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<tr>
<td>Author(s)</td>
<td>Sekine, Katsuhisa; Hanai, Tetsuya; Koizumi, Naokazu</td>
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Dielectric Behavior of Liposomes of Large Size

Katsuhisa Sekine, Tetsuya Hanai, and Naokazu Koizumi*

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By using liposomes prepared by the method of reverse-phase evaporation, dielectric behavior was studied of the liposomes of large size. The liposomes showed a marked dielectric relaxation with the distribution of relaxation frequencies. From the dielectric observations on filtered specimens, it was inferred that the distribution of relaxation frequencies was not caused by the distribution of the diameter of suspended particles. Assuming that the electrical conductivity of the shell phase of the liposomes is much lower than that of the outer medium and that of the inner phase, observed data were analyzed in the light of a theory of interfacial polarization in suspensions of shelled spheres. Relative permittivities and electrical conductivities of the shell phase and the inner phase were calculated by using formulas derived from the theory. The specific membrane capacitance of the shell phase was estimated to be 1.3 μF cm⁻². Liposomes of small size were produced by sonication. Dielectric properties of the suspended particles of the liposomes remained unchanged regardless of dilution. The values of the volume fraction of diluted specimens were consistent with the dilutions in the preparation of specimens. Under different osmolarity in the outer medium varied by changing glucose or KCl concentration, the volume of suspended particles changed following the van’t Hoff equation. From this result, the shell phase is seen to be a semipermeable membrane through which water can permeate but glucose and KCl cannot. The conductivity of the inner phase was linearly proportional to that of the outer medium.

KEY WORDS: Conductivity/ Dielectric property/ Interfacial polarization/ Liposomes/ Permittivity/

I. INTRODUCTION

Colloid and coarse dispersions show dielectric behavior characteristic of their structures.¹⁻³ In many instances, the dielectric behavior of the dispersions was interpreted in terms of theories of interfacial polarization. From the theories of interfacial polarization, it was deduced that W/O/W emulsions exhibited a couple of dielectric relaxations.⁴⁻⁵ In certain cases, only one dielectric relaxation was observed because one of the relaxations was much larger than the other.

Natural products in the W/O/W type such as biological cells and subcellular organella exhibited remarkable dielectric relaxations.⁶⁻⁷ Membrane capacitance, permittivity and conductivity of the cell interior were evaluated from these dielectric data on the basis of the theory of interfacial polarization.

In contrast to these examples, few dielectric measurements have been carried out so far for artificial W/O/W emulsions.⁸ Several techniques to obtain stable W/O/W emulsions of large size were reported recently by many workers.⁹⁻¹³ Zhang et al. reported dielectric observations on suspensions of polystyrene microcapsules,¹⁴ which were prepared by means of the interfacial polymer deposition technique.¹³

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In this paper, dielectric behavior is reported of liposomes of large size prepared by the method of reverse-phase evaporation. The liposomes show remarkable dielectric relaxations, which are characteristic of the treatments for the specimens. The observed data are analyzed in the light of the theory of interfacial polarization.

II. THEORETICAL

1. Theoretical Formulas for a Suspension of Shelled Spheres

Liposomes are considered to be a suspension in which spheres (the complex relative permittivity $\varepsilon_r^*$) covered with a shell phase ($\varepsilon_s^*$, and the thickness $d$) are suspended in an outer medium ($\varepsilon_a^*$) with a volume fraction $\Phi$.

According to the previous paper, the complex permittivity of such a suspension ($\varepsilon^*$) is given by

$$\frac{\varepsilon^* - \varepsilon_a^*}{\varepsilon_a^* - \varepsilon_q^*} = 1 - \Phi,$$

where $\varepsilon_q^*$ is the equivalent complex permittivity of the shelled sphere (the outer diameter $D$), being given by

$$\varepsilon_q^* = \frac{2(1-\nu)\varepsilon_s^* + (1+2\nu)\varepsilon_l^*}{2(1+\nu)\varepsilon_s^* + (1-\nu)\varepsilon_l^*}.$$

The complex permittivities $\varepsilon^*$, $\varepsilon_i^*$, $\varepsilon_s^*$, $\varepsilon_a^*$ and $\varepsilon_q^*$ are defined by an equation of the following form with subscripts $i$, $s$, $a$ and $q$.

$$\varepsilon_i^* = \varepsilon - j \frac{\kappa c}{2\pi f \varepsilon_0},$$

where $\varepsilon$, $c$, $\kappa$ and $\varepsilon_0$ are the relative permittivity, imaginary unit $\sqrt{-1}$, the electrical conductivity, the permittivity of vacuum and the frequency, respectively.

