CHITIN AND CHITOSAN

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PHYSICAL CHEMISTRY AND FUNCTIONAL PROPERTIES

PHYSICAL PROPERTIES OF CHITIN SHEET FROM LOLIGO PEN

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ABSTRACT

Chitin sheet from Loligo pen gel has better physical properties than that from crab origin. Direct sheet preparations from the jel is economically of greater advantage than nonwoven sheet of short cutting fiber from crab shell. The pen sheet of higher breaking length, 6.9Km and higher bursting factor, 7.4, was obtained by the evacuated paper-making process, which is also shows lower stiffness, relative low permeability O_2 and moisture, a strong affinity for blood proteins and slow biodegradation by lysozyme. Thus the pen sheet seems to be more suitable for temporarily artificial skin.

INTRODUCTION

Chitin is a widely distributed component in nature as well as cellulose. From structural point of view, it can be regarded as a substituent of cellulose with N-acetyl group instead of a hydroxyl group at C_2. Two of the crystal structure, α-chitin(crab shell or tendon) and β-chitin(Loligo pen) are already well known[1,2]. Their x-ray diffraction patterns(Fig.1) and IR spectra(Fig.2) can be distinguished each other. In the present work, the swelling properties of β-chitin, Loligo pen, was noted and related to a possibility of direct sheet preparation to use a biocompatible material, especially, temporarily artificial skin. While the inability of α-chitin, crab shell or tendon, to swell on soaking in water is explained by the crystal

structure of extensive intermolecular hydrogen bonding. Thus, in the α-chitin, we should firstly prepare a fiber from the chitin dope and then make nonwoven sheet from the short cutting fiber. Obviously the process is economically more disadvantageous than that of β-chitin, and also somewhat trouble with the residual organic solvent and the binder.

EXPERIMENTAL SECTION

Purified Loligo pen was disintegrated in small amount of water(3g sample on dry base/50ml H_2O) and then poured into large amount of water(one third of the gel/100ml H_2O) to suspend them and to make testing sheets by evacuated paper-making process for reason of considerably low freeness of the gel. Physical properties were measured by TAPPI standard methods for usual cellulose pulp sheet [3]. Dynamic Young modulas was calculated from a vibrating reed method [4]. Permeability of O_2 or water vapor was measured by JIS methods [5].

RESULTS AND DISCUSSION

Physical properties of the chitin sheets were summarized in Table 1. β-chitin sheet from Loligo pen shows a high bursting factor of 7.4 and breaking length of 6.9Km, compared with those of α-chitin sheet from crab shell, 1.0 and 3.0Km, respectively. While the static Young modulus of the pen sheet is 2.0×10^{-3} Kg/mm^2, which is one fifth of that of the shell sheet. Nevertheless the dynamic Young modulus is about the same in both chitin sheets to show the same rigidity of the chain molecule. Tearing factor of the pen sheet is about the same as that of news paper in the machine direction. The pen sheet has fatigue property of folding endurance superior to that of the shell sheet, in addition, relative low permiability of O_2 or water vapor, 20~40ml/m^2/24hr or 6000~8000gH_2O/cm^2,hr. Furthermore the sheet has a good biological properties which is a strong affinity for blood proteins(adsorption %; albumin 67.5%, fibrinogen 78.6%, globulin 69.5%) and slow biodegradation by lysozyme. The SEM observations clearly showed the difference in the morphology of both chitin sheets. The surface views of the sheets are shown in Figs. 3 and 4. The shell sheet has a well developed and very pronounced microfibril structure of about 100nm width(Fig.3). In contrast, the pen sheet shows a lamellae structure at high magnification regardless of observing a straight array of the fiber at low magnification.

These morphological differences may result in the different physical properties as mentioned above. Thus the pen sheet seems to be more suitable for temporarily artificial skin.

