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## POSSIBILITY OF USING CHITIN SUPPORT IN CHROMATOGRAPHIC CONTROL OF ENVIRONMENT POLLUTION

This paper describes a thin-layer chromatographic process on chitin layers in which separation of mixtures of inorganic ions occurs. The possibility of the separation of Ag(I), Ni(II), Cu(II), Co(II), Cd(II) and Hg(II) ions using buffer aqueous mobile phase has been examined. The behaviour of individual inorganic ions during chromatographic process allows establishing the principles of separation mechanism on chitin. The experiments proved that chitin can be equally suitable to chromatographic separation of ions such as stationary phases like cellulose and silica gel. Results of experiment show that chitin can selectively bond metal ions of some rare elements.

### 1. INTRODUCTION

In the last twenty years, waters in nature are being continuously polluted with metal ions. Concentration of metal ions in sea water systematically increases and therefore, simple and rapid methods guaranteeing its purification from metallic impurities are being sought. It has been stated that chitin — a biopolymer widespread in animal organisms — is capable of bonding metal ions.

The pioneering works on affinity of metal ions with chitin and its derivatives were undertaken by MUZARELLI and co-workers [1]–[4] in late sixties. These works concern the behaviour of almost all metal ions existing either in the form of simple cations or in the form of more complex ions. It was stated that chitin and its derivatives do not show any affinity with alkaline and alkaline earth metal ions independently of their concentrations in the solution. Such ions as Li(I), K(I), Na(I), Rb(I), Cs(I), Mg(II), Ca(II) and Sr(II) practically are not bonded by chitin and its fully

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deacetylated form – chitosan. The metals of other even series and odd series react with chitin in very different way. The amount of bonded ions is the quality characteristic of given ions. Individual ions can be bonded either by chitin or chitosan in various degree.

## 2. MATERIALS AND METHODS

When various factors influencing behaviour of chitin and its derivatives are considered, it becomes obvious that there arises a problem of inventing a simple and cheap method of wastewater purification and ion separation. The method which fulfils these requirements is thin-layer chromatography. Simple and cheap apparatus, relatively short time of measurements and possibility of chitin and chitosan layer formation on the glass plates have induced us to use this method as a pilot one for defining conditions of separation of inorganic ions on chitin layer.

Chromatographic process was carried out in conventional Stahl's chambers using the method described in [5]–[6]. Aqueous–methanolic solutions (1:1 v/v) of  $\text{CuCl}_2 \cdot \text{H}_2\text{O}$ ,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CdCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{HgNO}_3 \cdot 2\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$  and  $\text{AgNO}_3$  were used as the substances chromatographed. The concentration of each solution was  $0.05 \text{ mole/dm}^3$ .

Aqueous–methanolic (3:1 v/v) solution of ammonia or acetic acid containing also ammonium nitrate or acetate were used as mobile phases. Electrolyte concentrations were changed as follows:

mole concentration of ammonium: 0.33, 0.67, 1.00, 1.33,

mole concentration of acetic acid: 0.17, 0.33, 0.50, 0.67,

mole concentration of ammonium nitrate or ammonium acetate: 0.20, 0.35, 0.70.

Chitin and chitosan were used as stationary phases.

## 3. RESULTS AND DISCUSSION

In our investigations chromatographic systems containing chitin and chitosan as stationary phase were considered. Water containing methanol, buffering salts (ammonium nitrate or ammonium acetate) and complexing agents (ammonia or acetic acid) was used as mobile phase. Effect of individual substances on behaviour of inorganic cations during chromatographic process has been investigated.

