

## Sodium Cholate Sorption on *N*-Octadecylpectinamide in Comparison with Cholestyramine

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### Abstract

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*N*-Octadecylpectinamide is hydrophobically modified HM citrus pectin. Previously, it had been prepared by heterogeneous amino-de-alkoxylation of initial pectin with *n*-octadecylamine in dimethylsulphoxide and characterised as potential hydrophobic sorbent and cholesterol lowering agent. The sorption properties of *N*-octadecylpectinamide were analysed in comparison with cholestyramine, an effective bile acid sequestrant. Sorption experiments were carried out using sodium cholate as a model bile acid. Cholate concentration was estimated by enzymatic spectroscopic method. Sorption kinetics curves and sorption isotherms of both sorbents were constructed and analysed.

**Keywords:** *N*-octadecylpectinamide; cholestyramine; sodium cholate; sorption kinetics; sorption isotherm

Bile acids are amphiphilic molecules that participate in the digestion of fats by the formation of micelles and micellar aggregates (MUKHOPADHYAY & MAITRA 2004). They are synthesised in the liver by cholesterol oxidation and, therefore, play a key role in cholesterol metabolism. The sequestering of bile acids in the gastrointestinal tract by polymeric sorbents is an effective tool in reducing the total and low-density lipoprotein (LDL) serum cholesterol levels, hence decreasing the risk of cardiovascular diseases.

Cholestyramine (Figure 1a) is a known bile acid sequestrant and antilipemic agent (LÉONARD *et al.* 1993; ZHU *et al.* 2000). This is a strongly basic anion-exchange synthetic resin containing cationic trimethylbenzylammonium groups attached to a long styrene-divinylbenzene copolymer backbone.

The administration of cholestyramine reduces cholesterol absorption by sequestering bile acid conjugates and decreasing the micellarisation of exogenous cholesterol (HASSAN & RAMPONE 1979; MCNAMARA *et al.* 1980). Owing to its interrupting the enterohepatic circulation of bile acids, cholestyramine increases their faecal excretion and triggers a compensatory increase in the cholesterol oxidation in the liver. However, this polymeric resin also binds anionic drugs, vitamins, and salts (JOHANSSON *et al.* 1978; HARMON & SEIFERT 1991). The competition for cholestyramine binding sites in the digestive tract decreases the binding capacity for bile acids, so large doses are required for the effective sorption (STEDRONSKY 1994). In some patients, the large doses of cholestyramine required to produce the desired cholesterol level

