within membranes, the faster transport indicates that copolymer hydrogel membrane contains the larger amount of water than poly (HEMA) membrane does. This result is not due to the higher water affinity of AEMA including amino group than that of HEMA including hydroxy group, but due to the lower degree of aggregation of copolymer hydrogel membrane than that of poly (HEMA) membrane. So one may expect that the copolymerizing HEMA and AEMA produce the more porous part in the hydrogel.

Acknowledgement. One of the authors (M. S. Jhon) expresses appreciation for financial support to Korea Research Center for Theoretical Physics and Chemistry.

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Mechanism of Metal Ion Binding to Chitosan in Solution. Cooperative Inter- and Intramolecular Chelations

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(Receivd September 15, 1983)

Interactions between metal ions and chitosan in solution were studied by spectroscopic and viscometric measurements. Cu^{++}-chitosan complex exhibited an absorption band at 265 nm, whereas D-glucosamine compex showed one at 245 nm. The difference in λ_{max} was attributed to the different amine to Cu^{2+} ratios of the complexes, that is, 2: 1 for chitosan and 1:1 for D-glucosamine. The molar absorptivities and binding constants of the complexes were evaluated. The binding of Cu^{2+} to chitosan was cooperative near pH 5, and both intra- and intermolecular chelations depending on chitosan and Cu^{2+} concentrations were observed, The intermolecular chelation was stabilized by addition of salts. The cooperative intermolecular chelation of Ni^{++} was also observed at pH 6.2. No significant binding of other divalent ions was observed. The reported high adsorption abilities of chitosan particles for these ions were attributed to the deposition of metal hydroxide aggregates in pores of chitosan particles rather than chelation to amine groups.

Introduction

Chitosan is the deacetylated product of chitin, poly-(N-acetyl-D-glucosamine), which is the most abundant naturally occuring polysaccharides containing amino sugars. Recently, chitin and chitosan have drawn a great amount of interests, because of their wide range of applicabilities.[1] The chelation of various metal ions by chitosan has demonstrated the effectiveness of the polymer in inorganic chromatography and in heavy metal removal from polluted water. Muzzarelli has given excellent summaries of works in this field.[2,3] The adsorption (chelation) ability of chitosan for various metal ions was reported to be high, but it was shown that the ability depends on the method of preparation of chitosan sample, and is not directly proportional to the degree of deacetylation of chitin.[4] Masri *et al.*[5] also have shown that the relative adsorption ability of chitosan for different metal ions is significantly different from that of poly-(p-aminostyrene).

Despite of extensive studies on interaction of metal ions with chitosan, there is a paucity of data as to how they in-

teract. This seems to arise from lack of fundamental information on the interaction properties between metal ions and chitosan. Since most of studies were made on chitosan films and/or solid suspension, the crystallinity of sample, temperature, stirring rate, etc. might have greatly affected the result. In a previous paper[6], we reported the result of acid–base titration of chitosan solution in the presence of Cu^{2+}, which implied that the metal ion binds to chitosan cooperatively. Thus, we thought that studies in solution could provide valuable information on the nature of the interaction between metal ions and chitosan. This paper deals with spectroscopic and viscometric studies in this respect.

Experimental Methods

Chitosan, obtained from Tokyo Kasei, was purified and chitosan solutions were prepared as described in our previous paper.[6] The pH of solutions were adjusted with 0.01 *M* cacodylate buffer unless otherwise specified. UV spectra were recorded at ambient temperature using cells of 1 cm light path length with a Gilford 2600 spectrophotometer, and viscosity of solutions were measured at 25 °C using an Ostwald type viscometer. The concentration of amino group of chitosan was calculated from average equivalent weight of 253 based on 64 % deacetylation[6] of chitin.