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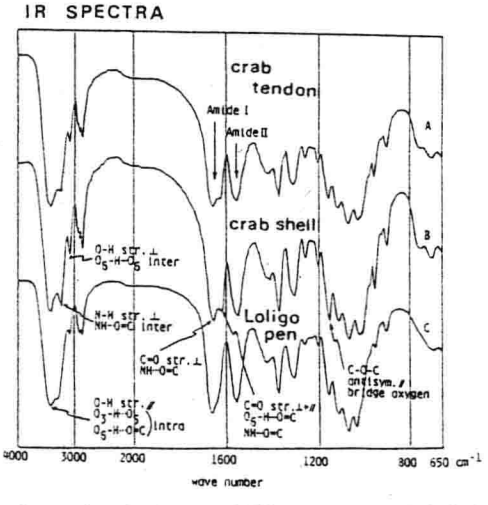

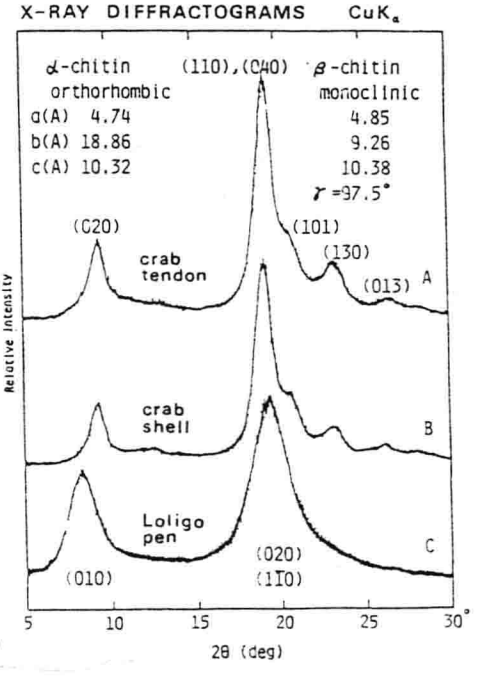

Figs. 1, 2 X-ray diffractograms(right) and IR spectra(left) of chtins, (A)crab tendon, (B)crab shell, and (C)Loligo pen.

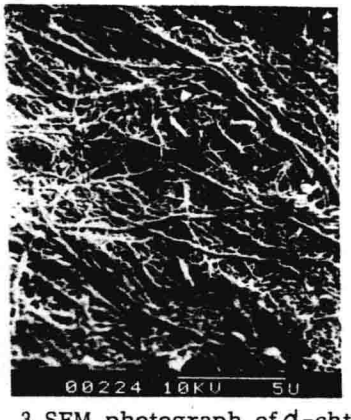

Fig. 3 SEM photograph of α-chtin sheet from crab shell.

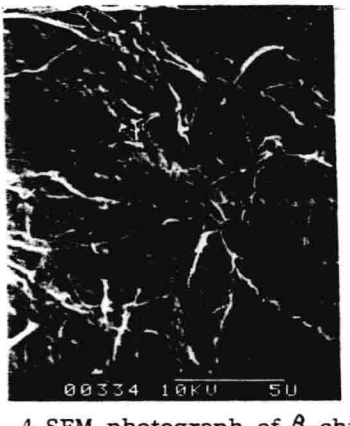

Fig. 4 SEM photograph of β-chitin sheet from Loligo pen.

Table 1. Physical Properties of Chitin Sheets from Loligo pen and Crab Shell Compared with Ohters

Samples	Basis Weight (g/m^2)	Young Modulas (GPa)	Rigidity E'I	Stiffness E'I/W	Breaking Length (Km)	Bursting Factor	Tearing Factor	Permeability O_2 ($ml/m^2.24hr$)	Permeability moisture ($g/cm^2.hr$)	Colony Formation
Loligo Pen	11	9.3	49	4	6.6	5.3	39		7800	–
	22	11.3	271	12	6.9	6.9	37	30	6600	–
	44	8.9	738	17	6.3	7.4	40	35	6100	–
Crab Shell	85	5.1	5620	66	3.0	1.0	-			–
Nonwoven Sheet from crab shell	31	4.3	7600	243	4.1	2.1	96	free	free	+
from collagen	27				4.9	3.2		free	free	+
Bacterial Cellulose	11	50.2	195	17	7.9	5.5				
Pulp Sheet (KP)	50	8.0	2720	36	7.4	5.9	139			
News Paper MD	47	8.6	3150	67	4.6	1.5	42			
CD	47	1.4	523	11	2.0	1.6	67			
Kevler Sheet	50	4.2	2730	55	2.1	1.7	211			
Glass Fiber Sheet	50	9.6	140000	2800	6.3	4.6	376			

CHITOSAN GELS: PART 4. CHITOSAN-BASED THERMALLY REVERSIBLE GELS.