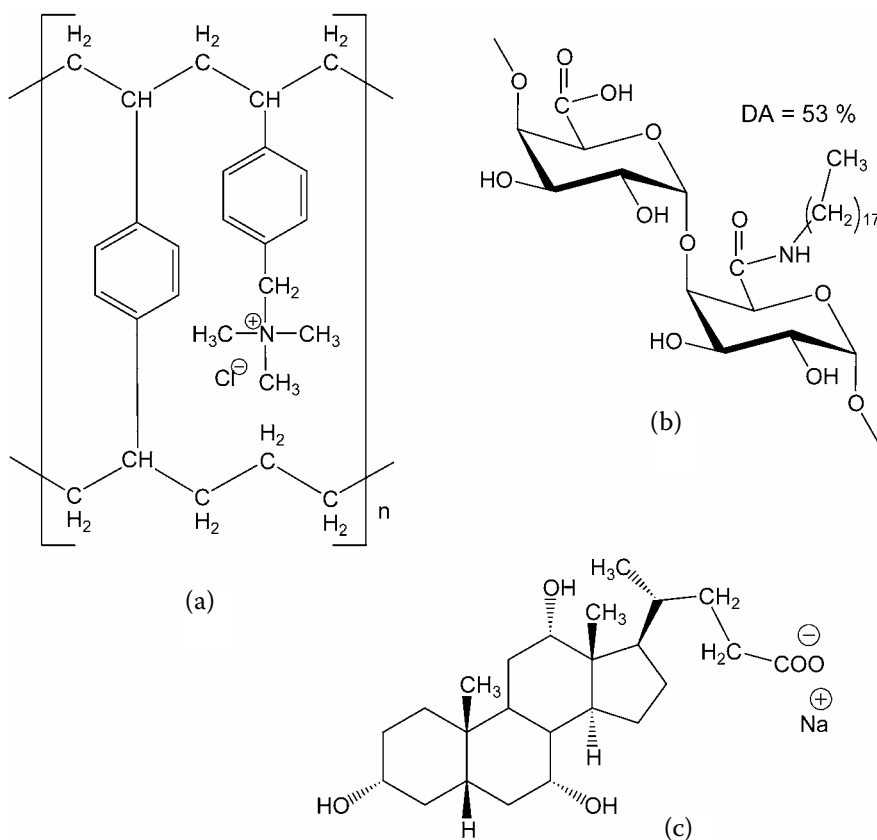


Figure 1. Structure of cholestyramine (a), *N*-octadecylpectinamide (b), and sodium cholate (c)

lowering can cause serious side effects. Therefore, there is a continuing need for new sorbents able to bind bile acids more effectively.

Another way of cholesterol level lowering is the consumption of dietary fibres that can remove bile acids from the digestive tract (SPILLER 1994). The dietary fibres consist mainly of plant cell wall polysaccharides, which are not hydrolysed by the enzymes of the small intestine. Some water soluble polysaccharides such as Arabic and guar gums, agars, pectins, or oat  $\beta$ -glucans are able to make viscous solutions and, therefore, increase the viscosity of the digestive contents. These polysaccharides are presumed to be cholesterol level lowering agents (KELLEY & TSAI 1978; BRAATEN *et al.* 1994; GONZALEZ *et al.* 1998; MORICEAU *et al.* 2000). Partially, pectins, a polysaccharide component of the dietary fibres, have shown some hypocholesterolemic activity dependent on their structural properties (JUDD & TRUSWELL 1985; YAMAGUCHI *et al.* 1995). The combination of cholestyramine with well-tolerated natural pectin has been shown to be an effective agent in the treatment of patients with familial hypercholesterolemia (SCHWANDT *et al.* 1982). Contrary to these, some other authors have reported the absence of

significant cholesterol-lowering effects of pectin as well (VONDERHEYDE *et al.* 1993; TRAUTWEIN *et al.* 1998; YAMADA *et al.* 2003).

The chemical modification of pectin (amidation, trans-esterification) is relatively easy and it modifies in a significant way physicochemical properties of pectin. The introduction of non-polar groups increases the hydrophobic character of pectin macromolecules. It has been reported that alkyl esters of pectin are able to adsorb bile acids, fat, and cholesterol (MÜLLNER *et al.* 1993; KLAVONS & BENNET 1995). *N*-Alkylpectinamides have some advantages in comparison with other alkylated derivatives of pectin because the amide bond is sufficiently resistant to hydrolysis by acids or alkali. The yields of *N*-alkylpectinamides prepared by the reaction of pectin with aliphatic non-branched amines are relatively high (SINITSYA *et al.* 2000; SYNITSYA *et al.* 2003; SIHELNÍKOVÁ *et al.* 2004).

Among the alkylamidated pectin derivatives obtained, water insoluble *N*-octadecylpectinamide (Figure 1b) is interesting due to its marked amphiphilic properties (SYNITSYA *et al.* 2004). This modified pectin is able to absorb selectively non-polar molecules (alkanes, alkylbenzenes and

polyaromates) as well as polar molecules with non-polar parts (*n*-alkyl and branched alcohols). Due to its long alkyl groups, *N*-octadecylpectinamide is able to bind to non-polar or amphiphilic biomolecules such as bile acids, fats, or cholesterol better than natural highly methylated (HM) pectin or other food polysaccharides. Feeding experiments on rats have concluded (MAROUNEK *et al.* 2007) that 30% and 53% substituted *N*-octadecylpectinamides significantly increased faecal content of cholesterol and decreased hepatic concentrations of cholesterol and fat. The effects of a more alkylamidated pectin were more pronounced. Highly substituted (53%) pectin derivative significantly decreased total serum cholesterol, while *N*-octadecylpectinamide of a lower degree of amidation (30%) increased serum HDL cholesterol at the expense of other cholesterol fractions. In contrast, the effects of initial citrus pectin on cholesterol homeostasis were absent or marginal. Both pectin derivatives were water insoluble and, therefore, were not able to increase the viscosity of the digestive contents. Therefore, cholesterol-lowering action of *N*-octadecylpectinamides can be based on the direct sorption of bile acids, exogenous cholesterol and fat.

