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# Dielectric monitoring method for the drug release mechanism of drug-loading chitosan microspheres

FANG Ming, GAO JinLong, WANG Sheng, LIAN YiWei & ZHAO KongShuang*

*College of Chemistry, Beijing Normal University, Beijing 100875, China*

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The chitosan microspheres loaded with model drug, salicylate molecular, are prepared, and the dielectric spectroscopy of this kind of suspension is measured, meanwhile a significant relaxation is observed for the microspheres suspension. By the modeling analytic of the relaxation, the parameters, which can reflect the properties of microspheres and continuous medium, are obtained. Furthermore, the releasing process of microspheres in aqueous phase is monitored real-timely, namely, by analyzing the time-dependent variation of the parameters reflecting the relaxation characteristics and electrical parameters in each phase the releasing process is detected. The research shows that the controlled release process of chitosan microspheres loaded with salicylate in aqueous phase is divided into three phases according to different releasing mechanisms. At the release stage, the quantitative relation between the phase parameters obtained by dielectric analysis and the amount of the salicylate carried in microspheres was derived, and a real-time monitoring method was established through releasing material in the microspheres at different times obtained by measuring and analyzing the dielectric spectroscopy.

**chitosan microspheres, dielectric analytic, sodium salicylate, releasing mechanism, dielectric detection**

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Drug release function based on an appropriate drug carrier decreases the minimum effective dose of drug and reduces the risk of side-effects [1]. Therefore, it is essential to select a suitable and high efficiency drug carrier. Considering its fine biocompatibility and degradability, chitosan has been regarded as an ideal drug carrier [2]. In recent years, there have been various reports concerning theoretical and practical aspects of chitosan as a drug carrier [3]. There is not yet a definite explanation for the release mechanism of chitosan in releasing any given drug. This is because chitosan contains various active groups each of which might have separate reaction mechanisms with drug molecules, and these mechanisms might be altered when such condition as temperature or pH change. Various methods exist for investigating the chitosan release system or analogous drug release systems, such as UV-Visible absorption photometry, fluorescence [3–6], the infrared spectrum, X-ray diffraction, scanning electron microscopy, and electrical titration [7–10]. These methods monitor the change of drug concentration deriving static information about the reaction between the drug molecule and chitosan in the releasing system. Despite being used to monitor the drug release process, these methods are limited by their relative shortage of information, and are thus unable to accomplish effective real-time monitoring of the drug release system.

The dielectric relaxation spectroscopy (DRS) method, due to its non-invasive detection of the dynamics and electronic information of heterogeneous systems, is used to explore the system change process online and provide parameters which are unavailable by other means [11,12]. DRS has been widely used for such tasks as dielectric monitoring [13–17]. It is for the above salient features, particularly the obtaining of information about the constituent phase inside heterogeneous systems by the dielectric analy-

*Corresponding author (email: zhaoks@bnu.edu.cn)

sis method based on a physical model, that the DRS method compensates for deficiencies of other methods [18–20]. In our earlier research, the dietetic spectra of microspheres made of cross-linked chitosan were studied, with the mechanism of dielectric relaxation and the rationality of theoretically calculated phase parameters also being interpreted [21]. These previous works lay a solid foundation for performing farther exploring study on the present drug releasing system by DRS method.

In present work, we used sodium salicylate (because sodium salicylate contains carboxyl, a hydroxide radical and a hydrophobic group and is present in most organic drug molecules) as a model drug, chitosan microspheres containing a salicylic suspension were both theoretically and experimentally investigated. Especial attentions ware focused on the analysis of time dependent dielectric spectra to gain information about inner matter releasing and absorbing, based on these information we can speculate the reciprocity between mode molecular and chitosan by evaluating diffusion mechanism. Another motivation of this study is to develop a monitoring method by which the change of model drug content in microsphere can be monitored in real-time by detecting the physical parameters and dialectic parameters of the chitosan microsphere, thereby, to provide a theoretical foundation for finding a new way to monitor the drug release behavior in actual process.

## 1 Experimental

### 1.1 Chemicals and materials

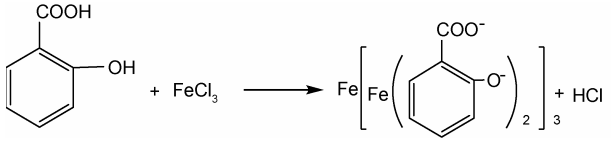
All the chemicals and reagents were of analytical reagent grade unless otherwise stated. Chitosan, powder, deacetylation 90.7%, was purchased from AoKang BioTech Ltd. Glutaraldehyde, with a mass fraction of 50%, was purchased from Tianjin Kermel Chemical Reagent Ltd. Salicylate was purchased from the Beijing Chemical Reagent Company. Such other reagents as NaOH and acetic acid were purchased from the Beijing Beihua Fine Chemicals Co., Ltd.