Comparison of the results obtained for the chromatographic systems, where water is used as mobile phase, shows great differences in chromatographic values of the ions considered (table 1A). In the case of copper and silver ions, high  $R_M$  values indicate that there are strong interactions between these ions and chitin surface. In the case of other ions investigated, i.e., Cd(II), Co(II) and Ni(II), these interactions are smaller (lower  $R_M$  values). Partial displacement of water by methanol (3:1 v/v)

Table 1

 $R_M$  values of metal ions on chitin (A) and chitosan (B) layers

Metal ions	Mobile phase		
	Water	Water+methanol (3:1 v/v)	Water+methanol (3:1 v/v)
Cu(II)	1.28	1.51	2.00
Co(II)	0.43	0.69	0.69
Cd(II)	0.69	1.00	1.38
Hg(II)	1.51	1.69	
Ni(II)	0.55	0.75	1.28
Ag(I)	1.20	1.38	

causes increase of all  $R_M$  values (table 1A). The results obtained for aqueous systems containing chitosan are not very reliable because water poorly wets chitosan layers which enables formation of broad front. Addition of methanol (25%) increases wettability of chitosan by mobile phase which results in formation of more sharp

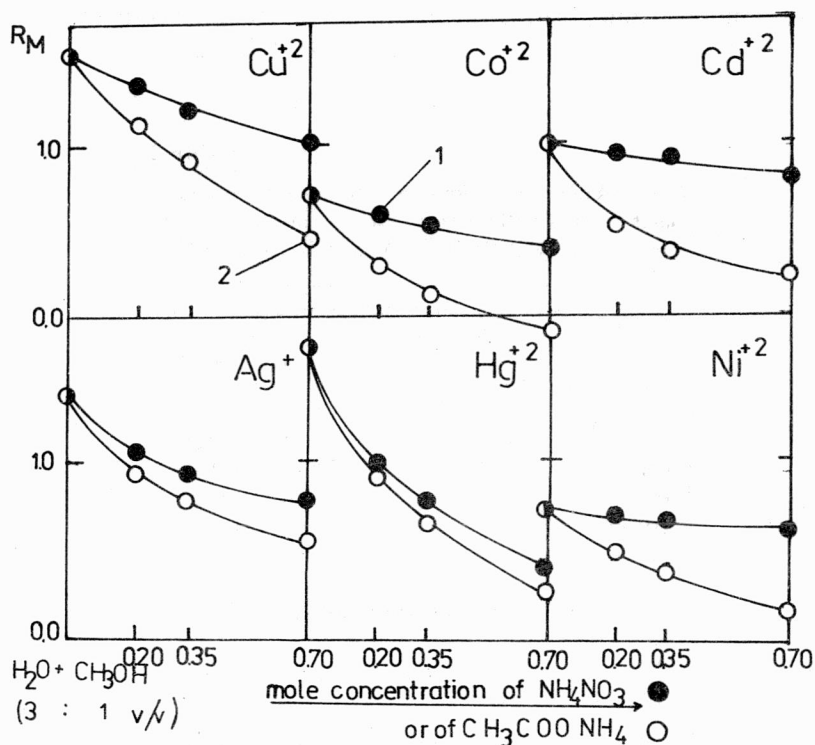


Fig. 1. Effect of addition of inorganic salts (ammonium nitrate (1) and ammonium acetate (2)) on chromatographic process on chitin layers

solvent front. In comparison to chitin, chitosan bonds much stronger metal ions (higher  $R_M$  values in comparison to chitin, table 1B). Copper and silver ions remain on the start line, cadmium and nickel ions show minimal tendency towards migration.

In the next stage, we have investigated the effect of addition of inorganic salts (ammonium nitrate or ammonium acetate) on chromatographic process. These salts affect chromatographic values of the cations investigated and cause the decrease of  $R_M$  values (figure 1) in comparison to  $R_M$  values obtained in methanol-water systems. Addition of salt to mobile phase (water-methanol) causes sharper changes of  $R_M$  values in the case of ammonium acetate than those observed in the case of ammonium nitrate. This proves that ammonium acetate interacts with the cations investigated more strongly than ammonium nitrate. Ammonium acetate on chitin support interacts more intensively with mercury ions (the greatest decrease of  $R_M$  values) and most weakly with nickel ions (the smallest decrease of  $R_M$  values). In relation to Ni(II) ion, addition of ammonium nitrate does not affect the changes in  $R_M$  values and the strength of bond formed between cation and chitin.

