10. INVESTIGATION OF BILE ACID SALTS BINDING CAPACITY BY VARIOUS KINDS OF CHITOSANS

Jan Meler, Janusz Pluta

Department of Dispensing Pharmacy, Medical University,
Szewska 38/39 50-139 Wrocław, Poland
e-mail: meler@bf.uni.wroc.pl

The primary task of bile acids present in the digestive tract is to emulsify lipids and provide a broad digestive surface for the action of pancreatic lipase. They derive from cholanic acid and are possess the ability of complexing (one molecule of fatty acid per 6-8 molecules of bile) with fatty acids from diet.

1. Aim of the study

The aim of the study was to investigate in vitro the capability of binding lipids and bile acid salts (sodium salts) by various kinds of non-modified chitosans and those which underwent radiation degeneration and thus to confirm the hypothesis that chitosans may significantly affect the bioavailability of lipids in human organism by their adsorption of lipids and bile acid salts properties.

2. Materials

The investigation was performed using chitosans from various manufacturers with deacytelation degree from 85 to 95%. They were submitted to radiation degradation at 5 to 30 KGy.

3. Method

The phenomenon of bile acid salts adsorption was investigated by means of a dynamic method in a biopharmaceutical model imitating conditions *in vitro* [1]. The amount of bile salt adsorbed by chitosan was calculated from the difference in concentration of the investigated salt prior to and after absorption [2 - 4]. In order to calculate the amount of bile salt adsorbed by various kinds of chitosans, a biopharmaceutical model of the digestive tract was used.

The investigation was performed in water bath with a shaker and attention has been paid to maintain conditions imitating those in the human digestive tract. The shaking amplitude as well as rate and temperature of the process (37 °C) were determined.
2 ml of a spare chitosan solution at pH 2 (fasting pH in the stomach) were added to 5 ml centrifuge vials. The volume corresponded to 0.05 g of chitosan. Next 1 ml of a solution containing 100 mg, 150 mg, 200 mg of bile salts was added. The mixture was shaken for 2 hours at 37 °C (imitation of gastric movements). Next, 0.2 M Na_2CO_3 was added in drops to obtain pH of duodenal juice at pH 6.4 and shaken (300 rpm) for 0.5 hrs. The sample with pH 7.0 - 7.6, corresponding to intestinal juice of the small intestine and the colon was incubated at 37 °C, shaking (300 rpm), for 2.5 hrs. The whole mixture was supplemented with phosphate buffer to the volume of 4 ml.

The system was brought to room temperature and centrifuged (2100 × g) for 20 minutes, and next left to stabilize for 0.5 h. 1.5 ml sample was collected from above the sediment, transferred to Eppendorfa tubes and centrifuged (15000 × g) for 6 min. Determination of the bile salts was performed spectrophotometrically in UV range [5, 6].

4. Results and discusion

Tables 1 and 2 present the measurements of cholic and deoxycholic acids adsorption by non-degradated chitosan and chitosan degradated with 5, 10, 15, 20 and 30 kGy.

The analysis of the effect of radiation degradation indicates that a decrease of mean molecular weight of chitosan increases its bile salts binding capacity. In case of cholic acid, chitosan with deacetylation degree of 85 was found to bind the biggest volume of cholic acid and its value decreases with the increase in the deacetylation degree.

On the other hand, in case of deoxycholic acid, a relationship was observed between the dose of degradating irradiation and the amount of adsorbed bile acid. Increase in the dose of degenerating irradiation resulted in increase of the adsorption properties in comparison to sample not submitted to degradation.

The obtained results prove that bile acids salts are adsorbed by chitosans at investigated pH ranges and the binding capability depends both, on environmental pH and on the kinds of applied chitosan. Mean adsorption of bile salts on chitosan ranged from 60 to 91% depending on environmental pH. The highest adsorption rate was observed above pH 7.

5. Conclusion

Chitosan degradation increases its capacity of binding bile salts. Molecular mass of the applied chitosan affects its bile salts binding capability. Bile salts binding rate depends on the degree of chitosan deacetylation. 1 g of chitosan is able to bind up to 3 g of bile acid, what may have an effect the amount of bile acids necessary to develop digestive surface for the digestive enzymes, and in particular for lipase.

Table 1. *Adsorption of cholic acid salts; Initial concentration 100 mg/4 cm³.*

Kind of chitosan	Irradiation dose used for degradation, KGy	Equilibrium concentration of cholic acid salt solution, mg / 4 cm³	Concentration of adsorbed cholic acid salt. mg / 4 cm³	% of adsorption
Chitosan (92% deacetylation)	0	29.479	70.521	70.52
	5	28.631	71.369	71.37
	10	27.742	72.258	72.26
	15	25.346	74.654	74.65
	20	22.368	77.632	77.63
	30	25.744	74.256	74.26
Chitosan (95% deacetylation)	0	38.548	61.452	61.45
	5	36.748	63.252	63.25
	10	33.675	66.325	66.32
	15	31.747	68.253	68.25
	20	30.855	69.145	69.14
	30	31.877	68.123	68.12
Chitosan (85% deacetylation)	0	10.744	89.256	89.26
	5	10.438	89.562	89.56
	10	10.548	89.452	89.45
	15	9.877	90.123	90.12
	20	8.747	91.253	91.25
	30	9.380	90.620	90.62

Standard deviation S - from 0.48 mg/4 cm³ to 1.12 mg/4 cm³ Relativity index Wz - 1.28 to 3.86.

