1. Introduction

The combination of polymers and biopolymers with bioactive small molecule compounds has recently been intensively researched. Small molecule active substance in combination with a polymer reveals in many instances a modified activity. On the other hand, the use of improper polymers may lead to drug-polymer incompatibility. Of special significance are interactions in the form of adsorption and formation of complexes which diminish the effect of the drug. Chitosan in acid environment forms a universal hydrogel and is a good vehicle for active substances with varied physicochemical and pharmacological properties, which are the components of preparations for local use [1 - 3].

For this reason the aim of the study was to investigate in in vitro conditions the effect of certain physicochemical factors on the adsorption capability of various kinds of chitosans and to evaluate the premise that the use of chitosan preparations has a significant influence on the bioavailability of concomitant orally administered active substances as well as to explain the mechanism of interactions of anti-inflammatory drugs: acetylsalicylic acid, sodium diclofenac and ibuprofen with dietary supplements containing chitosan.

2. Materials and method

2.1. Materials

Natural chitosans with deacetylation of 85% to 95%, degraded by 5 to 30 kGy radiation dose, manufactured by various producers were used in the study (Table 1 see page 84).
Ibuprofen (FP VI) (nonsteroid anti-inflammatory drug NSDAID, white crystalline powder easily soluble in ethanol (760 g/l), practically insoluble in water, Hasco Poland) [4]; Sodium diclofenac salt (European Pharmacopoeia 2005) (therapeutic compound, while or light yellow crystalline powder, slightly hygroscopic, hardly soluble in water, soluble in alcohol (760 g/l), methanol and acetone, Polpharma) [5]; Acetylsalicylic acid (FP VI) (white, crystalline powder with slightly acid smell, hardly soluble in water, soluble in ethyl ether, easily soluble in ethanol (760 g/l), Pharma Cosmetic) [4].

2.2. The effect of chitosans on NSAIDs solubility
Nonsteroid anti-inflammatory drugs are hardly soluble in water. In literature there are reports on the effect of microcrystalline chitosan (MCCH) on NSAIDs solubility in water. As high as a 10-fold increase in active substance solubility at MCCH concentration levels from 0.00% to 0.15% was demonstrated in case of ketoprofens. Further increase in the amount of MCCH did not have any effect on the solubility of ketoprofens in water [6].

The study was a trial to evaluate the effect of a chitosan solution (Chitosan type 352, 20 kGy) on the solubility of the investigated NSAID. The trial was performed in gastric environment (pH 2) with the use of two samples: A and B. Sample A contained only active substances, while sample B contained active substances in the presence of a polymer.

**Sample A:** weighed portions of active substances – 500 mg of acetylsalicylic acid, 200 mg of ibuprofen and 200 mg of sodium diclofenac salt (the amounts present in generally available drugs) were added and reduced to pH 2 with 0.05 n HCl.

**Sample B:** 300 mg of chitosan were added and shaken until dissolved; next the sample was reduced to pH 2 with 0.05n HCl and weighed portions of active substances were added.

The mixtures were shaken (300 r.p.m.) for 2 hours at 37 °C, what imitates the conditions in the stomach. Next they were cooled to room temperature, centrifuged (2,100×g) for 20 minutes and left to stabilize for 0.5 hours. 1.5 ml samples were collected from above the sediment, transferred to Eppendorf’s tubes and repeatedly subjected to centrifugation (15,000×g) for 10 minutes. Next, a definite amount of the sample from above the sediment was transferred to empty test tubes and a definite amount of solvent was added to determine the sample:
- 1.5 ml of acetylsalicylic acid sample was transferred to a test tube and 2.0 ml of methanol were added;
- 1.5 ml of ibuprofen sample was transferred to a test tube and 2.0 ml of 0.1 n HCl were added, next 0.5 ml of the contents was removed and 0.1 n HCl was added to 2.0 ml;
- 0.5 ml of sodium diclofenac salt sample was transferred to a test tube and 4.0 ml of methanol were added. Next 0.04 ml of the contents was removed from the test tube and made up with methanol to 1.0 ml.

After stirring, the test tubes contents were evaluated spectrophotometrically.