In the case of chitosan, changes of  $R_M$  values after addition of salts to developing systems are negligible. High positive values of  $R_M$  are remained constant and some of them even increase (e.g., for Co(II), Ni(II), Cd(II), table 2).

Table 2

$R_M$  values of inorganic ions on chitosan layers in mobile phases: water + methanol (3:1 v/v) +  $d$  ( $d$ -mole concentration of  $\text{CH}_3\text{COONH}_4$  (A) and  $\text{NH}_4\text{NO}_3$  (B))

Metal ions	Concentration of ammonium acetate - $d$ (A)			
	0.00	0.20	0.35	0.70
Cu(II)	2.00	2.00	1.82	1.59
Co(II)	0.69	1.51	1.44	1.38
Cd(II)	1.38	2.00	1.82	1.69
Hg(II)	$\infty$	1.28	1.16	0.95
Ni(II)	1.28	2.00...	2.00	1.82
Ag(I)			2.00	2.00

Concentration of ammonium nitrate - $d$ (B)				
Cu(II)	2.00	$\infty$	$\infty$	$\infty$
Co(II)	0.69	2.00	1.82	1.69
Cd(II)	1.38	$\infty$	$\infty$	$\infty$
Hg(II)	$\infty$	2.00	1.69	1.51
Ni(II)	1.28	$\infty$	$\infty$	$\infty$
Ag(I)	$\infty$	$\infty$	$\infty$	2.00

In the next stage, we have investigated such chromatographic systems where various amounts of  $\text{NH}_3$  or  $\text{CH}_3\text{COOH}$  were added to mobile phase. Such composition of developing systems was chosen because of several reasons. In literature there are data concerning complexation mechanism during interactions between metal ions and chitin or chitosan [1], [7]–[10]. In order to evaluate preliminarily this phenomenon, ammonia was added to mobile phases. Ammonia can form competing coordinating bonding with metal ions and, moreover, ammonia is also known as a complexing agent for metal ions. Parallel investigations were made also in the presence of acetic acid. In order to minimize the differences in pH values of individual mobile phases, to these phases there were added ammonium buffering salts, i.e. ammonium nitrate or ammonium acetate, in order to form buffer systems. The presence of methanol facilitates the wetting of supports.

Figures 2 and 3 present the effect of  $R_M$  values of the cations investigated on the concentration of ammonia or acetic acid in the mobile phase (water–methanol