From Eqs. (1) and (2), the suspension of shelled spheres is seen to exhibit two dielectric relaxations termed P-relaxation for lower frequencies and Q-relaxation for higher frequencies. Limiting values of relative permittivity $\varepsilon_l$ and $\varepsilon_h$ and electrical conductivity $\kappa_l$ and $\kappa_h$ at low (subscript $l$) and high (h) frequencies are given by

$$\varepsilon_l = \frac{3 \varepsilon_s^* - \varepsilon_a^*}{K_l - K_{eq}^1},$$

$$\varepsilon_h = \frac{3 \varepsilon_s^* - \varepsilon_a^*}{K_h - K_{eq}^1},$$

$$\kappa_l = \frac{3 \varepsilon_s^* - \varepsilon_a^*}{\varepsilon_s^* - \varepsilon_h^*},$$

$$\kappa_h = \frac{3 \varepsilon_s^* - \varepsilon_a^*}{\varepsilon_s^* - \varepsilon_h^*}.$$
Fig. 1. Change in the values of the relative permittivity $\varepsilon_m$ at intermediate frequency and the limiting values $\varepsilon_l$ and $\varepsilon_h$ at low and high frequencies with the change in the conductivity $\kappa_a$ in the outer medium. They were calculated from Pauly-Schwan’s theory using the following phase parameters: $\varepsilon_l=70$, $\varepsilon_h=60$ µS cm$^{-1}$, $\varepsilon_s=7$, $\kappa_s=0$ S cm$^{-1}$, $\varepsilon_a=80$, $D=1$ µm, $d=5$ nm and $\Phi=0.26$.

where $\varepsilon_{el}$, $\varepsilon_{eh}$, $\kappa_{el}$ and $\kappa_{eh}$ are the limiting values at low and high frequencies of the equivalent relative permittivity and the equivalent electrical conductivity of the shelled sphere. They are given by

$$
\varepsilon_{el} = \varepsilon_e \frac{\kappa_{el}}{\kappa_e} + \frac{9(\varepsilon_e \kappa_3 - \varepsilon_s \kappa_1) \kappa_2 \varepsilon_0}{[(2+\nu)\kappa_2 + (1-\nu)\kappa_1]^2},
$$

$$
\varepsilon_{eh} = \varepsilon_e \frac{2(1-\nu)\varepsilon_2 + (1+2\nu)\varepsilon_s}{(2+\nu)\varepsilon_2 + (1-\nu)\varepsilon_s},
$$

$$
\kappa_{el} = \kappa_e \frac{2(1-\nu)\kappa_2 + (1+2\nu)\kappa_1}{(2+\nu)\kappa_2 + (1-\nu)\kappa_1},
$$

and

$$
\kappa_{eh} = \kappa_e \frac{\varepsilon_{eh}}{\varepsilon_s} + \frac{9(\kappa_e \varepsilon_2 - \kappa_s \varepsilon_1) \varepsilon_0}{[(2+\nu)\varepsilon_2 + (1-\nu)\varepsilon_1]^2}.
$$

2. Behavior of the Intensity of the P-relaxation and the Q-relaxation

In order to examine the behavior of the intensity of the P-relaxation $\Delta \varepsilon_p$ and the Q-relaxation $\Delta \varepsilon_Q$, relative permittivity $\varepsilon_m$ at intermediate frequencies, $\varepsilon_l$ and $\varepsilon_h$ were calculated by Pauly-Schwan’s theory, which is valid in dilute suspensions. Figure 1 shows the change in $\varepsilon_l$, $\varepsilon_m$ and $\varepsilon_h$ with the change in $\kappa_a$. The value of $\Delta \varepsilon_p$ is given by $\Delta \varepsilon_p = \varepsilon_l - \varepsilon_m$, while $\Delta \varepsilon_Q$ is given by $\Delta \varepsilon_Q = \varepsilon_m - \varepsilon_h$. In the case of $\kappa_a < \kappa_i$, $\Delta \varepsilon_Q$ is in the same order of magnitude as that of $\Delta \varepsilon_p$. In the case of $\kappa_a > \kappa_i$, $\Delta \varepsilon_Q$ turns out negligibly small compared with $\Delta \varepsilon_p$.