Results and Discussions

Spectroscopic Studies. Chelation of Cu^{2+} by chitosan showed two absorption bands near 265 nm and 700 nm. While the molar absorptivity of 700 nm band is less than 100 M^{-1} cm^{-1}, that of 265 nm band was ca. 4000. Thus, chelation of Cu^{2+} by chitosan was followed in UV region. In Figure 1, spectra of chitosan (0.5 mg/m*l*), cupric ion (5×10^{-4} *M*) and the mixture containing both chitosan and Cu^{++} (0.5 mg/m*l* chitosan and 5×10^{-4} *M* Cu^{2+}) at pH 5.5 are shown. The difference spectrum of Cu^{2+}–chitosan chelate, calculated from the absorbance of the mixture and its components, is also included. The shape and position of the difference spectrum were virtually unchanged with variation of pH and Cu^{2+} to chitosan ratio. The absorption band of the Cu^{2+} complex involving amine group as a ligand usually appears near 265 nm,[7,8] and thus the 265 nm band of the difference spectrum can be attributed to a charge transfer transition involving amine group of chitosan and metal ion, in analogy to 237 nm band of Cu^{2+}–carboxylate complexes.[7] Cu^{2+} chelation by D-glucosamine, monomer of chitosan, exhibited an absorption band positioned at 245 nm rather than 265 nm. The difference in spectral position between D-glucosamine-Cu^{2+} complex and chitosan-Cu^{2+} complex supports our previous proposal of stoichiometries of the complexes, that is, 2:1(amine to Cu^{2+}) for chitosan and 1:1 for D-glucosamine.[6]

Since the chelation of Cu^{++} by amine group of chitosan competes with protonation of the amine group, the concentration of Cu^{2+} chelated is expected to depend on pH. In Figure 2, the variations of absorbance of the difference spectra (ΔA) of D-glucosamine-Cu^{2+} and chitosan-Cu^{2+} mixtures with pH are shown, and the results are compared

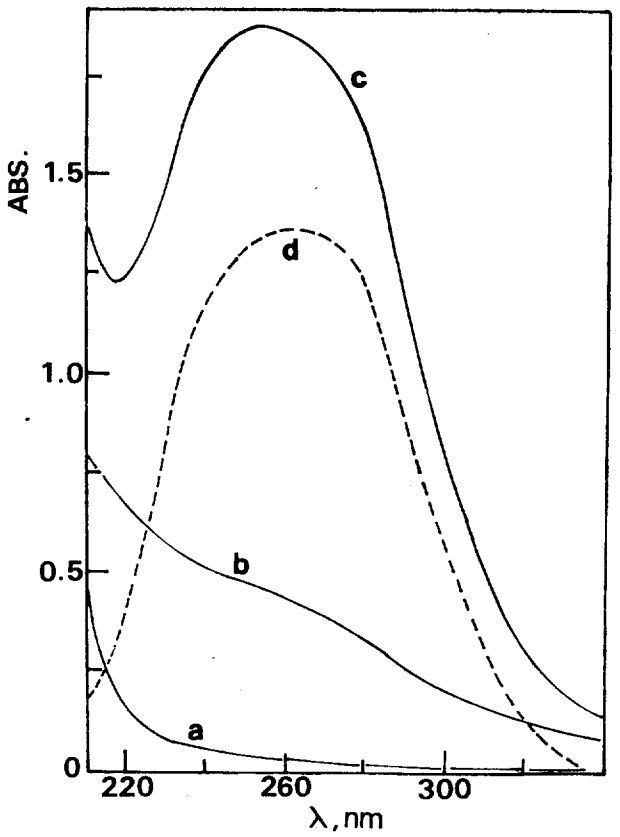

Figure 1. Absorption spectra of 0.5mg/m*l* chitosan(a), 5×10^{-4} *M* Cu^{2+}(b), and 0.5 mg/m*l* chitosan–5×10^{-4} *M* Cu^{2+} mixture (c) at pH 5.5. (d) is the difference spectra calculated by c–a–b.

