ATORVASTATIN ADSORPTION STUDIES ON CHITOSANS IN AN *in vitro* PHARMACEUTICAL MODEL

Jan Meler, Bożena Grimling, Maria Szcześniak, Bożena Karolewicz, Paweł Biernat

Faculty of Pharmacy, Department of Pharmaceutical Technology
Wrocław, Medical University
Borowska 211, 50-556 Wrocław, Poland.
Email: jan.meler@umed.wroc.pl

Abstract

During the pharmacological therapy of specific diseases, drugs are used which, with other preparations or foods, can create connections, in many cases changing or even blocking their action. On the other hand, the use of unsuitable polymers as excipients may result in drug-polymer incompatibilities. Interactions consisting mainly of the occurrence of the adsorption phenomenon and on the formation of complex bonds that reduce the effect of the drugs are of particular importance.

The aim of the study was to investigate whether the active substance atorvastatin is incompatible with dietary supplements containing chitosan.

The phenomenon of the adsorption of the drug was examined using a static model of a pharmaceutical gastrointestinal tract, in the concentration range generally ingested in a single dose. Measurement results of the amount of bound drug were used to determine the average percentage of adsorbed drug dose. The results of the study prove that the anticholinesterase drug is adsorbed on chitosan in the pH ranges used, and that the binding capacity depends on the chitosan variety, which indirectly affects the reaction of the environment. It was observed that the average size of sorption depending on the chitosan variety ranged from 38% to 86%.

The fact that the lowest value of adsorption was at pH 6.4 can be explained by the chemical properties of chitosan, which shows a charge only at pH >6.7. Under such conditions, the phenomenon of electrostatic adsorption may occur in relation to the healing substances of weak acids.

At a pH above 7.6, corresponding to the intestinal fluid-filled intestine, the mean sorption for the highest dose of chitosan was from 38–86%. The increase in the adsorbed amount of anticholinesterase drugs on the polymer along with the increase in pH from 7.6 to 8.0 can be explained by the chitosan swelling properties, which increase with an increase in the pH.

As a result, the specific surface area of the polymer and its sorption capacity increase. Based on the above considerations, it can be concluded that there is an antagonistic interaction between the drug and the polymer studied, which involves the adsorption of a drug from this group on the polymer (chitosan) and a decrease in its bioavailability.

***Key words**: Atorvastatin, adsorption, chitosan*

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1. Introduction

Nowadays, polyapharmacotherapy is a common phenomenon that carries a great danger of interaction. This phenomenon is exacerbated by the uncontrolled admission by patients of dietary supplements produced from ingredients with lower standards than pharmaceutical raw materials.

The presence of chitosan in dietary supplements poses a threat of reducing the therapeutic effect of medicinal substances taken by patients.

At the same time, in other centres, attempts have been made to use chitosan as a carrier of active substances, e.g. atorvastatin. Thanks to its high adsorption properties, depending on the length of the polymer chain, it will be possible to create a drug carrier with a modified release profile of the substance [1].

The recommended treatment regimens of obesity currently use many natural compounds whose activity is based on the ability to absorb nutrients. During the intestinal passage, a polymeric gel is produced that has the ability to adsorb nutrients.

The aim of the study was to examine, *in vitro*, the influence of selected physicochemical factors on the adsorption capacity of various types of chitosans and to assess the assumption that the use of chitosan preparations is important for the oral bioavailability of the drug and explain the mechanism of interaction of atorvastatin with dietary supplements, which include chitosan.

The high adsorption capacity of polymers such as chitosan may reduce the bioavailability of the therapeutic agents used.

2. Materials and method

2.1. Materials

Natural chitosans, with a very high degree of deacetylation ranging from 85% to 95%, were used for the tests. The chitosans were exposed to radiation before use to reduce molecular weight. Radiation doses from 5 to 30 kGy were applied (Table 1 in the study "Norfloxacin adsorption on chitosan" Progress on Chemistry and Application of Chitin and Its Derivatives Volume XVII, 2012, 102).

