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Copper binding capacity and physicochemical properties of pectins with different degrees of esterification. Approach to standardization of pectin preparations

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SUMMARY

Metal binding activity of the pectin samples with different physicochemical properties was studied. It was found that *in vitro* copper binding capacity of pectins is depending on the following factors: degree of esterification, content of non-methylated anhydrogalacturonic acid, and pH of solution. There was found that the maximum copper uptake capacity increases correspondingly to reduction of the degree of esterification of pectin, rise of the non-methylated anhydrogalacturnic acid content and the solution pH. It is proposed to use for standardization of pectin samples such parameters as the degree of esterification, content of anhydrogalacturonic acid, and intrinsic viscosity.

Key words: Pectin; Heavy metals; Sorption; Polysaccharides; Standardization

INTRODUCTION

Pectins are the ionic plant polysaccharides functioning as hydrating agents and cementing substances for cellulose network (Thakur *et al.*, 1997). They are widely used in the food industry because of their gelling and thickening properties (May, 1990). The main structural features of pectins consists of the linear chains containing more than 100 of (1→4)-linked α-D-galacturonic acid units (Thibault *et al.*, 1993) presenting non-branched blocks of molecule. These "smooth" homogalacturonic regions are interrupted with "hairy" rhamnogalacturonic parts, in which galacturonic acids are interspersed with (1→2)-linked α-L-rhamnopyranosyl residues carrying neutral side sugar chains (Schols and Voragen, 1996). The long linear chains of homogalacturonan are partially esterified with methanol. Natural pectins are mainly highly esterified while pectins with lower degree of esterification can be prepared. The linear regular structure is interrupted with the presence of neutral sugar side chains (Ridley *et al.*, 2001).

A number of pharmacological effects of pectins and pectin-containing products have been described in studies with laboratory animals. These effects include the decrease of serum cholesterol level (Gonzalez *et al.*, 1998; Vergara-Jimenez *et al.*, 1998), the lowering of sphingomielin concentration in very low density lipoproteins (Bladergroen *et al.*, 1999), an enhance of fecal bile acid excretion, an increase of hepatic synthesis of bile acids, depletion of cholesterol in liver (Garcia-Diez *et al.*, 1996), suppression of colon carcinogenesis (Heitman *et al.*,

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1992) and interaction with metal ions (Dongowski *et al.*, 1997).

In clinical studies pectin had been shown to exert beneficial effects on human health. Dietary supplementation of pectin have shown to induce decreased level of blood cholesterol in subjects with mild and moderate hypercholesterolemia (Groudeva *et al.*, 1996; Knopp *et al.*, 1999), and reduced serum glucose concentration in patients with diabetes mellitus (Levitt *et al.*, 1980). It exerted therapeutic effects in children with persistent diarrhea and had a significant influence resulted in slowed rate of catch-up body weight gain (Rabbani *et al.*, 2001).

Exertion by pectins of pharmacological effects similar to those of medicinal drugs makes the premises for elaboration of drugs on the base of pectins. Because pectins as a majority of polysaccharides are heterogeneous compounds according to their structure, molecular weight, and physicochemical properties, the important pharmaceutical problem is standardization of the pectin samples with different physicochemical properties and intensity of pharmacological effects (Khotimchenko *et al.*, 2001).

This study was designed to describe physicochemical properties of pectin samples with different degrees of esterification and their capacity to bind copper ions under *in vitro* conditions. We offer to use as the main parameters for standardization of the pectin samples such physical and chemical properties as molecular weight, galacturonan content, intrinsic viscosity, and methyl ester group content, i.e. degree of esterification.

MATERIALS AND METHODS

Materials

High-esterified citrus pectin without additives was obtained from Copenhagen Pectin A/S, Lille Skensved, Denmark. The degree of esterification of this preparation was 60.2%. The pectin preparation contained no acetyl or amide groups. All the reagents used were of analytical-reagent grade. Double distilled water used to prepare all the solutions.

