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ORIGINAL CONTRIBUTION

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# Dielectric analysis of chitosan microsphere suspensions: study on its ion adsorption

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**Abstract** The dielectric properties of chitosan microsphere beds in aqueous electrolyte solutions were investigated in a frequency range of 1–500 MHz. Distinct dielectric relaxation was observed around 10 MHz in weakly acidic solutions (pH 4–6), and the relaxation intensity depended on electrolyte concentration. The dielectric relaxation was analyzed on the basis of interface polarization, and the relative permittivity and conductivity of the microsphere interior were estimated from the relaxation parameters using Hanai's mixture equation. The internal conductivity of the microspheres was much higher than the conductivity of the external medium, and was attributed to counterions accumulated around fixed charges of chitosan and unknown ion conduction mechanisms, for example, protonic transport through the ionized sites. The adsorption abilities of the microspheres were also examined for $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, and $La^{3+}$ ions, which showed that $Cu^{2+}$ ions are more tightly bind to chitosan than the other ions.

**Keywords** Chitosan · Microsphere · Dielectric relaxation · Interface polarization · Ion adsorption

## Introduction

Recently, controlled-release technology has rapidly developed in various fields, and its related products now span medical, agricultural, and biochemical applications [1]. One of the key points in the applications is how to evaluate the ability to retain and release target materials. Chitosan, poly(1,4-β-D-glucopyranosamine), has many potential applications in controlled-release technology because it is a ubiquitous biopolymer and can adsorb various materials. Although the properties of chitosan have been investigated by several methods [2, 3, 4], a few studies have been reported for its adsorption properties, all of which have been done using chitosan films. In practical applications, chitosan microspheres in suspension may have advantages over the films because they have huge surfaces that can efficiently adsorb materials, for example, Nigalaye et al. [5] reported the sustained release of theophylline, as a target drug, from chitosan tablets.

When target drugs are ions, dielectric spectroscopy is available for exploiting the interactions between ions and chitosan matrices, which are important for controlled release of ions. Dielectric spectroscopy has been successfully applied to some particles in suspension, such as ion-exchange beads [6, 7, 8] and microcapsules [9]. Although dielectric spectroscopy has been used for analyzing chitosan solutions, as polyelectrolytes, [10] and solid films [2], chitosan microspheres in suspension have never been studied by dielectric spectroscopy. In order to evaluate the adsorptive activity of some ions on chitosan particles, we studied the dielectric behavior of chitosan microspheres in electrolyte solutions at various pH and concentrations.

## Theory

A microsphere having a homogeneous inner material suspended in a medium can be modeled as follows.

Microspheres having equivalent complex permittivity, $\varepsilon_i^*$, are dispersed in a continuum phase of $\varepsilon_a^*$ at volume fraction $\Phi$. The complex permittivity is defined as $\epsilon^* = \epsilon - j\kappa/\omega\epsilon_0$, where $\epsilon$ and $\kappa$ are the relative permittivity and the conductivity, respectively, $\epsilon_0$ is the permittivity of a vacuum ($\epsilon_0 = 8.8541$ pF/m), and $\omega$ is the angular frequency ($\omega = 2\pi f$, $f$ is the frequency). For a concentrated suspension of spherical particles, the complex permittivity of the whole system, $\varepsilon_s^*$, is given by Hanai [11, 12]:

$$\frac{\varepsilon_s^* - \varepsilon_i^*}{\varepsilon_a^* - \varepsilon_i^*}\left(\frac{\varepsilon_a^*}{\varepsilon_s^*}\right)^{1/3} = 1 - \Phi. \tag{1}$$

This equation predicts a dielectric relaxation with dielectric parameters $\epsilon_l$, $\kappa_l$, $\epsilon_h$ and $\kappa_h$, where the subscripts l and h refer to the low- and high-frequency limits, respectively.