III. EXPERIMENTAL

1. Preparation of Liposomes

Liposomes were prepared by the method of reverse-phase evaporation reported by Szoka, Jr. et al. A mixture of egg lecithin (Merck) and cholesterol (Standard for
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clinical work, Wako Pure Chemical Industries) in molar ratio 1:1 was used for the preparation of the liposomes. Aqueous solutions prepared for the inner phase of the liposomes contain KCl and glucose, whose concentrations were controlled to adjust the electrical conductivity and the osmolarity of the inner phase. Ficoll-400 was also added to the inner phase in 17 wt.% to facilitate the sedimentation of the suspended particles in the specimen. By means of centrifugation, the liposomes prepared were washed with aqueous solutions whose composition was the same as those used for the inner phase of the liposomes except for absence of Ficoll-400.

2. Dielectric Measurements

Capacitance and conductance were measured with a TR-1BK Transformer Ratio Arm Bridge made by Ando Electric Co., Ltd. over a frequency range 100 Hz to 1 MHz, and with a Model 4191A RF Impedance Analyser made by Hewlett-Packard Co., Ltd. over a range 1 to 200 MHz.

A measuring cell consisted of two concentric platinum cylinders, which was the same as used in a previous paper. The cell constant of 1.16 pF was determined by using air and several standard liquids.

The dielectric measurements were carried out at 25°C. Observed data were subjected to corrections for the errors arising from residual inductance caused by the cell assembly. The increase in capacitance due to electrode polarization was corrected by use of the observed data at low frequencies.

IV. RESULTS AND DISCUSSION

1. Dielectric Observations on the Liposomes and Estimation of Phase Parameters from Dielectric Parameters Observed

Figure 2(A) shows the frequency dependence of the relative permittivity \( \varepsilon \) and the electrical conductivity \( \kappa \) for the liposomes prepared with a 1 mM KCl solution for both the inner phase and the outer medium. Complex plane plots of the same data are shown in Fig. 2(B). As seen in Fig. 2(A) and Fig. 2(B), the liposomes showed a marked dielectric relaxation with the distribution of relaxation frequencies. Limiting values of the permittivity \( \varepsilon_I \) and \( \varepsilon_S \) at low and high frequencies were obtained by extrapolating the observed data to low and high frequencies with circular arcs on the complex plane plots, respectively. In this paper, the relaxation frequency \( f_0 \) was defined as the frequency corresponding to \( \varepsilon = (\varepsilon_I + \varepsilon_S)/2 \). Following the definition, the value of \( f_0 \) was determined graphically.

By the use of observed values of the dielectric parameters such as \( \kappa_I \), \( \varepsilon_I \), \( \varepsilon_S \) and \( f_0 \), the values of the phase parameters such as the volume fraction \( \Phi \), the relative permittivity \( \varepsilon_S \) of the shell phase, the relative permittivity \( \varepsilon_I \) and the electrical conductivity \( \kappa_I \) of the inner phase were estimated by means of a curve fitting method reported in the previous paper. Since conductivities of lipid membranes are very low in comparison with the aqueous phase, the electrical conductivity \( \kappa_S \) of the shell phase is assumed to be much lower than that of the inner phase \( \kappa_I \) and that of the outer medium \( \kappa_S \). Phase parameters estimated from the data shown in Fig. 2 are listed in Table I. The relative permittivity \( \varepsilon_S \) and the electrical conductivity \( \kappa_S \) of the outer medium were obtained by the dielectric measurement of the supernatant after centrifuging the liposomes at 2000 × g for 2 h.
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![Graphs showing frequency dependence of permittivity and electrical conductivity, and complex plane plots of dielectric relaxation for liposomes prepared with a 1 mM KCl solution.](image)

Fig. 2. (A) Frequency dependence of the relative permittivity $\varepsilon$ and the electrical conductivity $\kappa$, and (B) the complex plane plots of the dielectric relaxation for the liposomes prepared with a 1 mM KCl solution for both the inner phase and the outer medium. The solid curves are calculated from Eqs. (1) and (2) by using the phase parameters obtained.

The value of $\kappa_i (=58 \mu\text{S cm}^{-1})$ for the present liposomes was lower than $\kappa_a (=151 \mu\text{S cm}^{-1})$. In this case, only the P-relaxation can be observed because of $\Delta\varepsilon_i \ll \Delta\varepsilon_F$ as discussed in Section II-2. The relaxation frequency $f_0$ observed in Fig. 2 is interpreted as that of the P-relaxation.