Preparation of pectin samples with different degree of esterification

Preparation of the pectin samples with the degree of esterification less than 60.2% was executed using the method of alkali de-esterification of high esterified pectin in a water ethanol solution. Initially the raw high esterified pectin was washed with 50% ethanol solution for purification. In this process 200 g of pectin was suspended in 2 l of the ethanol solution for 30 min, then pectin was separated by filtration, rinsed on filter with 1.5 l of the 50% ethanol solution and 1 liter of the 95% ethanol solution, consecutively, and then dried at 70°C. Pectin obtained was pounded and fractioned according to the particle size. In the study was used a fine disperse fraction sifted through a sieve with a mesh size 74 μm. It was made purposefully to minimize the possibility of obtaining the heterogeneous samples regarding degree of esterification due to a different rate of de-esterification process in pectin molecule outside and inside granules caused by different velocity of diffusion of hydrolyzing agent.

Process of alkali de-esterification of pectin included initial neutralization of free carboxyl groups of anhydrogalacturonic acid and then, during increase of pH of the media higher than 8.5, proper pectin de-esterification. Amount of alkali, necessary for neutralization of carboxyl groups, was calculated as the follows: 1 g of pectin was suspended in 10 ml of 50% ethanol solution and in the presence of Hintone indicator titrated with 1 M NaOH solution in 50% ethanol until an indicator color changed. According to the titration results the amount of alkali necessary for pectin neutralization was determined using the following equation:

$$V_1 = m \cdot V_0$$

where V_1 is the volume of 1 M NaOH solution necessary for pectin neutralization (ml), m is the

weight of a pectin sample (g), V_0 is the volume of 1 M NaOH solution used during titration of 1 g of pectin (ml).

Amount of alkali necessary for achievement of a set degree of esterification of pectin was calculated using the following equation:

$$V_2 = \frac{(m \cdot A)}{176 \cdot 100} \cdot \frac{DE_{in} - DE_{fin}}{100} \cdot 1000$$

where V_2 is the volume of 1 M NaOH solution necessary for de-esterification of pectin (ml), m is the weight of a pectin sample (g), A is the anhydrogalacturonic acid content in pectin (%), 176 is the molar weight of anhydrogalacturonic acid, DE_{in} is the initial degree of esterification of a pectin sample (%), DE_{fin} is the set degree of esterification of a pectin sample (%).

The total volume of alkali necessary for the whole process of pectin de-esterification was found by summarizing of V_1 and V_2 volumes determined earlier.

The proper de-esterification process was carried out as follows. 20 g of pectin powder was suspended in 200 ml of 50% ethanol solution intensively stirring and then gradually 1 M NaOH solution in 50% ethanol was added. For the visual control of the process, the indicator phenolphthalein was added into reactive mixture. The color of phenolphthalein changes at pH 8.2 - 10.0. The next portion of alkali was added into the reactive volume only after discoloration of indicator. When 95% of calculated amount of alkali was added into reactive volume, the sampling was carried out for determination of the degree of esterification. According to results of this analysis the quantity of alkali was precised. When the preliminary set degree of esterification was achieved the reaction mixture was acidified with 1 M HCl solution in 50% ethanol achieving pH of the media 5 - 6 under intensive stirring. Obtained pectin preparation was separated from water-ethanol solution with filtration and consecutively rinsed with 300 ml of 50% ethanol solution and 150 ml of 95% ethanol. Rinsed pectin was dried at 70°C.

Total anhydrogalacturonic acid content, non-methylated (de-esterified) anhydrogalacturonic acid content, degree of esterification, and intrinsic viscosity were estimated in the pectin samples obtained.

Pectin analysis

The total anhydrogalacturonic acid content and non-methylated anhydrogalacturonic acid content of the pectin preparations was determined colorimetrically by the *m*-hydroxydiphenyl method (Blumenkrantz and Asboe-Haunsen, 1973). The degree of esterification was characterized using titrimetric analysis (Afanasyev *et al.*, 1984). Intrinsic viscosity of pectin was determined in 0.05 M NaCl/0.005 M Na-oxalate at 25°C and pH 6 using an Ubbelohde viscosimeter. The intrinsic viscosity is related empirically to the molecular weight by the Mark-Howink relation (Kravtchenko and Pilnik, 1990).

