Chemical composition and sorption properties of chitosan produced from fly larva shells

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Chitosans recovered from fly larva shells have been investigated by the elemental analysis, potentiometric titration and FT-IR spectrometry methods. The molecular weight of chitosans was determined by measuring their viscosity. The initial stage of removal of fatty compounds and proteins is the crucial one in the recovery of chitosan from fly larva shells. The chitosans' sorption capacity of Ni(II) ions as well as citrate depends on free amino groups or deacetylation degree (DD). With an increase in DD the sorption capacity increases. The Ni(II) sorption on chitosans from its citrate solution proceeds according to the pseudo-second order reaction.

Key words: chitosan, Ni(n), citrate, sorption

INTRODUCTION

Chitin, poly[β-(1-4)-2-acetamido-2-deoxy-D-glucopyranosel], is the most important natural polysaccharide after cellulose found in nature. Chitin and its deacetylated derivative chitosan are produced as a rule from seawater and freshwater crustaceans (crab, krill, shrimp, etc.). These raw materials along with chitin contain rather big amounts of side substances, viz. organic, such as fatty compounds and lipids, and inorganic, such as calcium phosphate and carbonate. The biggest amounts of chitin are found in insects. The content of side materials here is low. However, the insects are overspread in the environment and can hardly be used for chitin production [1].

The fly (*Musca domestica*) larva shells, which form in rather big amounts as a waste product in the process of biological treatment of food industry wastes, can be used as a raw material for chitin production [2]. These shells contain about 40% of chitin; for comparison, the prawn *(Nephrops norvegicus)* shells contain approximately 16% of chitin [3]. Chitosan from fly larva shells may be produced in the same way as from the crustacean exoskeletons. The key steps in the extraction of chitin from shells are the removal of proteins and minerals, such as calcium carbonate and phosphate, by treatment with alkaline and acidic solutions in turn. Chitosan, poly[β-(1-4)-2-amino-2-deoxy-D-glucopyranosel], is a partially or fully deacetylated derivative obtained by alkaline treatment of chitin. Chitin can be converted to chitosan by either homogeneous or heterogeneous alkaline N-deacetylation or by enzymatic means [4]. Despite numerous attempts, N-acetyl groups cannot be completely removed. Chitin is insoluble in many solvents; it is difficult to isolate from natural sources in a pure form. Chitosan is soluble in aqueous acidic solutions due to the presence of amino groups. The solubility is also controlled by the distribution of the acetyl groups remaining along the chain.

The polysaccharide chain undergoes some degradation during treatment with acids, alkali and high temperature when chitosan is produced. The solubility of chitosan also depends on the structure and molecular weight of chitosan. Therefore, to produce chitosan of desirable properties the treatment conditions are varied [4].

The characterization of chitin and chitosan is difficult, and numerous related studies have been carried out [5–10]. NMR or IR spectroscopy only can characterize chitin in solid state due to its lack of solubility. For chitosan, due to its solubility, more methods such as potentiometry, UV spectroscopy, viscosimetry can be used. Elemental analysis can be used when chitosan is in the pure form [9].

In the recent decade chitosan has received much attention because of its properties. Chitosan is used

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in medicine, biotechnologies, industry for wastewater treatment because of its high capacity to fix molecules such as pesticides, proteins, dyes and to form complexes with heavy metals [11]. These chelating properties are turned to account for wastewater treatment to recover metals from dilute solutions by using chitosan as an adsorbent. The use of biosorbents is preferable because of their high sorption capacity and selectivity for heavy metal ions. The alkaline and alkaline earth metals are not sorbed by chitosan. Chitosan acts also as a good sorbent for organic acids due to the interaction of $-NH_2$ group with carboxylic groups of acids [12].

The shape of fly larva shells is very suitable for sorption. They are of oblong hollow-balls with very thin walls (5–10 μm), having a large surface per weight. Therefore chitosan produced from them might be successfully used as a sorbent for wastewater treatment, if the structure of walls has remained similar to the initial one.

The current work was done with the purpose to produce chitosan from fly larva shells under different deacetylation conditions, to characterize their chemical composition and sorption capacity for the Ni(II)-citrate complex used in electroless plating solutions.