By separating the real and imaginary parts of Eq. (1), Hanai [11, 13] derived relations between the phase parameters ($\epsilon_i$, $\kappa_i$, $\epsilon_a$, $\kappa_a$, $\Phi$) and the dielectric parameters ($\epsilon_l$, $\kappa_l$, $\epsilon_h$, and $\kappa_h$ ). Since $\epsilon_a$ is usually known, we can determine the phase parameters $\epsilon_i$, $\kappa_i$, $\kappa_a$, and $\Phi$ from the observed dielectric parameters $\epsilon_l$, $\kappa_l$, $\epsilon_h$, and $\kappa_h$. Hanai et al. [14] further rearranged the relations to obtain the following relations favorable to analysis with a computer:

$$\begin{aligned} J(\kappa_a) \equiv {} & \left[3 - \left(2 + \frac{\varepsilon_a}{\varepsilon_h}\right)C\right](1 - DC)\kappa_h \\ & - 3\{\kappa_l - [\kappa_a(D-1) + \kappa_l]C\}(1 - C) \\ & + \kappa_a\left(1 - \frac{\varepsilon_h}{\varepsilon_a}\right)C(1 - DC) = 0, \end{aligned} \tag{2}$$

$$\Phi = 1 - \left(\frac{\varepsilon_a}{\varepsilon_h}\right)^{1/3} C, \tag{3}$$

$$\varepsilon_i = \frac{\varepsilon_h - \varepsilon_a C}{1 - C}, \tag{4}$$

$$\kappa_i = \frac{\kappa_l - \kappa_a DC}{1 - DC}, \tag{5}$$

where the quantities $C$, $D$, $P$, $Q$ and $R$ are given by

$$C = \frac{-Q - \sqrt{Q^2 - 4PR}}{2P}, \tag{6}$$

$$D = \left(\frac{\varepsilon_a \kappa_l}{\varepsilon_h \kappa_a}\right)^{1/3}, \tag{7}$$

$$P = \left(\frac{\kappa_a}{\kappa_l} + 2\right)\varepsilon_l D - 3[\varepsilon_h D - \varepsilon_a(D-1)]D + \left(\frac{\kappa_l}{\kappa_a} - 1\right)\varepsilon_a D, \tag{8}$$

$$Q = 3[2\varepsilon_h D - \varepsilon_a(D-1)] - \left[\left(\frac{\kappa_a}{\kappa_l} + 2\right)D + 3\right]\varepsilon_l D, \tag{9}$$

and

$$R = 3(\varepsilon_l - \varepsilon_h). \tag{10}$$

With the observed values of $\epsilon_a$ and the dielectric parameters $\epsilon_h$, $\kappa_h$, $\kappa_l$, and $\epsilon_l$, we can determine the value of $\kappa_a$ by solving Eq. (2) numerically. Once the value of $\kappa_a$ has been determined, the values of $\Phi$, $\epsilon_i$, and $\kappa_i$ are calculated from Eqs. (3), (4), and (5), respectively.

## Experimental

### Materials

Chitosan (80% deacetylated chitin) was purchased from Yuhuan Sea Co., China. The average molecular weight was 430,000, which was determined from the viscosity of the chitosan solutions using the Mark–Houwink equation [15]. The viscosity was measured using a Ubbelohde viscometer at 35 ± 0.5 °C. Glutaraldehyde was a product of Shanghai Chemical Co.

### Preparation of microspheres

Chitosan microspheres with smooth surfaces were prepared by cross-linking chitosan particles in paraffin oil with glutaraldehyde [16]. Chitosan was dissolved in an acetic acid solution, and the solution was filtered to remove undissolved impurities. The chitosan solution was added to paraffin oil under stirring. The resulting suspension of chitosan droplets was mixed with a glutaraldehyde solution and incubated at 40 °C for 2 h. After the glutaraldehyde treatment, the chitosan particles were resuspended in an ammonium hydroxide solution (pH 9) and allowed to stand for 2 h at 60 °C. The chitosan particles were thoroughly washed with petroleum ether, isopropyl alcohol, and distilled water in turn, and finally were fractionated through a set of no. 200 China standard test sieves.

The particles obtained were dried under reduced pressure at 60 °C and then were observed using a scanning electron microscope (SEM) (XL-30ESEM, Philips Co., The Netherlands). The SEM photographs of the particles used for the dielectric measurements are shown in Fig. 1. The microspheres have smooth surfaces and the diameter was about 90 μm (Fig. 1a). The cross-section of a particle, shown in Fig. 1b, indicated that the interior was homogeneous except for cracks and cavities. It should be emphasized that the homogeneous model adopted here is suited to analyze our system although the particles in suspension may be somewhat different from the dry particles which were photographed.