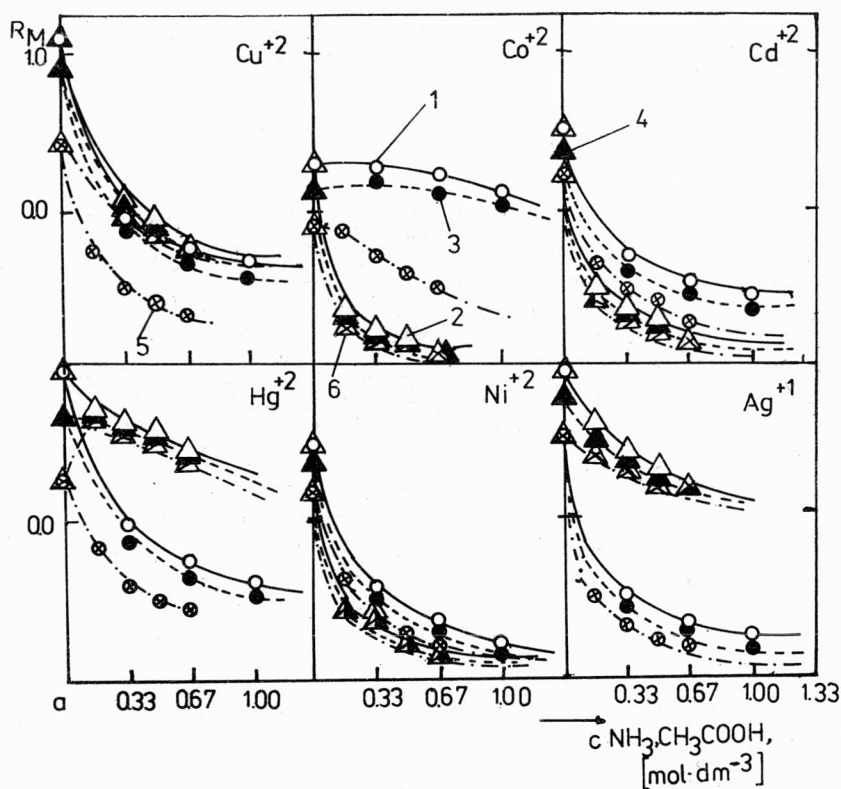


Fig. 2. Relationship between  $R_M$  values and mole concentration ( $c$ ) of  $\text{NH}_3$  (1) and  $\text{CH}_3\text{COOH}$  (2) in buffer mobile phase. Buffering salt,  $\text{CH}_3\text{COONH}_4$

$a$  = water + methanol (3:1 v/v) +  $d$  ( $d$  – mole concentration of ammonium acetate); 1, 2,  $d = 0.20$ ; 3, 4,  $d = 0.35$ ; 5, 6,  $d = 0.70$   
Stationary phase, chitin

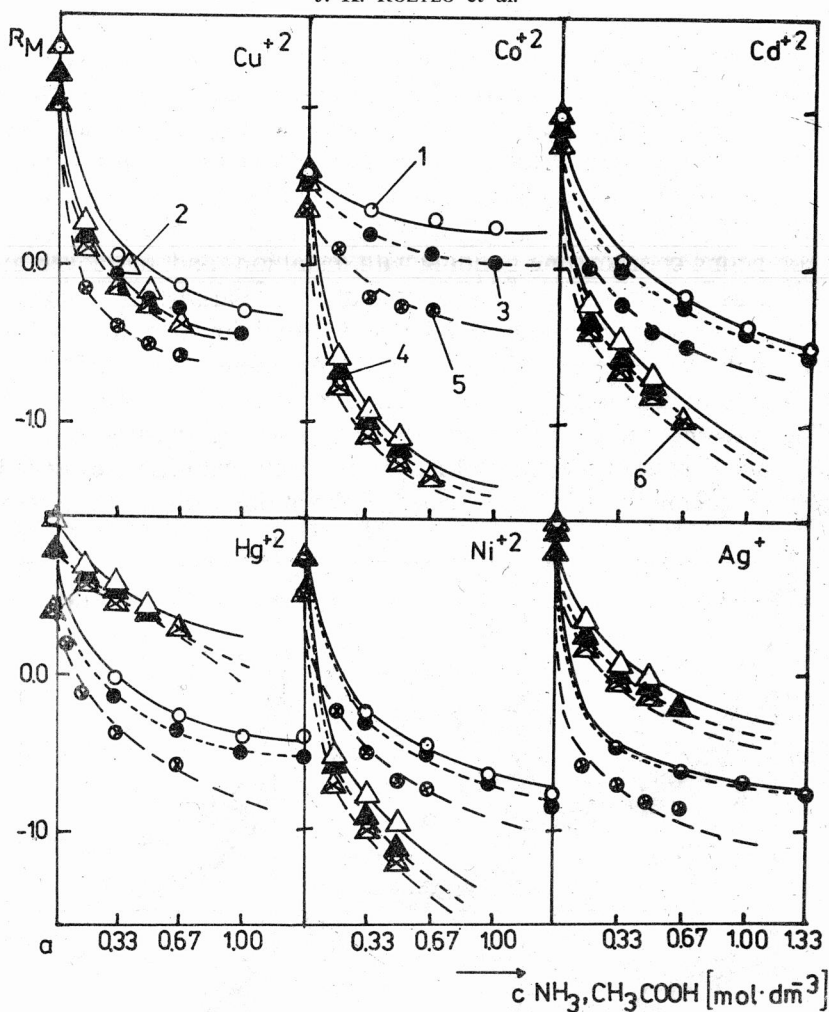


Fig. 3. Relationship between  $R_M$  values and mole concentration ( $c$ ) of  $\text{NH}_3$  (1) and  $\text{CH}_3\text{COOH}$  (2) in buffer mobile phase. Buffering salt,  $\text{NH}_4\text{NO}_3$