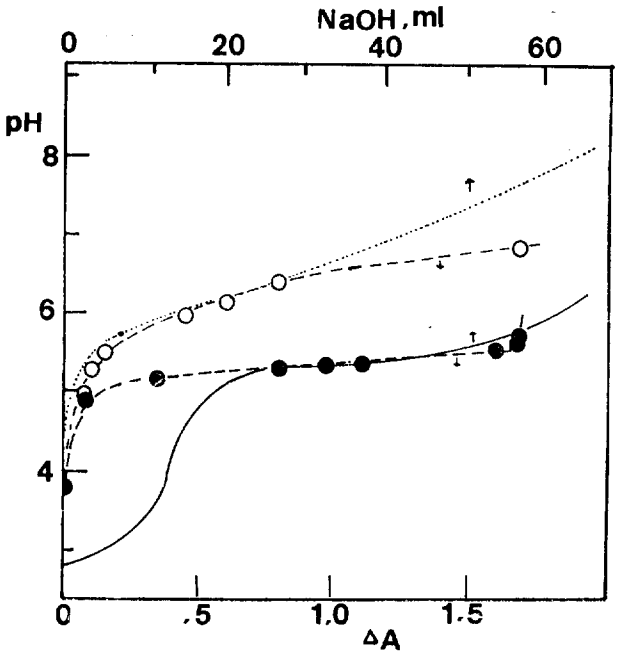

Figure 2. pH dependence of absorbances of the difference spectra of 0.5mg/m*l* chitosan–5×10^{-4} *M* Cu^{2+} at 265 nm (○), and of 0.5 mg/m*l* D-glucosamine–HCl–5×10^{-4} *M* Cu^{2+} at 245 nm (●). Titration curves of 50 m*l* of 2 mg/m*l* chitosan (——) and 2mg/m*l* D-glucosamine–HCl (·····) in the presence of 2×10^{-3} *M* Cu^{2+} with 0.01 *M* NaOH are taken from Ref. 6 and included for comparison.

with titration curves[6] of the corresponding mixtures. This figure reveals the parallelism between the amounts of $-NH_3^+$ group titrated (amounts of base used) and Cu^{2+} chelated (ΔA). The small difference in pH's, at which $-NH_3^+$ is titrated and Cu^{2+} is chelated, can be attributed to the difference in concentrations of Cu^{2+} and the amino sugar in the two sets of experiments. The chelation of Cu^{2+} by chitosan in narrow pH range, compared to D-glucosamine, confirms our previous proposal[6] of cooperative binding of Cu^{2+} to the polymer. The variations of ΔA (amount of Cu^{2+} chelated) at given pH's with ligand (D-glucosamine or chitosan) concentration exhibited typical binding curves (Figure 3). The molar absorptivities of Cu^{2+} complexes calculated from saturated absorbance values and total Cu^{2+} concentrations were $3.2\times10^3\ M^{-1}\ cm^{-1}$ for D-glucosamine at pH 6.3, and $4.0\times10^3\ M^{-1}\ cm^{-1}$ for chitosan at pH 5. 8.

In contrast to Cu^{2+}, addition of other divalent cations such as Mg^{2+}, Ca^{2+}, Zn^{2+}, Ni^{2+}, $Co^{2+}$and Cd^{2+} to chitosan solution of pH$<$5.8 and to D-glucosamine solution of pH$<$ 6.3 does not show noticeable spectral change above 240 nm. The binding strength of these ions, however, can be estimated from the decrease of absorbance of Cu^{2+}–ligand complexes (ΔA) by addition of these ions to Cu^{2+}–ligand solutions: binding of added ions to the ligand shifts Cu^{2+}–ligand equilibrium resulting dissociation of Cu^{2+}–ligand complexes, which manifests itself in the decrease of ΔA.[9] The decreases of $(\Delta A)_{265\,nm}$ of $2.0\times10^{-4}\ M\ Cu^{2+}$ $-4.0\times10^{-4}\ M$ chitosan solutions caused by the presence of $2.0\times10^{-4}\ M$ metal chlorides at pH 5.8 were 19.6 % (from 0.556 to 0.498) for Co^{2+}, 9.9 % for Cd^{2+}, 5.2 % for Zn^{2+}, 5.0 % for Ni^{2+}, 4.0 % for Mg^{2+} and 2.6 % for Ca^{2+}.