### Dielectric measurements

The cell used for the dielectric measurements consisted of a methacrylate resin spacer with a cylindrical sample cavity and a pair of platinum disc electrodes. The electrodes were coated with platinum black to reduce electrode polarization effects. The effective area of the electrodes was 78.5 mm$^2$ and the electrode distance was 8 mm. Dielectric measurements were carried out over a frequency range of 1–500 MHz using a 4191A radio-frequency impedance analyzer (Hewlett-Packard) controlled by a personal computer. Before the measurements, the chitosan microspheres were equilibrated in aqueous electrolyte solutions for 4 h. All the measurements were

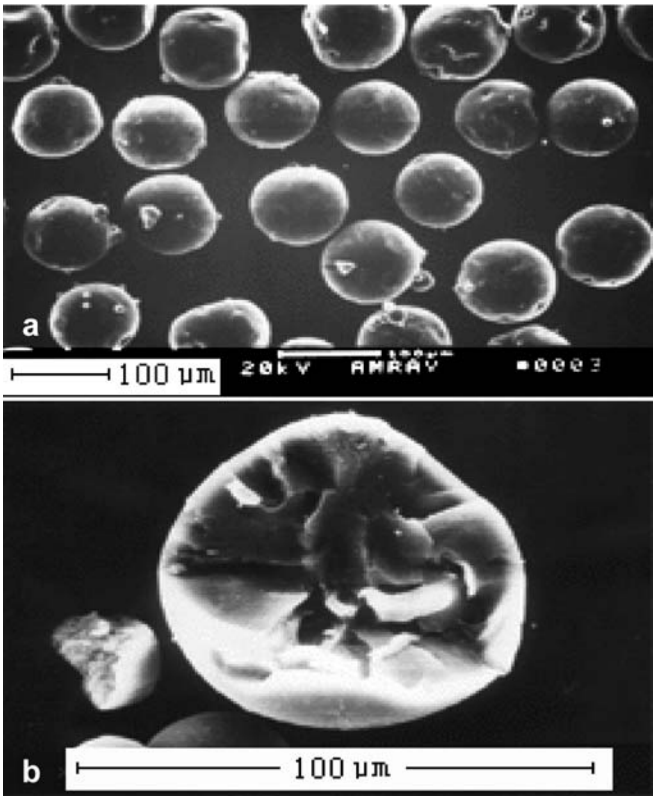

**Fig. 1a,b.** Scanning electron microscope photographs of chitosan microspheres. **a** Low-magnification view, **b** cross-section of a microsphere

carried out at room temperature (21 ± 1 °C). The measurement errors due to electrode polarization at low frequencies were corrected by the method used previously [17].

## Results and discussion

### pH dependence of dielectric relaxation

The dielectric measurements were carried out for chitosan microsphere beds in water, the pH of which was varied from 1.5 to 13 by adding HCl or KOH solutions. When the pH was between 2 and 6, dielectric relaxation was clearly observed at frequencies between 1 and 100 MHz. The dielectric relaxation was represented by a Cole–Cole function:

$$\varepsilon^* = \varepsilon_h + \frac{\varepsilon_l - \varepsilon_h}{1 + (j\omega\tau)^{1-\alpha}} + \frac{\kappa_l}{j\omega\varepsilon_0}, \tag{11}$$

where $\epsilon_h$, $\epsilon_l$, and $\kappa_l$ are the dielectric parameters described earlier and $\tau$ is the relaxation time ($\tau = 1/2\pi f_0$, $f_0$ is the relaxation frequency). $\alpha$ is a coefficient characterizing the distribution of relaxation times. The pH dependence of the relaxation intensity, which had a maximum around pH 4, is shown in Fig. 2. This can be

