

Dielectric Properties of Protoplasm, Plasma Membrane and Cell Wall in Yeast Cells

Koji ASAMI*

Received August 27, 1977

Dielectric behavior of yeast cell suspensions has been studied to elucidate dielectric properties of protoplasm, plasma membrane and cell wall. Conductivity of the cell wall showed the behavior similar to that of an ion-exchange resin membrane owing to ionized compounds in the cell wall. The concentration of the ionized compounds was estimated to be 0.011 equiv/l. Since the cell wall had the same conductivity as that of the suspending medium with KCl concentrations ranging from 10 mM to 80 mM, an electrical model of single-shell spheres may substantially be applied to the yeast cells. An analysis of the dielectric data was carried out by use of Pauly and Schwan's theory. The membrane capacitance was estimated to be $1.1 \mu\text{F}/\text{cm}^2$ and was unaffected by changes in salt concentration and osmolarity of the suspending medium. No change in the conductivity of the protoplasm was observed with variation of KCl concentration of the outer medium. This suggests that the salt concentration in the aqueous phase of the protoplasm is kept unchanged irrespective of the KCl concentration of the outer medium. The conductivity and the dielectric constant of the protoplasm was lower in comparison to the outer electrolyte solution. This may be attributed to the presence of intracellular organelles and proteins. The osmotic behavior of the yeast cells was also studied by dielectric technique. Plasmolysis was found to occur in suspending mediums with osmolarities higher than 1 osM.

INTRODUCTION

Biological cell suspensions are known to show dielectric dispersions due to interfacial polarization. Many examples are summarized in excellent reviews by Schwan¹⁾ and Cole.²⁾ By the application of an appropriate analysis to the dielectric dispersion, it is possible to derive important information upon dielectric properties of protoplasm, plasma membrane and cell wall.

Fricke and Curtis³⁾ first carried out dielectric measurement of yeast cells in suspension and calculated their membrane capacitance to be $0.6 \mu\text{F}/\text{cm}^2$. Recently, Sugiura, Koga, and Akabori⁴⁾ confirmed that the dielectric dispersion of yeast suspensions can be interpreted in terms of the Maxwell-Wagner mechanism by assuming the cell membrane to be less conductive. Unfortunately, electrical properties of the protoplasm and the cell wall were not examined in their study except for the membrane capacitance.

The purpose of this paper is to propose an electrical model for yeast cells and to clarify dielectric properties of cell wall, protoplasm and plasma membrane of the yeast cells. Osmotic behavior of the yeast cells is also studied by a dielectric technique.

* 浅見耕司 : Laboratory of Dielectrics, Institute for Chemical Research, Kyoto University, Uji, Kyoto.

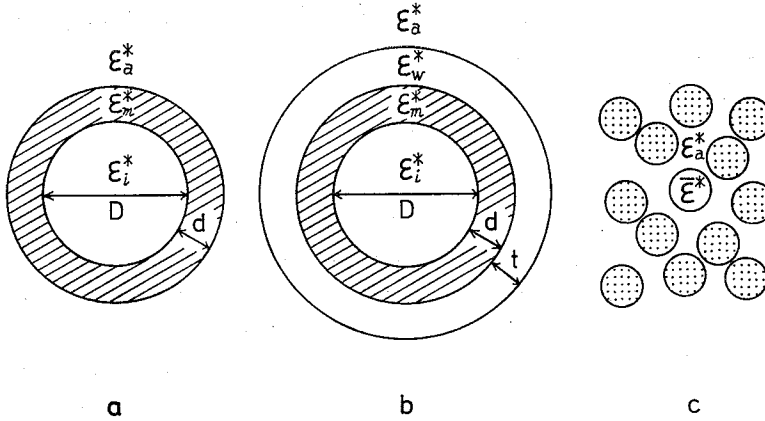


Fig. 1. a. An electrical cell model having no regard for cell wall. b. An electrical cell model taking into consideration both cell membrane and cell wall. c. A suspension of a homogeneous sphere as an equivalent of the electrical cell model.

THEORETICAL

We shall consider a complex dielectric constant ϵ^* of a biological cell suspension with a model that the cell is a sphere (ϵ_i^*) covered with a membrane phase (ϵ_m^*) and a wall phase (ϵ_w^*) as shown in Fig. 1b.