### 1.2 Preparation of drug-loaded chitosan gel beads

In order to prepare the chitosan gel beads without salicylate, 4 g chitosan powder was dissolved in a mass fraction of 0.5% of the acetic acid to prepare a concentration of 1.0% chitosan solution. After the bubble burst, the chitosan solution was dripped into a 500-mL solution with a mass fraction of 0.5% NaOH utilizing a syringe, generating white chitosan balls. After 2 h, when the filtration of sodium hydroxide solution was completed, the balls were rinsed with twice-distilled water until the washing liquid was neutral (tested by pH test strips). The cross-linked chitosan gel balls were prepared using the mass fraction of 0.5% glutaraldehyde as a crosslinker for 1 h, after which the balls were immersed in twice-distilled water for 10 min. The above steps were repeated three times. After removing the water, the balls were slowly dried at 40°C thereby completing the preparation of cross-linked chitosan microspheres. By the same method, 4 g chitosan powder and 4 g salicylate were dissolved in HAc. Following the anti-foaming, cross-linking and drying, the cross-linked chitosan microspheres containing sodium salicylate were obtained. The diameter of the microspheres varied from 1.25 mm to 1.75 mm.

### 1.3 Detection of sodium salicylate

Salicylic acid and ferric chloride occurred following a characteristic color reaction, with a newly created complex being purple. By this reaction, the presence of sodium salicylate was semi-quantitatively detected [22].




Sodium salicylate was detected by the following method: 0.5 g microspheres were placed in a beaker containing 3 mL twice-distilled water. After soaking for respectively 2, 4, 6, 8 h, a small amount (about 0.3 mL) of the immersion from the beaker was dropped into a 0.01-mol/L solution of $FeCl_3$. The solution was purple and the color gradually deepened with increased soaking time. This indicated that the sodium salicylate had been wrapped into the microspheres, and would be released immersed in the water.

### 1.4 Dielectric measurements

Dielectric measurements were carried out with HP 4294A (Agilent Technologies) Precision Impedance Analyzer over a frequency range from 40 Hz to 110 MHz and the amplitude of the applied alternating field was 500 mV. The values of capacitance (C) and conductance (G) at varying frequencies were obtained. The cell used for dielectric measurements consisted of concentrically cylindrical platinum electrodes as shown in Figure 1. The cell constant ($C_l$) and stray capacitance ($C_r$) at 25°C were respectively 0.0253 pF and 0.0128 pF, which were determined by using several standard liquids, pure water andethanol, and air. All of the experimental data was corrected for errors arising from $C_l$ and $C_r$. The directly measured capacitance ($C$) and conductance ($G$) were translated into permittivity $\varepsilon$ and conductivity $\kappa$ by respectively using the formulas: $C=\varepsilon C_r+C_r$ and $G=\kappa C/\varepsilon\varepsilon_0$, where $\varepsilon_0$ is the vacuum dielectric constant ($\varepsilon_0$=8.8541 pF/m). All of the measurements were carried out at (25±1)°C. After 0.2 g chitosan microspheres containing salicylic acid were put into a measuring cell filled with approximately 5 mL of deionized water, the dielectric spectra data was acquired every 10 min until the dielectric spectroscopy showed no change.

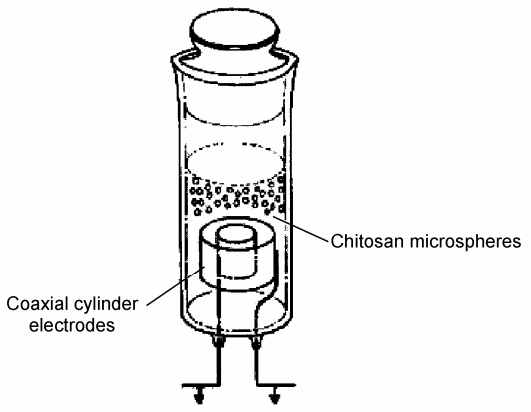



**Figure 1** Measuring cell for chitosan microsphere suspension, with the electrodes of the cell connected to the 4294A. Analyzer for dielectric measurements.

