THE EFFECT OF PHYSICOCHEMICAL FACTORS ON ABSORPTION PROPERTIES OF CERTAIN SPASMOLYTICS IN THE PRESENCE OF DIETARY SUPPLEMENTS CONTAINING CHITOSAN

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Abstract

Chitosan is used in the treatment of obesity due to its lipid, cholesterol, fatty acids, triglycerides, bile acids binding capacity. A molecule of a cationic polymer, such as chitosan, is capable of binding acid drugs. The aim of the study was to determine the capability of binding spasmyotics: 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride (Papaverinum hydrochloridum) and [3(S)-endo]-8-methyl-8-azabicyclo[3.2.1]oct-3-nyl ester of α-(hydroxymethyl)-bensoacetic acid (Butylscopolamini butylobromidum) by chitosans present in slimming drugs. Absorption of spasmyotics was investigated with the dynamic method on a biopharmaceutical model imitating in vitro conditions. Mean absorption of drugs on chitosan in relation to environmental pH ranged from 0.1328 g to 0.265 g per 1 g of chitosan for papaverine, and from 0.033 g to 0.330 g per 1 g of chitosan for scopolamine. The highest absorption rates were observed at pH above 7. Concluding, the studies have shown that an interaction occurs between spasmyotics, what decreases the amount and affects the bioavailability of these drugs.

Key words: papaverine hydrochloride; Scopolamine butylbromide; Butylscopolamine; chitosan; absorption
1. Introduction

Chitosan is used in the treatment of obesity due to its lipid, cholesterol, fatty acids, triglycerides, bile acids binding capacity. It is an effective source of soluble dietary fibers. It is indigestible; it dissolves in the acid environment in the stomach, where it binds many water molecules, forming stable adsorptive gel. A molecule of cationic polymer, such as chitosan, is capable of binding acid drugs [1, 2].

The aim of the study was to determine the capability of binding spasmolytics: 1-(3,4-dimethoxybenzyl)-6,7-dimethoxyisoquinoline hydrochloride (Papaverinum hydrochloridum) and [3(S)-endo]-8- methyl-8-azabicyclo[3.2.1]oct-3-yl ester of α-(hydroxymethyl)-benzoacetic acid (Butylscopolamine - scopolamine butylbromide) by chitosans present in slimming drugs.

Papaverine is a spasmolytic drug (spasmolytica) acting directly on smooth muscles. Direct spasmolytics, also referred to as muscelotrophic spasmolytics, exert their effect through inhibition of phosphodiesterase, what leads to an increase in the level of cyclic AMP (cAMP), and in consequence, to relaxation of smooth muscles Scopolamine is used in the treatment of visceral spasms (intestinal, renal, hepatic colic, painful menstruation in women) and in combined therapy of gastric and duodenal ulceration [3, 4].

The absorption of spasmolytics was investigated by means of the dynamic method in a biopharmaceutical model in vitro conditions [4]. The quantity of drug absorbed by chitosan was calculated from differences in concentration prior to and after sorption. The findings were used to calculate mean percentage of absorbed dose.

2. Materials and method

Natural chitosans with deacetylation of 85% to 95%, degraded by 5 to 30 kGy radiation dose were used in the study.

Also, dietary supplements containing chitosan were used (Vitana®, Hitec Nutrition®, Chromdiet®, Bio-Active Tech-Food Trading®). The investigated therapeutic agents included spasmolytics: 1-(3,4-dimethoxyphenyl)methyl]-6,7-dimethoxy-isoquinoline hydrochloride (Papaverinum hydrochloridum) as well as the [7(S)-(1α,2β,4β,5α,7β)]-9-butyl-7-(3-hydroxy-1-oxo-2-phenylpropoxy)-9-methyl-3-oxa-9-azonitricyclo[3.3.1.0(2,4)] nonane (Scopolamine butylbromidum - Butylscopolamine).

The absorption of spasmolytics was investigated by means of the dynamic method in a biopharmaceutical model imitating in vitro conditions [5, 6]. The quantity of drug absorbed by chitosan was calculated from differences in concentration prior to and after sorption. The findings were used to calculate mean percentage of absorbed dose.
0.03 g portions of chitosan were weighed and put to 5 ml glass centrifuge vials and next 2 ml of 0.05 M HCl were added to achieve pH 2 of the solution, what corresponds to natural fasting gastric pH.

Next 0.1 M Na$_2$CO$_3$ were added to the vials to achieve pH 6.4 (pH in the duodenum) and stirred for 0.5 hour (300 r.p.m.), next it was alkalized with sodium carbonate to achieve pH 7.0 - 7.6, corresponding to pH of the intestinal juice. The samples were then incubated at 37 °C in a shaker (300 rpm) for 2.5 hours.

Next the samples were brought to room temperature and centrifuged (2100 × g) for 20 minutes. The vials were then left for 30 minutes to stabilize and next, depending on the kind of investigated drug, certain amount of the solution was collected from over the sediment and determined spectrophotometric analysis.