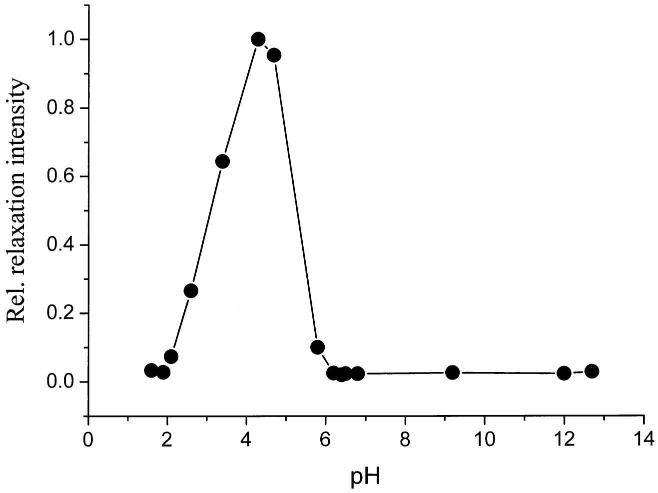

**Fig. 2.** pH dependence of the relaxation intensity of chitosan microspheres in water. The pH of the suspending medium was changed by adding HCl and KOH solutions

interpreted as follows. In an acidic medium, chitosan exhibits polyelectrolyte behavior and a reaction equilibrium occurs between the ionized group $-NH_3^+$ and the neutral group $-NH_2$ on the main chain [10]. Since there is an equilibrium between the ionized group $-NH_{3+}$ and the neutral group $-NH_2$ on the main chain, the protonation of $-NH_2$ proceeds with increasing pH and chitosan exhibits polyelectrolyte behavior in acidic media. In the present system, bulk chitosan, the same protonation trend as that for the molecule in solution can be considered, although the number of neutral $-NH_2$ on the bulk was influenced somewhat by crosslinking in the preparation of the chitosan microspheres. The dielectric behavior in the pH range between 2 and 6 can be suggested to be in relation to the protonation which took place partially in the pH range. The dielectric measurements were carried out to obtain reliable dielectric parameters and to achieve a good analysis.

The dielectric behavior of the chitosan microspheres was different from that of chitosan solutions found at frequencies below 1 MHz [10]. The dielectric relaxation of chitosan solutions was expressed by the sum of two Cole–Cole relaxation functions. The intensity of the low-frequency relaxation increased with increasing pH from 5 to 7 and leveled off above pH 7, whereas that of the high-frequency relaxation was independent of pH. The relaxation phenomena observed here can be considered to be due to interface polarization because of the heterogeneous structure of the particle suspension, so we can discuss and analyze the relaxations using interface polarization theory.

The chitosan microspheres consist of polymer matrices and intermatrix spaces. Since the matrices have fixed positive charges, $-NH_3^+$ at acidic pH, the microspheres behave like ion-exchange resin beads. Indeed, the dielectric relaxation is similar to that found for ion-exchange resin beads in water [6, 7, 8].

## Dielectric behavior in KCl electrolyte solutions

The frequency dependences of the relative permittivity and the conductivity of chitosan microsphere beds in distilled water and KCl solutions are shown in Fig. 3. The pH of the external media, examined before the dielectric measurements, was within 3.9–5.0. The intensity of the dielectric relaxation rapidly decreased with increasing KCl concentration (Fig. 4); this behavior was similar to that of ion-exchange beads in electrolyte solutions [8]. Since chitosan is regarded as a polyelectrolyte in acidic media, the decrease in relaxation intensity with KCl concentration can be interpreted in terms of the Donnan equilibrium. In water, mobile ions in the microsphere are only conterions of $-NH_{3+}$ and, with increasing KCl concentration, $K^+$ and $Cl^-$ distribute between the microsphere and the medium according to the Donnan equilibrium. Thus the conductivity ratio of the microsphere to the medium decreases with KCl concentration, which diminishs the interfacial polarization at the microsphere interface.

The dielectric parameters were estimated by fitting Eq. (11) to the observed dielectric relaxation as shown in Fig.5. The best-fit values of the dielectric parameters for the data shown in Fig. 3 are listed in Table 1. It should be pointed out that the values of $\kappa_h$ in the table were calculated by using the equation $\kappa_h = (\epsilon_l - \epsilon_h) 2\pi f_0 \epsilon_v + \kappa_l$ [13] because it cannot be determined with Eq. (11). The values of $\alpha$ were greater than 0, indicating that the relaxation curves were broader than Debye-type relaxation with a single relaxation time. The relaxation times decreased (or the relaxation frequencies increased) with increasing KCl concentration, being characteristic of interface polarization [13, 18].