The absorbance value of 0.556 of 2.0×10^{-4} Cu^{2+} $-4.0\times10^{-4}\ M$ chitosan solution at pH 5.8 corresponds to 1.39×10^{-4} M Cu^{2+}–chitosan complex, and thus uncomplexed Cu^{2+} and amine group concentration are 0.61×10^{-4} and 1.22×10^{-4} M, respectively. The concentration of unprotonated amine group, $[RNH_2]$, is related to total concentration of amine group by the following equation:

$$[RNH_2]=\frac{Ka}{Ka+[H^+]}[RNH_2]_{total}$$

The pKa of $-NH_3^+$ group of chitosan was reported to be 6.1,[6] and the apparent binding constant of Cu^{2+} to a pair of amine groups of chitosan, expressed as $K_{app}=[Cu^{2+}]_{bound}/([Cu^{2+}]_{free}[RNH_2]/2)$, was found to be $1.1\times10^5\ M^{-1}$ in the above mentioned condition. Since binding of Cu^{++} to chitosan is cooperative, K_{app} value depends strongly on Cu^{2+} to chitosan ratio giving larger values as Cu^{2+} to chitosan ratio increases. However, K_{app} value of 1:1 Cu^{++}–D-glucosamine complex formation was found to be $1.1\times10^4\ M^{-1}$, virtually independent of Cu^{2+} to D-glucosamine ratio.

If one assumes the decrease in ΔA of Cu^{2+}–chitosan complex caused by addition of other ions is solely due to dissociation of Cu^{2+}–chitosan complex by competition of Cu^{2+} and added ions to common binding site, RNH_2, the apparent bindig constant of added ions to chitosan can be estimated by the method reported[9]. The ΔA value of 0.498 of $2.0\times10^{-4}\ M\ Cu^{2+}$ $-4.0\times10^{-4}\ M$ chitosan $-2.0\times10^{-4}\ M\ Co^{2+}$ solution corresponds to the ΔA of 2×10^{-4} M Cu^{2+} $-3.63\times10^{-4}\ M$ chitosan solution in the absence of Co^{2+}. This suggests that $0.37\times10^{-4}\ M$ of chitosan is complexed to Co^{2+}. The apparent binding constant of Co^{2+} to chitosan evaluated from this data was 5.5×10^3. As evident from smaller decrease in ΔA for other ions, the binding constants of other ions to chitosan are expected to be much smaller than 5.5×10^3. These results are in good agreement with much higher stability of Cu^{2+}–D-glucosamine complex compared to other divalent metal ion–D-glucosamine complex.[10] Therefore, the reported strong adsorption ability[2,3] of solid chitosan particle to various metal ions studied in this work except Cu^{2+} cannot be ascribed to chelation of the metal ions by amine groups of chitosan. The deposition of metal hydroxide aggregates as speculated by Eiden *et al.*[11] from ESCA studies on interaction of Pb^{2+} with chitin and chitosan can explain the observed high adsorption ability of chitosan particles for the metal ions. The metal hydroxide aggregates may deposit in pores of chitosan particles in similar fashion to capillary condensation of vapor. This mechanism can also successfully explain the sensitivity of adsorption ability and rate on sample crystallinity, stirring rate, temperature, *etc.*