Table I. The phase parameters calculated for the liposomes prepared with a 1 mM KCl solution and the change in the values of the phase parameters with the change in $D/d$

<table>
<thead>
<tr>
<th>$D$ (µm)</th>
<th>$d$ (nm)</th>
<th>$D/d$</th>
<th>Phase Parameter Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>5</td>
<td>400</td>
<td>$\phi$, $\varepsilon_s$, $\frac{DC_M}{pF \text{ cm}^{-1}}$, $\varepsilon_i$, $\frac{\kappa_i}{\mu\text{S cm}^{-1}}$</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>5</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Dielectric parameters observed: $\varepsilon_i=454$, $\varepsilon_a=75.6$, $\kappa_i=96.5 \mu\text{S cm}^{-1}$, $f_0=106 \text{ kHz}$. Outer medium: $\varepsilon_a=80.1$, $\kappa_a=151 \mu\text{S cm}^{-1}$.

(303)
The change in the values of phase parameters with the change in $D/d$ is summarized in Table I. Since the values of $\varepsilon_s$ are inversely proportional to $D/d$, the capacitive property of the shell phase is appropriately represented by $DC_M$ (the product of the outer diameter $D$ of the shelled spheres and the specific membrane capacitance $C_M$ of the shell phase given by $C_M=\varepsilon_s\varepsilon_0/d$). Other phase parameters such as $\Phi$, $\varepsilon_i$ and $\kappa_i$ are seen to be independent of the values of $D/d$.

According to electron microscopic observation, the mean diameter of the suspended particles was 1.0 $\mu$m. If we assume $D=1.0$ $\mu$m, $C_M$ can be evaluated from the value of $DC_M$ to be 1.3 $\mu$F cm$^{-2}$, which is consistent with those of biological cells (about 1 $\mu$F cm$^{-2}$). The values of $\varepsilon_i$ are close to the relative permittivity of water. The values of $\kappa_i$ are about 1/3 of the conductivity of 1 mM KCl solutions.

2. Effect of Filtration

Liposomes were prepared with a 200 mM glucose solution for both the inner phase and the outer medium of the liposomes. They were fractionated by use of filters (Uni-Pore membrane filter, Bio-Rad Laboratories) of the pore size 1 $\mu$m and 0.6 $\mu$m. Before the dielectric measurements, the specimens were concentrated by centrifuging at $2000 \times g$ for 2 h. In the case of the specimen sieved through the filter of the pore size 0.6 $\mu$m, the precipitate was loosely packed compared with the other specimens. The values of $s_o$ and $k_o$ were obtained by the dielectric measurements of the supernatants.

The results of dielectric measurements of a control specimen and the fractionated specimens are shown in Fig. 3 and Table II. As seen in the figure, the observed data are represented satisfactorily by circular arcs given by

$$\varepsilon - j\frac{\kappa}{2\pi f\varepsilon_0} = \varepsilon_b + \frac{(\varepsilon_i-\varepsilon_b)(1+[j/(j\omega)])^\beta}{\varepsilon_b} - j\frac{\kappa_i}{2\pi f\varepsilon_0}; \quad 0 < \beta \leq 1,$$  

where $f_o'$ is the most probable relaxation frequency and $\beta$ is the distribution parameter of relaxation frequencies.\(^{21}\)

According to electron microscopic observation, the diameter of the suspended particles was distributed over a range of 0.1 $\mu$m to 3 $\mu$m. If the distribution of relaxation frequencies is caused by the distribution of the diameter of the suspended particles, the dielectric relaxation is to tend toward the system with a single relaxation time as the liposomes approach a monodispersion, giving the value of $\beta$ closer to
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Table II. The dielectric parameters observed and the phase parameters calculated for the liposomes fractionated by use of filters

<table>
<thead>
<tr>
<th>Filter Pore Size μm</th>
<th>Outer Medium Dielectric Parameter Observed</th>
<th>Phase Parameter Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>ε₀</td>
<td>κ₀</td>
</tr>
<tr>
<td>Control</td>
<td>78.8</td>
<td>29.6</td>
</tr>
<tr>
<td>1</td>
<td>78.3</td>
<td>30.8</td>
</tr>
<tr>
<td>0.6</td>
<td>77.4</td>
<td>40.5</td>
</tr>
</tbody>
</table>

a) The distribution parameter of relaxation frequencies β is given in Eq. (13).

unity. In order to make the diameter of the suspended particles uniform, large particles suspended were removed by filtration. In spite of the unification of the particle size, the values of β shown in Table II remain unchanged regardless of the filtration treatment. This result suggests that the distribution of relaxation frequencies is not caused by the distribution of the diameter of the suspended particles.

Provided that the value of \( C_M \) is 1.3 μF cm⁻², the values of \( D \) can be estimated from the values of \( DCM \) to be 1.3 μm for the control, 1.0 μm and 0.7 μm for the two specimens sieved through the filters of the pore size 1 μm and 0.6 μm. The decrease in the values of \( D \) by the filtration reflects the fact that the mean diameter of suspended particles is decreased by the filtration.