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SUMMARY

Thermally reversible gels have been produced by the addition of suitable co-solutes, principally naphthalene sulphonic acid derivatives, to solutions of chitosan in dilute acetic acid. The principal factor controlling gel melting temperatures (Tm) is the co-solute concentration, whilst chitosan concentration, acetic acid concentration, temperature of mixing or duration of cooling time prior to melting, within the limits examined, have little or no effect on Tm. Gels having Tm values of from 45-85°C have been produced and in all cases sharp transitions were observer. The Tm value for a given gel remains constant over a number of melting/cooling cycles.

Addition of NaCl, NaBr and NaI reduce the value of Tm, the effectiveness of this reduction being $Cl^- < Br^- < I^-$ in the case of gels formed with 1-naphthol-4-sulphonic acid (Na^+) as co-solute and $I^- < Br^- < Cl^-$ in the case of gels with 1-naphthylamine-4-sulphonic acid (Na^+) as co-solute.

INTRODUCTION

Chitosan-based gels have been reported by a number of workers[1-10] but the gels have mainly been thermally stable in character[1-9]. Thermally reversible gelation behaviour has been reported for solutions of chitosan in oxalic acid[10] but gelation times were prolonged, being of the order of 24 hours at room temperature for a 7% (w/v) solution of chitosan in 10% (w/v) aqueous oxalic acid and 500 hours for a 3% (w/v) solution in the same solvent. Hayes and Davis stated that the gels reformed much faster after they had been melted but no figures were given.

The author wishes to report a new type of thermelly reversible gel based on chitosan solutions containing appropriate co-solutes, typically naphthalene sulphonic acid derivatives.

EXPERIMENTAL

Materials

Two samples of chitosan were used during the course of this work:

a) a sample supplied by the Kypro Company (Seattlr, Washington) having[11] 79% deacetylation and $\bar{M}_v = 2.01 \times 10^6$;

b) a sample supplied by Protan Laboratories, Inc. (Redmond, Washington) having[12] 86.8% deacetylation and $\bar{M}_v = 7.46 \times 10^5$.

Both samples were purified as described previously[11] prior to use. The chemicals used were G.P.R. grade and used as supplied.

Preparation of solutions for gelation studies

Aliquots (10 g) of a 1% (w/v) chitosan solution in 0.1M acetic acid were weighed into boiling tubes and the required volumes of acetic acid (0.1M or 1.0M) and distilled water added. The tubes were sealed with Suba-seal caps and heated to 90°C in a waterbath, pressure in the tubes being relieved by insertion of a hypodermic needle. Once the chitosan solution had reached 90°C the required volume of a 0.4M solution of the appropriate gelling agent, also at 90°C, was added and the solution shaken to ensure adequate mixing. A portion of this final mixture was transferred to a screw neck culture tube fitted with cap and PTFE liner, capped and allowed to cool in a waterbath at 20°C for a minimum of 1 hour before measuring the gel melting temperature (Tm).

In the case of samples for investigating the effects of heating times on Tm the sample, after being transferred to the culture tube and capped, was kept at 90°C in the waterbath for the appropriate length of time before being cooled at 20°C.

Measurement of Tm

The capped tube, with the gel in the bottom half, was inverted and attached to a rod then positioned vertically in a 2 litre beaker of water which was heated at a rate of 1°C/minute. Tm was taken as the temperature at which the gel began to flow down the wall of the tube. As the melting transition is quite sharp this was always within 1°C of the temperature at which the first signs of melting could be detected.

RESULTS AND DISCUSSION

The first gel of this type was produced during an attempt to fractionate a chitosan sample by the method of Doczi[13], in which sodium salicylate is used as a precipitating agent. The cooled mixture was found to have formed a pink, opaque gel rather than the expected precipitate of chitosan salicylate and although the gel was soft and had very little mechanical strength, its formation did suggest that reversible gels of more acceptable strengths could be prepared by a suitable choice of co-solute.