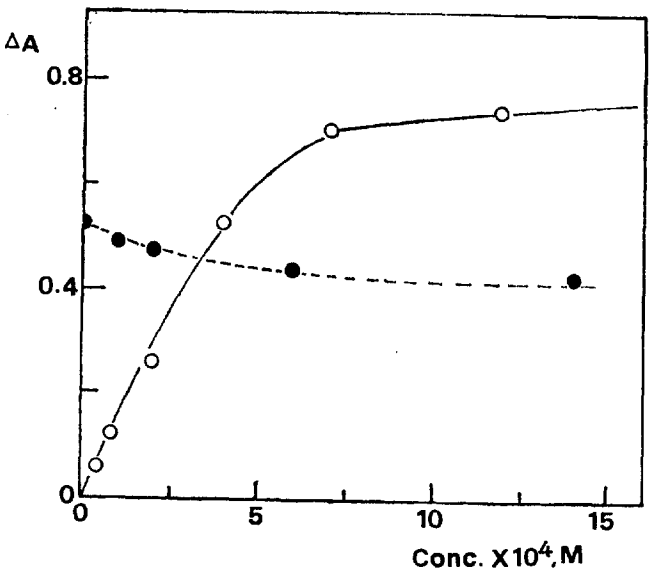

Figure 3. Variation of $(\Delta A)_{265nm}$ of $2\times10^{-4}\ M\ Cu^{2+}$ with concenrtation of chitosan at pH 5.8 (○) and of 2×10^{-4} M Cu^{2+}–$4\times10^{-4}\ M$ chitosan mixture with concentration of Co^{2+} (●).

Effect of Metal Ion Chelation on Viscosity. Changes in hydrodynamic volume and interaction properties associated with metal ion binding can be studied viscometrically. These studies are expected to provide information regarding the nature of metal ion–chitosan interaction. Figure 4 shows pH dependence of specific viscosity $(\eta_{solution}/\eta_{solvent}-1)$ of 1 mg/ml $(4.0\times10^{-3}M)$ chitosan solutions in the presence of 2.5×10^{-3} M Cu^{2+} and Mg^{2+} at 25 °C. In the presence of Mg^{2+}, η_{sp} of chitosan solution decreases only slightly with increasing pH, that is, more deprotonation of $-NH_3^+$ groups. This behavior agrees with our previous report,[6] which showed the independence of η_{sp} on pH at high NaCl concentration due

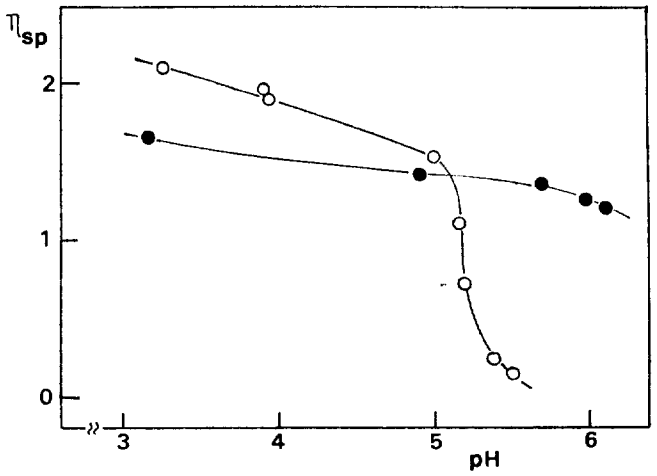

Figure 4. Specific viscosities of 1 mg/m*l* chitosan solutions in the presence of $2.5\times10^{-3}M$ Cu^{2+} (○) and Mg^{2+} (●) at 25 °C.

to effective charge shielding by anions. On the other hand, η_{sp} in the presence of Cu^{++} decreases remarkably near pH 5. This pH range coincides well with the pH of cooperative chelation of Cu^{2+} to chitosan observed by titration and UV absorption measurements (Figure 2). Thus, the η_{sp} decrease can be attributed to intramolecular chelation of Cu^{2+} by chitosan in the experimental condition. Intramolecular chelation seems to reduce chain flexibility and change orientation of glycosidic linkages, which lead to reduced hydrodynamic volume, and thus viscosity of chitosan solution.