Estimation of metal binding capacity of pectin preparations

Metal binding capacity of pectins with the different degree of esterification was assessed on the base of interaction with the copper ions. In this study we used an original device consisting of incubating cell with the filtering kapron fiber sieve and gathering bulk equipped with piston. The mesh size of kapron filter was about 100 ìm providing fast passage of experimental solution from one cell into another. During the whole process the pectin powder particles were permanently locating in the incubating cell. 50 g of pectin powder with the particle size varying between 125 - 177 μm was added into the incubating cell. Into the gathering bulk were added from 0.5 to 3 ml of 1 M $CuSO_4$ solution, 1 ml of 1 M buffer solution of the necessary pH, and distilled water up to 5 ml of total volume. To start a binding process incubating cell and gathering bulk was assembled together and the experimental solution was carried into incubating cell using a piston. Incubating cell was tightly closed and continually stirred for a set period of

time. At the end of incubation period the liquid phase was removed with the piston, 2.5 ml of supernatant was collected. The binding experiments were conducted at 25°C under digital control on a combined shaker table. The shaking rate was 50 rpm. After filtration, the concentration of copper ions in supernatant was analyzed by atomic absorption method ("Nippon Jarrel Ash", model AA-855). The amount of bound metal was calculated based on the differences of Cu^{2+} concentration in solution before and after sorption according to the following equation:

$$q = \frac{V(C_i - C_f)}{m}$$

where q is the metal uptake capacity (mg metal/g dry weight (dw) of pectin), V is the volume of solution in the incubating cell (liters), C_i is the initial concentration of metal in solution (mg/l), C_f is the final equilibrium concentration of metal in solution (mg/l), and m is the initial pectin weight (g).

Investigation of dynamic characteristics typical of binding processes between pectin and copper ions was assessed in the following periods of time: 10 s, 20 s, 40 s, 1 min, 2 min, 3 min, 5 min, 10 min, 15 min, and 60 min.

For estimation of relationship between metal binding capacity of pectins and pH of the media, uptake of the copper ions by samples was measured at the range of pH between 2 and 8. The maintaine of the needed pH meanings the buffer solutions such as follows were used: glycin buffers (glycin/HCl) were used to control the pH in the range of 2 - 3; acetate buffers (acetic acid/NàÎÍ) were used for experiments in the 4 - 6 pH range, and tris-buffers (tris/HCl) were used for experiments in the pH 7 - 8 range. The proper buffer capacity of pectin was also taken to consideration for precise achievement of the necessary pH meanings. With this purpose, solution containing 1 g of pectin sample with known degree of esterification was brought to desirable pH meaning in the 2 - 8 range using titration with 1 M solutions of HCl and NaOH. According to the results obtained during titration the necessary volumes of 1 M acid and alkali solutions were calculated and then added into experimental solution at the same time with the buffer solution.

All results were expressed as the mean analysis from three replicate samples.

RESULTS

There were obtained seven samples of pectins by means of alkali de-esterification method. The samples were ranged according to more or less gradual reduction of degree of esterification from 60.2% to 1.2%. Intervals characterizing changes of the degree of esterification in the adjoining samples of pectin were approximately 10%. Physicochemical parameters of the pectin samples obtained are shown in Table 1. It was found that the pectin samples, which were subjected to de-esterification procedure (samples II - VII), are characterized by similar contents of anhydrogalacturonic acid making from 66.6% to 73.7% of a sample mass. Simultaneously with the decrease of the degree of esterification from 60.2 to 1.2% was registered a relative rise of the non-methylated anhydrogalacturonic acid content from 31.6% to 72.8%, i.e. 2.3 times more. Generally intrinsic viscosity of pectin samples

Table 1. Physicochemical parameters of pectin preparations with different degrees of esterification