## 2 Results and discussion

### 2.1 Time dependence of dielectric spectroscopy for chitosan microsphere suspension

(1) Three-dimensional representations of dielectric spectra. For any substances or systems, their complex permittivity $\varepsilon^*$ is defined by the real part $\varepsilon$ and the imaginary part $\varepsilon''$ of $\varepsilon^*$ as:

$$\varepsilon^* = \varepsilon - j\varepsilon'' = \varepsilon - j\frac{\kappa}{\omega\varepsilon_0}, \tag{1}$$

where $\omega$, $\varepsilon_0$ and $\kappa$ have been defined above. Figure 2 shows the results of the dielectric measurements for the chitosan microspheres containing a salicylic acid suspension. From the Figure 2, it can be clearly seen shows that dielectric relaxation (permittivity decreasing with the increment of measuring frequency), which is caused by interfacial polarization between the microspheres and the solution, exists at the frequency range of $10^4$–$10^6$ Hz. The relaxation appears at a maximum after about 200 min of the chitosan microspheres being placed in water, as indicated by the arrows.

(2) Determination of dielectric parameters. To examine the dielectric spectra in detail and explore what changed inside the chitosan microspheres during the release of the salicylic acid, it is necessary to theoretically analyze the dielectric spectra in order to obtain the electrical parameters of the dispersed phase which reflected the change inside the microspheres. The dielectric relaxation parameters, i.e., the relaxation frequency $f_0$, the limiting values of ε and $\kappa$ at low (subscript 1) and the high (subscript h) frequencies, were obtained by fitting the following Cole-Cole empirical equation (eq.(2)) to the experimental data, with a part of the results listed in Table 1.

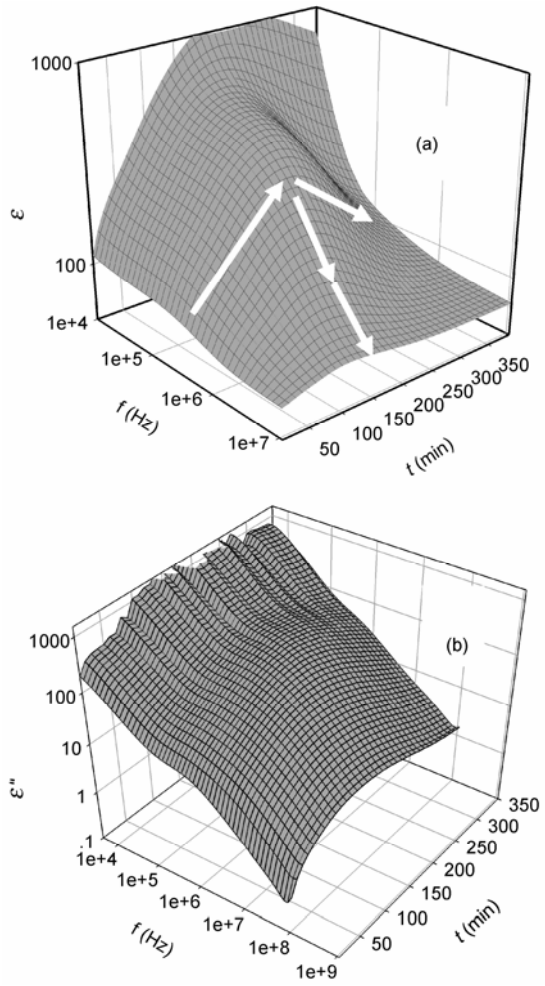



**Figure 2** Three-dimensional representations of the time dependence of complex permittivity for chitosan microspheres containing a salicylic suspension. (a) The permittivity spectrum and (b) the dielectric loss spectrum.

$$\varepsilon^* = \varepsilon - j\varepsilon'' = \varepsilon_h + \frac{\varepsilon_l - \varepsilon_h}{1 + (jf/f_0)^{\beta}}. \tag{2}$$

Three parameters in Table 1, reflecting the relaxation characteristics, are particularly noteworthy: the increment of the dielectric constant (or relaxation strength) $\Delta\varepsilon$, the relaxation time $\tau$ ($\tau = 1/2\pi f_0$), and the distributed parameter of the relaxation time $\beta$. The values of $\beta$ slightly change with the variation of time, being between 0.82–0.88, suggesting that the sizes and the structures of the microspheres as well as the distribution of the electrical properties have very limited variation after water entered into them, suggesting that structural and property variation are uniform for all microspheres. Secondly, the time-dependent relationship of $\Delta\varepsilon$ and $\tau$ are different, which is explained by the structural variation and the electrical properties of the microspheres after the aqueous solution entered into them. For the sake of enhanced clarity, $\Delta\varepsilon$ and $\tau$ were plotted against time as shown in Figure 3. From Figure 3, it is apparent might be considered