Besides intramolecular chelation, intermolecular chelation is also possible, especially at high chitosan concentration. The viscosities of 4 mg/m*l* chitosan solution with various concentration of Cu^{2+} (pH 5.0 and 3.5) and Mg^{2+}(pH 5.0) are plotted in Figure 5. This figure shows that η_{sp} of 4 mg/m*l* chitosan solution decreases slightly with addition of Cu^{2+} at pH 3.5 and Mg^{2+} at pH 5.0 reflecting increased ionic strength of solution. However, addition of Cu^{2+} at pH 5.0 decreases η_{sp} initially, but increases η_{sp} at high $[Cu^{2+}]$. This result can be interpreted as intramolecular chelation at low $[Cu^{2+}]$ and intermolecular chelation at high $[Cu^{2+}]$. Interestingly, the increase in η_{sp} was found to be closely related to deepening of blue color of solutions at high Cu^{2+} and chitosan concentrations. The amine to Cu^{2+} ratio may not be same in intra-and intermolecular chelations. Involvement of two adjacent amine groups to a Cu^{2+} ion for intramolecular chelation, and two pairs of amine groups (each pair from different chitosan chain) to a Cu^{2+} ion for intermolecular chelation can be proposed. For given $[Cu^{2+}]$ and chitosan concentrations at which intermolecular chelation is possible, the viscosity of the solution was observed to increase with addition of salts, such as NaCl, $MgCl_2$ and $CaCl_2$. For example, when concentration of $MgCl_2$ was higher than $1\times10^{-3}M$, $1\times10^{-3}M$ Cu^{2+} −4 mg/m*l* chitosan solution at pH 5.0 formed gel. Although $CaCl_2$, $MgCl_2$ and NaCl do not bind to chitosan directly, these salts seem to promote intermolecular chelation of Cu^{2+} resulting gel. This result can be interpreted by reduced electrostatic repulsion between chitosan chains by association of counter ions (Cl^-), enhancing intermolecular chelation of Cu^{2+}.

Figure 6 shows viscosities of 4 mg/m*l* chitosan solutions containing Cu^{2+}, Ni^{2+} and Mg^{2+} at various pH values. This figure demonstrates that the intermolecular chelations,

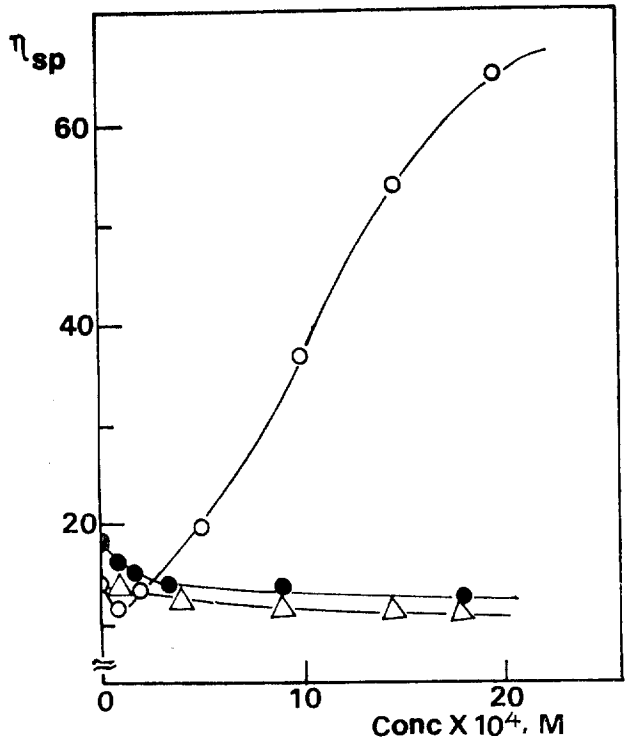

Figure 5. Variation of specific viscosities of 4 mg/m*l* chitosan with added metal salts: Cu^{2+} at pH 5.0 (○); Mg^{2+} at pH 5.0 (△); Cu^{2+} at pH 3.5 (●).