Initial studies

a) The first suitable co-solute examined was the sodium salt of 1-naphthol-4-sulphonic acid (NSA) which was found from preliminary tests to form firm gels having sharp melting temperatures in the approximate temperature range 50-85°C. The effect on Tm of variation in NSA concentration at several concentrations of chitosan was examined and the results are given in Figure 1. This shows that for chitosan concentrations in the range 2.0-5.0 g.dm^{-3}, and at a constant ratio of acetic acid: chitosan, the Tm value is independent of the chitosan concentration and depends only on the NSA concentration. However one effect of decreasing the

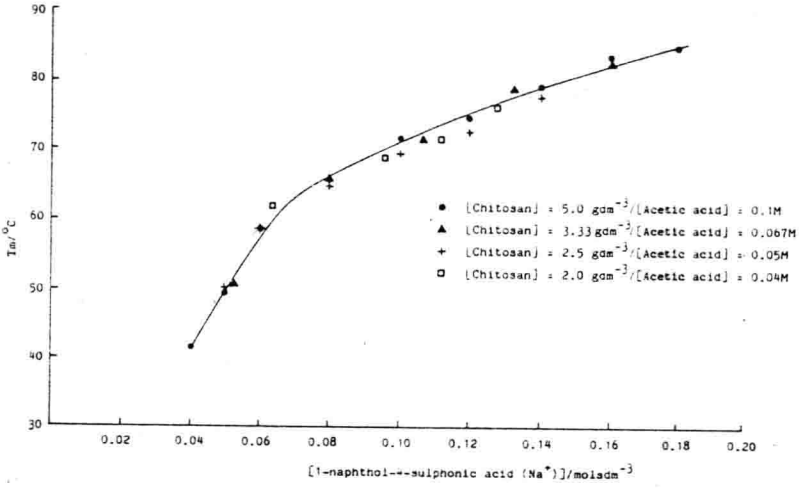

Figure 1. Gel melting temperatures as a function of NSA concentration at constant chitosan:acetic acid ratio.

chitosan concentration is that there is a decrease in the NSA concentration at which, on mixing, a precipitate is formed which does not dissolve on continued heating at 90°C. Since the main factor controlling Tm is the NSA concentration this means that the highest Tm value obtainable decreases with decreasing chitosan concentration.

b) The second co-solute examined in detail was the sodium salt of 1-naphthylamine -4-sulphonic acid (NASA) and the results are given in Figure 2. This shows that the Tm values depend to some extent on the chitosan concentration, the results for 5.0 and 3.3 g.dm^{-3} coinciding as do those for 2.5 and 2.0 g.dm^{-3}. Again the results plotted in Figure 2 are for mixtures having a constant ratio of acetic acid: chitosan.

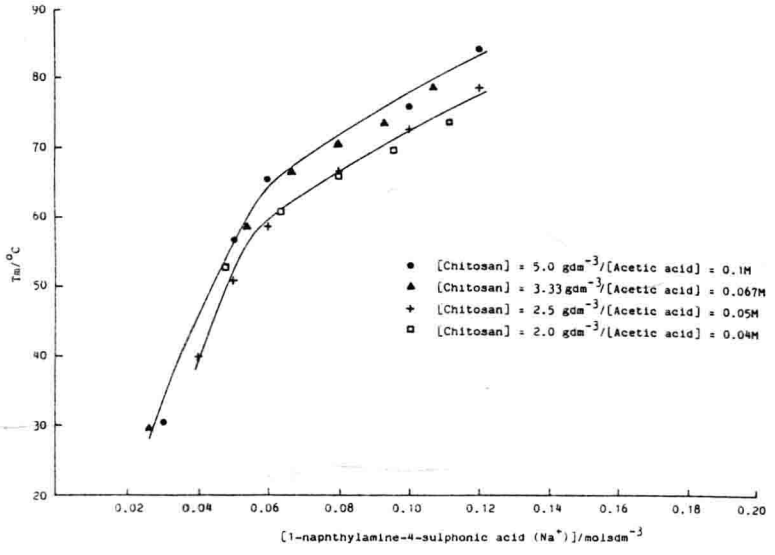

Figure 2. Gel melting temperatures as a function of NASA concentration at constant chitosan:acetic acid ratio.

Variation in chitosan concentration

The effect of varying the chitosan concentration whilst retaining both the acetic acid concentration and the co-solute concentration constant was studied for both NSA and NASA gels and the results are given in Table 1. Those for NSA-

Table 1. Effect on Tm of variation in chitosan concentration at constant acetic acid concentration and of variation in acetic acid concentration at constant chitosan concentration for both NSA and NASA gels.