EXPERIMENTAL

Characterization of chitosans

Chitosan from fly larva shells was produced in the same way as from crustacean exoskeletons [3, 13]. For chitin extraction from shells, proteins were removed by treatment with NaOH and minerals with HCl solutions. For bleaching, $K_2S_2O_8$ was used. Chitosan from chitin was recovered by deacetylation with concentrated NaOH solutions at a high temperature. After each procedure the shells were thoroughly washed. The treatment conditions are indicated when discussing the results.

The content of ash, carbon, nitrogen and phosphorus in these biopolymers was determined according to [14]. Incineration was carried out at 600 °C for 3 h, total nitrogen was determined by the Kjeldahl method, organic carbon – after oxidation by chromic acid, organic and inorganic phosphorus and sulphur – by oxidation with H_2O_2 and dissolving in HCl.

The molecular weight of chitosans was determined by measuring the viscosity of 0.05–0.15% chitosan dissolved in 0.5 mol/l acetic acid and 0.2 mol/l sodium acetate solutions using an Ostwald viscometer. The molecular weight was calculated by the Mark–Houwink equation:

$$\eta = K_m M^{\alpha},$$

where $K_m = 3.5 \cdot 10^{-4}$ and $\alpha = 0.76$ [4].

For pH-metric titration, dried chitosan was crushed to powder. The aqueous suspension of this powder was titrated with HCl.

The infrared spectra of chitosan were recorded in KBr pellets on a Fourier transformation infrared spectrometer (Hartman & Braun, Canada) with 2 cm^{-1} scale resolution. The spectra were recorded in the region of wave number between 4000 and 500 cm^{-1}.

Sorption experiments

The metal ion adsorption was investigated at room temperature under batch conditions by mixing chitosan with metal ion containing solutions. The load was 10 g of dry sorbent per 1 l of Ni–citrate complex containing solution. pH was adjusted with NaOH or H_2SO_4 solutions and checked every day.

In order to determine the amount of sorbed components, their concentrations in solutions before and after sorption on chitosan were determined. Ni(II) in the solutions was determined complexometrically by using EDTA as a titrant and murexide as an indicator. The low Ni(II) concentrations were determined photometrically at $\lambda = 490$ nm, using dimethylglyoxime. The citrate concentration in the solutions was determined after oxidation in alkaline solutions with $KMnO_4$, its excess being retitrated in acidic solutions with oxalic acid. In the kinetic investigations Ni(II) concentration in solutions was determined by means of the radioactive tracer method using $^{63}Ni(II)$. The radioactivity of solutions was measured in a UMF-1500M system with an SBT-13 counter (Russia).

RESULTS AND DISCUSSION

The conditions of chitosan production were varied with the purpose to produce chitosan mostly suitable for sorption of metal complexes from wastewater. They are indicated in Table 1. The initial stage in chitosan production is the removal of fatty compounds in alkaline solutions in order to achieve wetting of the surface. During this treatment proteins are removed as well. Usually this process is slow. The wetting is achieved more rapidly at higher NaOH concentrations and temperature. The subsequent treatment with HCl enables to remove mineral compounds. HCl concentration and temperature were selected according to [3]. The obtained material after such treatment must be chitin. However, it is brown in color. Production of white chitin requires bleaching.

According to literature data [4], considerable changes in the chitin chain proceed during its dea-

Table 1. **Conditions of chitosan production, its molecular weight and deacetylation degree (DD)**

Chitosan	Removal of fatty compounds	Removal of mineral compounds	Bleaching	Deacetylation	Molecular weight, Da	Deacetylation degree (DD)
No 1	40 g/l NaOH, 50 g/l shells, room temperature, 3 days	200 ml HCl + + 500 ml H_2O + + 100 g shells, 40 °C, 3 h	500 ml H_2O + + 50 ml H_2SO_4, $K_2S_2O_8$ – – 100 g/l.	600 g/l NaOH, 200 g/l shells, 110 °C, 5 h	96000	65
No 2	As No 1	As No 1	As No 1	600 g/l NaOH, 200 g/l shells, 3 g/l $NaBH_4$, 110 °C, 5 h	115000	72
No 3	As No 1	As No 1	As No 1	As No 1, additionally deacetylation repeated twice	85000	80
No 4	As No 1	As No 1	As No 1	As No 2, additionally deacetylation repeated twice	90000	82
No 5	40 g/l NaOH, 50 g/l shells, 70 °C, 1h	As No 1	As No 1	As No 3,		30
No 6	As No 5	As No 1	As No 1	As No 2,but 6 g/l $NaBH_4$		45

The amount of shells is indicated for the initial stage of treatment.

cetylation. The high temperature and strong alkaline solutions cause the chain breaks and abridging. The use of BH_4^- for radical trapping enables to obtain chitosan of higher molecular weight. The increase in treatment time, temperature and NaOH concentration increases the degree of deacetylation (DD), *i.e*. the ratio of the number of amino groups in chitosan to the sum of amino- and acetamidogroups.

