EVALUATION OF PREPARATIONS PHARMACEUTICAL SALAZOSULFAPIRIDIN DEPENDING USED FOOD SUPPLEMENTS CONTAINING CHITOSAN IN MODEL "*IN VITRO*"

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1. Introduction

In curing the obesity at present many natural large-molecular associations are obeying, of which action is based on aiding slimming down. These Centers expanding in the digestive tract are creating the arrangement gel polymer which has abilities to adsorb till 8 times of more lipids with respect to own mass. Using healing substances having an anti-inflammatory effect mainly to the mucous membrane in inflammatory conditions of intestines together with supplements containing diets to purposes the chitosan can lead bioavailability of the cure to the shift. Salazosulfapiridin (Sulfasalazine : 5-[4-(2-Pirydylsulfamoyl)phenylazo]salicylic acid) is acting immunosuppressive mainly in the range of the connective tissue, the wall of intestines and in serums, probably he is also binding oxygen free radicals and an influence on the production of prostaglandins has, leucotriens and of lighting different mediators [1]. With purpose our of work was epithet abilities bonds Salazosulfapiridin depending on of variables of factors physico-chemicals, appearing in of model of digestive tract through chitosans appearing in cures aiding slimming down.

2. Materials and method

2.1. Materials

It takes advantage in work about degree from deacetylation 85 for 95% natural chitosans; from 5 for 30 kGy degrade dose radiation (***Table 1***). Salazosulfapirydin (Sulfasalazine) - Sigma 50883,

2.2. Method

The phenomenon of the adsorption of the cure was being examined with dynamic method in the pharmaceutical model imitating conditions *in vitro*. Amount absorptions of cure through chitosans they calculated from the difference of checked concentrations of preparations before and after sorption.

The examinations were performed spectrophotometrically at wavelength of 359 nm (the regression line determined for Sulfasalazine was $y = 62.699x + 0.0005$), using NaOH solution (4 g/l), acetic acid (6.0 g/l) and purified water at 3:2:1 ratio as a reference.

Sulfasalazine are hardly soluble in water. In literature there are reports on the effect of microcrystalline chitosan (MCCH) on drugs 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 ketoprofen in water [2].

The study was a trial to evaluate the effect of a chitosan solution (Chitosan type 352, 20kGy) on the solubility of the investigated Sulfasalazine. 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 – 150 mg of sulfasalazine (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 (2100×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 (15000×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 sulfasalazine sample was transferred to a test tube and 8,5 ml of reference were added;

After stirring, the test tubes contents were evaluated spectrophotometrically.

2.3. Examining the adsorption of Sulfasalazine

Adsorption of Sulfasalazine was investigated by means of a dynamic method in the range of concentrations in a generally administered single dose using a pharmaceutical model of the alimentary tract on the basis of a modification of the test according to Polish Pharmacopoeia for such preparations [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 150 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 Na_2CO_3 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 rpm) for 2.5 hours.

The investigated sample was brought to room temperature and centrifuged (2100×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 references was added.

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. Since the Mark-Houwink parameters used to recalculate intrinsic viscosity into viscosity-average molecular weight are known for chitosan in this solvent composition ($K = 1.81 \times 10^{-6}$ dm^3 g^{-1}, $\alpha = 0.93$) [4].

Table 1. *Intrinsic viscosity [η] and viscosity - average molecular weight $M_{[\eta]}$ of the investigated chitosans (* - deacetylation degree).*

Chitosan	Dose of degrading radiation, kG	Intrinsic viscosity [η], dm^{3}g^{-1}	Viscosity-average molecular weight $M_{[\eta]}$, kDa
PRIMEX (85)*	0	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)*	0	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)*	0	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)*	0	0.2117	282
	5	0.1949	258
	10	0.1696	222
	15	0.1639	214
	20	0.1575	177
	30	0.1497	194
Chromdiet®	0	0.1872	242
Bio – active®	0	0.1576	205
Witana®	0	0.1774	229

3. Results and discusion

3.1. The effect of chitosan on Sulfasalazine 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 Sulfasalazine solubility (round 10%) 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 Sulfasalazine solubility, and the process of Sulfasalazine adsorption in the gastric environment mitigates the harmful effect of the drugs on gastric mucous membrane.