$a$  = water+methanol (3:1 v/v)+ $d$  ( $d$ —mole concentration of ammonia nitrate); 1, 2,  $d = 0.20$ ; 3, 4,  $d = 0.35$ ; 5, 6,  $d = 0.70$   
Stationary phase, chitin

3:1 v/v) in the presence of ammonium acetate or ammonium nitrate. With the increase of ammonia or acetic acid content, polarity of mobile phase also increases which leads to the decrease of  $R_M$  values of the cations (stronger interactions between mobile phase and substance chromatographed). From the diagrams presented it can be seen that even low content of ammonia or acetic acid in the mobile phase causes serious decrease in  $R_M$  values. Further increase of  $\text{NH}_3$  and  $\text{CH}_3\text{COOH}$  concentrations has significantly smaller influence on migration distance. These diagrams also prove that in the systems investigated concentration of buffering

salt does not affect significantly chromatographic values. Comparison of the results obtained leads to some conclusions concerning the change in behaviour of cations when ammonia is replaced by acetic acid. The individual cation influence is very different.  $R_M$  values of copper as well as of cadmium and nickel ions do not depend on the medium nature. In acetic acid medium considerable increase of  $R_M$  values for Hg(II) and Ag(I) ions is observed due to inhibition of migration (the curves obtained for acetic acid lie above those for ammonia). In the case of Co(II) cations the same phenomenon takes place in ammonia medium. The curves illustrating the dependence of  $R_M$  values on ammonia content for individual cations are very similar which indicates that there are similar interactions between ammonia and individual ions.  $R_M = f[x(\text{CH}_3\text{COOH})]$  curves have different shapes which is probably due to variety of phenomena occurring in these systems. A great increase of  $R_M$  values for Hg(II) and Ag(I) ions in comparison to the increase occurring in ammonium medium appears to be a distinct anomaly. In the neighbourhood of other cations, Hg(II) and Ag(I) cations do not interact practically with acetic acid or interactions between these substances are very poor.

Figures 4 and 5 present graphical dependences of  $R_M$  values of the cations investigated on concentration of ammonia in the mobile phase obtained for the systems containing chitosan as stationary phase (experiments in the presence of the acetic acid were inexpedient because chitosan is soluble in acetic acid). The course of the curves is similar to the course of the curves plotted for chitin, taking into account

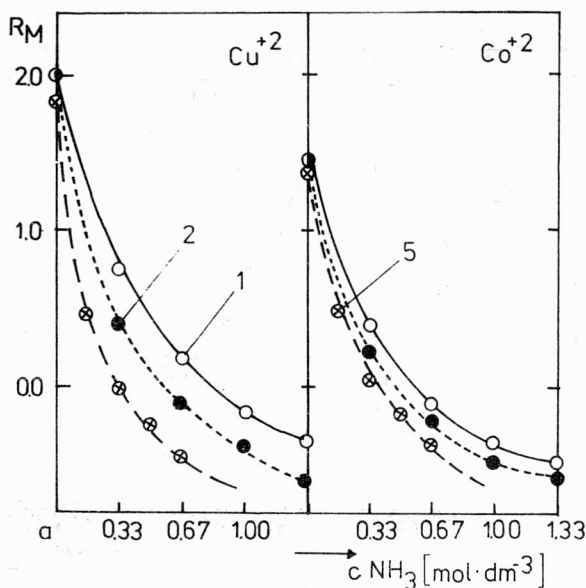


Fig. 4. Relationship between  $R_M$  values of inorganic ions and mole concentration of  $\text{NH}_3$  ( $c$ ) in buffer mobile phase