The complex dielectric constant $\bar{\epsilon}_1^*$ of the homogeneous sphere which is equivalent to the membrane-bounded sphere (shown in Fig. 1a) is given by

$$\bar{\epsilon}_1^* = \epsilon_m^* \frac{2\epsilon_m^* + \epsilon_i^* - 2(\epsilon_m^* - \epsilon_i^*)v}{2\epsilon_m^* + \epsilon_i^* + (\epsilon_m^* - \epsilon_i^*)v} \quad (1)$$

where $v = \left(\frac{D}{D+2d}\right)^3$. This equation was first derived by Maxwell.⁵⁾

If the membrane-bounded sphere (protoplast) is further covered with a cell wall as shown in Fig. 1b, the equivalent complex dielectric constant $\bar{\epsilon}^*$ of the membrane-bounded sphere covered with the cell wall is given by

$$\bar{\epsilon}^* = \epsilon_w^* \frac{2\epsilon_w^* + \bar{\epsilon}_1^* - 2(\epsilon_w^* - \bar{\epsilon}_1^*)u}{2\epsilon_w^* + \bar{\epsilon}_1^* + (\epsilon_w^* - \bar{\epsilon}_1^*)u} \quad (2)$$

where $u = \left(\frac{R}{R+2t}\right)^3$.

For a disperse system (shown in Fig. 1c), where the spheres ($\bar{\epsilon}^*$) are suspended in a continuous medium (ϵ_a^*) with a volume fraction Q , the complex dielectric constant ϵ^* of the whole system is given by the following equation which was derived by Wagner⁶⁾.

$$\epsilon^* = \epsilon_a^* \frac{2\epsilon_a^* + \bar{\epsilon}^* - 2(\epsilon_a^* - \bar{\epsilon}^*)Q}{2\epsilon_a^* + \bar{\epsilon}^* + (\epsilon_a^* - \bar{\epsilon}^*)Q} \quad (3)$$

Substitution of Eqs. (1) and (2) for Eq. (3) yields an equation which expresses the

complex dielectric constant of the suspension of cells covered with walls. If $\epsilon_w^* = \epsilon_a^*$, the equation for a suspension of cells with walls is simplified to the following equation.

$$\epsilon^* = \epsilon_a^* \frac{(1+2P)\epsilon_m^*[(1+2v)\epsilon_i^* + 2(1-v)\epsilon_m^*] + 2(1-P)\epsilon_a^*[(1-v)\epsilon_i^* + (2+v)\epsilon_m^*]}{(1-P)\epsilon_m^*[(1+2v)\epsilon_i^* + 2(1-v)\epsilon_m^*] + (2+P)\epsilon_a^*[(1-v)\epsilon_i^* + (2+v)\epsilon_m^*]} \quad (4)$$

where $P = Qu$. This equation, which is the same as a general equation of Pauly and Schwan's theory⁷⁾ for a suspension of single-shell spheres, is characterized by two relaxation times. Under the conditions $d \ll D$, $\kappa_m \ll \kappa_a$ and $\kappa_m \ll \kappa_i$ which are usually the case for biological cell suspensions, Eq. (4) reduces in effect to a single relaxation system.

LIST OF SYMBOLS

- ϵ dielectric constant
- κ electrical conductivity
- ϵ_0 dielectric constant of free space
- ϵ^* complex dielectric constant given by $\epsilon^* = \epsilon' - j\epsilon'' = \epsilon - j \frac{\kappa}{2\pi f \epsilon_0}$
- f measuring frequency
- f_p characteristic frequency of the P -dispersion
- D diameter inside the cell membrane
- R outer diameter of membrane-bounded sphere (protoplast), $D + 2d$
- d, t thickness of cell membrane and cell wall
- C_M specific membrane capacitance
- Q volume fraction of cells including cell wall in suspension
- P volume fraction of protoplasts in suspension, $P = Qu$
- v ratio of volume of cell interior (protoplasm) to protoplast, $v = \left(\frac{D}{D+2d}\right)^3$
- u ratio of volume of protoplast to whole cell, $u = \left(\frac{R}{R+2t}\right)^3$
- V volume of protoplast

Subscripts

- a outer phase
- i inner phase of cell (protoplasm)
- m, w membrane phase and wall phase
- l limiting values at low frequencies
- h limiting values at high frequencies

MATERIALS AND METHODS

Yeast cells (*Saccharomyces cerevisiae*) were grown in shaken cultures at 27°C in a medium containing 10 g yeast extract, 10 g polypepton and 20 g D-glucose per liter. The cells were collected at their early stationary phase and washed twice with

distilled water, then suspended in KCl solutions of concentrations ranging from 1 mM to 80 mM. The suspensions were incubated for at least one hour at room temperature so as to attain an ionic equilibrium between the inner and the outer phase. After incubation the collected yeast cells were resuspended in a KCl solution containing 0.1% agar. The addition of agar was effective in avoiding sedimentation of the cells during dielectric measurements and had no influence on the dielectric constant and conductivity of the medium.