Atorvastatin (FARMAKOPEA POLSKA) was used as a multifunctional organic chemical compound derived from natural lovastatin, a prodrug with a lactone ring structure in its molecule. It is hydrolysed, together with other statins in the form of an active pharmacophore group, to the β-hydroxy carboxylic acid chain. Atorvastatin is used as a lipid-lowering medicine. It also has an additional pleiotropic effect on the circulatory system by affecting endothelial function, the stabilisation of atherosclerotic plaques, inhibition of the coagulation system, stimulation of the fibrinolytic system, the inhibition of inflammatory reactions and immunomodulatory effects. It works by inhibiting the enzyme 3-hydroxy-3-methyl-glutarylcoenzyme A reductase (HMG-CoA) [2,3].

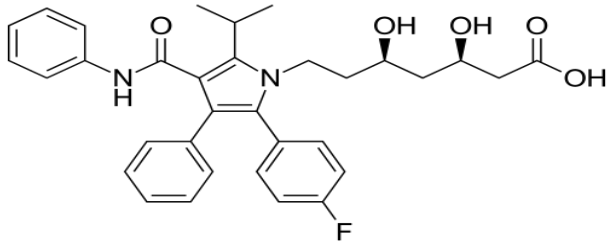

Figure 1. Atorovastatin

Table 1. List of physicochemical properties of Atorovastatin

Commercial preparations	Chemical name	Molar mass [g/mol]	Solubility in water [g/l]	Melting point [°C]
Atoris, Atorpharm, Atorvasterol, Atractin, Atrox, Corator, Lipitor, Sortis, Torvacard, Tulip	[R- (R *, R *) - 2- (4-fluorophenyl) -β, δ- dihydroxy-5- (1) methylethyl) -3-phenyl-4 - [(phenylamino) carbonyl] -1H-pyrrole-1- heptanoic acid	558,64	$4.95 \cdot 10^{-4}$	159.2-160.7

The study used 3 types of chitosan with variable viscosity to compare their adsorptive properties.

Table 2. Types of chitosan

No.	Chitosan type	Intrinsic viscosity [η] [dm^3g^{-1}]
1	Chito-Clear TM 1015	0.5100
7	Chitosan type 352 food grade	0.2117
13	Chitosan type 652 sample	0.3132

2.2. Method

2.2.1 Studies of the adsorption of atorvastatin

The atorvastatin adsorption effect was investigated by a static method in the range of concentrations of a generally-used single dose using a gastrointestinal pharmaceutical model based on the modification of the test according to Polish Pharmacopoeias for this type of preparation [4-6].

The study was carried out in a shaker water bath, maintaining conditions resembling the conditions prevailing in the gastrointestinal tract as much as possible. The amplitude of vibrations imitated peristaltic motions (300 rpm) and the process temperature (37ºC). In 5 ml centrifuge vials, 2 ml of the respective chitosan solutions were measured and adjusted to pH 2 (0.05M HCl), which is similar to the fasting stomach pH. The volume of solution used corresponded to 0.03 g of chitosan (the dose used as a slimming supplement). The quantities of drug substances corresponding to 0.08 g of atorvastatin (the dose used for the therapeutic treatment) were then added to the vials and mixed at 300 rpm for 2 hours. Then, the contents of the tubes were adjusted with 0.1M Na_2CO_3 to pH

7.0–7.6, which corresponds to the reaction of the intestinal juice and the colon. Samples were incubated at 37°C shaking (300 rpm) for 2.5 hours.
The test mixture was brought to room temperature and centrifuged at 2100 x g for 20 minutes before being allowed to stabilise for 0.5 hours. Then, 1.5 ml of the supernatant was collected in clean tubes and spectrophotometrically determined (λ = nm) in 1 cm quartz cuvettes using a standard curve determined from the concentrations of the drug to be determined.

3. Results and discussion

3.1. The influence of viscosities important for the adsorption of atorvastatin by various types of chitosans

The analysis of the effect of radiation dose degradation, which affects the intrinsic viscosity of the obtained system on the adsorption capacity of atorvastatin by chitosans, shows that the decrease in intrinsic viscosity of chitosan affects the increase in the amount of drug listed in Fig. 2.and Tab. 3.