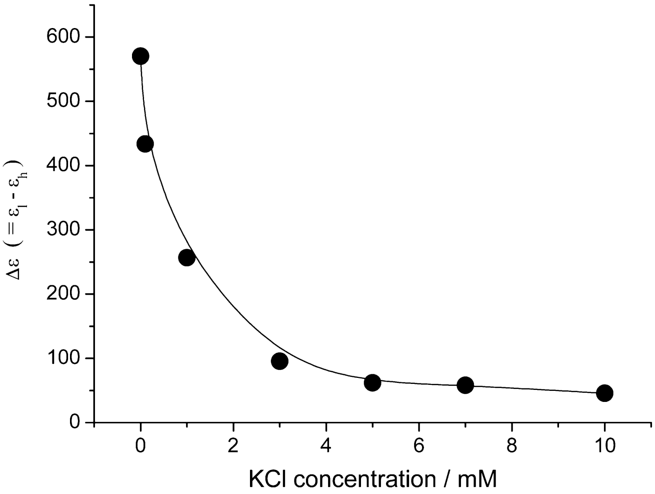

**Fig. 4.** Relations between the relaxation intensity and the ion concentration of the external medium

## Theoretical analysis based upon interfacial polarization theory

As described in the Theory section, the phase parameters, $\epsilon_i$, $\kappa_i$, $\kappa_a$, and $\Phi$ can be determined from the observed dielectric parameters, $\epsilon_l$, $\epsilon_h$, $\kappa_l$, and $\kappa_h$, and $\epsilon_a = 80$ (permittivity of water at 20 °C) using Eqs. (2), (3), (4), and (5). The phase parameters estimated from the dielectric parameters listed in Table 1 are shown in Table 2. The values of $\Phi$ lie between 0.62 and 0.70 and

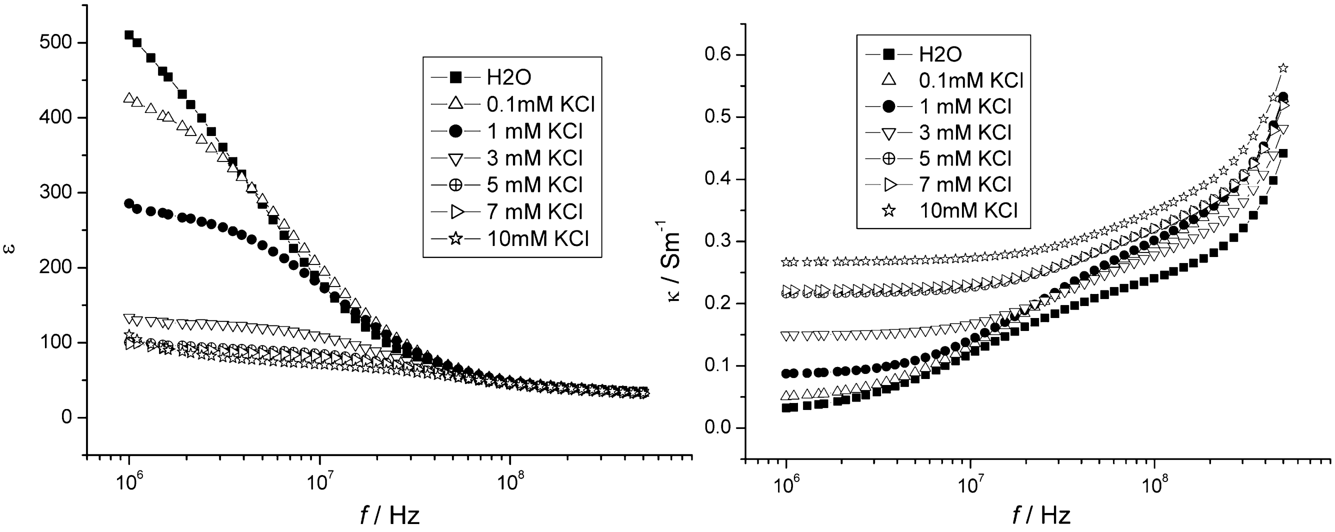

**Fig. 3.** Frequency dependence of the relative permittivity, $\epsilon$, and the conductivity, $\kappa$, of chitosan microsphere beds in distilled water and KCl solutions