- In case of papaverine hydrochloride solution, 3 doses were used: 40 mg/5ml, 60 mg/5ml, 80 mg/5ml, what corresponded respectively to: 0.8 ml, 1.2 ml, 1.6 ml of papaverine hydrochloride solution.

- In case of scopolamine butylbromide solution, 3 doses were used: 10 mg/5ml, 50 mg/5ml, 100 mg/5ml, what corresponded respectively to: 0.05 ml, 0.25 ml, 0.5 ml of scopolamine butylbromide solution.

\[\text{H}_3\text{CO} \begin{array}{c}
\text{H}_3\text{CO} \\
\text{N} \\
\text{OCH}_3 \\
\text{OCH}_3 \\
\text{HCl}
\end{array}\]

\textbf{Schema 1.} 1-[(3,4-dimethoxyphenyl) methyl]-6,7-dimethoxy-isoquinoline hydrochloride (Papaverinum hydrochloridum) as well as (-)-(S)-3-Hydroxy-2-phenyl-propionic acid (1R,2R,4S,7S,9S)-9-methyl-3-oxa-9-aza-tricyclo[3.3.1.0$^{2,4}$]non-7-yl ester of acid.

\[\text{O} \begin{array}{c}
\text{N} \\
\text{O} \\
\text{O}
\end{array}\]

\textbf{Schema 2.} Scopolamine butylbromide [7(S)-(1α, 2β, 4β, 5α, 7β)-9-butyl-7-(3-hydroxy-1-oxo-2-phenylpropoxy)-9-methyl-3-oxa- 9-azonitricyclo [3.3.1.0(2, 4)] nonane.
The measurements were 5 times repeated and the findings were thoroughly evaluated statistically, standard deviations and relativity coefficient were determined.

Measurements were led at constant temperature of 25 °C in automatic Ubbelohde viscometer. Water solution of 0.1 M acetic acids was employed and it was filtered to separate insoluble fraction of 0.2 M sodium chloride. The time of outflow through the viscometer gauge was measured for all the solutions and their dilutions. At least five measurements were executed for each concentration. Since the Mark-Houwink parameters used to recalculate intrinsic viscosity into viscosity-average molecular weight are known for chitosan, it was determined in this solvent composition \((K = 1.81 \times 10^{-6} \text{ dm}^3 \text{ g}^{-1}, \alpha = 0.93)\) [7].

3. Results and discussion

3.1. The effect of radiation degradation on intrinsic viscosity of chitosans

The analysis of the effect of radiation degradation rate on intrinsic viscosity shows that a decrease in mean molecular weight of chitosan causes a decrease of this parameter (Table 1).

The highest decrease in viscosity-average molecular weight under the effect of an increased dose of degradation radiation (0 - 30 kGy) was observed in case of Huasu chitosan and it was 680 kDa.

The lowest change in intrinsic viscosity was observed for Chito Clear TM 1015, and it was 81 kDa. The remaining chitosans demonstrated a moderate decrease in viscosity with increased dose of degradation radiation 0 - 30 kGy, which in case of chitosan 352 was 88 kDa, for chitosan 652 - 219 kDa and for chitosan 343 - 569 kDa (Figure 1).

3.2. Papaverine hydrochloridum and Scopolamine butylbromidum binding by degraded and non-degraded chitosans

Examination of absorption of the investigated drugs: Papaverine hydrochloride and Scopolamine butylbromide confirm that the Mount of bounded drug depends on the degradation rate of chitosan and its origin.

The analysis of the effect of intrinsic viscosity on the capability of absorption of the investigated drugs by chitosans reveals linearity of the drug binding. In case of papaverine hydrochloride, the amount of absorbed drug increases with an increase in intrinsic viscosity for all the investigated chitosans [8].

In scopolamine butylbromide, higher absorption was observed in case of Huasu chitosan. Chito Clear chitosan absorbs with increased viscosity initially larger and later smaller amounts of scopolamine butylbromide. Absorption of the drug drops with increased viscosity for chitosans 343, 352 i 652 (Figures 2 - 4).
Table 1. The value of intrinsic viscosity and the viscosity-average molecular weight in studied chitosans and chitosan preparations.