Measurements of capacitance and conductance were carried out with a TR-1C Transformer Ratio-Arm Bridge of Ando Electric Co., Ltd. and with a 250A RX-Meter of Boonton Radio Corporation over a frequency range of 10 kHz to 3 MHz and 1 MHz to 100 MHz respectively. The measuring cell is a parallel plate condenser consisting of two platinized platinum plates and a lucite spacer, the cell constant being about 0.03 pF. The measuring cell is illustrated in Fig. 2. Above several tens of MHz, the bridge readings of the capacitance and the conductance were seriously affected by residual inductance arising from the terminal leads and the measuring cell. The residual inductance was estimated by Schwan's method⁸⁾ and a new method⁹⁾ to be 2.8×10^{-8} H. Correction for the residual inductance was made with Schwan's method.⁸⁾

Diameters of yeast cells were measured over about 500 cells under an optical microscope, being shown by a histogram for relative cell number in Fig. 3. Yeast cells were of a spheroid ranging from 1 to 6 μm in minor diameter and from 1 to 7 μm in major diameter. The mean diameter of the cells was calculated to be 4.3 μm from the mean volume of the cells by assuming a sphere of the same volume

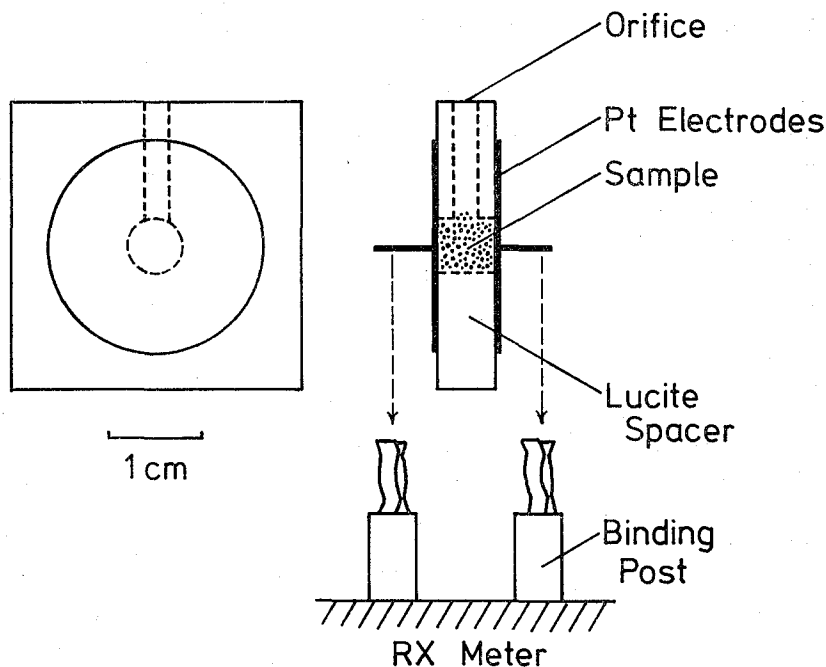


Fig. 2. A cell for dielectric measurement

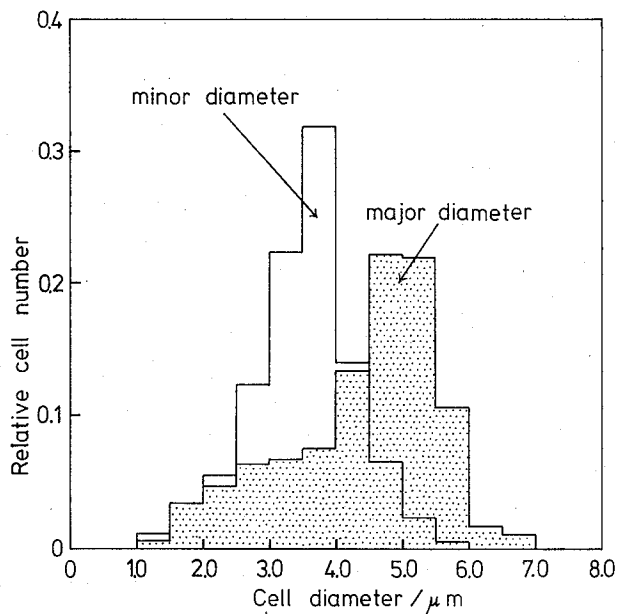


Fig. 3. Distribution of the diameter of yeast cells obtained by microscopic observation.

as that of the spheroid. The mean diameter of protoplast, R , was assumed to be $3.8 \mu\text{m}$ by using a value of $0.25 \mu\text{m}$ for the thickness of the cell wall, t , reported by Agar and Douglas.¹⁰⁾ The number of the cells in suspensions was counted by a Micro-Cell Counter Model CC-1002 of Toa Medical Electronic Co., Ltd.