In this work, we describe sorption experiments with *N*-octadecylpectinamide and cholestyramine as sorbents, and sodium cholate, a model bile acid anion, as a sorbate.

## MATERIAL AND METHODS

**Materials.** Highly substituted *N*-octadecylpectinamide (DA = 53%) was prepared by heterogeneous amino-de-alkoxylation (aminolysis) of HM citrus pectin (type XSS, Danisco, Denmark; DM = 68%) according to SYNITSYA *et al.* (2003). Sodium cholate and cholestyramine resin were purchased from Sigma, Germany. Sodium phosphate buffer (pH 7) was prepared using dibasic and monobasic sodium phosphates (puriss., Riedel-deHaën, Germany). Bile acid enzymatic set (Trinity Biotech, Ireland) was used for the determination of sodium cholate contents.

**Sorption experiments.** The sodium cholate sorption experiments were carried out in sodium phosphate buffer (pH 7). The dry sorbent (*m*, 0.5 g), i.e. *N*-octadecylpectinamide or cholestyramine, was suspended in the buffer solution (*V*, 50 ml) with a given initial concentration of sodium cholate  $C_0$ , covering the range of 0.1–0.5 mmol/l. The mix-

ture was rigorously stirred for 2–120 min at 37°C. Then the suspension was filtered and the filtrate (*x* ml aliquot) was analysed by UV-Vis spectrophotometry (530 nm) using bile acid enzymatic set (Trinity Biotech, Ireland). The absorption measurement was made by double beam UV4 UV-Vis spectrophotometer (Unicam, Great Britain) in 1 cm cuvette against deionised water. All these procedures were repeated 6 times for every cholate concentration  $C_0$  and for every time interval *t*. The absorption values obtained were used for the calculation of the residual cholate concentration in solutions after sorption  $C_t$  (mmol/l) and the amount of cholate  $q_t$  (mmol/g) sorbed at every time interval *t* (min):

$$q_t = (C_0 - C_t) \frac{V}{m} \quad (1)$$

Vision 32 (Unicam, Great Britain) and ORIGIN 6.0 (Microcal Origin, USA) software were used in the data processing and preparation of the graphs.

## RESULTS AND DISCUSSION

**Sorption kinetics.** The sorption kinetics curves obtained for the binding of cholate by *N*-octadecylpectinamide and cholestyramine are shown in Figure 2. It is evident that amidated pectin absorbs cholate slower than cholestyramine, especially during the first 15 minutes. However, after 75 min of the sorption process, *N*-octadecylpectinamide

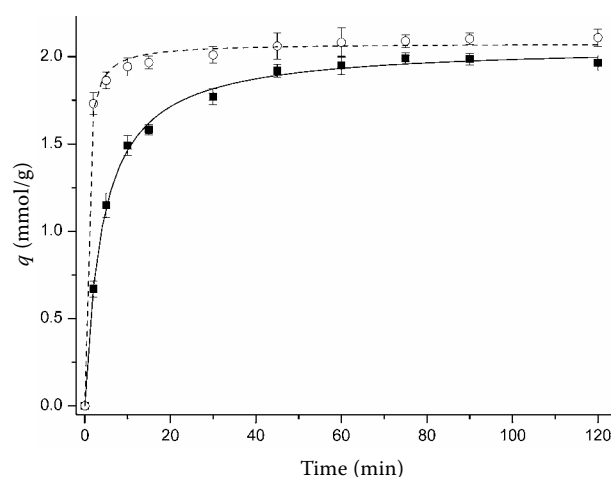


Figure 2. Kinetic curves obtained for the cholate sorption from phosphate buffer solutions (pH 7) at 37°C by *N*-octadecylpectinamide (—■—) and by cholestyramine (--○--). Lines represent fits to equation (4) (pseudo-second-order rate model)

demonstrated the level of the absorbed cholate ( $\sim 2$  mg/g) similar to that of cholestyramine.