<table>
<thead>
<tr>
<th>Chitosan</th>
<th>Dose of degradation radiation, kGy</th>
<th>Intrinsic viscosity $[\eta]$, dm$^3$ g$^{-1}$</th>
<th>Viscosity-average molecular weight $M_{\eta}$, kDa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chito Clear TM 1015</td>
<td>0</td>
<td>0.5100</td>
<td>725</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4172</td>
<td>584</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.2550</td>
<td>344</td>
</tr>
<tr>
<td>Chitosan 652</td>
<td>0</td>
<td>0.3132</td>
<td>429</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.2725</td>
<td>369</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.1615</td>
<td>210</td>
</tr>
<tr>
<td>Chitosan 343</td>
<td>0</td>
<td>0.6402</td>
<td>925</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.4588</td>
<td>647</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.2700</td>
<td>366</td>
</tr>
<tr>
<td>Chitosan 352</td>
<td>0</td>
<td>0.2117</td>
<td>282</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.1949</td>
<td>258</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.1497</td>
<td>194</td>
</tr>
<tr>
<td>Chitosan HUASU</td>
<td>0</td>
<td>0.7437</td>
<td>1087</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>0.5843</td>
<td>839</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.2986</td>
<td>407</td>
</tr>
<tr>
<td>Vitana ®</td>
<td>Sample</td>
<td>0.1774</td>
<td>229</td>
</tr>
<tr>
<td>Chromadiet ®</td>
<td>Sample</td>
<td>0.1872</td>
<td>242</td>
</tr>
<tr>
<td>Chitosan Nutrisearch®</td>
<td>Sample</td>
<td>0.1576</td>
<td>205</td>
</tr>
<tr>
<td>Bio-Active ®</td>
<td>Sample</td>
<td>0.1576</td>
<td>205</td>
</tr>
</tbody>
</table>

Figure 1. The value of viscosity-average molecular weight in studied chitosans and chitosan preparations.
Figure 2. The graph of relation between binding Scopolamine butylbromide near dose 0.01 g/g and essential viscosity $[\eta]$.  

Figure 3. The graph of relation between binding scopolamine butylbromide near dose 0.05 g/g and essential viscosity $[\eta]$.  

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The absorption of papaverine hydrochloride increases with an increase in viscosity in case of Chito Clear and Huasu chitosans, and decreases in case of chitosans 652, 343, 352 (Figures 5 - 7).

**Figure 4.** The graph of relation between binding scopolamine butylbromide near dose 0.5 g/g and essential viscosity $[\eta]$.

**Figure 5.** The graph of relation between binding papaverine hydrochloride near dose 0.04 g/g and essential viscosity $[\eta]$. 
The significant of relation between the total percentage of mean adsorption investigate drugs on chitosans evaluated statistically by means of a uni-factorial Anova?Manova analysis, post hoc Nir test, confirmed statistically significant differences in the percentage...
of value adsorption of drugs on chitosans. Standard deviations of mean adsorbance levels were in the limits from 0.06 mg/cm$^3$ to 0.043 mg/cm$^3$ and variation coefficients were from 0.35% to 4.81%.

The analysis of viscosity-average molecular weights demonstrated that absorption of individual drugs increases with decrease in molecular weight of all the investigated chitosans. This is presumably associated with lower strength of the polymer chain and the capability of formation of larger branches in the polymer network.

### 3.3. Investigation of Papaverine hydrochloridum and Scopolamine butylbromidum binding by chitosans present in dietary supplements

The binding of papaverine hydrochloridum and scopolamine butylbromidum by chitosans contained in dietary supplements confirm the hypothesis of aggregative character of chitosans in relation to these drugs. The chitosans used in the study present in dietary supplements available on the slimming products market are capable of binding on an average of 0.1328 – 0.265 g of Papaverine hydrochloride (Figure 8) and 0.033 – 0.330 g of Scopolamine butylbromide (Figure 9) per 1 g of chitosan.

![Figure 8](image.png)

**Figure 8.** The amount of Papaverine hydrochloride bound by 1 g of chitosans present in dietary supplements.
Figure 9. The amount of Scopolamine butylbromidum bound by 1 g of chitosans present in dietary supplements.

The highest absorption rate was observed at pH above 7. Standard deviation ranged from 0.006 to 0.042 and determined relativity coefficient ranged form 0.53% to 4.89%, what confirms high accuracy of measurements [7].

4. Conclusion

The above considerations lead to a conclusion that the investigated spasmolytical drugs interact with chitosan. The interaction has an antagonistic character and consists in adsorption of the investigated drugs on chitosan.

Thus we can presume that adsorption has a physical and chemical character.

Papaverine hydrochloride was found to be the best adsorbed drug of the investigated digestive tract motility stimulants, while the adsorbance capability of Scopolamine butylbromide was much lower.

Among the tested spasmolytic drugs Scopolamine butylbromide adsorps best, papaverine hydrochloride adsorps significantly less.

Investigations carried out so far on the character and the degree of mutual interactions of gastroenterological agents (spasmolytics) in the presence of chitosan confirm an antagonistic interaction consisting in adsorption. The highest value was observed in test performed at pH < 6.4. The adsorption of tested drugs on chitosans depends on drug concentrations and the form of chitosans. The above analysis of results of our studies confirm the significance of the problem and allows to complete the biopharmaceutical informa-
tion concerning the investigated preparations as well as enables control of the efficiency of polypragmasy. In conclusion, the studies have shown that there is an interaction between spasmytics, what decreases their quantity and affects the bioavailability of these drugs.

5. References