**Table 1** Dielectric parameters of chitosan microspheres containing salicylic acid suspension at varying times

| *t*/min | $\varepsilon_l$ | $\varepsilon_h$ | $\Delta\varepsilon$ | $\beta$ | $f_0$(Hz) | $\kappa_l$(S m$^{-1}$) | $\kappa_h$(S m$^{-1}$) |
|---|---|---|---|---|---|---|---|
| 10 | 106.10 | 78.29 | 27.80 | 0.876 | 280009.5 | 0.00095 | 0.00138 |
| 20 | 106.61 | 78.07 | 28.54 | 0.851 | 536455.9 | 0.00153 | 0.00238 |
| 30 | 116.32 | 78.05 | 38.27 | 0.859 | 670092.5 | 0.00199 | 0.00342 |
| 40 | 131.01 | 77.74 | 53.27 | 0.835 | 721150.9 | 0.00242 | 0.00456 |
| 50 | 146.19 | 77.47 | 68.71 | 0.826 | 724313 | 0.00285 | 0.00562 |
| …… | …… | …… | …… | …… | …… | …… | …… |
| 160 | 285.24 | 77.05 | 208.19 | 0.878 | 444117.6 | 0.0104 | 0.0155 |
| 170 | 283.88 | 77.16 | 206.73 | 0.878 | 414247.4 | 0.0112 | 0.0159 |
| 180 | 279.04 | 77.27 | 201.77 | 0.878 | 388132.8 | 0.0119 | 0.0163 |
| 190 | 269.37 | 77.38 | 191.98 | 0.878 | 365585.9 | 0.0126 | 0.0165 |
| 200 | 256.76 | 77.50 | 179.26 | 0.875 | 344738.2 | 0.0132 | 0.0166 |
| …… | …… | …… | …… | …… | …… | …… | …… |
| 260 | 168.74 | 77.89 | 90.85 | 0.827 | 248093.9 | 0.0145 | 0.0158 |
| 270 | 156.01 | 77.91 | 78.10 | 0.809 | 230885.3 | 0.0143 | 0.0153 |
| 280 | 145.60 | 77.88 | 67.72 | 0.789 | 214598.6 | 0.0142 | 0.0150 |
| 290 | 134.49 | 77.93 | 56.55 | 0.771 | 205104.3 | 0.0138 | 0.0144 |
| …… | …… | …… | …… | …… | …… | …… | …… |

that because the amino groups exist on the chitosan chain, the electrical properties of the electrical double layer on the surface of microspheres are determined by the hydrogen bonds, which are constructed by the amino groups in microspheres with the hydrogen ions in the solution or the hydrogen atoms existing in amino groups with the carbonyl oxygen existing in the salicylate anion. With the entering process of the water molecules, the sizes and the structures of the microspheres varied according to the gelation of the chitosan. Both the electrical and the structural properties of the electrical double layer were changed, which caused the variation of the parameters of dielectric relaxation. Concerning the relaxation strength $\Delta\varepsilon$, the maximum value of $\Delta\varepsilon$ was observed at around 180 min as presented in Figure 3(a), which means that there was no relaxation when the dry microspheres were added into the water phase at the initial stage, but with the absorption of the water, the sodium salicylate adsorbed by the chitosan microspheres was dissolved. The structures of the microspheres also varied, which caused the formation of the electrical double layer, so that the relaxation strength was enlarged. About 3 h later, the immersion of a sufficient number of water molecules resulted in the gelating swelling of the chitosan microspheres, the structure of which was become loose. The variation of the thickness of the electrical double layer resulted in the reduction of the relaxation strength (Figure 3 (a)). Concerning the time dependence of relaxation $\tau$, with the prolongation of the time at which microspheres were added into the water, $\tau$ monotonously increased. This phenomenon occurred according to the dielectric relaxation theory of particle dispersion [23,24], because relaxation time which is caused