[Chitosan] $g.dm^{-3}$	[NSA] $mol.dm^{-3}$	[Acetic acid] $mol.dm^{-3}$	Tm °C	[NASA] $mol.dm^{-3}$	[Acetic acid] $mol.dm^{-3}$	Tm °C
5.0	0.12	0.1	57.5	0.12	0.07	57.0
3.5	0.12	0.1	58.5	0.12	0.07	57.0
2.5	0.12	0.1	57.0	0.12	0.07	53.0
2.0	0.12	0.1	50.5	0.12	0.07	53.5
5.0	0.1	0.075	52.5	0.1	0.05	52.5
5.0	0.1	0.125	52.0	0.1	0.10	52.0
5.0	0.1	0.225	50.0	0.1	0.20	50.5
5.0	0.1	0.325	48.5	0.1	0.30	50.0

induced gels confirm that Tm is independent of chitosan concentration over the range 5.0-2.5 $g.dm^{-3}$ but that there is a considerable drop in Tm on reducing the chitosan concentration further to 2.0 $g.dm^{-3}$. However this concentration is on the borderline for gel formation; on reduction to 1.6 $g.dm^{-3}$ chitosan no gel was formed at the same NSA concentration.

The results for the NASA gels also confirm the previous conclusion that there is some dependence of Tm on chitosan concentration, the results for 5.0 and 3.5 $g.dm^{-3}$ being in agreement, as are the results for 2.5 and 2.0 $g.dm^{-3}$.

Variation in acetic acid concentration

Variation in acetic acid concentration, with constant chitosan and co-solute concentrations, was studied for both co-solutes (Table 1). Both show a trend towards lower Tm values with increasing acid concentration.

Variation in time of cooling

Aliquots of a chitosan/NSA solution were cooled at 20°C for times ranging from 1 - 6 hours prior to determining Tm. No dependence on cooling time was observed, all the gels melting within a 1°C range.

Variation in temperature of preparation

Chitosan/NSA solutions were prepared at 5°C intervals between 70° and 90°C, then cooled for 1 hour at 20°C. The Tm values obtained showed no dependence on the preparation temperature within the range examined.

Variation in heating time at 90°C

A chitosan/NSA solution was prepared at 90°C, aliquots transferred to tubes, then heated at 90°C for varying times before being cooled at 20°C and the Tm values measured. The results below show that there is an increase in Tm on heating for 15 minutes after mixing but that no further increase occurs on extending the heating period to up to 120 minutes. This behaviour suggests that

Time of heating/mins	0	15	30	60	120
Tm/°C	55.5	58.5	58.0	58.5	58.0

the initial heating period allows the formation of a more uniform solution and hence a more uniform gel, resulting in a higher Tm value, with no further improvement in uniformity on extending the heating period.

Repeatability of Tm

Three chitosan/NSA gels were put through a series of melting/cooling cycles to determine the constancy of Tm. The results show that Tm remains constant over a number of such repeat cycles.

Cycle number	1st	2nd	3rd	4th
Tm/°C - Sample 1	48.5	49.0	48.0	48.5
Tm/°C - Sample 2	52.5	52.0	51.5	52.5
Tm/°C - Sample 3	57.0	56.5	57.0	-

Addition of electrolyte

Although the mechanism of gelation in these systems has not been established it is reasonable to assume that electrostatic interaction between the sulphonic acid groups and the protonated amine groups of the chitosan is involved. The effect of adding electrolytes which could compete with the naphthalene sulphonic acid anions for the cationic groups on the chitosan was therefore investigated.

The effect of a gradual increase in electrolyte concentration on Tm was determined using NaCl, NaBr and NaI with both NSA and NASA gels. The results (Figures 3 and 4) show that both gel systems exhibit a gradual decrease in Tm with increase in electrolyte concentration.

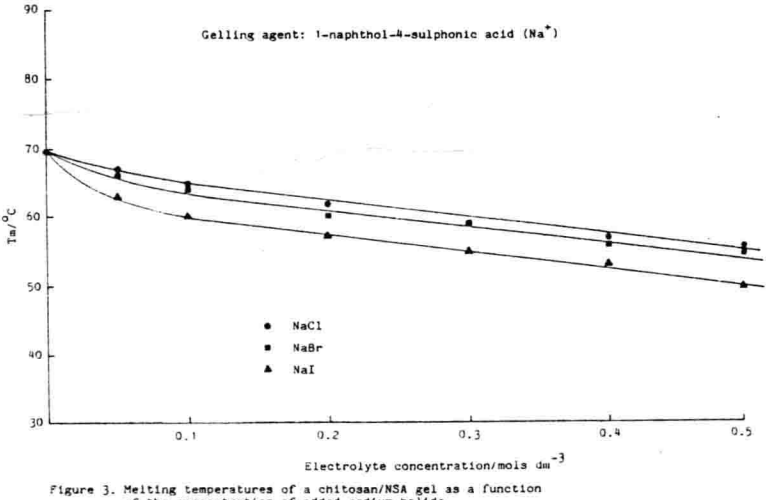

Figure 3. Melting temperatures of a chitosan/NSA gel as a function of the concentration of added sodium halide.