$a$  = water + methanol (3:1 v/v) +  $d$  ( $d$  - mole concentration of ammonia acetate); 1,  $d = 0.20$ ; 2,  $d = 0.35$ ; 5,  $d = 0.70$   
Stationary phase, chitosan

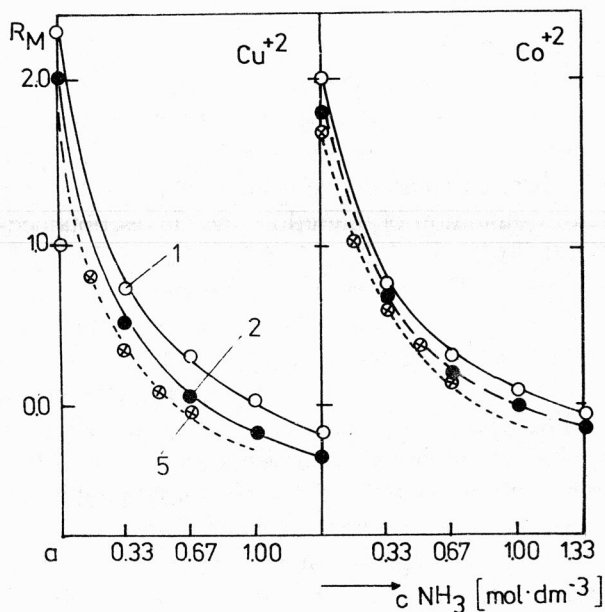


Fig. 5. Relationship between  $R_M$  values of inorganic ions and mole concentration of  $\text{NH}_3$  ( $c$ ) in buffer mobile phase on chitosan layers

$a = -\text{water} + \text{methanol} (3:1 \text{ v/v}) + d$  ( $d$  - mole concentration of ammonia nitrate); 1,  $d = 0.20$ ; 2,  $d = 0.35$ ; 5,  $d = 0.70$

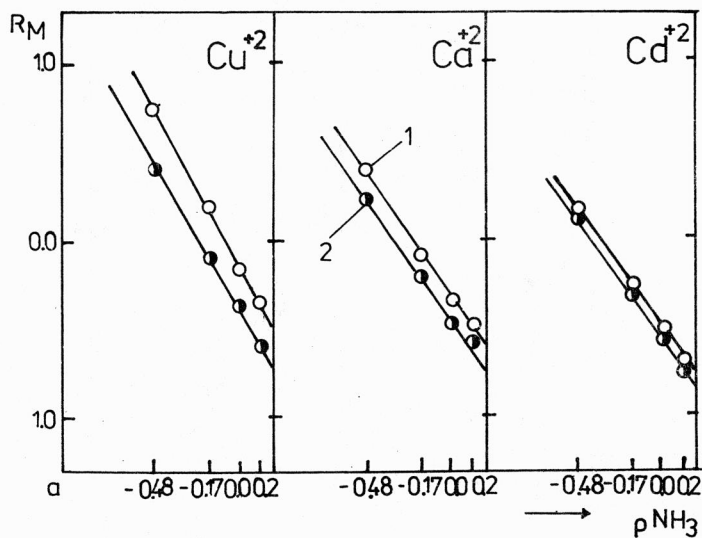


Fig. 6. Relationship between  $R_M$  values of inorganic ions and  $-\log c$  ( $c$  - mole concentration of  $\text{NH}_3$  -  $p \text{ NH}_3$ ) on chitosan layers in buffer mobile phase

$a = \text{water} + \text{methanol} (3:1 \text{ v/v}) + d$  ( $d$  - mole concentration of ammonia acetate); 1,  $d = 0.40$ ; 2,  $d = 0.65$