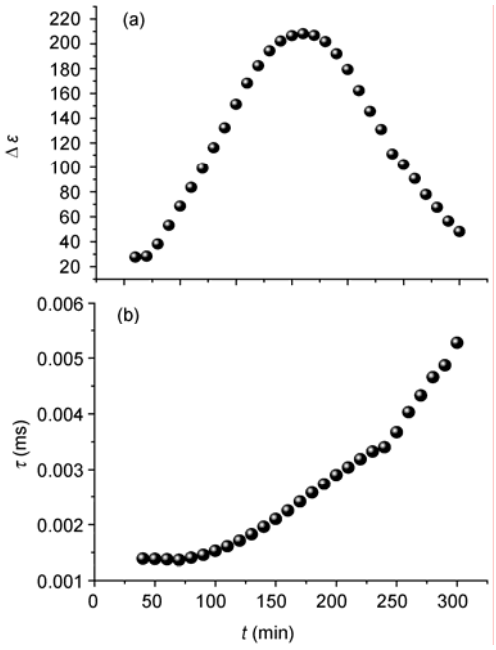



**Figure 3** Time dependence of (a) dielectric increment and (b) relaxation time for chitosan containing a salicylic suspension.

by interfacial polarization is related to the thickness of the electrical double layer and the sizes of the microspheres, the immersion of copius amounts of water molecules into the microspheres caused the swelling of the microspheres. Not only was the size of the microspheres incresed but also the thickness of electrical double layer was enhanced, which was related to the degree of the swelling, so that the relaxation frequency or the relaxation time monotonically incre-

sed.

(3) Mechanism of dielectric relaxation. According to the Maxwell interfacial polarization theory, for a heterogeneous system which consists of $n$ constituent phases, when the conductivities and permittivities of the constituent phases meet the following relation: $\varepsilon_1/\kappa_2 \neq \varepsilon_2/\kappa_2 \ldots\ldots \neq \varepsilon_n/\kappa_n$, the number of relaxations which are observable will be $\leqslant n-1$ [25], and these relaxations caused by interfacial polarization will appear between a frequency range of 105–106 Hz [23,26]. In this work, because the chitosan microsphere suspension was composed of two phases, i.e., a microsphere phase and a water phase, the relaxation observed at about 104 –106 Hz originated from the interfacial polarization mechanism between the microspheres and the water medium. According to the low-frequency dielectric theory [24], the radial or tangential diffusion of counter-ions in an electrical double layer of a microsphere surface will yield counter-ions by which the relaxation, in general, appears at frequencies given by the following formula:

$$f_0' = \frac{ukT}{\pi R^2 e}, \tag{3}$$

where the $R$, $e$, $u$, $\kappa$ and $T$ are respectively the radii of particles, the electric charge of the counter ions, ionic mobility, Boltzmann's constant and absolute temperature. The radii of a chitosan microsphere is about (1.5±0.2)mm, and the mobility of a hydroxide ion, which is the largest among all of the anions, salicylate ion and hydroxide ion, in our system, was adopted for use in calculation. The value of $f_0'$ calculated using this set of data was in the range of 0.55–1.07 mHz. Therefore, it was evident that the relaxation found in a chitosan microsphere suspension was not attributable to counter-ion polarization because the relaxation frequency $f_0$ determined by our experiment exceeded the value of $f_0'$, and was attributed to the interfacial polarization. The chitosan microsphere suspension may be reasonably analyzed utilizing the interfacial polarization theory.

## 2.2 Calculation of phase parameters and their time dependence

In order to obtain the phase parameters reflecting the electrical properties of the dispersed microsphere phase and the continuous water phase, and to depict the drug release behaviors of chitosan microspheres based on the dielectric model shown in Figure 4 and the M-W interfacial polarization theory, the phase parameters were calculated by means of the Hanai equation [11,27] and the corresponding analytical method [28,29] from the dielectric parameters given in Table 1, with the results listed in Table 2.

$$\frac{\varepsilon^* - \varepsilon_i^*}{\varepsilon_a^* - \varepsilon_i^*}\left(\frac{\varepsilon_a^*}{\varepsilon^*}\right)^{1/3} = 1-\phi\,. \tag{4}$$

The symbols $\varepsilon^*$, $\varepsilon_i^*$, $\varepsilon_\alpha^*$ in Figure 4 and eq.(4) respectively indicate the complex permittivity of the entire suspension, microsphere and water medium, with $\phi$ being the microsphere volume fraction in suspension.

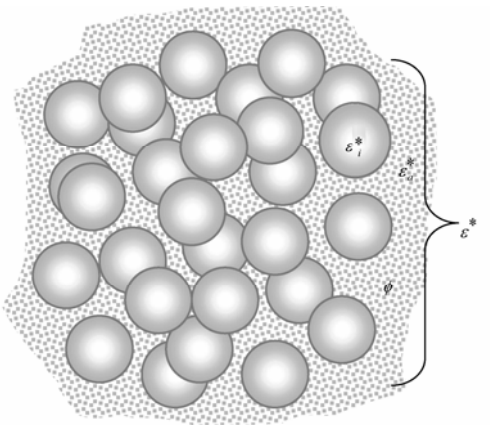


**Figure 4** Dielectric model of chitosan microspheres dispersed in a twice-distilled water medium with volume fraction $\phi$.