Parameter	Pectin sample						
	I	II	III	IV	V	VI	VII
Degree of esterification, %	60.2	52.0	40.1	27.4	18.8	9.6	1.2
Total anhydrogalacturonic acid content, %	79.4	72.0	70.4	67.8	66.6	72.5	73.7
Non-methylated anhydrogalacturonic acid content, %	31.6	34.6	42.2	49.2	54.1	65.5	72.8
Intrinsic viscosity, ml/g anhydrogalacturonic acid	915	570	533	452	433	427	408

reduces with the decrease of the degree of esterification. But at the very beginning of the de-esterification process a dramatic reduction of intrinsic viscosity was found. Lowering of the degree of esterification by 8.2% from 60.2% typical of sample I to 52.0% typical of sample II was associated with 37.7% reduction of intrinsic viscosity from 915 ml/g to 570 ml/g. Further course of the de-esterification process was associated with milder reduction of intrinsic viscosity. Lowering of the degree of esterification from 52.0% typical of sample II to 1.2% typical of sample VII was found to contribute to reduction of intrinsic viscosity by 28.4%. There was found 2.4-fold difference between sample VII possessing the lowest degree of esterification (1.2%) and the least intrinsic viscosity and initial high esterified pectin (sample I) regarding intrinsic viscosity. In correspondence with Mark-Howink equation relating intrinsic viscosity and molecular weight of pectin the difference found in the study conforms to 2.8-fold reduction of an average molecular weight. Because molecular weight of initial high esterified pectin was substantially high (more than 200 kDa), the sample with the degree of esterification 1.2% and intrinsic viscosity 408 ml/g may be considered as a high molecular pectin.

In order to determine the contact period required for the binding equilibrium studies, initially experiments estimating sorption kinetics were conducted. It should be noted that sorption kinetics are important physicochemical parameters for evaluation of the basic qualities of a good sorbent. Copper binding capacities of pectin with the degree of esterification 1.2% as a function of time at pH 6.0 and at initial Cu (II) concentration 30 mmol/l is shown in Fig. 1. The dynamic chart showed that accumulation begins with a rapid phase (until about 2 min) followed by a relatively slow phase (until 10 min) and further significantly does not alter. The results also indicate that 50% amount of binding capacity were achieved in approximately 30 sec of interaction process. Therefore, the sorption of Cu (II) increases with agitation

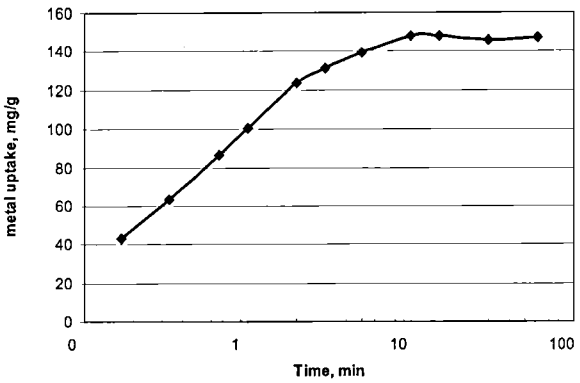

Fig. 1. Copper binding capacity of pectin with the degree of esterification 1.2% at various periods of contact time.

period and attains equilibrium in about 10 min. It shows that the sorption of Cu (II) remained constant indicating the equilibrium is achieved. The rest of the pectin samples differed with the degree of esterification was characterized with similar dynamic parameters, so the results of their interaction with the metal are not illustrated. This provided a guide for the sorption contact period to be used in the following equilibrium experiments, and during our further study the contact time was 30 min.

On the base of quantitative parameters obtained through sorption experiments, the equilibrium sorption isotherms showing the metal uptake of pectin in dependence on the final metal concentration in solution at various pH ranges were calculated and built. Fig. 2 shows the isothermal sorption curves of copper uptake by pectin with degree of esterification 1.2% at various pH ranges. Affinity constant and maximum uptake capacity of pectins to copper ions were determined by equation using the Langmuir sorption model (Volesky, 2003) as follows:

$$q = q_{\max}\frac{C_f}{K + C_f}$$

where q is the metal accumulation (mg/g dw), C_f is the final concentrations of the metal in solution (mg/l), K is a coefficient related to the affinity