The process of cholate sorption was modelled using a pseudo-second-order rate equation of chemical kinetics (VOET & VOET 1990):

$$\frac{dq_t}{dt} = k(q_e - q_t)^2 \quad (2)$$

where:

$k$  – rate constant of sorption (g/mmol/min)

$q_e$  – amount of cholate sorbed at equilibrium (mmol/g)

Taking into account the initial sorption rate  $v_0$  (mmol/g/min):

$$v_0 = k q_e^2 \quad (3)$$

the kinetic equation (2) can be rearranged to obtain:

$$q_t = \frac{v_0 q_e t}{q_e + v_0 t} \quad (4)$$

The values of  $q_e$  and  $v_0$  were determined experimentally by plotting  $t/q_t$  versus  $t$ :

$$\frac{t}{q_t} = \frac{1}{v_0} + \frac{1}{q_e} t \quad (5)$$

The kinetic parameters, obtained for cholate sorption on *N*-octadecylpectinamide and cholestyramine using linearisation of a pseudo-second-order rate model are shown in Table 1. The initial sorption rate  $v_0$  was about four times higher with cholestyramine (2.11 mmol/g/min) than with *N*-octadecylpectinamide (0.53 mmol/g/min), while the equilibrium amounts of the sorbed cholate  $q_e$  were quite similar with both these sorbents (2.05 mmol/g with *N*-octadecylpectinamide and 2.12 mmol/g with cholestyramine). The equilibrium time  $t$  was deduced from the kinetic experiments and fixed at 75 min with both sorbents. The experimental  $q_{75}$  values obtained with *N*-octadecylpectinamide (1.99 mmol/g) and cholestyramine (2.09 mmol/g) are close to the corresponding parameters  $q_e$  derived from the second-order kinetic model (Table 1).

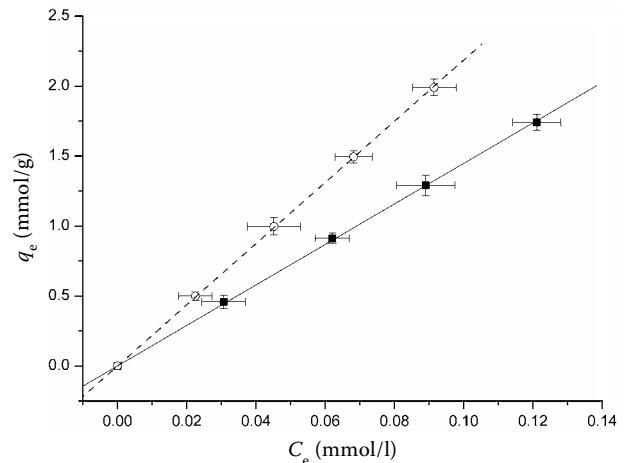


Figure 3. Sorption isotherms obtained for the equilibrium cholate sorption from phosphate buffer solutions (pH 7) at 37°C by *N*-octadecylpectinamide (—■—) and by cholestyramine (--○--). Lines represent fits to equation (6) (linear sorption model)

**Sorption isotherms.** Sorption isotherms plot the sorption capacity ( $q_e$ ) versus the residual concentration of the metal in the solution at equilibrium ( $C_e$ ) (GUIBAL 2004). This equilibrium distribution of cholate between the solid and the liquid phase was obtained by varying the initial cholate concentration ( $C_0$ ) from 0.1 to 0.5 mmol/l. Figure 3 represents the equilibrium sorption data for *N*-octadecylpectinamide and cholestyramine.