Table 2. *Deoxycholic acid salts adsorption; Initial concentration 100 mg/4 cm³.*

Kind of chitosan	Irradiation dose used for degradation, KGy	Equilibrium concentration of deoxycholic acid salt solution, mg / 4 cm³	Concentration of adsorbed deoxycholic acid salt, mg / 4 cm³	% of adsorption
Chitosan (92% deacetylation)	0	30.479	69.521	69.52
	5	29.676	70.324	70.32
	10	28.437	71.563	71.56
	15	27.354	72.646	72.65
	20	24.367	75.633	75.63
	30	26.855	73.145	73.14
Chitosan (95 % deacetylation)	0	36.644	63.356	63.36
	5	34.875	65.125	65.12
	10	33.675	66.325	66.32
	15	32.744	67.256	67.26
	20	28.731	71.269	71.27
	30	30.877	69.123	69.12
Chitosan (85 % deacetylation)	0	9.745	90.255	90.25
	5	14.464	85.536	85.54
	10	12.744	87.256	87.26
	15	9.877	90.123	90.12
	20	9.873	90.127	90.13
	30	10.855	89.145	89.14

Standard deviation S - from 0.54 mg/4 cm³ to 1.29 mg/4 cm³ Relativity index Wz - 1.37 to 4,21

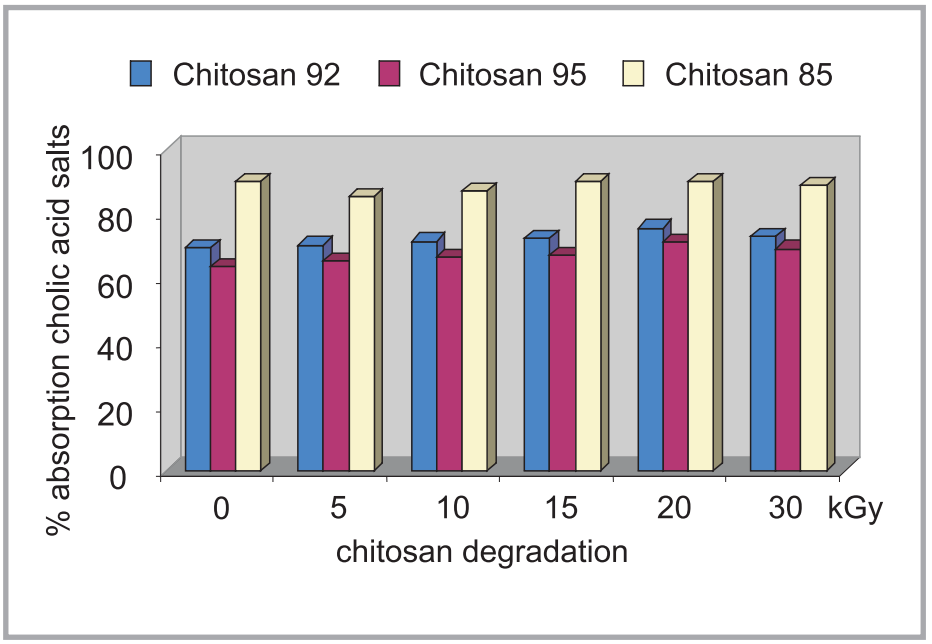

Figure 1. *Cholic acid salts binding in relation to chitosan degradation.*

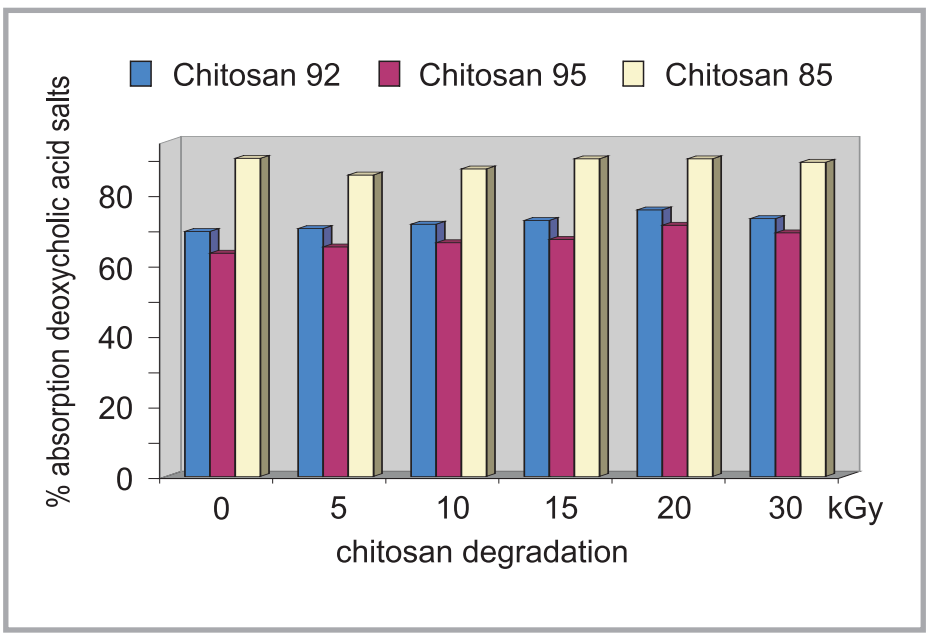

Figure 2. *Deoxycholic acids salts binding in relation to chitosan degradation.*

6. References

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