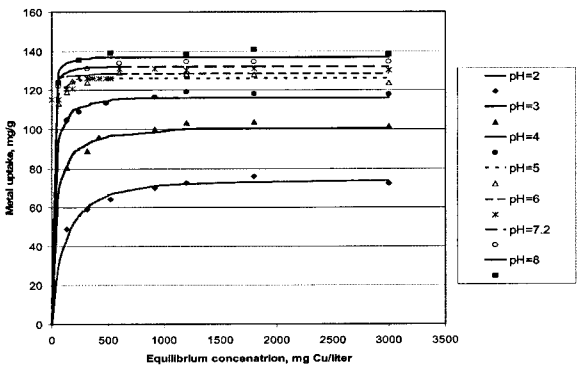

Fig. 2. Copper binding capacity of pectin with the degree of esterification 1.2% at various pH.

between sorbent and sorbate, q_{max} is the maximum sorbate uptake under the given conditions (mg/g).

The following linearized plot of the Langmuir equation was used in this study:

$$\frac{C_f}{q_e} = \frac{C_f}{q_{\max}} + \frac{1}{q_{\max} b}$$

which gives q_{max} and b.

Results for parameters of pectin with degree of esterification 1.2% are shown in Table 2. Maximum uptake capacity at pH 8 was 138.6 mg/g dw and it is approximately 3.9, 8.0, 8.2, 17.5, 34.6 and 74.8% higher than that of pHs 7.2, 6.0, 5.0, 4.0, 3.0, and 2.0, respectively. Also, affinity coefficient at pH 8 was 0.2522 l/mg and it is approximately 1.1, 1.2, 1.5, 3.6, 8.2, and 25 times higher than that of pH 7.2, 6.0, 5.0, 4.0, 3.0, and 2.0, respectively. These values indicate

Table 2. Experimental Langmuir equation constants and correlation coefficients for Cu^{2+} onto pectin with degree of esterification 1.2% at various pH

pH	Langmuir equation constants	
	q_{max} (mg/g)	b (l/mg)
2.0	79.3	0.0101
3.0	103.0	0.0308
4.0	118.0	0.0706
5.0	128.1	0.1658
6.0	128.3	0.2090
7.2	133.4	0.2381
8.0	138.6	0.2522

the uptake capacity of the pectin regarding copper ions is strongly depending on the solution pH.

The essential features of a Langmuir isotherm can be expressed in terms of a dimensionless constant separation factor or equilibrium parameter, R_L that is used to predict if an adsorption system is "favourable" or "unfavourable". The separation factor, R_L is defined by:

$$R_L = \frac{1}{1 + bC_9}$$

where C_0 is the initial Cu (II) concentration (mg/l) and b is the Langmuir adsorption equilibrium constant (l/mg).

The results of the R_L factor calculation showed that based on the effect of separation factor on isotherm shape, the R_L values of all compounds studied were in the range of $0 < R_L < 1$, which indicates that the sorption of Cu (II) by these susbtances are favorable. Thus, pectins are favorable materials for binding of the Cu (II) ions. Analysis of equilibrium sorption isotherm shows that at final concentration of Cu (II) ions 10 mM the condition considered as saturation is achieved regardless pH of solution. In other words, in experiments with final copper concentration 10 mM and more, all the pectin samples bound the maximum amount of the copper ions. Table 3 shows the uptake capacity of pectins with various degrees of esterification regarding copper ions at equilibrium concentration of Cu (II) 10 mM. These results display two patterns. First, maximum metal binding capacity of all the pectin samples with different degree of esterification is 2 - 2.1-fold decreased in accordance with the rise of the solution pH from 2.0 to 8.0. Second, at every pH meaning reduction of the degree of esterification from 60.2% to 1.2% did result in 2.3 - 2.4-fold increased maximum metal binding capacity of pectin.