**Table 2** Phase parameters calculated by using eq. (4) and the dielectric parameters in Table 1

| $t$(min) | $\kappa_a$(S m$^{-1}$) | $\varepsilon_i$ | $\kappa_i$(S m$^{-1}$) |
|---|---|---|---|
| 10 | 0.001430 | 51.605 | 0.011 |
| 20 | 0.001680 | 58.591 | 0.022 |
| …… | …… | …… | …… |
| 60 | 0.001938 | 66.971 | 0.051 |
| 70 | 0.001966 | 67.792 | 0.058 |
| 80 | 0.001939 | 69.584 | 0.064 |
| …… | …… | …… | …… |
| 140 | 0.001729 | 71.338 | 0.075 |
| 150 | 0.001685 | 71.877 | 0.076 |
| 160 | 0.001619 | 71.579 | 0.075 |
| 170 | 0.001598 | 71.607 | 0.076 |
| …… | …… | …… | …… |
| 200 | 0.001435 | 71.999 | 0.073 |
| 210 | 0.001466 | 72.671 | 0.072 |
| 220 | 0.001393 | 72.620 | 0.070 |
| …… | …… | …… | …… |
| 340 | 0.002095 | 72.164 | 0.056 |
| 350 | 0.002610 | 71.979 | 0.055 |

For clarity, the parameters in Table 2 were plotted against time as shown in Figure 5. Figure 5 shows the change of electrical properties inside the release system. The permittivity of the dispersed phase, i.e., the inner permittivity of the chitosan microspheres containing the drug, $\varepsilon_i$ sharply increased from about 51 to about 60 in the first 50 min after the microspheres were put in water, and then the increment became slow. After 180 min, $\varepsilon_i$ reached a steady value of about 73 (Figure 5 (a)), being close to the value 78.3 of pure water at 25°C. This showed that the

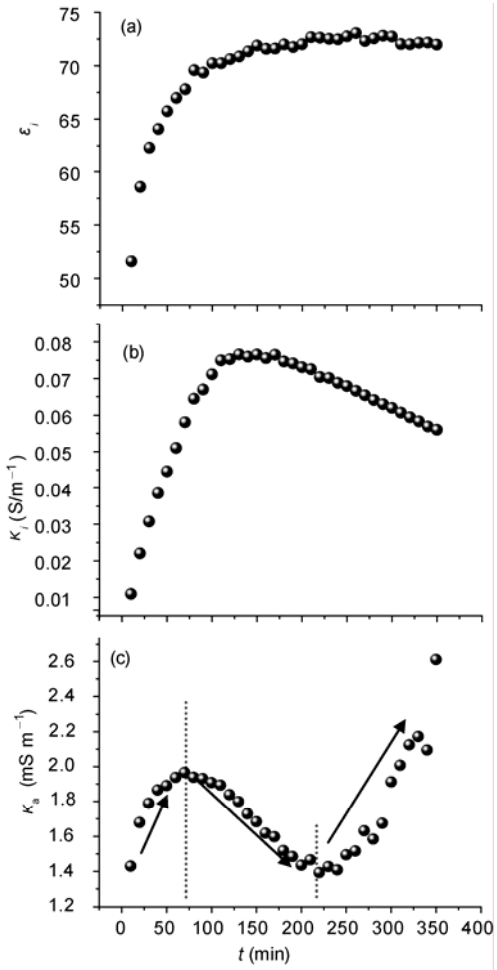



**Figure 5** Phase parameters ((a) permittivity of microspheres, (b) conductivity of microspheres, (c) conductivity of medium water) for chitosan containing a salicylic suspension as a function of time.

swelling of chitosan microspheres had attained an equilibrium state, i.e., the microspheres were water-saturated after the microspheres were immersed in water for about three hours. Figure 5(b) shows the change of the conductivity $\kappa_i$ of chitosan microspheres corresponding to the permittivity $\varepsilon_i$: the value of $\kappa_i$ increases as sharply as $\varepsilon_i$ during the initial stage when the microspheres were initially immersed in water. After attaining a relatively steady state $\kappa_i$ monotonically decreases.