Viscosimetric measurements (Table 1) show a considerable influence of treatment conditions on the molecular weight of chitosans recovered. With an increase in treatment time the molecular weight decreases. It increases somewhat with the use of BH_4^-.

Chitosan in acidic media becomes a polyelectrolyte because of protonation of the – NH_2 groups. The ionization proceeds according to the equation

$$Chit–NH_2 + H^+ \rightarrow Chit–NH_3^+.$$

This property of chitosan was proposed to use for determination of deacetylation degree [9]. The pH-metric titration of soluble chitosans gives rather correct results. In case of suspension titration the obtained DD is somewhat lower than the actual DD. The amount of H^+ necessary for titration of chitosan suspension at pH 6.5–6 corresponds to the number of $–NH_2$ groups in chitosan. The DD values obtained from pH-metric titration (Fig. 1) are given in Table 1.

Shell treatment at a high temperature at the initial stage in alkaline solutions (chitosans Nos 5 and 6) has the negative influence on DD. The molecular weight of these chitosans was not determined due to their low solubility.

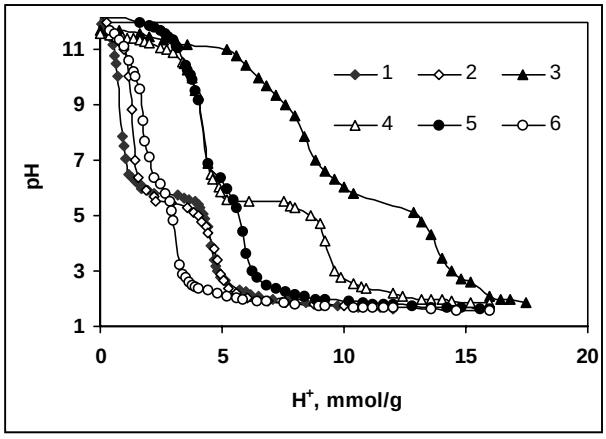

Fig. 1. pH-metric titration of chitosan suspensions. The number of curves corresponds to the number of chitosans from Table 1

The results obtained have shown that shell treatment conditions such as the use of BH_4^- and prolonged times have some influence on molecular weight or deacetylation degree. The treatment at the initial stage, i.e. removal of proteins and lipids, has the decisive influence on the properties of recovered chitosans.

The chemical composition of the sorbents recovered from fly larva shells under different conditions is tabulated in Table 2. Organic carbon and nitrogen are the essential elements of chitin. The decrease of nitrogen by treatment with alkali indicates the removal of proteins. Ashes indicate the content of inorganic components in fly larva shells. The shells contain calcium carbonate and phosphate. Organic phosphorus is present mostly in fatty compounds, such as phospholipids. The proteins such as cerotins contain sulfur. The proteins, fatty compounds and inorganic compounds are removed by treatment with alkaline and acidic solutions. Comparison of the composition of chitosans obtained with that of a raw material (fly larva shells) indicated that the recovered chitosans were rather pure and contained no lipids, proteins or inorganic substances. The differences in nitrogen and carbon content in chitosans produced under different conditions may be related to their different chemical composition as well as to the deviations of determination.

Table 3. **FT-IR vibration bands in chitin and chitosan**

Assigments	Chitin	Chitosan
ν OH	~3500	~3450; 3340
ν_{as} NH (-NH_2)	–	~3500
ν_s NH (-NH_2)	–	~3400
ν NH (-$NHCOCH_3$)	3265; 3100	–
ν_{as} CH (CH_3)	2961w	–
ν_{as} CH (-CH_2-)	2928m	2926s
ν_s CH (-CH_2-)	2871m	2864s
ν C=O (-$NHCOCH_3$)	1655s (I)	1650w
δ NH + ν CN (-$NHCOCH_3$)	1560s (II)	–
	1310	–
δ NH (R-NH_2)	–	1596s
δ_{as} CH (-CH_2-)	1426	1418
δ_s CH (-CH_2-)	1378	1377
ν CN	1220-1020	1200-1020
ν_{as} C-O (-C-O-C-)	1077	1082
ν_s C-O (-C-O-C-)	1024	1033