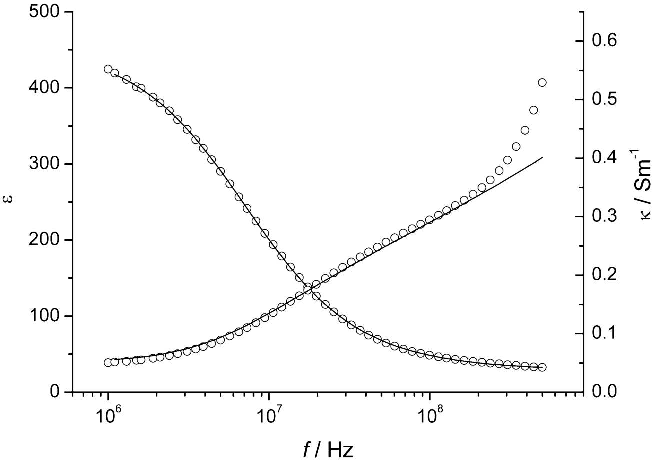

**Fig. 5.** Curve-fitting of the dielectric relaxation for chitosan microsphere suspensions by the Cole–Cole equation. The *lines* are theoretical curves calculated from the Cole–Cole equation. The best-fit parameters are $\epsilon_l = 461 \pm 1$, $\epsilon_h = 27.5 \pm 0.4$, $\tau = 22.4 \pm 0.1$ ns, $\kappa_l = 50.7 \pm 0.9$ mS/m, and $\alpha = 0.200 \pm 0.002$

increase slightly with KCl concentration. This shows that the microspheres are closely packed in the microsphere beds. The internal permittivity, $\epsilon_i$, was 10–20 and was almost independent of KCl concentration above 3 mM. If we assume that the microsphere is a mixture of water and polymers, the relative permittivity of the microsphere is simply expressed by

$$\varepsilon_i = f_w \varepsilon_w + (1 - f_w)\varepsilon_p, \tag{12}$$

where $f_w$ is the volume fraction of water in the microsphere and $\epsilon_p$ is the permittivity of the polymer itself. With $\epsilon_i = 20$ and $\epsilon_w = 80$, the value of $f_w$ was calculated from Eq. (12) to be 0.23 for $\epsilon_p = 2$ and 0.14 for $\epsilon_p = 10$. The volume ratio of water in the microsphere, therefore, is at most 20%.

The internal conductivity, $\kappa_i$, is much larger than the external conductivity, $\kappa_a$. The $\kappa_i/\kappa_a$ ratio decreases with increasing KCl concentration and tends to have a limiting value at high concentrations (Fig. 6). The limiting value of $\kappa_i/\kappa_a$ was estimated to be 6.9 by fitting an exponential decay function to the data. $\kappa_i/\kappa_a$ plotted against $\kappa_a$ is similar to that found for ion-exchange resin beads, although the limiting value of $\kappa_i/\kappa_a$ is larger than 1. The high internal conductivity of the ion-exchange resin beads at low electrolyte concentrations is mainly due to the contribution of counterions accumulated around the fixed charges following the Donnan equilibrium. When the concentration of the electrolyte solution is much higher than that of the fixed charges, the electrolytes distribute almost equally between the internal water phase and the external phase, and thus the $\kappa_i/\kappa_a$ ratio becomes less than 1 because of the dead space due to the polymer matrices. That the limiting value of $\kappa_i/\kappa_a$ is more than 1 for chitosan micropheres suggests

**Table 2.** The phase parameters of chitosan microspheres calculated from the dielectric parameters shown in Table 1 using Eqs. (2), (3), (4), and (5)

| KCl concentration (mM) | $\kappa_a$ (mS/m) | $\Phi$ | $\epsilon_i$ | $\kappa_i$ (mS/m) |
|---|---|---|---|---|
| Distilled water | 2.49 | 0.625 | 8.91 | 171 |
| 0.1 | 4.95 | 0.638 | 9.63 | 219 |
| 1.0 | 12.0 | 0.621 | 13.5 | 290 |
| 3.0 | 34.8 | 0.645 | 17.4 | 310 |
| 5.0 | 57.4 | 0.695 | 18.8 | 363 |
| 7.0 | 62.9 | 0.686 | 17.8 | 370 |
| 10 | 85.6 | 0.698 | 17.3 | 415 |