DISCUSSION

Analysis of complete spectrum of pharmacological effects exerted by pectins and elaboration of

Table 3. Uptake capacity for copper ions of pectins with various degrees of esterification at initial Cu^{2+} concentration 10 mM and various pH

pH	Uptake capacity (mg copper/g dw pectin)						
	DE = 60.2	DE = 52.0	DE = 40.1	DE = 27.4	DE = 18.8	DE = 9.6	DE = 1.2
2.0	29.0	32.8	39.6	46.6	59.2	63.0	69.6
3.0	40.6	45.6	55.4	64.5	71.8	87.7	95.2
4.0	49.5	54.8	68.1	77.6	86.5	107.8	116.4
5.0	57.5	62.6	74.8	87.0	96.9	117.1	129.7
6.0	57.9	62.6	76.1	87.7	97.4	119.8	130.9
7.2	59.1	64.2	78.6	89.4	99.4	122.3	134.9
8.0	60.1	65.7	80.3	91.9	102.0	124.7	140.0

DE: degree of esterification

medicine preparations based on pectins make the interesting direction of pharmacy (Khotimchenko *et al.*, 2001). Some recent studies suggest that beneficial effects of pectins are closely related to their structural characteristics and physical and chemical properties. For example, pectins with greater methoxyl content and higher molecular weight or higher viscosity are considered as better cholesterol-lowering agents (Yamaguchi *et al.*, 1995; Terpstra *et al.*, 1998). The viscosity of pectins may affect cholesterol absorption and fecal excretion of neutral sterols and bile acids (Judd and Truswell, 1985; Terpstra *et al.*, 1998). Interaction of pectins with bile acids is diminished according to the decrease of the degree of esterification (Dongowski, 1995). There were shown different efficacy of pectins with high and low degree of esterification as well as with high and low molecular weight regarding ferric iron solubility and absorption of solubilized iron in intestine of rats (Kim, 1998). Briggs (1997) suggested that pectin with less methoxyl content and low molecular weight (< 10,000 Da) is more efficient for cancer metastasis prevention. At last, activity of pectin inhibiting the process of attachment of fibroblast growth factor to its receptor was shown to correlate significantly with sugar content, methoxyl content, and molecular weight of pectin (Liu *et al.*, 2001).

As a majority of polysaccharides, pectins are heterogeneous compounds regarding structure, molecular weight, and physicochemical properties. These parameters of pectins vary from one fruit species to another and also during the different developmental stages of the fruit (Fishman *et al.*, 1991; Chang *et al.*, 1994). They also depends on the process of chemical and enzymatic modifications (Ralet *et al.*, 2001; Hotchkiss *et al.*, 2002). Therefore, in experiments on the pharmacologic efficacy of different pectins, it is of great important to have the most complete characteristic of structural and physicochemical properties of the pectin samples studied. Analysis of papers devoted to exploration of correlation between structure of pectins and their pharmacological activities showed that the most significant and suitable parameters, which may be applied for standardization of pectin samples, are molecular weight, intrinsic viscosity, the degree of esterification, and non-methylated anhydrogalacturonic acid content. In investigation of some pharmacological effects such parameters as total neutral sugar content and relative ratio of individual sugars may have some influence. Besides, due to the presence of rhamnose interrupting the regularity of the linear structure of the anhydrogalacturonic acid backbone, the percentage of rhamnose may play an impotent role in the pharmacological activities of pectins.

The present work was devoted to the study of the relationship between physicochemical properties

of pectins and their metal binding capacity using as an example interaction of pectins with copper. At the first stage the main goal was in obtaining of pectins samples with different degree of esterification. Comparison of binding properties of pectins with different degree of esterification should be carried out with comparing samples having all other characteristics similar. The pectin samples we obtained using alkali method of de-esterification differed from each other by 10% within the range from 1 to 60%. Therefore, the difference between pectin samples with lowest and highest relative contents of methoxylated carboxyl groups was 50.2 times. At the same time the total anhydrogalacturonic acid content of samples differed only by 6.5%. The results indicating that pectin samples with different degree of esterification have almost similar amount of total anhydrogalacturonic acid prove that pectin molecules was not subjected to substantial structural degradation during de-esterification process. This conclusion may be considered as correct regarding the side molecular chains responsible for branched structure of pectin because broken side chains consisting of neutral saccharides would inevitably induce the rise of polygalacturonic acid content making the main feature of linear molecular chain. At the same time the changes of intrinsic viscosity of pectins having place as a result of de-esterification does not indicate significant degradation of the main pectin chain. Lowering of the degree of esterification from 52.0% to 1.2% was associated with 28.4% reduction of molecular weight calculated with the use of Mark-Howink equation.