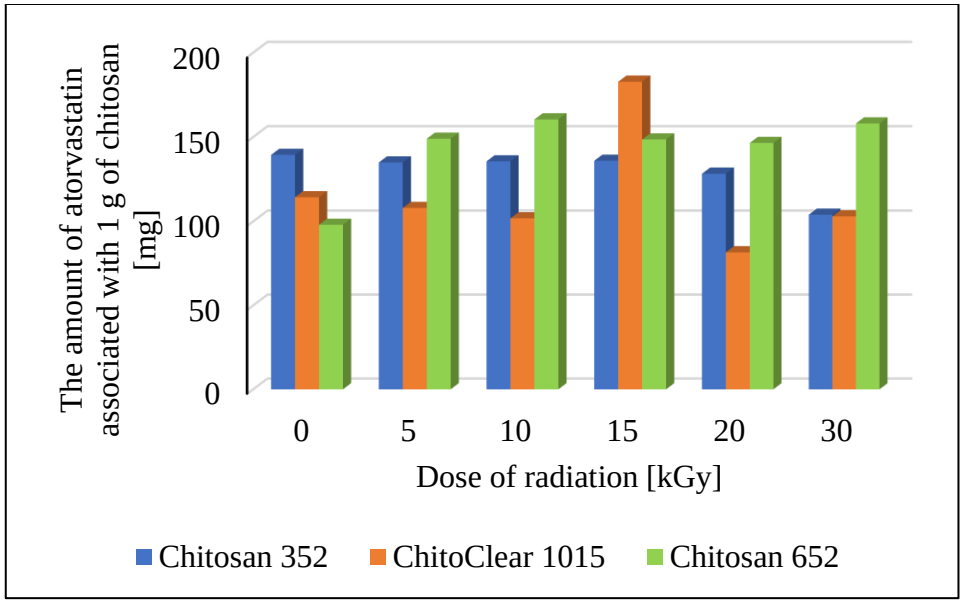

Figure 2. Amount of attached Atorovastatin by different types of chitosans depending on the intrinsic viscosity η (dm^3g^{-1})

Table 3. Dependence of the amount of bound atorvastatin on chitosan in relation to the intrinsic viscosity of the polymer used

No.	Chitosan type	**Intrinsic viscosity [η] [dm^3g^{-1}]**	Average amount of bound statin [mg]	Average amount of binding statin by 1g of chitosan [g]	Standard deviation (DS) [g]	Relative standard deviation (RDS) [%]
1	Chito-Clear TM 1015	0.5100	3.42	114.30	3.00	2.62
2	Chito-Clear TM 1015 (5)	0.4172	3.23	107.95	3.12	2.89
3	Chito-Clear TM 1015 (10)	0.3440	3.05	101.84	1.50	1.47
4	Chito-Clear TM (15)	0.2910	5.48	182.77	5.00	2.74
5	Chito-Clear TM 1015 (20)	0.2580	2.44	81.58	3.44	4.22
6	Chito-Clear TM 1015 (30)	0.2550	3.08	102.97	3.45	3.35
7	Chitosan type 352 food grade	0.2117	4.18	139.44	2.34	1.68
8	Chitosan type 352 food grade (5)	0.1949	4.04	134.95	3.67	2.72
9	Chitosan type 352 food grade (10)	0.1696	4.06	135.59	4.12	3.04
10	Chitosan type 352 food grade (15)	0.1639	4.07	135.94	5.11	3.76
11	Chitosan type 352 food grade (20)	0.1575	3.84	128.27	5.98	4.66
12	Chitosan type 352 food grade (30)	0.1497	3.11	103.95	3.66	3.52
13	Chitosan type 652 sample	0.3132	2.93	97.97	4.25	4.34
14	Chitosan type 652 (5)	0.2725	4.46	148.99	3.45	2.32
15	Chitosan type 652 (10)	0.2345	4.81	160.44	2.44	1.52
16	Chitosan type 652 (15)	0.2133	4.45	148.56	4.25	2.86
17	Chitosan type 652 (20)	0.1775	4.39	146.48	5.34	3.65
18	Chitosan type 652 (30)	0.1615	474	158.06	3.16	2.00

Analysis of viscosity mean molecular weight determinations showed that these values for chitosans changes depending on the degree of radiation degradation of the polymer, which is adequate to the intrinsic viscosity (Tab. 4).