This might be interpreted as follows: the rising of $\kappa_i$ in the initial stage is due to the rapid permeation of water into chitosan microspheres, leading to the electrolyte being dissolved from the microspheres participating in electrical conduction process. Thus when a dissolved electrolyte attains equilibrium, $\kappa_i$ tends towards stability. After about three hours, because swelling attains equilibrium and the salicylate ion is released from the chitosan microspheres, the conductivity $\kappa_i$ of the microspheres began gradually declining. This implies that by observing the change of the inner conductivity of microspheres over time, the drug adsorption or release process within the drug-loaded microspheres system was monitorable on line.

The change of conductivity $\kappa_a$ of a water medium in the chitosan microspheres containing a salicylic suspension was analyzed with reference to the length of time that the microspheres maintained in water. As shown in Figure 5(c), the curve had two separate flex points at about 60 and 220 minutes, and the curve was thereby divided into three parts. The release behaviors of sodium salicylate in each of the three parts may be due to the following: in the initial 50–60 minutes after the microspheres were put in water, because the microspheres adsorbed sodium salicylate, the inner conductivity $\kappa_i$ increased. Salicylate ions in the water phase also participated in the electrical conductance, leading to the conductivity $\kappa_a$ of the water medium rapidly increasing. Within about 120 min, $\kappa_a$ linearly decreased and then increased again, which may be attributed to the release of salicylate ions from chitosan microspheres into the water. The mechanism of controlled release in this system might be decided by the change in the structure of the microspheres, the dissolution of electrolytes that participated in the electrical conduction process such as salicylate ions and the adsorption equilibrium.

### 2.3 Mechanism of the controlled-release process

According to the analysis of the relationship of phase parameters with time as described in the above section, the drug release of chitosan gel beads is a complex controlled-process. For this process, we tried to analyze and give quantitatively following explanations with the aid of picture below: Because the crude chitosan molecule has a complex double coiled spiral structure, extensive twisting occurred [30]. On the other hand the amino group of the chitosan reacted with glutaraldehyde, gradually forming a three-dimensional network structure of which the main chains connected by glutaraldehyde molecules were chitosan molecules [31], and the ions in the microspheres spread outside through such structures. Also, it was reported that the cross-linked chitosan microspheres showed gaps that existed within the pores as shown in Figure 6 [32], with channels used to exchange ions. It was reported that the interaction between the positively charged chitosan polymer and the salicylate ion was very weak [33]. Therefore, we believed that salicylic ions were adsorbed onto the chitosan backbone. We divided them into two parts, with some adsorbed onto chitosan microsphere pore walls, rapidly spreading outside through the pores, while the others were adsorbed onto chitosan chains, passing through the pores by diffusion or directly spread outside.

According to results given by Figure 5(c), we divided the process of the release of salicylic acids into three stages. In the initial 0–80 min that microspheres were placed in twice-distilled water, salicylic acid rapidly dissolved, referred to as the phase burst release stage. At this stage, the

**Figure 6** Schematic representation of chitosan microsphere with pore structure.

water rapidly entered the microspheres through the pores, which led to an increment in the permittivity of the microspheres stabilized (Figure 5(a)). The salicylic acid absorbed on pore walls dissolved and spread outside through the pores, which caused a linear increase in the conductivity of both the microsphere and the water ($\kappa_l$, $\kappa_a$)(Figure 5(b) and (c)). In about 80–200 min, due to the large quantity of water entering the microspheres in the first 100 min or so, the microspheres swelled, with pores narrowing or even disappearing, so that the material transmission channels were blocked, and the conductivity stopped rising. At the same time, because the entry of the water molecules in the medium into the microspheres became slower, the permittivity of the microspheres stabilized (Figure 5(a)). After 200 min, the fact that the three parameters changed with time showed the slow release of salicylic acid (the permittivity of the microspheres had stabilized, the conductivity of the microsphere slowly decreased, while the conductivity of the water began to rise), and it was explained as being the result of the continuous diffusion of salicylic acid absorbed onto the chitosan chain.

### 2.4 The real-time dielectric measuring of the amount of released drugs

Suppose that following 200 min the salicylate release process was determined by diffusion, then based on the literature [34], the following equation could be satisfied by the total amount of loaded drugs $m_0$(i.e., the amount of drugs before release) and the amount of drugs at the time point $t$ after release as well as the coefficient of diffusion $D$.

$$\frac{m_t}{m_0}=\frac{6}{r}\sqrt{\frac{D}{\pi}}\cdot t^{\frac{1}{2}}, \tag{5}$$

where $r$ is the radius of the microsphere.