RESULTS

Dielectric Behavior of Suspensions of Yeast Cells

Figure 4 shows an example of dielectric measurement for the suspension of yeast cells. The dielectric constant ϵ and electrical conductivity κ of the suspensions showed remarkable dependence on frequency. The dielectric dispersion is assigned to the P-dispersion¹¹⁾ or the β -dispersion following a nomenclature by Schwan.⁸⁾ The complex plane plots of the dielectric data shown in Fig. 4 was found to be a circular arc as seen in Fig. 5.

Sugiura *et al.*⁴⁾ reported similar results for yeast cell suspensions, and suggested that the dielectric dispersions in question are explained in terms of the Maxwell-Wagner mechanism by regarding a yeast cell as a conducting sphere covered with a less conducting shell. In addition, they¹²⁾ concluded that the less conducting shell corresponds to a cell membrane and not to a cell wall, because a marked reduction of the dielectric dispersions was observed by treating the cells with cetyl trimethyl ammonium bromide which acts directly on lipid components in the cell membranes. In our previous paper,¹³⁾ similar results were obtained also by the treatment with dodecyl dimethyl benzyl ammonium chloride and sodium dodecyl sulfonate. The question, however, still remains as to whether the presence of cell wall has no in-

Dielectric Properties of Intact Yeast Cells

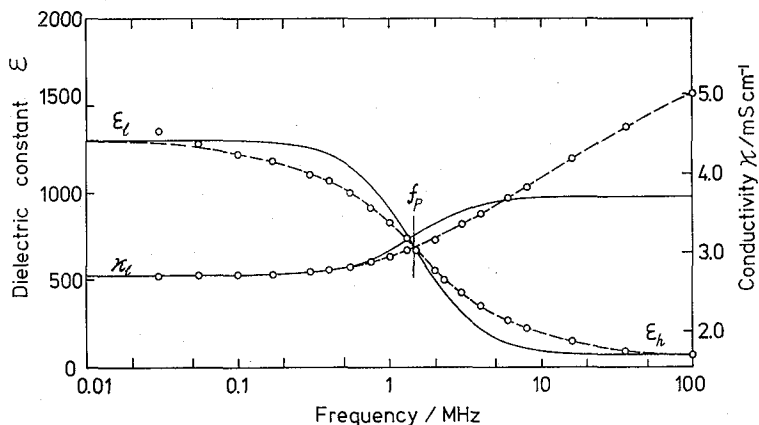


Fig. 4. An example of dielectric dispersion of a yeast cell suspension. The dielectric constant ϵ and conductivity κ were plotted against logarithm of measuring frequency. The solid curves were calculated from Eq. (4) with the estimated phase parameters (see text).

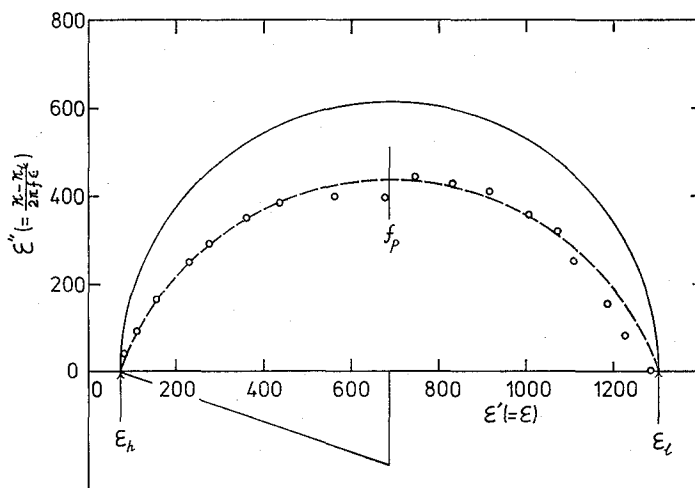


Fig. 5. Complex plane plots of the dielectric data shown in Fig. 4. The theoretical curve obtained from Eq. (4) is indicated by the solid line.

fluence on the dielectric dispersions at all, even though the dielectric dispersions may result dominantly from the cell membranes. Prior to the analysis of the present dielectric data, we must consider whether or not the cell wall may be ignored for the electrical model of the yeast cells.