Table 4. Intrinsic viscosity of chitosan

No	Chitosan type	Dose of radiation (kGy)	**Intrinsic viscosity [η] [dm^3g^{-1}]**
1	Chito-Clear TM 1015	0	0.5100
2	Chito-Clear TM 1015	5	0.4172
3	Chito-Clear TM 1015	10	0.3440
4	Chito-Clear TM 1015	15	0.2910
5	Chito-Clear TM 1015	20	0.2580
6	Chito-Clear TM 1015	30	0.2550
7	Chitosan type 352 food grade	0	0.2117
8	Chitosan type 352 food grade	5	0.1949
9	Chitosan type 352 food grade	10	0.1696
10	Chitosan type 352 food grade	15	0.1639
11	Chitosan type 352 food grade	20	0.1575
12	Chitosan type 352 food grade	30	0.1497
13	Chitosan type 652	0	0.3132
14	Chitosan type 652	5	0.2725
15	Chitosan type 652	10	0.2345
16	Chitosan type 652	15	0.2133
17	Chitosan type 652	20	0.1775
18	Chitosan type 652	30	0.1615

The research results prove that atorvastatin is adsorbed on chitosan in the pH ranges used, and that the binding capacity depends on the chitosan variety and its degradation [6].

The results of the adsorption of Atorovastatin by chitosan contained in the concomitantly used formulations that are generally available on sale without a prescription as dietary supplements confirmed the hypothesis that the adsorption shows great variation for individual preparations. It is most strongly bound by preparations containing chitosans with an intrinsic viscosity between 0.14 and 0.34 (dm^3g^{-1}), while an absorption between 0.34 and 0.54 (dm^3g^{-1}) is the weakest to increase after crossing the 0.54 limit up to 0.74 (dm^3g^{-1}).

The amounts of bound Atorovastatin by individual slimming market formulations show similar values, but are significantly higher compared to the adsorption of this drug by chitosans from different manufacturers. Chitosan contained in medicinal preparations has the ability to bind almost 86% of the administered dose of the drug, and thus significantly affects the bioavailability of the concomitantly used atorvastatin.