First of all, in exploration of sorption activity it was necessary to know the duration of period sufficient for a whole binding process between pectin and metal. According to the different sources these periods vary from 10 - 30 min for water soluble compounds to 7 days for insoluble substances (Jodra and Mijangos, 2001). This was used to make an order of the first series of experiments. The results showed that equilibrium state between copper ion bound with the agent and free ions in solution occurs in 10 min after the start of interaction. So, experiments on binding of all the metal ions with every pectin sample tested were carried out with the duration of incubation period at least 30 min.

Sorption capacity is one of the most important characteristics of any sorbent objectively reflecting its capacity of binding a sorbate and a suitable criterion for comparative assessment of efficacy of different sorbents. Comparative estimation of different binding agents regarding their sorption properties is rather complicated task, in particular, in the cases when it is necessary to compare substances of different chemical structure or substantially different regarding their water solubility. One of the important factors in assessment of binding capacity of a sorbent is a sorbate water concentration. Sorption capacity of a sorbent is depending on it. Sorption process continues until equilibrium between concentrations of bound ions and free ions in experimental solution have a place, which is characteristic of each species of sorbents (Volesky, 2003). In our study there were pectins differed regarding their degree of esterification.

For characterization of a sorption capacity of different sorbents usually a Langmuir method is in use (Jang *et al.*, 1992; Murakami *et al.*, 2003; Iqbal and Edyvean, 2004). Analysis of a curve reflecting sorption activity allows defining of sorption capacity at any concentration of a sorbate. Usage of Langmuir equation permits calculating of a maximum possible sorption capacity at infinitely high sorbate concentration, which may be used as one of the criteria for comparative assessment of different sorbents. Often for comparative assessment of the sorbents is used a Freindlich sorption model (McKay *et al.*, 1989). But Freindlich model is rather suitable for estimation of sorption capacity of absorbents, in which binding process have place onto single superficial heterogeneous layer of a sorbent (Schmuhl *et al.*, 2001).

In our work for comparative assessment of different pectins regarding their metal binding

capacity we defined their sorption capacity in solutions with the same sorbate concentration. The results obtained in experiments studying a correlation between pectin sorption of cooper ions and final equilibrium copper concentration in solution showed that sorption curve rises and becomes flat at final copper concentration about 10 mM for the whole pH range from 2 to 8. Therefore, we have taken to consideration only quantitative meanings of uptake capacity registered for all pectin samples studied, which were obtained in solution with mentioned copper concentration. Obtained results showed that efficacy of the binding of copper ions by pectins under *in vitro* conditions depends on the degree of esterification and non-methylated galacturonic acid content in the polysaccharide molecule. In spite of tested pectin samples being different regarding degree of esterification as well as intrinsic viscosity, which is correlatively related to molecular weight, the role of intrinsic viscosity in metal binding process may be only indirectly judged. Probably, parameters of intrinsic viscosity insignificantly influence binding processes, at least regarding ions of copper. For more complete assessment of the role of intrinsic viscosity in efficacy of the metal binding activity of pectins it is necessary to obtain the pectin samples with the same degree of esterification but with different molecular weight.

Also binding processes between pectins and metal ions are substantially dependent on pH of media. Alkali shift of pH results in increased sorption capacity of pectins with any degree of esterification. We did not study interaction with copper at pH lower than 2 because polysaccharides consisting of uronic acids sediments at pH lower than 2 (Fishman *et al.*, 2001). Increase of the pH meanings more than 8 would obviously result in polysaccharides become unstable (Schmuhl *et al.*, 2001; Lofgren *et al.*, 2002) and, hence, in dramatically reduced metal binding capacity of them. The model of interaction between metals and pectins, which was described before (Kohn, 1987), states that metal binding is caused generally by formation of firm ionic and covalent bonds between the metal cations and free carboxyl groups of anhydrogalacturonic acids. Decrease of metal binding activity because of the pH reduction may be explained by protonation of carboxyl groups of anhydrogalacturonic acids resulting in them losing their charge and, consequently, their capacity to firm ionic bonds with metal cations.

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