Electrical Model for Yeast Cells

A cell of yeast has usually an ellipsoidal shape, and the protoplasm is enclosed with a cytoplasmic membrane and a cell wall. The wall is possessed of comparatively narrow pores which are impermeable to molecules such as dextran with a molecular weight higher than about 4500.¹⁴⁾ The pores are large enough to permeate small

molecules such as inorganic ions, amino acids and sugars. The barrier of permeation to such small molecules is the cytoplasmic membrane. As an electrical model of yeast cells, we assume a conducting sphere covered with two shells corresponding to the cytoplasmic membrane and the cell wall: the former is less conductive, and the latter more conductive. The model is schematically shown in Fig. 1b. If the cell wall has nearly the same conductivity and dielectric constant as those of the suspending medium, the electrical model of the cells may be simplified to a conducting particle covered with a less conducting shell as shown in Fig. 1a. In such a case the present data on dielectric dispersion can be analyzed by means of the procedure which was proposed by Hanai *et al.*¹¹⁾ for a single-shell sphere on the basis of Eq. (4).

Conductivity of Cell Wall

In order to consider the conductivity of the cell wall, we shall first derive a relation between the conductivities of the suspension and the cell wall. If we confine our discussion to the characteristics at low frequencies, an equation of conductivity of the suspension is derived from the general equation for a suspension of cells with walls by putting $f=0$, and is simplified to the following equation because of low values of κ_m .

$$\frac{\kappa_l}{\kappa_a} = \frac{2(1+2Q)(1-u)\kappa_w/\kappa_a + 2(1-Q)(2+u)}{2(1-Q)(1-u)\kappa_w/\kappa_a + (2+Q)(2+u)} \quad (5)$$

When Q and u are kept unchanged in Eq. (5), a change in κ_l/κ_a reflects only in κ_w/κ_a . Figure 6 shows the ratio κ_l/κ_a of suspensions for the same volume fraction plotted as a function of KCl concentration and a function of conductivity of the suspending medium. The constant volume fraction was obtained by keeping the same cell concentration and the same osmolarity of suspending medium for each suspension. In the KCl concentrations ranging from 10 mM to 80 mM, the ratio κ_l/κ_a appears to be constant irrespective of κ_a , while in the concentrations below 5 mM, κ_l/κ_a increases with decrease in κ_a . Thus the ratio κ_w/κ_a can be regarded as constant throughout the concentration range from 10 mM to 80 mM, κ_w being proportional to κ_a .

Next we estimate the constant value of κ_l/κ_a at higher KCl concentrations. The dependence of κ_l/κ_a on volume fraction at a fixed κ_a value (40 mM KCl solution) is shown in Fig. 7. The observed values fitted, within experimental errors, to a curve which was drawn according to Eq. (5) for a case where $\kappa_w/\kappa_a=1$. It may be concluded from these results that κ_w is nearly the same value as κ_a at the KCl concentrations ranging from 10 mM to 80 mM. The model of the single-shell sphere shown in Fig. 1a, therefore, can be accepted in such a range of KCl concentration as far as ε_w is in the same order of magnitude as ε_a or ε_m .

By assuming that $\kappa_w/\kappa_a=1$ at the outer medium concentrations higher than 10 mM, Eq. (5) is simplified to the following equation.

$$\frac{\kappa_l}{\kappa_a} = \frac{2(1-Qu)}{2+Qu} = \frac{2(1-P)}{2+P} \quad (6)$$