Three models were applied to describe the cholate sorption. These are the linear (Equation 6), the Freundlich (Equation 7), and the Langmuir (equation 8) isotherms:

$$q_e = K_d C_e \quad (6)$$

$$q_e = K_F C_e^{1/n} \quad (7)$$

$$q_e = \frac{q_m b C_e}{1 + b C_e} \quad (8)$$

$K_d$  (l/g) is defined as the distribution coefficient in the linear isotherm,  $K_F$  (mmol $^{1-1/n}$  l $^{1/n}$ /g) and  $1/n$  (dimensionless) are related to the sorption capacity and the

Table 1. Kinetic parameters for cholate sorption on *N*-octadecylpectinamide and cholestyramine

Sorbent	$v_0$ (mmol/g/min)	$q_e$ (mmol/g)	$q_{75}$ (mmol/g)
<i>N</i> -Octadecylpectinamide	$0.53 \pm 0.07$	$2.05 \pm 0.39$	$1.99 \pm 0.03$
Cholestyramine	$2.11 \pm 0.02$	$2.12 \pm 0.01$	$2.09 \pm 0.04$

Table 2. Coefficients of the sorption isotherms obtained for *N*-octadecylpectinamide and cholestyramine

Sorbent	Linear $K_d$ (l/g)	Freundlich		Langmuir	
		$K_F$ (mmol <sup>1-1/n</sup> l <sup>1/n</sup> /g)	1/ $n$	$q_m$ (mmol/g)	$b$ (l/mmol)
<i>N</i> -Octadecylpectinamide	14.47 ± 0.07	13.50 ± 0.09	0.97 ± 0.003	30.8 ± 2.6	0.493 ± 0.0011
Cholestyramine	21.86 ± 0.06	20.89 ± 0.20	0.98 ± 0.004	90.1 ± 11.6	0.247 ± 0.0003

energy distribution of the sorption sites, respectively, in the Freundlich isotherm

It is evident that the linear isotherm is a special case of the Freundlich isotherm where the exponent  $n$  is equal to 1. In the Langmuir isotherm,  $b$  (l/mmol) is the affinity of the sorbent for cholate and  $q_m$  (mmol/g) is the maximum sorption capacity at saturation of the monolayer.

The values of  $q_m$  and  $b$  were determined by plotting  $1/q_e$  versus  $1/C_e$ :

$$\frac{1}{q_e} = \frac{1}{q_m b} \times \frac{1}{C_e} + \frac{1}{q_m} \quad (9)$$

For the cholate sorption evaluation, the parameters obtained with each model are shown in Table 2. In the range of concentrations studied, the sorption is depicted by a linear isotherm. Although the Freundlich model is also suitable and presents a better correlation, the linear model was preferred due to its simplicity. With both sorbents, the value of  $1/n$  is very close to the unity, so the linear isotherm seems to be an acceptable adjustment. A distribution coefficient  $K_d$  obtained with *N*-octadecylpectinamide was 14.47 l/g, while cholestyramine showed about 1.5 times higher value (21.86 l/g). Similar relationship was found with the sorption capacities  $K_F$  of the Freundlich model. The Langmuir isotherm cannot be considered a suitable model to fit the experimental points since they are far away from the saturation region. This is reflected in the relatively large errors associated with the Langmuir parameter  $q_m$ .

Amphiphilic nature of *N*-octadecylpectinamide, i.e. polar polysaccharide backbone and non-polar side substituents, is an important prerequisite for its sorption properties (SYNYTSYA *et al.* 2004). This sorbent demonstrates a high binding selectivity to non-polar and alkyl containing polar compounds, while small polar molecules are also retained by it. Cholate sorption on *N*-octadecylpectinamide

needs no ion exchange, as it was reported for cholestyramine (LÉONARD *et al.* 1993), because the polysaccharide chains contain anionic groups that cannot support the sorption of cholate anion, but may inhibit it due to the electrostatic repulsion. On the other hand, *in vivo* experiments on substituted and non-substituted pectins confirm that *N*-octadecylpectinamide, but not initial HM citrus pectin, is able to remove alimental cholesterol and fats in faeces owing to direct sorption (MAROUNEK *et al.* 2007). Thus, we suggest that the mechanism of *in vitro* sorption of sodium cholate by this alkylamidated pectin can be based on hydrophobic interactions with the pendant *n*-octadecyl groups rather than on polar interactions with the polysaccharide backbone.