3. Effect of Sonication

The liposomes were sonicated at 0°C. The frequency dependence of \( ε \) and \( κ \) of the control specimen and those sonicated for 5 and 30 min are illustrated in Fig. 4. Phase

Fig. 4. Frequency dependence of \( ε \) and \( κ \) for the sonicated liposomes. The relaxation frequencies of each specimen are represented by arrows.
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Table III. The dielectric parameters observed and the phase parameters calculated for the sonicated liposomes

<table>
<thead>
<tr>
<th>Sonication</th>
<th>Dielectric Parameter Observed</th>
<th>Phase Parameter Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\varepsilon_i$</td>
<td>$\varepsilon_h$</td>
</tr>
<tr>
<td>Time min</td>
<td>$\mu$ S cm$^{-1}$</td>
<td>kHz</td>
</tr>
<tr>
<td>Control</td>
<td>456</td>
<td>75.1</td>
</tr>
<tr>
<td>5</td>
<td>376</td>
<td>75.2</td>
</tr>
<tr>
<td>30</td>
<td>155</td>
<td>76.0</td>
</tr>
</tbody>
</table>

Outer medium: $\varepsilon_a=80.2$, $\kappa_a=325$ $\mu$ S cm$^{-1}$.

parameters evaluated from these data are listed in Table III. The sonicated liposomes were hardly precipitated by the centrifugation. Hence, the values of $\varepsilon_a$ and $\kappa_a$ were obtained by the dielectric measurement of the supernatant after the centrifugation of the liposomes which were not sonicated.

The values of $\varepsilon_i$ decreased and those of $\kappa_i$ increased with the sonication. These changes in dielectric parameters seem to result from the decrease in $\Phi$ and $DC_M$. If we assume that $C_M$ remains unchanged by the sonication, the decrease in $DC_M$ suggests the decrease in $D$. As reported by Huang,22) liposomes of small size (mean diameter: about 25 nm) were produced by sonication of lipid suspended in water. Provided that the liposomes of small size are produced by the sonication of the liposomes of large size, the volume fraction $\Phi$ and the diameter $D$ are to decrease by the sonication. From the present results of the dielectric measurements on the sonicated liposomes, it is suggested that the liposomes of small size are produced by sonicating the liposomes of large size.

4. Change in Volume Fraction

The liposomes prepared with a 2 mM KCl solution for both the inner phase and the outer medium were kept in a cellulose tube for dialysis (Visking) and stored overnight at room temperature in an aqueous solution of the same composition as the outer medium. The liposomes were then diluted with the medium used in the dialysis. The values of $\varepsilon_a$ and $\kappa_a$ were obtained by the dielectric measurements of the medium used in

Table IV. The dielectric parameters observed and the phase parameters calculated for the liposomes in different dilutions

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Dielectric Parameter Observed</th>
<th>Phase Parameter Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$\varepsilon_i$</td>
<td>$\varepsilon_h$</td>
</tr>
<tr>
<td></td>
<td>$\mu$ S cm$^{-1}$</td>
<td>kHz</td>
</tr>
<tr>
<td>1</td>
<td>652</td>
<td>71.4</td>
</tr>
<tr>
<td>5/4</td>
<td>558</td>
<td>73.1</td>
</tr>
<tr>
<td>5/3</td>
<td>456</td>
<td>75.1</td>
</tr>
<tr>
<td>5/2</td>
<td>335</td>
<td>77.0</td>
</tr>
</tbody>
</table>

Outer medium: $\varepsilon_a=80.2$, $\kappa_a=325$ $\mu$ S cm$^{-1}$.

a) The relative volume fraction $\Phi_R$ is defined as the ratio of the volume fraction of the diluted specimens to that of the undiluted specimen.

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Figure 5 and Table IV show the results of the dielectric measurements of the liposomes in different dilutions. The volume fraction $\Phi$ decreased with the dilution of the liposomes. Relative volume fraction $\Phi_R$ is defined as the ratio of the volume fraction of the diluted specimens to that of the undiluted specimen. The values of $\Phi_R$ shown in Table IV are consistent with the dilutions in the preparation of specimens. Other phase parameters, $DCM$, $\varepsilon_i$ and $\kappa_i$ remain unchanged regardless of the dilution. These results are reasonable because these phase parameters should be inherent in the suspended particles themselves and be independent of the volume fraction.

5. Change in Osmolarity in the Outer Medium

The dielectric measurements of the liposomes in different osmolarity in their outer medium were carried out by the following procedure.