This equation is Wagner's equation for the case that non-conducting spheres are

Dielectric Properties of Intact Yeast Cells

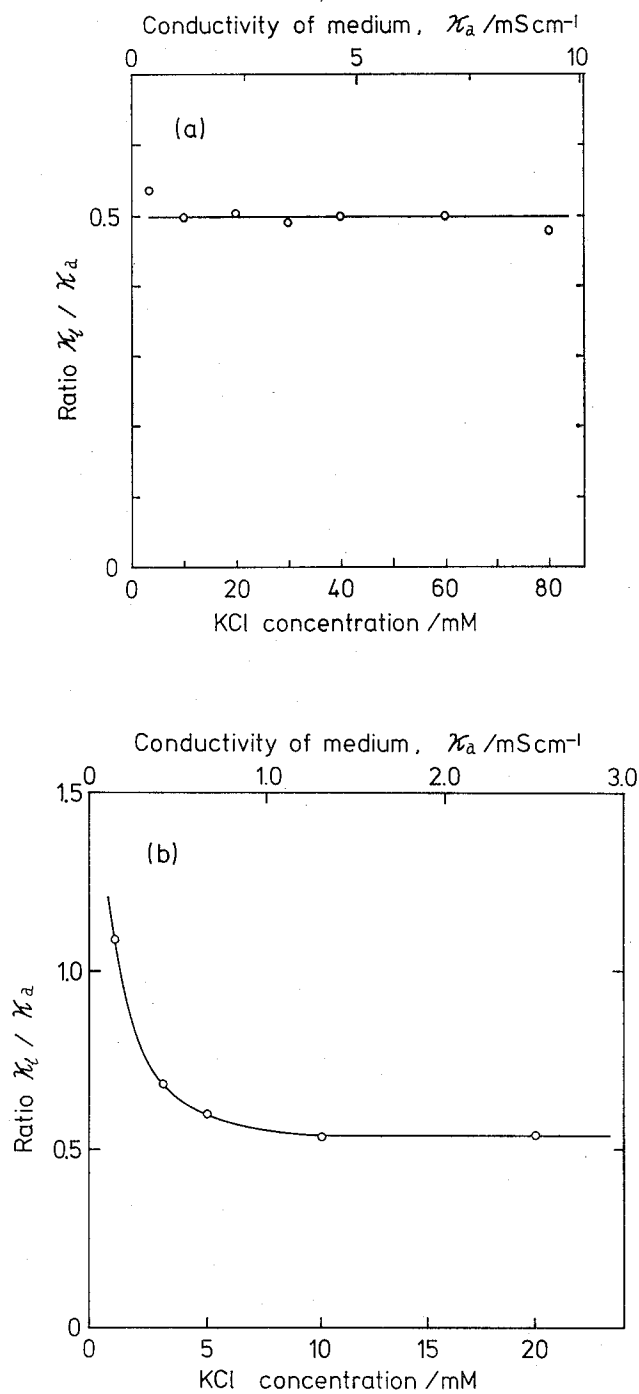


Fig. 6. Plots of κ_l/κ_a versus KCl concentration and conductivity of the suspending medium, (a) for a higher, and (b) for a lower range of the salt concentration. Osmolarities were kept constant in the suspending medium by addition of sorbitol. Cell concentrations were kept constant.

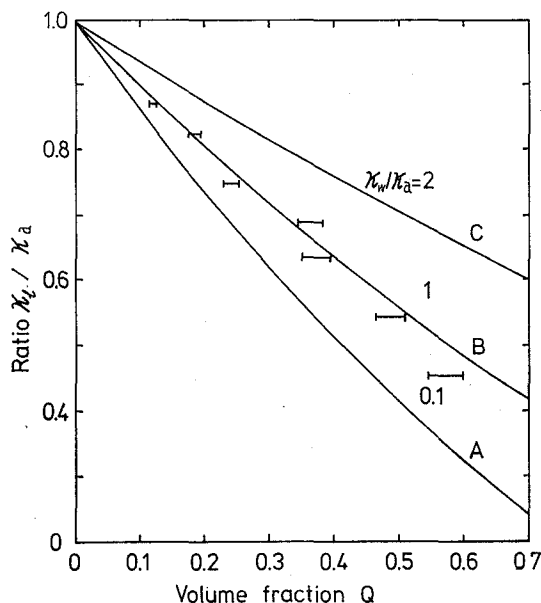


Fig. 7. Plots of κ_i/κ_a versus volume fraction Q . For each suspension, the volume fraction was calculated from average cell volume and cell number, which was obtained by optical microscopic observation and by a Cell Counter, respectively. The curves were calculated from Eq. (6) with $\kappa_w/\kappa_a=0.1$ for Curve A, 1 for Curve B and 2 for Curve C. The suspending medium was 40 mM KCl solution.

suspended in conducting medium. By using Eq. (6), volume fraction P of the suspension is calculated from the observed values of κ_a and κ_i .

When the KCl concentration in the suspending medium was below 5 mM, the conductivity of cell walls was not proportional to that of suspending medium (Fig. 6b). Estimation of these conductivities of the cell walls was carried out as follows. As the volume fraction P of each specimen shown in Fig. 6b is kept constant, the value of P which are estimated by Eq. (6) for the specimens with suspending medium of 10 and 20 mM KCl may be used for the other specimens. The value of u was assumed to be 0.69, which was calculated from the value of R and t of the cell described in Materials and Methods. By using the obtained values for P and u and the observed values of κ_i and κ_a of each specimen, the conductivities κ_w of cell walls were calculated by means of Eq. (5). Figure 8 shows the estimated wall conductivity plotted against the KCl concentration and the conductivity of suspending medium.