ν – streching, ν_s – symmetric stretching, ν_{as} – asymmetric stretching, δ – bending
s – strong, m – medium, w – weak

Table 2. **Content of the main elements in chitosans recovered under conditions indicated in Table 1**

Sorbent	Ash, %	N, %	C, %	P, %		S, %	
				Total	Inorganic	Total	Inorganic
Raw material	9.2	8.2	31.7	0.85	0.75	0.32	0.12
Chitosan No 1	0.08	7.8	41.8	0	0	0	0
No 2	0.07	6.3	41.2	0.011	0	<0.005	0
No 3	0.1	7.1	41.5	0.005	0	<0.005	0
No 4	0.07	6.9	42.3	0.006	0	<0.005	0
No 5	0.08	7.4	42.4	0.004	0	<0.005	0
No 6	0.06	7.6	42.6	0.004	0	<0.005	0

IR investigations are widely used for studies of chitin and chitosan [15]. IR spectra enable to evaluate the DD and molecular weight of chitosan. FT-IR investigations show that chitin has the characteristic absorption bands at 1560, 1655 cm^{-1} and in the vicinity of 3265 and 3100 cm^{-1} and corresponds to the stretching vibration ν C=O and ν NH (-$NHCOCH_3$), respectively (Table 3). In chitosans the band emerging at 1650 cm^{-1} (amide I) becomes weaker, and the new ab-

Table 4. **Ni(II) sorption from Ni(II)–citrate solutions on different chitosans at pH 6**

Composition of solution, mmol · l^{-1}	Sorption capacity of chitosan, mmol/g					
	No1	No 2	No 3	No 4	No 5	No 6
Ni(II) – 10	0.85	0.77	0.88	0.92	0.47	0.22
Ni(II) –100	2.8	1.4	2.3	2.7	0.5	0.4
Ni(II) – 10 Citrate –5	0.8	0.96	0.9	0.9	0.73	0.29
Ni(II) – 10 Citrate –10	0.5	0.61	0.6	0.4	0.2	0.09
Ni(II) – 10 Citrate – 20	0.2	0.22	0.38	0.21	0.16	0.08

sorption feature observed around 1596 cm^{-1} (bending vibration δ NH of R-NH_2) indicates an increase in DD. The degree of deacetylation might be determined by using adsorption ratios A_{1655}/A_{3450} in the connection of the calibration curve [16]. C–H stretching vibrations at 2926 and 2864 cm^{-1} and bending vibrations at 1418 and 1377 cm^{-1} in chitosans indicating the molecular weight correspond well with measurements of viscosity.

The chitosans recovered under different conditions have the different sorption capacity for Ni(II) ions (Table 4). Chitosans with the highest DD, *i.e.* containing the biggest amount of free –NH_2 groups, exhibit the best sorption capacity. The sorption of Ni(II) on chitosans with low DD (Nos 5 and 6) is poor. The sorption of Ni(II) and citrate on chitosan No 4, which possesses the highest DD, indicates that chitosan sorbs both Ni(II) and citrate ions from solutions in a rather wide range of pH (5–9) (Fig. 2).

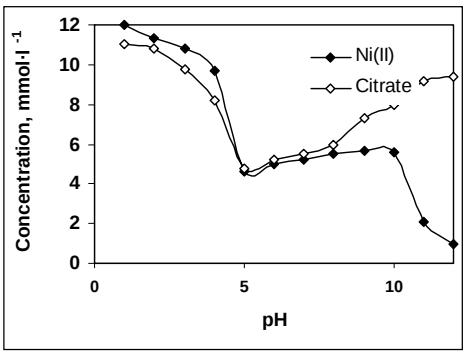

Fig. 2. Influence of pH on the residual concentrations of Ni(II) and citrate after sorption onto chitosan No 4