$Step\ 1 \ Liposomes \ prepared \ with \ a \ solution \ whose \ osmolarity \ was \ adjusted \ to \ C_s \ were \ divided \ into \ several \ fractions \ in \ equal \ volume. \ The \ volume \ of \ each \ of \ the \ fractions \ was \ V_s.$

$Step\ 2 \ Solutions \ whose \ osmolarity \ was \ adjusted \ to \ C_s \ were \ added \ to \ each \ of \ the \ fractions \ up \ to \ equal \ volume \ V_s. \ At \ this \ stage, \ the \ osmolarity \ in \ outer \ medium \ was \ C_s. \ The \ values \ of \ C_s \ were \ varied \ by \ changing \ C_s.$

$Step\ 3 \ Dielectric \ measurements \ of \ each \ of \ the \ fractions \ were \ performed \ within \ one \ hour \ after \ changing \ the \ osmolarity.$

$Step\ 4 \ The \ values \ of \ \varepsilon_s \ and \ \kappa_s \ were \ obtained \ from \ dielectric \ measurements \ of \ the \ supernatant \ of \ each \ of \ the \ fractions \ after \ centrifugation.$

Liposomes were prepared with a 1 mM KCl solution whose osmolarity was adjusted to 20 mOsm by adding glucose. The unit of osmolarity Osm means Osmol l$^{-1}$. The
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Fig. 6. Frequency dependence of ε and κ for the liposomes with different osmolarity in the outer medium $C_0$. The liposomes were prepared with a 20 mOsm solution, $C_0$ being changed by using glucose.

osmolarity in their outer medium was changed by using glucose. Figure 6 and Table V show the results of the dielectric measurements in which the osmolarity in the outer medium was changed by using glucose. By use of the liposomes prepared with a 2 mM KCl solution (its osmolarity was 4 mOsm), a series of measurements was carried out in which the osmolarity in the outer medium was changed by use of KCl. Their results are given in Fig. 7 and Table VI.

The change in the volume of the suspended particles was analyzed in the light of the

Table V. The dielectric parameters observed and the phase parameters calculated for the liposomes prepared with a 20 mOsm solution in different osmolarity in the outer medium changed by using glucose

<table>
<thead>
<tr>
<th>Outer Medium</th>
<th>Dielectric Parameter Observed</th>
<th>Phase Parameter Calculated</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_0^a$ mOsm$^b$</td>
<td>ε</td>
<td>κa</td>
</tr>
<tr>
<td>--------------</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>8.11</td>
<td>79.8</td>
<td>156</td>
</tr>
<tr>
<td>10.1</td>
<td>79.7</td>
<td>157</td>
</tr>
<tr>
<td>20.0</td>
<td>80.1</td>
<td>151</td>
</tr>
<tr>
<td>29.7</td>
<td>79.9</td>
<td>144</td>
</tr>
<tr>
<td>59.7</td>
<td>79.8</td>
<td>136</td>
</tr>
<tr>
<td>120</td>
<td>79.6</td>
<td>133</td>
</tr>
</tbody>
</table>

a) The osmolarity in the outer medium.
b) The unit of osmolarity Osm means Osmol l$^{-1}$.
c) The relative particle volume $V_R$ is defined as the ratio of the volume of the suspended particles in changed osmolarity to that under isotonic condition.
van't Hoff equation. If the shell phase of the liposomes possesses the nature of a semi-permeable membrane, the volume $V_q$ of the suspended particles changes following the relation,

$$V_q = \frac{A}{C_o} + V_d,$$

(14)

where $A$ is a constant independent of $V_d$, $C_o$ the osmolarity in the outer medium and $V_d$ the osmotically dead volume of the suspended particles.

In our experiments, the volume of the suspended particles is represented by relative particle volume $V_R$ in place of $V_q$. The $V_R$ is defined as the ratio of $V_q$ under changed

<table>
<thead>
<tr>
<th>Outer Medium $C_o$ mOsm</th>
<th>Dielectric Parameter Observed $\epsilon_a \mu_S \text{cm}^{-1}$</th>
<th>Phase Parameter Calculated $\epsilon_i \mu_S \text{cm}^{-1}$</th>
<th>$\kappa_i \text{cm}^{-1}$</th>
<th>$f_0$ kHz</th>
<th>$\Phi$</th>
<th>$V_R$ pF cm$^{-1}$</th>
<th>$\alpha$ pF cm$^{-1}$</th>
<th>$\kappa_i$ mS cm$^{-1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.32</td>
<td>79.5 122</td>
<td>452 75.7 76.8 122</td>
<td>0.265 1.03 129 73 0.071</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.00</td>
<td>79.4 306</td>
<td>450 75.7 196 222</td>
<td>0.257 1.00 132 73 0.12</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>5.95</td>
<td>79.4 443</td>
<td>419 75.7 298 268</td>
<td>0.232 0.90 133 71 0.14</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.63</td>
<td>79.8 558</td>
<td>401 75.9 406 268</td>
<td>0.191 0.74 150 66 0.15</td>
<td></td>
<td></td>
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<tr>
<td>13.2</td>
<td>79.5 943</td>
<td>329 76.3 712 402</td>
<td>0.171 0.67 131 68 0.20</td>
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</table>