2.3. Examining the adsorption of nonsteroid anti-inflammatory drugs (NSAIDs)
Adsorption of nonsteroid anti-inflammatory drugs (NSAIDs) was investigated by means of a dynamic method in the range of concentrations in a generally administered single dose
using a biopharmaceutical model of the alimentary tract on the basis of a modification of the test according to Polish Pharmacopoeia for such preparations [1 - 3.7]. The investigation was performed in water bath with a shaker, maintaining the conditions maximally resembling those in the alimentary tract. Shaking amplitude was set at 300 rpm and the temperature at 37 °C.

2 ml solutions of chitosans were measured to 5 ml shaker vials and reduced to pH 2, what corresponds to fasting gastric pH. The applied volume of the solution was equivalent to 0.03 g of chitosan. Next amounts of active substances corresponding to 100 mg of the substance (amount of the active substance in a therapeutic dose) were added and shaken (300 r.p.m.) for 2 hours. Next 0.2 n Na₂CO₃ was added to the vial contents to reduce it to pH 7.0 – 7.6, what corresponds to the intestinal juice and colon. The samples were incubated at 37 °C, shaking (300 r.p.m.) for 2.5 hours.

The investigated sample was brought to room temperature and centrifuged (2,100×g) for 20 minutes, and next left for 0.5 hours to stabilize. Next a definite amount of the sample from above the sediment was collected to empty test tubes and a definite amount of determination solvent was added:
- 1.5 ml of acetylsalicylic acid was transferred to a test tube and 2.0 ml of methanol were added;
- 1.5 ml of ibuprofen sample was transferred to a test tube and 2.0 ml of 0.1 n HCl were added, next 0.5 ml of the contents was removed and 0.1 n HCl was added to 2.0 ml;
- 0.5 ml of sodium diclofenac salt sample was transferred to a test tube and 4.0 ml of methanol were added. Next 0.04 ml of the contents was removed from the test tube and made up with methanol to 1.0 ml.

After stirring, the test tubes contents were evaluated spectrophotometrically.

2.4. Measurement of viscosity and determination of average molecular weight

Measurements were led at constant temperature 25 °C with Ubbelohde viscometer [Polish Pharmacopoeia VI]. Water solution of 0.1 M acetic acids was employed and it filter solution for separating insoluble fraction 0.2 M sodium chloride. For all solutions and time of outflow gauge them three with solutions of viscometer. At least five measurements were executed for each concentration (Table 1 see page 84). Since the Mark-Houwink parameters used to recalculate intrinsic viscosity into viscosity-average molecular weight are known for chitosan in this solvent composition (K = 1.81×10⁻⁶ dm³ g⁻¹, α = 0.93) [8].

3. Results and discussion

3.1. The effect of chitosan on NSAIDs solubility

The applied concentrations of chitosan were equivalent to those commonly used in medical preparations. The investigation was performed in strongly acid environment of the stomach, and in these conditions drugs which are weak acids are weakly dissociated and hardly soluble (Figure 1). In the experiment imitating the natural gastric environment, chitosan occurs in the form of gel and its enhancing effect on NSAIDs solubility cannot be excluded, as this possible property of the polymer may be masked by more pronounced adsorption. Thus it may be assumed that in the investigated concentration ranges chitosan no longer affects NSAIDs solubility, and the process of NSAIDs adsorption in the gastric environment
mitigates the harmful effect of the drugs on gastric mucous membrane.

3.2. The effect of intrinsic viscosity and viscosity-average molecular weight on NSAIDs adsorption by chitosans

The analysis of the effect of intrinsic viscosity on NSAIDs adsorption by chitosans reveals non-linear active substance binding, which depends on the kind of polymer (deacetylation degree) Figures 2, 3 and 4.

Certain regularity may be demonstrated in case of acetylsalicylic acid, as the amount of bound substance increases with increasing intrinsic viscosity, with the exception for Chitosan 352, in which an increase in intrinsic viscosity results in the adsorption of increasing, to be followed by decreasing, amounts of ASA.