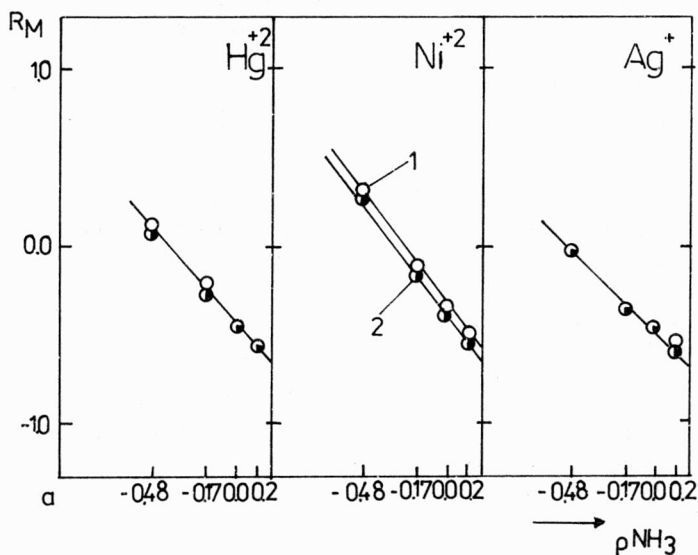


Fig. 7. Relationship between  $R_M$  values of inorganic ions and  $-\log c$  ( $c$  - mole concentration of  $\text{NH}_3 - p \text{NH}_3$ ) on chitosan layers in buffer mobile phases

$a$  = water + methanol (3:1 v/v) +  $d$  ( $d$  - mole concentration of ammonia nitrate); 1,  $d = 0.40$ ; 2,  $d = 0.65$

the fact that changes in concentration of ammonia in the mobile phase influence more significantly retention of cations on chitosan in comparison to chitin. The curves plotted for chitosan are more sharp.

According to suggestions proposed in [8], the efforts were made in order to establish a relationship between  $R_M$  values of the ions investigated and the logarithm of ammonia concentration in the mobile phase. Similarly as in the case of chitosan-cellulose layers, these relationships  $R_M = \text{const} + a p\text{NH}_3$  are represented by straight lines (figs. 6, 7). Slopes of these straight lines may be related to coordination number of cation complex.

The knowledge of behaviour of transition metal cations chromatographed on chitin and chitosan permits us to define possibilities of application of these polymers in the systems purifying wastewater and sea water as well as in the systems serving for recovery of these elements.

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#### MOŻLIWOŚCI UŻYCIA PODŁOŻA CHITYNOWEGO W CHROMATOGRAFICZNEJ KONTROLI ZANIECZYSZCZEŃ ŚRODOWISKA

Opisano proces chromatografii cienkowarstwowej na warstwach chitynowych umożliwiającą rozdział mieszanin prostych jonów metali d-elektronowych. Badano możliwości rozdziału jonów srebra, kadmu, kobaltu, niklu i rtęci przy użyciu buforowych faz ruchomych. Zachowanie się poszczególnych jonów nieorganicznych podczas procesu chromatograficznego pozwoli na wyciągnięcie wniosków odnośnie do mechanizmu rozdziału jonów na chitynie. Badania wykazały, że chityna może być stosowana w procesie chromatograficznym do rozdziału mieszanin jonów na równi z innymi popularnymi fazami stacjonarnymi, takimi jak żel krzemionkowy czy celuloza.

#### ВОЗМОЖНОСТИ УПОТРЕБЛЕНИЯ ХИТИНОВОГО ОСНОВАНИЯ ДЛЯ ХРОМАТОГРАФИЧЕСКОГО КОНТРОЛЯ ЗАГРЯЗНЕНИЙ СРЕДЫ

Описан процесс тонкослойной хроматографии на хитиновых слоях, дающий возможность разделения простых смесей ионов металлов d-элементов. Исследованы возможности разделения ионов серебра, кобальта, никеля и ртути при употреблении буферных подвижных фаз. Поведение отдельных неорганических ионов во время хроматографического процесса позволит сделать выводы относительно механизма разделения ионов на хитине. Проведенные исследования обнаружили, что хитин можно применять в хроматографическом процессе для разделения смесей ионов наравне с другими популярными стационарными фазами, как силикогель или целлюлоза.