption, chitosan.

## 1. Introduction

In the treatment of obesity, the use of natural compounds whose activity is based on the possibility of supporting weight loss through the absorption of food factors is now performed. These compounds form a polymeric gel in contact with water, and have the ability to adsorb nutrients and other components in their vicinity.

The aim of this study was to investigate the *in vitro* effect of selected physicochemical factors on the adsorption capacity of different types of chitosan and evaluation of the assumption of chitosan formulations which is important for the bioavailability of ingested and simultaneously orally administered drug substances and provide an explanation of the mechanism of drug interactions with dietary supplements containing erythromycin and chitosan. Applying various antibacterial substances for medicinal purposes, which act together with dietary supplements containing chitosan, may lead to changes in the bioavailability of the individual drug.

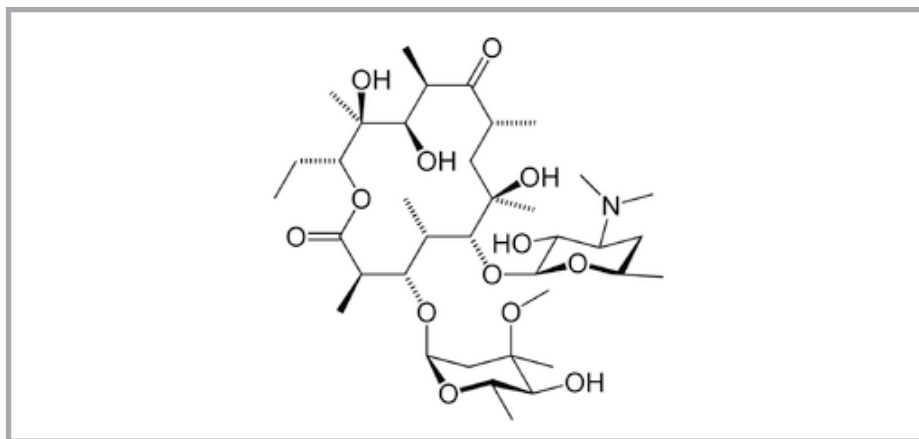
## 2. Materials and method

### 2.1. Materials

In the study, natural chitosans with a high degree of deacetylation (85% to 95%) were used, which had been treated with IR radiation doses in the range from 5 to 30 kGy; these were developed by different producers [1]. Erythromycin (Erythromycinum) was from FARM-IMPEX SP.J. Series FI 28052007, Wrocław, Poland.

### 2.2. Method

Erythromycin (lat. *Erythromycinum*) is a mixture of organic chemicals, and macrolides produced by *Streptomyces erythraeus*, the main components of which are erythromycin A, B and C (**Figure 1**) [2]. The mechanism of action is based on the inhibition of protein synthesis by binding to the 50S ribosomal subunit [3].



**Figure 1.** Erythromycin

The phenomenon of the adsorption of drugs was tested in the pharmaceutical dynamic model, simulating *in vitro* conditions. The amount of drug adsorbed by the chitosan concentration was calculated from the difference in the tested preparations before and after the sorption. Tests were carried out spectrophotometrically at a wavelength of  $\lambda$  - 220 nm.

### **2.3. Investigation of the erythromycin adsorption process on chitosan probes**

The adsorption phenomenon of erythromycin was studied by static and dynamic methods, in the range of concentrations for a single dose, using a pharmaceutical model of the gastrointestinal tract based on a modification (method adapted from probe of 900 ml to probe of 3 ml) of the Polish Pharmacopoeia method [4, 5].

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

Centrifuge vials (5 ml) containing 2 ml of chitosan solutions were measured after adjusting the environment to pH 2 (0.05 M HCl), which is equivalent to a fasting gastric pH. The volume of solution used corresponded to 0.03 g of chitosan (the dose used for dietary slimming). Then, an amount of medicinal substance erythromycin corresponding to 0.025 g (dose in therapeutic treatments) was added to the vials and shaken (300 r.p.m.) for 2 hours. The tubes were adjusted with 0.1 M Na<sub>2</sub>CO<sub>3</sub> 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.

The test layout was brought to room temperature and centrifuged (2100 × g) for 20 minutes before being allowed to stabilise for 0.5 hours. Then, the supernatants were collected into clean 1.5 ml tubes and assessed spectrophotometrically ( $\lambda$  = 220 nm) in 1 cm quartz cuvettes. Calibration curves were used for the analytical determination of the concentration of the antibiotic (the linear regression equation  $y = 05734x - 0.0261$ ;  $R^2 = 0.9972$ ).