<table>
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<tr>
<th>Chitosan</th>
<th>Dose of degrading radiation</th>
<th>Intrinsic viscosity ([\eta]), dm(^3)g(^{-1})</th>
<th>Viscosity-average molecular weight (M_\eta), kDa</th>
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<tr>
<td></td>
<td>5</td>
<td>0.2545</td>
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<td></td>
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<td>30</td>
<td>0.1576</td>
<td>205</td>
</tr>
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Influence of different changes on bioavailability of medicine chitosans antiphlogistic drugs

Figure 1. Changes in NSAIDs solubility (ASA- Acetylsalicylic acid, IB-Ibuprofen; DS- Diclofenac sodium) without the addition of a polymer (1) and with polymer addition (2) on the basis of changes in their concentration (C% in mg%).

Figure 2. Binding of ASA (Acetylsalicylic acid) by different kinds of chitosans in relation to intrinsic viscosity $[\eta]$.

Figure 3. Binding of Ibuprofen by various kinds of chitosan in relation to intrinsic viscosity $[\eta]$. 
An increase in the adsorption of ibuprofen and sodium diclofenac salt is observed when Chitosan 352 with highest intrinsic viscosity is compared to chitosan with the lowest intrinsic viscosity. Similar effect is observed when sodium diclofenac salt is bound by Primex 85 chitosan, and ibuprofen – by Chito – Clear TM 1015 chitosan. The increase rate does not follow a regular pattern, it fluctuates with increasing viscosity.

In case of Huasu chitosan, the adsorption of ibuprofen and sodium diclofenac decreases slightly for the highest viscosity polymer in relation to the lowest viscosity polymer, and the pattern of decrease in the drug binding capability assumes an irregular character.

A significant decrease of NSAIDs binding capability with increasing viscosity has been observed in case of ibuprofen adsorption by Primex 85 and in the adsorption of sodium diclofenac salt by chitosan Chito – Clear TM 1015.

The analysis of viscosity-average molecular weight revealed that the parameter for chitosans changes in relation to the degree of radiation degradation of the polymer. The measurements of the amount of bound active substance were used to determine the average percentage of adsorbed dose of the drug. The findings prove that nonsteroid anti-inflammatory drugs are adsorbed on chitosan in the applied pH ranges, and the binding capability depends on the kind of chitosan, i.e. indirectly, on the environmental pH.

The findings of the measurement of NSAIDs adsorption by chitosans contained in commonly available OTC drugs confirmed the hypothesis that acetylsalicylic acid is least adsorbed and its adsorption is highly differentiated for different preparations. The adsorption is the strongest in case of Bio – active®, and the weakest in case of Vitana®.

The binding of ibuprofen and sodium diclofenac salt by various chitosan preparations reveals similar values, which are markedly higher in relation to the adsorption of ASA.

**Figure 4.** Binding of Diclofenac sodium by various kinds of chitosans in relation to intrinsic viscosity $[\eta]$. 

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**Progress on chemistry and application of chitin and its derivatives in different areas of life science**

Volume XIII, 2008

86
Influence of different changes on bioavailability of medicine chitosans antiphlogistic drugs

The amount of bound inbuprofen is slightly lower in comparison to the amount of adsorbed sodium diclofenac salt. Chitosan contained in medical preparations has the capability of binding almost 100% of the administered active substance, thus it significantly affects the bioavailability of simultaneously administered NSAIDs (Figure 5).

Mean sorption in relation to the kind of chitosan ranged from 95% to 99%. The observation of the lowest adsorption at pH 6.4 may be explained by the chemical properties of chitosan, which is charged only at pH < 6.7 and only then can it reveal an electrostatic adsorption in relation to active substances which are weak acids [9].

At pH above 7.6, what corresponds to the environment of the intestine filled with food, mean sorption for the highest chitosan dose ranged from 98% to 100%.

4. Conclusion

The increased adsorption of anti-inflammatory drugs on polymer with pH increased from 7.6 to 8.0 may be explained by the swelling properties of chitosan, which increase with increasing environmental pH to alkaline levels, in which the compound occurs in the form of emulsion, what increases the surface of the polymer and its sorption capacity. Basing on the above considerations, it can be stated that the investigated drug interacts with the polymer; the interaction is of an antagonistic character and consists in adsorption of the drugs on a polymer such as chitosan.

5. References


4. Pharmacopeia Poland VI, PZWL, Warsaw, 2002