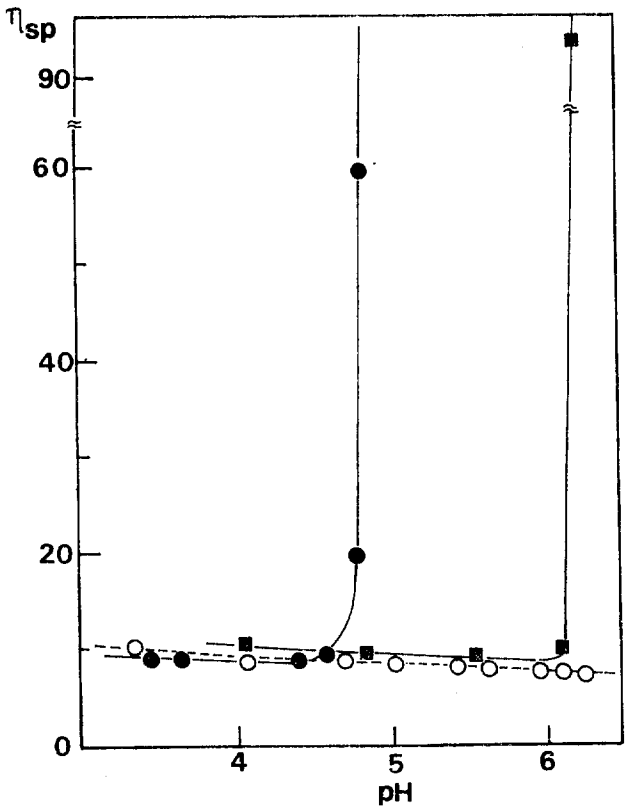

Figure 6. Variation of specific viscosities of 4 mg/m*l* chitosan solutions containing $2\times10^{-3}M$ metal ions with pH: Cu^{2+} (●); Mg^{2+}(○); Ni^{2+}(■).

revealed as sharply increased η_{sp}, also take place cooperatively at narrow pH range, 4.7 for Cu^{2+} and 6.2 for Ni^{2+}. The absence of abrupt η_{sp} change for Mg^{2+} indicates that the metal ion does not bind to chitosan in the experimental pH range. Other metal ions which form amine complexes are expected to chelate to chitosan at appropriate pH's. However, precipitation of chitosan and metal hydroxides at higher pH prevented the study. The difference in chelating properties among metal ions suggests that separation of a mixture of metal ions into its components is possible by ion chromatography or dialysis using chitosan gel matrix with variation of pH of eluent or medium. The chitosan gel suitable for this purpose can be prepared by partial N-acylation[12].

Acknowledgement. This work was supported by the Korea Science and Engineering Foundation. The authors gratefully acknowledge the support.

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The Pressure Effect on the Ionic Association of the 3, 5, N-trimethyl Pyridinium Iodide in Ethanol-Water Mixture

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The association constants (K) of 3,5,N-trimethyl pyridinium iodide in 95 volume percent ethanol-water mixed solvent were determined by a modified UV and conductance method at 25°, 30°, 40° and 50°C over the pressure range 1 to 2000 bars. The association process is enhanced with increasing pressure and decreasing temperature. From K values, we obtained the total partial molar volume change (ΔV) and some thermodynamic parameters. The electrostriction volume (ΔV_{el}) and intrinsic volume (ΔV_{in}) were also evaluated. The values of ΔV, ΔV_{el}, ΔV_{in} are negative, negative and positive, respectively, and the absolute values of all these three decrease with increasing pressure and temperature. The ion-pair size (a) were varied 3 to 6 Å with pressure and temperature. The solvation number (n) decreased from 2 to 0.5 with increasing temperature.

Introduction

In 1955, Kosower[1,2] showed that N-methyl pyridinium iodide (NMPI) made an ultraviolet charge transfer band in solution. This band supposedly arises due to the transfer of electron density from the highest occupied orbital of iodide to the lowest unoccupied orbital of the N-methyl pyridinium cation.

Recently, the thermodynamic investigation of the association process of NMPI in ethanol-water mixture was discussed by Paul Hemmes *et al.*[3]

Hitherto few pressure studies have been undertaken to analyze the electrostatic aspects of the dissociation process of ion pairs.[4,5,6]

We studied the pressure effect on the association of 2, N-dimethyl pyridinium iodide in 95 volume percent ethanol-water mixed solvent in the previous paper.[7]

In this report, we want to discuss the pressure dependence