## CONCLUSION

The sorption experiments of the present work confirmed that *N*-octadecylpectinamide can act as an effective sorbent for sodium cholate, being a good alternative to conventional sorbent cholestyramine, which is widely used in medicinal applications. In spite of the somewhat slower sorption kinetics and a lower sorption capacity, the alkylamidated pectin has some advantages and may therefore compete with synthetic sorbents: (a) it is a relatively cheap bioavailable material based on chemically modified natural polysaccharide; (b) it has no cationic groups and does not bind organic anions like cholestyramine; (c) due to long alkyl groups, it can also bind uncharged lipids like cholesterol and fats; (d) it can also possess positive side effects on the digestion, such as the production of volatile fatty acids in colon or binding of heavy metal cations by free carboxylic groups. The use of a mixture of these two bile acid sorbents could be interesting for the treatment of hypercholesterolemia. The mixture should allow a reduction of the amount of cholestyramine required and hence of the side effects associated with its use.

## References

- BRAATEN J.T., WOOD P.J., SCOTT F.W., WOLYNETZ M.S., LOWE M.K., BRADLEY-WHITE P., COLLINS M.W. (1994): Oat  $\beta$ -glucan reduces blood cholesterol concentration in hypercholesterolemic subjects. *European Journal of Clinical Nutrition*, **48**: 465–474.
- GONZALEZ M., RIVAS C., CARIDE B., LAMAS M.A., TABOADA M.C. (1998): Effects of orange and apple pectin on cholesterol concentration in serum, liver and faeces. *Journal of Physiology and Biochemistry*, **54**: 99–104.
- GUIBAL E. (2004): Interactions of metal ions with chitosan-based sorbents: a review. *Separation and Purification Technology*, **38**: 43–74.
- HARMON S.M., SEIFERT C.F. (1991): Levothyroxine-cholestyramine interaction reemphasized (Letter). *Annals of Internal Medicine*, **115**: 658–659.
- HASSAN A.S., RAMPONE A.J. (1979): Intestinal absorption and lymphatic transport of cholesterol and *p*-sitostanol in the rat. *Lipid Research*, **20**: 646–653.
- JOHANSSON C., ADAMSSON U., STIERNER U., LINDSTEN T. (1978): Interaction by cholestyramine on the uptake of hydrocortisone in the gastrointestinal tract. *Acta Medica Scandinavica*, **204**: 509–512.
- JUDD P.A., TRUSWELL A.S. (1985): The hypocholesterolaemic effects of pectins in rats. *British Journal of Nutrition*, **53**: 409–425.
- KELLEY J.J., TSAI A.C. (1978): Effect of pectin, gum arabic and agar on cholesterol absorption, synthesis, and turnover in rats. *Journal of Nutrition*, **108**: 630–639.
- KLAVONS J.A., BENNET R.D. (1995): Preparation of alkyl esters of pectin and pectic acid. *Journal of Food Science*, **60**: 513–515.
- LÉONARD D.L., CLAS S.-D., BROWN G.R. (1993): Polymeric sorbents for bile acids. 4. Treatment of cholestyramine as a Donnan system. *Reactive Polymers*, **20**: 131–140.
- MAROUNEK M., VOLEK Z., SYNITSYA A., ČOPÍKOVÁ J. (2007): Effect of pectin and amidated pectin on cholesterol homeostasis and caecal metabolism in rats fed a high-cholesterol diet. *British Journal of Nutrition*, submitted.