Table VI. The dielectric parameters observed and the phase parameters calculated for the liposomes prepared with a 4 mOsm solution in different osmolarity in the outer medium changed by using KCl.

(309)
osmolarity to $V_{\phi 0}$, which is the value of $V_{\phi}$ under isotonic condition. The value of $V_R$ is evaluated by

$$V_R = \frac{\Phi}{\Phi_0},$$

where $\Phi_0$ is the volume fraction under the isotonic condition. From Eq. (14), $V_R$ varies in the manner given by

$$V_R = \frac{A}{V_{\phi 0}C_\phi} + \frac{V_d}{V_{\phi 0}}.$$

On the other hand, the value of $C_\phi$ can be evaluated by

$$C_\phi = \frac{C_0(V_d - \Phi_0 V_d) + C_d(V_d - V_\phi)}{V_d(1 - \Phi)}.$$

In Fig. 8, $V_R$ is plotted against the reciprocal of $C_\phi$. Under hypertonic condition ($C_\phi > C_0$), the suspended particles shrink in the manner following Eq. (16). This result suggests that the shell phase of the liposomes is a semipermeable membrane through which water can permeate but glucose and KCl cannot. The volume of the suspended particles remain unchanged under hypotonic condition ($C_\phi < C_0$). It seems that the shell phase resists the expansion under hypotonic condition.

6. Change in Electrical Conductivity in the Outer Medium and the Inner Phase

Liposomes were prepared with solutions whose osmolarity was adjusted to 200 mOsm by adding glucose. The values of $k_1$ and $k_2$ were varied by changing KCl concentration. Figures 9 and 10 show the results of the dielectric measurements in different $k_2$ under both isotonic and hypotonic conditions. The osmolarity in their outer medium was controlled by using glucose. The results obtained from the liposomes prepared with a 1 mM KCl solution are given in Fig. 9 and those obtained from the liposomes prepared with a 10 mM KCl solution are given in Fig. 10. Phase parameters evaluated from these data are summarized in Table VII. The values of $\varepsilon_\phi$ and $\kappa_\phi$ were obtained by the dielectric measurements of the supernatants after centrifuging the liposomes.
Dielectric Behavior of liposomes of large size

![Graph showing frequency dependence of ε and κ for liposomes prepared with a 1 mM KCl solution in different Ks.](image)

Fig. 9. Frequency dependence of ε and κ for the liposomes prepared with a 1 mM KCl solution in different Ks.

The values of ε<sub>t</sub> and ε<sub>g</sub> remained unchanged regardless of the change in Ks. The relaxation frequency f<sub>0</sub> shifted to higher frequencies with the increase in Ks. The values of Φ, DC<sub>M</sub> and ε<sub>t</sub> remained unchanged regardless of the change in Ks, whereas the values of κ<sub>t</sub> were dependent on Ks. Figure 11 shows the relationship between κ<sub>t</sub> and Ks. The values of κ<sub>t</sub> are proportional to Ks, being independent of the concentration of KCl in the inner phase. The solid line in Fig. 11 is the empirical regression curve which is given by

\[
\kappa_t / \text{mS cm}^{-1} = 0.017 + 0.293 \kappa_s / \text{mS cm}^{-1}. \tag{18}
\]

Since the shell phase of the liposomes is impermeable to KCl as discussed in the

![Graph showing frequency dependence of ε and κ for liposomes prepared with a 10 mM KCl solution in different Ks.](image)

Fig. 10. Frequency dependence of ε and κ for the liposomes prepared with a 10 mM KCl solution in different Ks.
Katsuhisa Sekine, Tetsuya Hanai, and Naokazu Koizumi

Table VII. The dielectric parameters observed and the phase parameters calculated for the liposomes prepared with 200 mOsm solutions in different conductivity in the outer medium changed by using KCl