Estimation of Electrical Phase Parameters of Yeast Cells

Since yeast cells suspended in KCl solutions of 10 mM to 80 mM may be regarded as a sphere covered with a non-conducting shell as discussed in the preceding section, the dielectric data of such yeast suspensions may be treated with the procedure⁽¹⁾ based on Eq. (4). Phase parameters such as ϵ_m , ϵ_i , and κ_i are thus

Dielectric Properties of Intact Yeast Cells

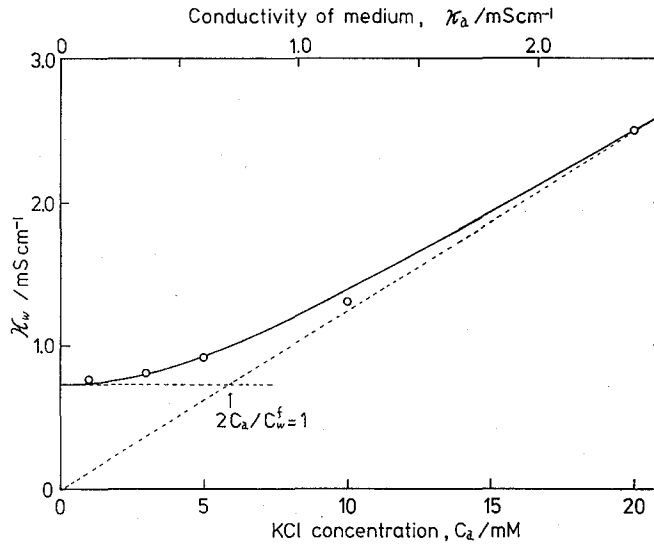


Fig. 8. Plots of the estimated conductivity of cell wall κ_w versus KCl concentration of suspending medium C_a . The solid line was calculated from Eq. (16) with $C_w^f = 0.011$ equiv/l and $\Lambda_w = 120$ S cm²/equiv.

determined so that the theoretical curves given by Eq. (4) may fit in with the observed data with respect to ϵ_i , ϵ_h , and f_p as shown in Fig. 4. The phase parameters obtained were as follow: $C_M = 1.17$ $\mu\text{F}/\text{cm}^2$, $\epsilon_m = 6.6$, $\epsilon_i = 51$, $\kappa_i = 2.5$ mS/cm. A volume fraction P was determined from the values of κ_a and κ_i by Eq. (6), being 0.29. Morphological parameters of yeast cells employed in this analysis are described in Materials and Methods. A value of 50 Å was assumed as the thickness of the cytoplasmic membrane. The membrane capacitance C_M was calculated from ϵ_m and d by means of the following equation, which was derived on the assumption that $d/D \ll 1$.

$$C_M = \frac{\epsilon_0 \epsilon_m}{d} \quad (7)$$

As pointed out by Hanai *et al.*,¹¹⁾ the value of C_M was not affected seriously by varying the value of d , while ϵ_m was directly dependent on d . The complex plane plots for the theoretical curve is featured by a semicircle as shown in Fig. 5.

Variation of Volume Fraction

Figure 9 shows frequency dependence of dielectric constant and conductivity for suspensions of yeast cells in various volume fractions. The complex plane plots of these dielectric data are illustrated in Fig. 10. The dilution of each suspension is the following: Specimen 1-A, dilution 1; 1-B, 3/5; 1-C, 2/5; 1-D, 1/5. In Table I are summarized the dielectric parameters of the dielectric dispersions shown in Figs. 9 and 10. The values of ϵ_i and κ_i are remarkably dependent on volume fraction. The values of ϵ_h is slightly lower than that of the suspending medium, showing a tendency to decrease with the increase in the volume fraction.