- MCMNAMARA D.J., DAVIDSON N.O., SAMUEL P., AHRENS E.H., Jr. (1980): Cholesterol absorption in man: effect of administration of clofibrate and/or cholestyramine. *Journal of Lipid Research*, **21**: 1058–1064.
- MORICEAU S., BESSON C., LEVRAT M.A., MOUNDRAS C., REMESY C., MORAND C., DEMIGNE C. (2000): Cholesterol-lowering effects of guar gum: changes in bile acid pools and intestinal reabsorption. *Lipids*, **35**: 437–444.
- MUKHOPADHYAY S., MAITRA U. (2004): Chemistry and biology of bile acids. *Current Science*, **87**: 1666–1683.
- MÜLLNER S., WEISNER M., AHLERS M. (1993): DE patent No. 41 36 322 A1, 6 May 1993.
- SCHWANDT P., RICHTER W.O., WEISWEILER P., NEUREUTHER G. (1982): Cholestyramine plus pectin in treatment of patients with familial hypercholesterolemia. *Atherosclerosis*, **44**: 379–383.
- SIHELNÍKOVÁ L., SYNITSYA A., ČOPÍKOVÁ J. (2004): Amino-dealkoxylation of HM citrus pectin with *n*-alkylamines: a kinetic study. *Czech Journal of Food Sciences*, **22**: 235–237.
- SINITSYA A., ČOPÍKOVÁ J., PRUTYANOV V., SKOBLYA S., MACHOVIČ V. (2000): Amidation of highly methoxylated citrus pectin with primary amines. *Carbohydrate Polymers*, **42**: 359–368.
- SPILLER R.C. (1994): Pharmacology of dietary fibre. *Pharmacology & Therapeutics*, **62**: 407–427.
- STEDRONSKY E.R. (1994): Interaction of bile acids and cholesterol with non-systemic agents having hypocholesterolemic properties. *Biochimica et Biophysica Acta*, **1210**: 255–287.
- SYNITSYA A., ČOPÍKOVÁ J., MAROUNEK M., MLČOCHOVÁ P., BLAFKOVÁ P., TKADLECOVÁ M., HAVLÍČEK J. (2003): Preparation of *N*-alkylamides of highly methylated (HM) citrus pectin. *Czech Journal of Food Sciences*, **21**: 162–166.
- SYNITSYA A., ČOPÍKOVÁ J., MAROUNEK M., MLČOCHOVÁ P., SIHELNÍKOVÁ L., SKOBLYA S., HAVLÁTOVÁ H., MATĚJKA P., MARYŠKA M., MACHOVIČ V. (2004): *N*-octadecylpectinamide, a hydrophobic sorbent based on modification of highly methoxylated citrus pectin. *Carbohydrate Polymers*, **56**: 169–179.
- TRAUTWEIN E.A., RIECKHOFF D., KUNATH-RAU A., ERBERSDOBLER H.F. (1998): Psyllium, not pectin or guar gum, alters lipoprotein and biliary bile acid composition and faecal sterol excretion in the hamster. *Lipids*, **33**: 573–582.
- VOET D., VOET J.G. (1990): *Biochemistry*. Wiley, New York: 330–332.
- VONDERHEYDE R., VONDERHEYDE M.E.D., WOSIACKI G. (1993): Relationship between dietary fibre, serum lipids and faecal excretion of biliary acids – An experimental study with rats. *Arquivos de Biologia e Tecnologia*, **36**: 207–218.
- YAMADA K., TOKUNAGA Y., IKEDA A., OHKURA K., KAKU-OHKURA S., MAMIYA S., LIM B.O., TACHIBANA H. (2003): Effect of dietary fibre on the lipid metabolism and immune function of aged Sprague-Dawley rats. *Bioscience Biotechnology and Biochemistry*, **67**: 429–433.
- YAMAGUCHI F., UCHIDA S., WATABE S., KOJIMA H., SHIMIZU N., HATANAKA C. (1995): Relationship between molecular weights of pectin and hypocholesterolemic

effects in rats. *Bioscience Biotechnology and Biochemistry*, **59**: 2130–2131.

ZHU X.X., BRIZARD F., PICHÉ J., YIM C.T., BROWN G.R. (2000): Bile salt anion sorption by polymeric resins: comparison of a functionalized polyacrylamide resin

with cholestyramine. *Journal of Colloid and Interface Science*, **232**: 282–288.

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