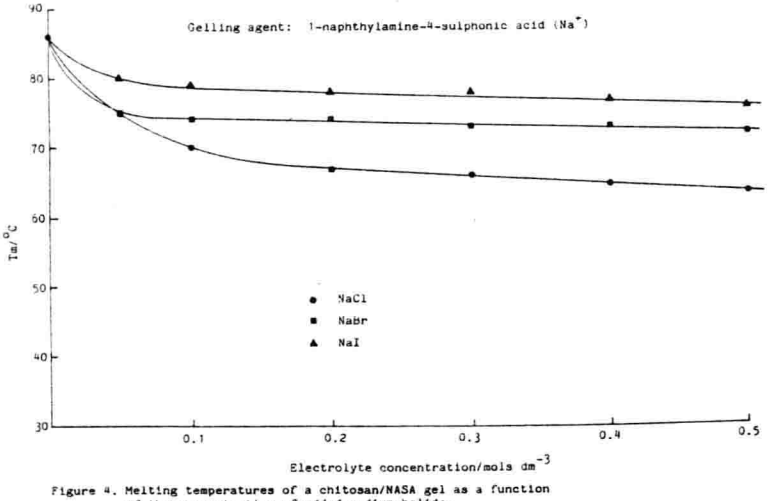

Figure 4. Melting temperatures of a chitosan/NASA gel as a function of the concentration of added sodium halide.

However the order of effectiveness in reducing Tm is $Cl^- < Br^- < I^-$ in the case of NSA-based gels but $I^- < Br^- < Cl^-$ NASA-based gels. If the halide anions are competing for the protonated amine groups according to the equilibrium

$$-\overset{+}{N}H_3\ \overset{-}{O}_3S\text{-}Ar + X^- \rightleftharpoons -\overset{+}{N}H_3\ X^- + Ar\text{-}SO_3^-$$

the order of effectiveness is the same in the case of the NSA gels as the order of affinity for the $-NH_3^+$ group through the operation of water-structure enforced ion pair formation, first proposed by Diamond[14]. No explanation can be given for the reversal of this order with NASA-based gels but may arise from the fact that the NASA itself contains a protonated amine group that could also interact with the halide anions. Further work is being carried out on this.

ACKNOWLEDGEMENTS

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DIFFERENT COPPER(II)-BINDING ABILITY OF AMINO SUGARS

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ABSTRACT

Potentiometric and spectroscopic studies have shown that D-glucosamine, D-galactosamine, D-mannosamine as well as methyl 2-amino-2-deoxy-D-glucopyranoside are very effective chelators of copper(II) ions. The stability constants for the complexes with D-mannosamine are distinctly higher than those with the other amino sugars. The primary binding site is the amino group for all the ligands, while the most effective secondary sites are the deprotonated hydroxyls C(1)-OH (for glucosamine and galactosamine) or C(3)-OH (in mannosamine). The comparative examination of the data suggests that, depending on the position, the different hydroxyl functions can be involved in the metal ion binding.

INTRODUCTION

According to literature (1-8), amino sugars and their polymeric derivatives, chitosan and chitin, are very efficient ligands forming stable complexes with metal ions. Therefore, their binding properties may be important in nature, e.g., for chelation of essential and toxic elements as well as in industrial applications, e. g., for the removal of metal ions from aqueous solutions. It is generally accepted that simple amino sugars bind copper(II) with the amino group as a basic donor, while the second donor is one of the hydroxyl groups. However, almost no information concerning the involved hydroxyl groups is available.

We report here a study on the complexes formed in aqueous solution by copper(II) with D-glucosamine, D-galactosamine and D-mannosamine, hereafter abbreviated as GLUAM, GALAM and MANAM, respectively. The coordination behaviour of methyl 2-amino-2-deoxy-D-glucopyranoside (Met-GLUAM) also has been examined.