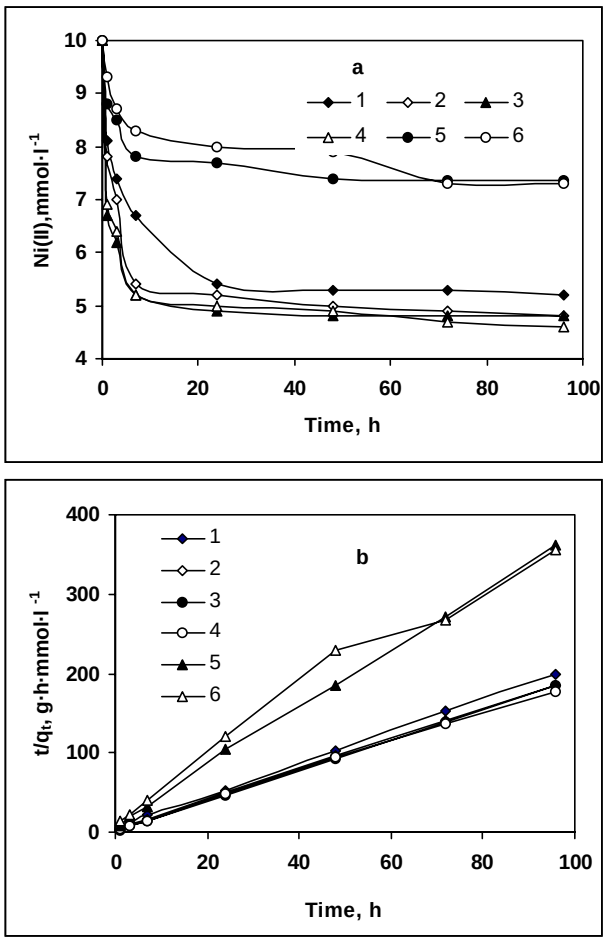

Fig. 3. The uptake of Ni(II) by chitosans. Initial concentrations 10 mmol · l^{-1} Ni(II) and 10 mmol · l^{-1} citrate, pH 7 (**a**) and test of pseudo-second order equation for adsorption of Ni(II) (**b**). Number of curves corresponds to the number of chitosans from Table 1

Fig. 3 shows the kinetics of Ni(II) sorption by chitosan prepared under different conditions. During sorption from a solution containing 10 mmol · l^{-1} each Ni(II) and citrate, Ni(II) uptake by chitosan makes up 0.5 mmol · g^{-1}, *i.e.* about 50%, if DD is rather high (>60%). The uptake of Ni(II) is rather rapid in the first 3 hours and practically is finished after 6–7 hours. Attempts to fit the first order kinetics equation indicate that the plots are approximately linear over the initial 6–7 hours only. The correlation coefficients in this case are very small. The application of the pseudo-second order model provides a much better correlation of the experimental data than the first-order model [17–20] (Table 5). The reaction order was calculated according to the equation

$$\frac{t}{q_t} = \frac{1}{k_2 Q^2} + \frac{t}{Q},$$

where Q is the amount of Ni(II) sorbed under equilibrium conditions, mmol · g^{-1}, q_t is amount of Ni(II) sorbed by chitosan at time t, mmol · g^{-1}, k_2 is the pseudo-second order sorption rate constant, g · mol^{-1} · h^{-1}.

The pseudo-second rate model fits over the whole range of time interval. The pseudo-second order model is based on the assumption that the rate-limiting

Table 5. **Rate constants and correlation coefficients in kinetic experiments**

Experiment conditions		Pseudo-second order reaction parameters	
		k_2, g · $mmol^{-1}$ · h^{-1}	R^2
Fig. 3	curve1	1.082	0.9992
	curve 2	1.061	0.9997
	curve 3	1.414	0.9999
	curve 4	1.612	0.9993
	curve 5	4.563	0.9986
	curve 6	0.839	0.9827

step may be chemical sorption involving valency forces through sharing or exchange of electrons between sorbent and sorbate [21]. In our case it may be the interaction of citrate and Ni(II) ions with the $–NH_2$ and –OH groups of chitosan.

Experiments have shown that the sorption capacity of chitosans (Table 4) depends on sorbate concentration in solutions. Ni(II) sorption from more concentrated solutions is more significant, however, its residual concentration in solutions is higher. An increase in citrate concentration in solutions decreases the sorption ability of chitosan. The further experiments were carried out with the purpose to investigate the sorption ability of the already used chitosan as a sorbent (Fig. 4), and fresh chitosan from used for sorption solution (Fig. 5).