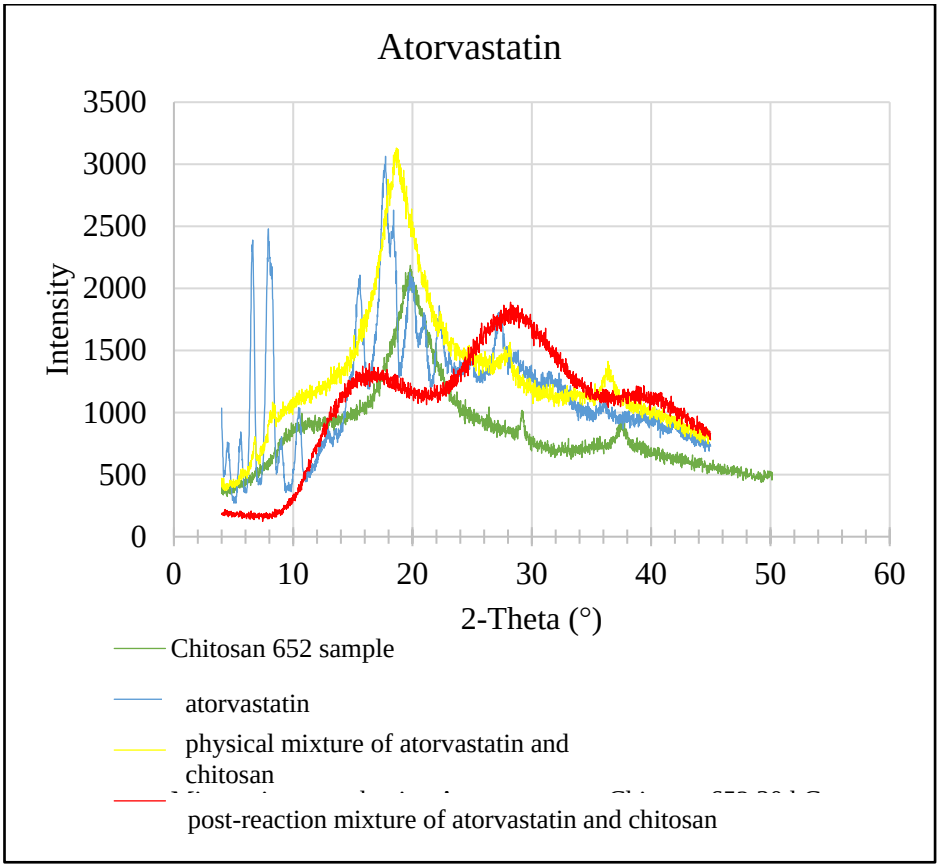

Figure 3. The result of a diffractometric test of chitosan samples, atorvastatin, a physical mixture of atorvastatin and chitosan, and post-reaction mixture of atorvastatin and chitosan.

The results of the diffraction analysis of the samples are presented in Figure 3. The image obtained after testing the pure statin, pure chitosan, chitosan and drug mixture as well as the post-reaction mixture of chitosan and drug were compared. The post-reaction mixture was obtained after the test in the gastrointestinal tract model; the sediment remaining after centrifugation of the solution containing the precipitated chitosan and the bound statin was used.

Chitosan prepared for testing directly from shrimp shells has two distinctive peaks at 10° and 20°; this spectrum is typical for semi-crystalline chitosans [7, 8]. The spectrum obtained in the Bruker diffractometer for Chitosan 652 samples also shows a strong peak at 20° and two weaker peaks at around 29° and 38°. At 10°, a slight rise in the spectral line is noticeable.

Figure 3, obtained for atorvastatin, allows the comparison of samples that have been tested in a biopharmaceutical model of the gastrointestinal tract with samples not subjected to such testing and with pure substances. The spectrum for the post-reaction mixture illustrates the changes that have occurred in the drug/chitosan mixture after the experiment. By comparing the spectra for the physical mixture of atorvastatin and chitosan, and for the pure drug, it can be seen that they do not show significant differences, both show similar sized peaks at about 28º; only the pure drug has additional peaks in the

initial spectrum at 6º and 8º. The spectrum of the post-reaction mixture indicates that atorvastatin was present in the sediment, because the elevation at 29° is visible, partially overlapping with the peak of pure drug at 28°. This indicates that part of the drug has been bound by chitosan. At 20º, there is a clear drop, which means that not all of the drug has been adsorbed, but that the remaining part is in the solution.

The lowest value of adsorption at pH 6.4 can be explained by the chemical properties of chitosan, which can occur only at pH >6.7 and can have an electrostatic voltage and the ability to show adsorption in relation to medicinal substances [9].

At a pH above 7.6, it is the environment of the intestine filled with the alimentary content; the average amount of adsorption for the highest dose of drug on chitosan was within 38.0–86.0%.

4. Conclusion

The increase in the adsorption level of atorvastatin on the polymer along with the increase in pH from 7.6 to 8.0 can be explained by the swelling properties of chitosan, which forms a conglomerate in the form of an emulsion.

Based on the obtained results, it can be concluded that the binding of statins, for example atorvastatin, depends on the viscosity of chitosan, which results from the particle size of the polymer. Only for chitosan type 352 is there a proportional relationship between the size of the polymer molecule and the amount of bound active substance. The studies show that the smaller the molecular weight of the 352 chitosan monomer, the lower the degree of binding to atorvastatin.

Based on the above considerations, it can be concluded that there is an antagonistic interaction between the drug and the polymer studied, which involves the adsorption of this drug on the polymer, chitosan, which reduces its bioavailability and therapeutic concentration.

Currently, there are many dietary supplements on the pharmaceutical market, in which there are different types of chitosan, characterised by different viscosity and different particle sizes. It is very important, in the context of the conducted research, to use chitosan with the weakest adsorption properties in such preparations.

In subsequent tests, the dependence of the level of adsorption between the types of chitosan and atorvastatin, with increased bioavailability, occurring in the form of nano-crystals, should also be considered.

5. References

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