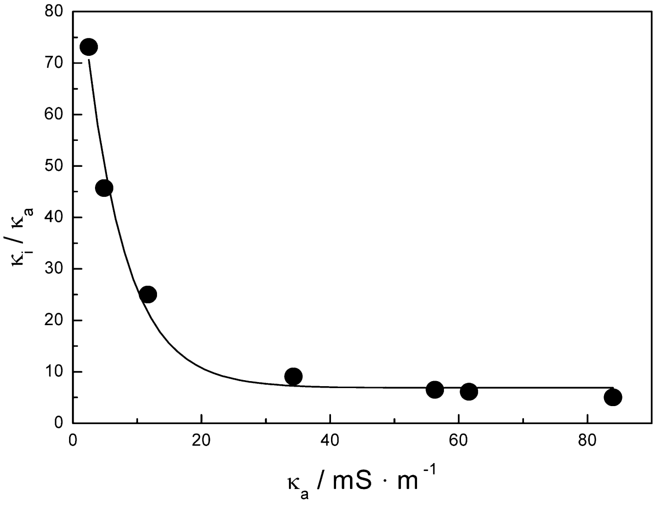

**Fig. 6.** Plots of the internal/external conductivity ratio, $\kappa_i/\kappa_a$, against the external conductivity, $\kappa_a$. The *line* indicates the curves obtained by fitting an exponential decay function

**Table 1.** The dielectric parameters estimated by fitting the Cole–Cole equation to the dielectric relaxations observed for chitosan microsphere beds in distilled water and KCl solutions

| KCl concentration (mM) | $\alpha$ | $\epsilon_l$ | $\epsilon_h$ | $\kappa_l$ (mS/m) | $\kappa_h$ (mS/m) | $\tau$ (nS) | $f_0$ (MHz) |
|---|---|---|---|---|---|---|---|
| Distilled water | 0.22 | 598 | 27.6 | 28.6 | 160 | 38.4 | 4.14 |
| 0.1 | 0.20 | 461 | 27.5 | 50.7 | 208 | 24.4 | 6.52 |
| 1.0 | 0.16 | 289 | 32.1 | 86.4 | 260 | 13.1 | 12.1 |
| 3.0 | 0.13 | 130 | 34.1 | 151 | 279 | 6.60 | 24.1 |
| 5.0 | 0.15 | 94.4 | 32.6 | 218 | 338 | 4.55 | 35.0 |
| 7.0 | 0.16 | 90.1 | 32.2 | 223 | 346 | 4.17 | 38.1 |
| 10 | 0.22 | 76.6 | 31.1 | 269 | 395 | 3.20 | 49.7 |

that the internal conductivity is determined not only by the conduction due to movable ions in the microsphere but also by unknown conduction mechanisms, such as proton transport through ionized sites proposed by Bordi et al. [10].

To examine the validity of the dielectric analysis based on Hanai's equations, the theoretical relaxation curves calculated from the estimated phase parameters were compared with the observed ones. The frequency dependences of the relative permittivity and the conductivity observed and calculated for the chitosan dispersed in 1 mM KCl solution are shown in Fig. 7. The theoretical curves are in good agreement with the observed data over the entire relaxation, indicating that the present analysis is appropriate.

### Effects of $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, and $La^{3+}$ on the electrical properties of chitosan microspheres

In order to examine the adsorption and release of $Ca^{2+}$, $Mg^{2+}$, $Cu^{2+}$, and $La^{3+}$ on and from chitosan microspheres, dielectric measurements were carried out for chitosan microspheres equilibrated in their chloride salt solutions and those rinsed with distilled water.

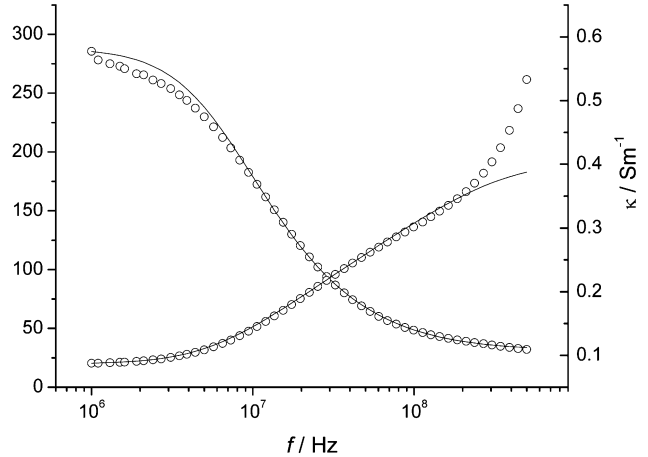

**Fig. 7.** Frequency dependences of the relative permittivity and the conductivity of chitosan in 1 mM KCl. The *curves* are theoretical values calculated from Eq. (1)