The first sorption of Ni(II) as well as citrate on fresh chitosan (Table 6) makes up about 0.5 mmol/g[1], *i.e*. 50%. When the second sorption proceeds on once used chitosans, it makes up only 20% (Fig. 4). The Ni(II) sorption from used solution on fresh chitosan makes up ~60%, but the amount of sorbed Ni(II) is only 0.3 mmol · g^{-1}. The complete removal of Ni(II)–citrate complex can be achieved only after 3–4 treatments with fresh chitosan (Table 6). On the other hand, the chitosan used for sorption from low concentration solutions possesses a rather high capacity for sorption from more concentrated solutions.

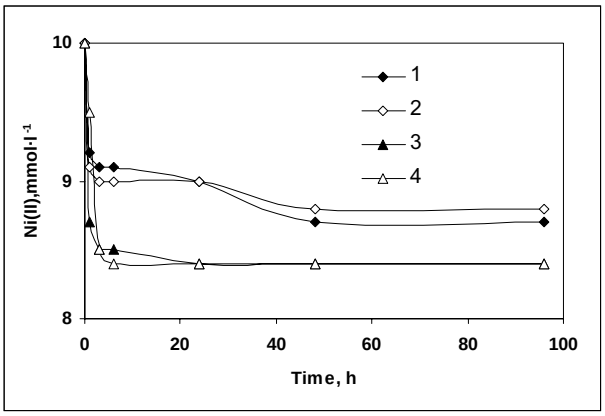

Fig. 4. The uptake of Ni(II) from fresh solution on chitosan once used under conditions of Fig. 3. pH 7. The number of curves corresponds to the number of chitosans from Table 1

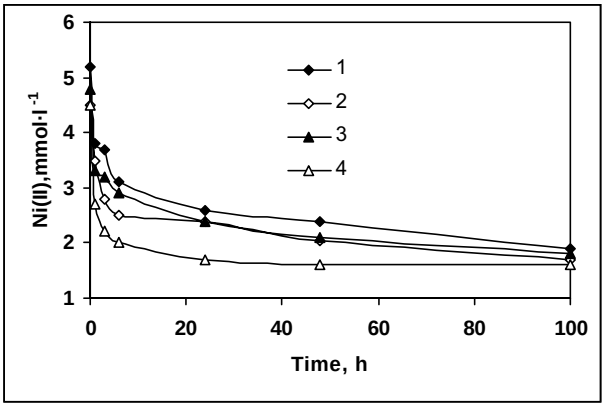

Fig. 5. The second uptake of Ni(II) that remained after treatment under the same conditions as in Fig. 3 with fresh chitosan. The number of curves corresponds to the number of chitosans from Table 1

Table 6. **Ni-citrate sorption onto chitosan No 4. Initial concentrations: Ni(II) 10.5 and citrate 10.2 mmol · l^{-1} at pH 6**

Number of batches	Residual concentrations, mmol · l^{-1}	
	Ni(II)	Citrate
1	4.8	5
2	0.8	2.4
3	0.5	1.1
4	0.004	0.01

CONCLUSIONS

The initial stage in production of chitosan from fly larva shells, i.e. the removal of fatty compounds and proteins determines its properties.

The use of BH_4^- additive during deacetylation enables to produce chitosan of a higher molecular weight and a higher degree of deacetylation.

Chitosans recovered from fly larva shells act as a sorbent for Ni(II) as well as for citrate ions. The Ni(II) sorption on chitosans from its citrate solution proceeds according to the pseudo-second order reaction.

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IŠ MUSIŲ LERVŲ IŠNARŲ PAGAMINTO CHITOZANO CHEMINĖ SUDĖTIS IR SORBCINĖS SAVYBĖS

S a n t r a u k a

Chitozanai, pagaminti iš musių lervų išnarų, buvo tiriami nustatant jų cheminę sudėtį, titruojant potenciometriškai, nagrinėjant FT-IR spektrus. Chitozanų molekulinis svoris nustatytas viskozimetriniu metodu. Lemiamos įtakos chitozano cheminėms ir sorbcinėms savybėms turi pradinės riebalų ir baltymų pašalinimo sąlygos. Chitozano geba sorbuoti Ni(II) ir citrato jonus iš kompleksinių tirpalų priklauso nuo chitozano deacetilinimo laipsnio, t. y. nuo laisvų aminogrupių chitozano molekulėje skaičiaus. Didėjant deacetilinimo laipsniui, chitozano sorbcinė geba didėja. Ni(II) sorbcija iš citratinių tirpalų vyksta pagal pseudoantro laipsnio reakcijos modelį.