<table>
<thead>
<tr>
<th>Inner Phase KCl mM</th>
<th>Outer Medium Current Density C_d mOsm</th>
<th>Dielectric Parameter Observed ( \kappa_d ) mS cm(^{-1})</th>
<th>( \varepsilon_d )</th>
<th>( \varepsilon_i )</th>
<th>( \varepsilon_h )</th>
<th>( \kappa_i ) mS cm(^{-1})</th>
<th>Phase Parameter Calculated ( \phi ) MHz</th>
<th>( \Phi ) pF cm(^{-1})</th>
<th>( \kappa_i ) mS cm(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>0.950</td>
<td>78.9</td>
<td>354</td>
<td>75.7</td>
<td>0.707</td>
<td>0.270</td>
<td>0.179</td>
<td>138</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>0.149</td>
<td>78.6</td>
<td>344</td>
<td>75.3</td>
<td>0.108</td>
<td>0.114</td>
<td>0.193</td>
<td>124</td>
</tr>
<tr>
<td>1</td>
<td>71</td>
<td>0.963</td>
<td>79.3</td>
<td>498</td>
<td>75.6</td>
<td>0.822</td>
<td>0.580</td>
<td>0.253</td>
<td>151</td>
</tr>
<tr>
<td>1</td>
<td>71</td>
<td>0.566</td>
<td>79.2</td>
<td>496</td>
<td>75.3</td>
<td>0.366</td>
<td>0.350</td>
<td>0.252</td>
<td>150</td>
</tr>
<tr>
<td>1</td>
<td>71</td>
<td>0.176</td>
<td>79.1</td>
<td>455</td>
<td>74.7</td>
<td>0.115</td>
<td>0.122</td>
<td>0.247</td>
<td>139</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>1.35</td>
<td>78.6</td>
<td>240</td>
<td>75.3</td>
<td>1.04</td>
<td>1.06</td>
<td>0.160</td>
<td>93</td>
</tr>
<tr>
<td>10</td>
<td>200</td>
<td>0.427</td>
<td>78.9</td>
<td>258</td>
<td>75.6</td>
<td>0.324</td>
<td>0.350</td>
<td>0.168</td>
<td>98</td>
</tr>
<tr>
<td>10</td>
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<td>1.32</td>
<td>78.6</td>
<td>353</td>
<td>74.8</td>
<td>0.934</td>
<td>0.980</td>
<td>0.206</td>
<td>121</td>
</tr>
<tr>
<td>10</td>
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<td>0.680</td>
<td>78.8</td>
<td>333</td>
<td>74.7</td>
<td>0.478</td>
<td>0.505</td>
<td>0.209</td>
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<tr>
<td>10</td>
<td>76</td>
<td>0.533</td>
<td>78.6</td>
<td>323</td>
<td>74.5</td>
<td>0.373</td>
<td>0.440</td>
<td>0.212</td>
<td>106</td>
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</table>

Fig. 11. Plots of the electrical conductivity in the inner phase \( \kappa_i \) against \( \kappa_d \). The \( \kappa_d \) of the liposomes prepared with the 1 mM KCl solution was changed under isotonic condition (\( \bullet \)) and under hypotonic condition (\( \circ \)). The \( \kappa_d \) of the liposomes prepared with the 10 mM KCl solution was changed under isotonic condition (\( \oplus \)) and under hypotonic condition (\( \triangle \)). The solid line is the regression curve given by Eq. (18).

preceding section, the values of \( \kappa_i \) are expected to remain unchanged regardless of the change in \( \kappa_d \). At the same time, the values of \( \kappa_i \) of the liposomes prepared with the 10 mM KCl solution should be ten times higher than those of the liposomes prepared with the 1 mM KCl solution. The cause of these anomalous observations on \( \kappa_i \) described above is not understood yet.

V. CONCLUSIONS

1. The value of \( C_M \) was 1.3 \( \mu \)F cm\(^{-2}\), which is consistent with those of biological cells.
2. The values of \( \beta \) remained unchanged regardless of the filtration of the liposomes. This result suggests that the distribution of relaxation frequencies is not caused by the
Dielectric Behavior of liposomes of large size
distribution of the diameter of the suspended particles.

3. Both $\Phi$ and $DC_M$ decreased by the sonication. It is suggested from this fact that the liposomes of small size are produced by the sonication.

4. The values of $DC_M$, $\epsilon_i$ and $\kappa_i$ remained unchanged regardless of the dilution of the liposomes. The values of $\Phi_R$ are consistent with the dilutions in the preparation.

5. Under different osmolarity in the outer medium changed by using glucose or KCl, the change in $V_R$ was successfully represented by the van't Hoff equation. It is suggested from this fact that the shell phase is a semipermeable membrane through which water can permeate but glucose and KCl cannot.

6. The $\kappa_i$ was linearly proportional to $\kappa_s$.

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