## **3. Results and discussion**

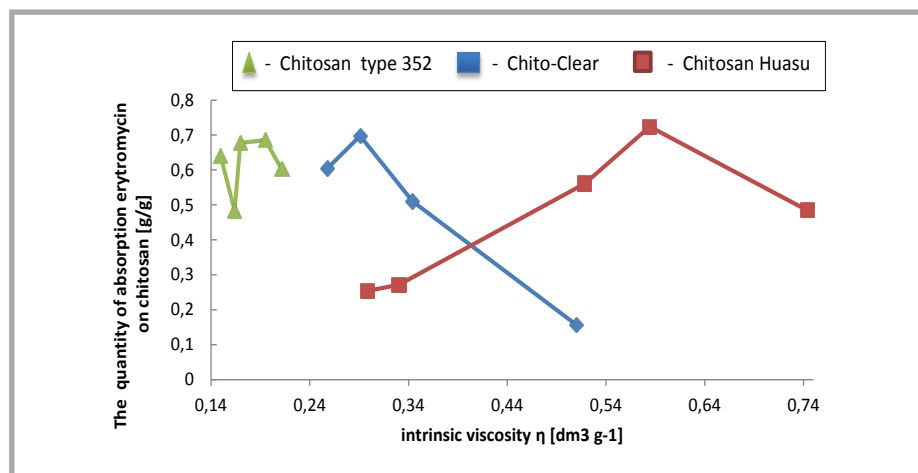
### **3.1. Impact of the intrinsic viscosities and viscosity average molecular weights on the adsorption process by chitosans and erythromycin**

Analysis of the impact of degrading radiation dose that has an important effect on the viscosity obtained at the adsorption capacity of the erythromycin by the chitosans shows that the reduction in the intrinsic viscosity of the chitosan causes an increase in the amount of drug bound in *Table 1* and *Figure 2*.

Analysis of the viscosity determinations of average molecular weights has shown that these values for the chitosan depend on the degree of degradation of the polymer radiation which is adequate for the intrinsic viscosity. The test results show that erythromycin is adsorbed onto the chitosan in the pH ranges used, and that the binding ability of the chitosan depends on the variety and its degradation.

**Table 1.** Influence on bond of exhighway of erythromycin by important degradation - average of chitosans.

Sample No.	Type of chitosan	Average amount of bound antibiotic [mg]	Average amount of antibiotic bound by 1 g of chitosan [g]	Standard deviation DS. [g]	The relative standard deviation of RDS [%]
1	Chito-Clear TM 1015	4.8033	<b>0.157</b>	0.003	1.91
2	Chito-Clear TM 1015 (10)	15.4138	<b>0.510</b>	0.021	4.12
3	Chito-Clear TM (15)	20.5573	<b>0.697</b>	0.015	2.15
4	Chito-Clear TM 1015 (20)	18.7818	<b>0.605</b>	0.034	5.62
5	Chitosan type 352 food grade	17.9107	<b>0.602</b>	0.014	2.32
6	Chitosan type 352 food grade (5)	19.9433	<b>0.686</b>	0.033	4.81
7	Chitosan type 352 food grade (10)	20.4192	<b>0.677</b>	0.018	2.66
8	Chitosan type 352 food grade (15)	14.3220	<b>0.483</b>	0.017	3.52
9	Chitosan type 352 food grade (30)	19.2808	<b>0.641</b>	0.018	2.81
10	Chitosan Huasu sample	14.2815	<b>0.485</b>	0.016	3.30
11	Chitosan Huasu sample (5)	22.4391	<b>0.724</b>	0.035	4.83
12	Chitosan Huasu sample (10)	16.4931	<b>0.561</b>	0.016	2.85
13	Chitosan Huasu sample (20)	8.3002	<b>0.271</b>	0.005	1.84
14	Chitosan Huasu sample (30)	7.8401	<b>0.254</b>	0.007	2.76



**Figure 2.** Erythromycin binding by various types of chitosan according to the intrinsic viscosity  $\eta$  ( $\text{dm}^3 \text{g}^{-1}$ ) (temp. 37 °C and pH 7.6).

The results of the measurement of the adsorption process of erythromycin by chitosan contained in concomitant preparations generally available for freehand purchase have confirmed the hypothesis that adsorption increases for different preparations. This is most strongly bound by preparations containing chitosans with an intrinsic viscosity between 0.14 and 0.34 ( $\text{dm}^3 \text{g}^{-1}$ ), while the absorption between 0.34 and 0.54 ( $\text{dm}^3 \text{g}^{-1}$ ) is the weakest.

Erythromycin binding by each slimming preparation shows similar values, but is much greater in comparison with the adsorption of the drug by various chitosan manufacturers.

Chitosan contained in the drug formulations has a binding capacity of nearly 99% of the dose, and thus significantly affects the bioavailability of erythromycin when used simultaneously.

The lowest value of adsorption at pH 6.4 can be explained by the chemical properties of chitosan, which shows the possibility of a charge until  $\text{pH} > 6.7$ , and it may have electrostatic voltage and the opportunity to demonstrate adsorption in relation to medicinal substances [6, 7].

The intestinal environment at a pH above 7.6 is filled; the adsorption of average size chitosan at the highest dose of the drug was in the range of 80% to 99.00%.

## 4. Conclusion

The increase in the size of adsorption of erythromycin onto the polymer with increasing pH from 7.6 to 8.0 can be explained by the swelling properties of chitosan which is present in the form of a conglomerate in the emulsion system. Based on the above considerations it can be concluded that there is an antagonistic interaction between the test drug and the polymer involving the adsorption of the drug on the polymer, which is chitosan, which reduces its bioavailability and therapeutic concentration.

## 5. References

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