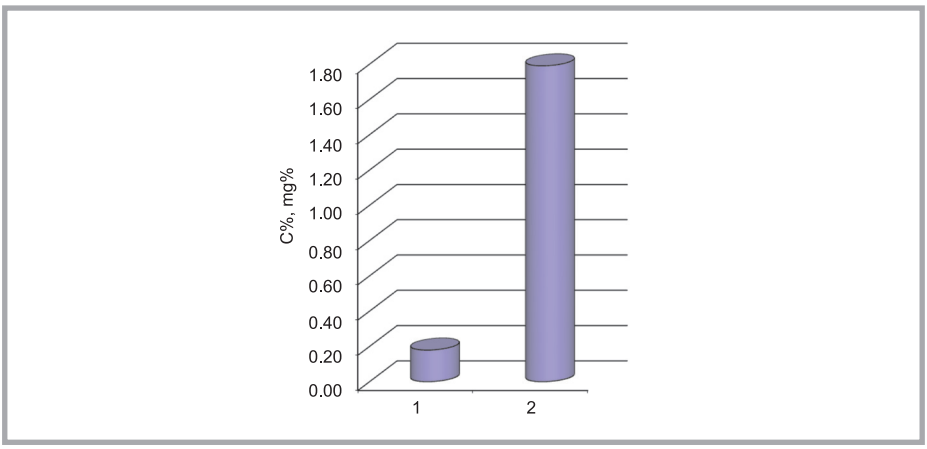

Figure 1. *Changes in Sulfasalazine solubility without the addition of a polymer (1) and with polymer addition (2) on the basis of changes in their concentration (C% in mg%).*

3.2. The effect of degradation radiation on Sulfasalazine absorption by chitosans

Analysis of the effect of degradation radiation rate on the capability of chitosans to absorb Sulfasalazine reveals certain regularity, in which a decrease in chitosan intrinsic viscosity is associated with increased volume of bound drug (***Figure 2***).

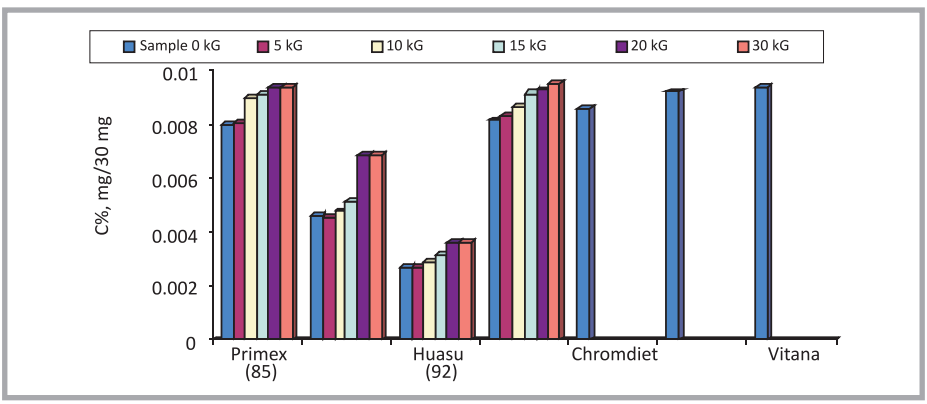

Figure 2. *Sulfasalazine binding by various kinds of chitosans in relation to degradation radiation rate in kG.*

Analysis of mean viscosity molecular mass measurements revealed that the values for chitosans change in relation to the polymer radiation degradation rate. The findings prove that sulfasalazine is absorbed on chitosan at applied pH ranges, and the binding capability depends on the kind of chitosan and its degradation.

The findings of measurements of sulfasalazine absorption by chitosan contained in simultaneously administered OTC preparations confirmed the hypothesis that the absorption varies significantly depending on preparation. It is the highest in case of Vitana®, and the weakest in case of Chromdiet® preparation.

The binding of sulfasalazine by individual preparations available on the market reveals similar rates, but they are significantly higher in comparison to absorption of this drug by chitosans from various manufacturers. Chitosan contained in medicinal preparations is capable of binding almost 100% of the administered drug dose, thus is affects markedly the bioavailability of simultaneously administered sulfasalazine (***Figure 2***).

Mean absorption rate was observed to range from 85% to 98% depending on the kind of chitosan.

The fact of lowest absorption at pH 6.4 may be attributed to chemical properties of chitosan, which reveals the charge only at pH < 6.7 and then it may reveal electrostatic absorption in relation to active substances with weak acid pH [6].

At pH above 7.6, corresponding to the intestinal contents environment, mean absorption for the highest dose of the drug on chitosan ranged from 98% to 100%.

4. Conclusion

An increase in sulfasalazine absorption on a polymer at increasing pH from 7.6 to 8.0 may be explained by the swelling properties of chitosan, which forms a conglomeration in the form of emulsion system.

Basing on the above considerations, it may be assumed that an antagonistic interaction occurs between the investigated drug and the polymer, which consists in absorption of the drug on a polymer such as chitosan.

5. References

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