The microspheres were dispersed in $CaCl_2$, $MgCl_2$, $CuCl_2$, and $LaCl_3$ solutions and were left for about 10–15 h to attain an equilibrium state. The suspensions were measured by dielectric spectroscopy. Successively, the solutions were removed from the suspensions and the microspheres specimens were rinsed 8–10 times with distilled water. After filtrating, they were dispersed afresh in distilled water for about 10 h, and then the suspensions were again subjected to dielectric measurement. The pH of the suspending medium was checked before the dielectric measurements, and was between 4 and 5. Although the measurements were made for specimens treated with salt solutions of three different concentrations (0.01, 0.1, and 1mM), the results with 1 mM salt solutions are listed in Table 3 because the same conclusion was obtained independent of salt concentration. The estimated values of $\Phi$, $\epsilon_i$, and $\kappa_i$ for $Cu^{2+}$ are different from those of $Ca^{2+}$, $Mg^{2+}$, and $La^{3+}$. This result indicates that $Cu^{2+}$ interacts with chitosan in a different way from $Ca^{2+}$, $Mg^{2+}$, and $La^{3+}$. The low values of $\epsilon_i$ and $\kappa_i$ for $Cu^{2+}$ suggest that in the chitosan microspheres the relative volume of the water phase decreases or the relative volume of the chitosan matrices increases. The low value of $\Phi$ for $Cu^{2+}$, the low packing of the chitosan microspheres in the bed, may suggest that $Cu^{2+}$ shrinks the microspheres to make the surfaces rough.

The release of ions from chitosan can be evaluated from the difference in the internal conductivity before and after washing with distilled water, namely, $\Delta\kappa_i = \kappa_{i,\mathrm{before}} - \kappa_{i,\mathrm{after}}$. The value of $\Delta\kappa_i$ for $Cu^{2+}$ was much smaller than that for $Ca^{2+}$, $Mg^{2+}$, and $La^{3+}$. This result suggests that $Cu^{2+}$ binds more tightly to chitosan than $Ca^{2+}$, $Mg^{2+}$, and $La^{3+}$, and the ability of chitosan to adsorb to metal ions depends on the chelating constant of the metal ion and not the charge number. This conclusion is supported by the chelating constant of $Cu^{2+}$ with $NH_3^{+}$ that is much larger than those of $Ca^{2+}$,$Mg^{2+}$, and $La^{3+}$ [19].

## Concluding remarks

In the present study, dielectric spectroscopy has proved to be a promising method for estimating the distribution

**Table 3.** Phase parameters of chitosan microsphere in 1 mM salt solutions and those after washing with distilled water. $\Delta\kappa_i = \kappa_{i,\mathrm{before}} - \kappa_{i,\mathrm{after}}$, the difference in the conductivity of the chitosan microspheres before and after washing with distilled water

| Salts | Washing with distilled water | $\kappa_a$ (mS/m) | $\Phi$ | $\epsilon_i$ | $\kappa_i$ (mS/m) | $\Delta\kappa_i$ (mS/m) |
|---|---|---|---|---|---|---|
| $CaCl_2$ | Before | 16.7 | 0.67 | 19 | 168 | 55 |
| | After | 2.00 | 0.60 | 10 | 113 | |
| $MgCl_2$ | Before | 16.3 | 0.67 | 18 | 174 | 57 |
| | After | 2.10 | 0.61 | 11 | 117 | |
| $LaCl_3$ | Before | 21.1 | 0.67 | 19 | 235 | 77 |
| | After | 2.96 | 0.61 | 9 | 158 | |
| $CuCl_2$ | Before | 1.96 | 0.59 | 8 | 101 | 1 |
| | After | 1.71 | 0.59 | 6 | 100 | |

and binding of ionic substances to particles in a noninvasive way. The information obtained by dielectric analysis is important for evaluating the quality of products in controlled-release technology. The present study also showed the feasibility of real-time monitoring of target ions distributed between the particle interior and the external medium using an automated instrument of dielectric spectroscopy.

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