The calibration curve was drawn by preparation of a series of sodium salicylate solutions with varying condensation. The linear correlation between the conductivity of the aqueous phase $\kappa_a$ and the condensation of sodium salicylate, shown by the equation $\kappa_a=\gamma C$ (where $\gamma$ and $C$ are respectively the proportion factor and the condensation of sodium salicylate), combined with the formula $m_t/m_t=c_tV_tM/c_0V_0M=c_tV_t/c_0V_0$ (where $M$ is the molecular weight of sodium salicylate), such that the following equation is achieved:

$$\frac{\kappa_{a,t}}{\kappa_{a,0}}=\frac{6}{r}\sqrt{\frac{D}{\pi}}\sqrt{t}, \tag{6}$$

where the $\kappa_{a,t}$ and $\kappa_{a,0}$ are respectively the conductivity of time point $t$ and the conductivity when all molecules were released into the water medium. Thus, by virtue of the calibration curve, the amount of salicylate at any certain time point is calculated by measuring the conductivity of the aqueous phase $\kappa_{a,t}$ at the same time point. The release velocity of a drug at an arbitrary time within chitosan microspheres in an aqueous phase is calculated using the release amount of the drugs at an arbitrary time. The diffusion equilibrium between the salicylic acid in chitosan microspheres and the aqueous solution may be hypothesized, so that the controlled drug release system is monitored in real time.

In consideration of the controlled release stage of salicylate shown in Figure 5(c), i.e., the variation of conductivity after 200 min when the gelation equilibrium was achieved, $\kappa_{a,t}/\kappa_{a,0}$ was plotted against $t^{1/2}$ and the linear correlation as shown in Figure 7 was obtained. The small fluctuation is due to the deviation, which is caused by the analysis of the phase parameters and the calculation steps mentioned above. According to the slope of the straight line, the diffusion coefficient $D$ was calculated, $D$=9.4×10$^{-9}$ m$^2$/min. This result further demonstrated the inference of the controlled releasing mechanism of the chitosan microsphere, i.e., the differences in the controlled release mechanism between the earlier 200 min and the later 200 min. The initial 200 min was the mass transference in the pore passages, while the final 200 min was the diffusion dominated release.

## 3 Conclusions

Through the dielectric measurements and the model analysis of the suspension of chitosan microspheres, which was

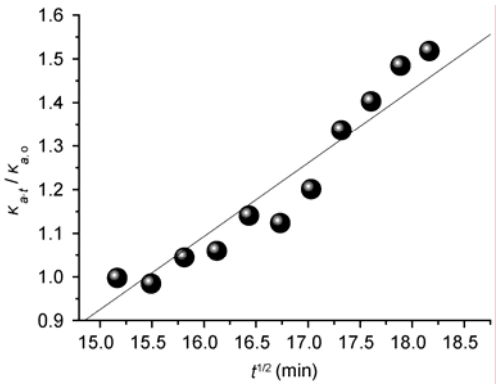



**Figure 7** $t^{1/2}$ dependence of $\kappa_{a,t}/\kappa_{a,0}$.

loaded into a model drug molecule, sodium salicylate, a significant dielectric relaxation, which appeared with the gelation of the microspheres, was observed. The dielectric parameters were achieved by using the Cole-Cole equation, and the phase parameters were obtained by means of Hanai-theory. The time dependent variation of the electrical parameters inside the chitosan microspheres and in the aqueous phase were obtained. The gelation of the microspheres and the controlled release process of salicylate were also traced as well as measured. The possible microscopic mechanism of the release process, which is divided into three stages, was proposed based on the experimental facts. Because of the inner structure of chitosan microspheres, which are full of pore canals, when drugs are released the microspheres have a self-blockade-process to them, and this self-blockade-process (the radius of the pore canals are decreased by swelling, such that the rapid release of salicylate is retarded) is the guarantee that the drug loaded microspheres, when they taken into human bodies, remain effective.

In addition, through the quantitative relationship between the amount of drugs and the conductivity obtained by dielectric analysis, the method of real-time monitoring for the release system was preliminarily established, i.e., the inner information concerning different phases is solely obtained by analyzing the dielectric spectroscopy, when the system is not interfered with. This method provides the scientific basis for obtaining detailed information about the controlled release process which can temperately not be replaced by other methods.

As mentioned above, the features and advantages of the dielectric spectroscopy method in non-invasive and *in situ* research are discussed in this article. References used in developing this method, which is used for the controlled release of drug loaded chitosan microspheres is provided. It is hoped that this process will be used in the actual drug controlled release system, so that the adsorption capacity of drugs or adsorbed substances may be evaluated, as a component of basic pharmacological research.

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