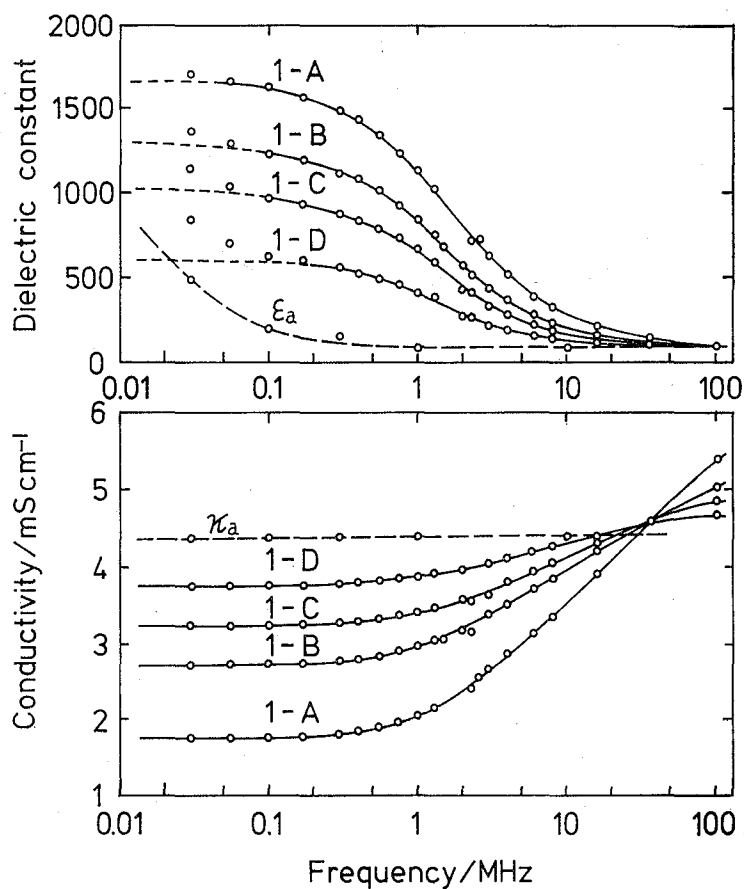


Fig. 9. Frequency dependence of dielectric constant and conductivity of yeast cell suspensions in various volume fractions. The suspending medium was a 40 mM KCl solution. Volume fraction was changed [by diluting a specimen] as : Specimen 1-A, dilution 1; 1-B, 3/5; 1-C, 2/5; 1-C, 1/5.

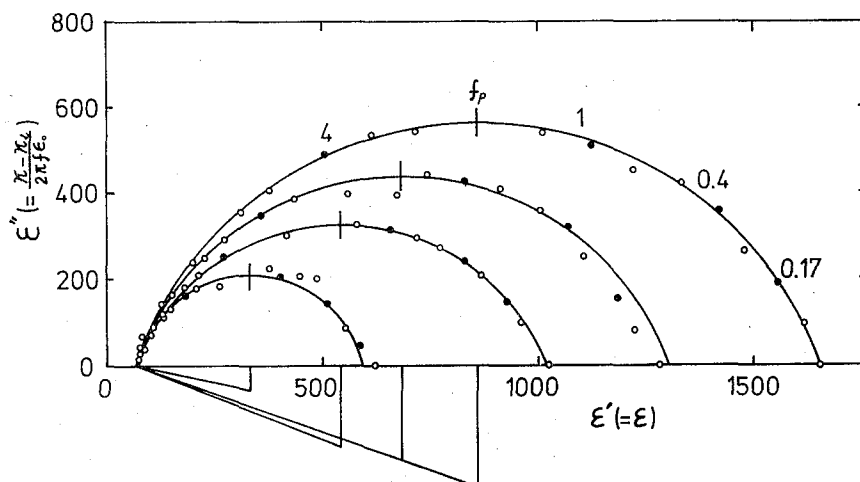


Fig. 10. Complex plane plots of the dielectric data shown in Fig. 9. Numbers beside the measured points are frequency in MHz.

Dielectric Properties of Intact Yeast Cells

Table I. Dielectric Parameters of Yeast Suspensions in Dilution Series and Estimated Phase Parameters of Yeast Cells

Specimen	Dilution	Dielectric parameters				Phase parameters				
		κ_l mS/cm	ϵ_l	ϵ_h	f_p MHz	P	C_M $\mu\text{F}/\text{cm}^2$	ϵ_m	ϵ_i	κ_i mS/cm
1-A	1	1.7	1659	64	1.9	0.50	1.05	5.9	51	2.6
1-B	3/5	2.7	1305	71	1.5	0.29	1.17	6.6	51	2.5
1-C	2/5	3.2	1017	74	1.4	0.19	1.24	6.9	49	2.5
1-D	1/5	3.7	592	77	1.4	0.10	1.20	6.8	52	2.5

$\kappa_a = 4.4$ mS/cm, $\epsilon_a = 80$, $d = 50$ Å, $R = 3.8$ μm , measuring temperature = 14°C.

The phase parameters, C_M , ϵ_m , ϵ_i , and κ_i were obtained by the analysis of the dielectric data for each specimen, being listed in Table I. From Table I, it is readily seen that C_M , ϵ_m , ϵ_i , and κ_i of the yeast cells remain unchanged regardless of dilution or change in volume fraction of the suspension, except for the value of C_M in Specimen 1-A. These results seem to be reasonable, because these parameters are supposed to be intrinsic in the cell itself and independent of volume fraction.

Variation of Salt Concentration in Outer Medium

In Fig. 11 is shown the frequency dependence of dielectric constant for yeast cell suspensions in varying KCl concentration of the suspending medium, each of the suspensions having approximately the same volume fraction. Osmolarity of the suspending medium were kept constant to prevent the cells from volume change by

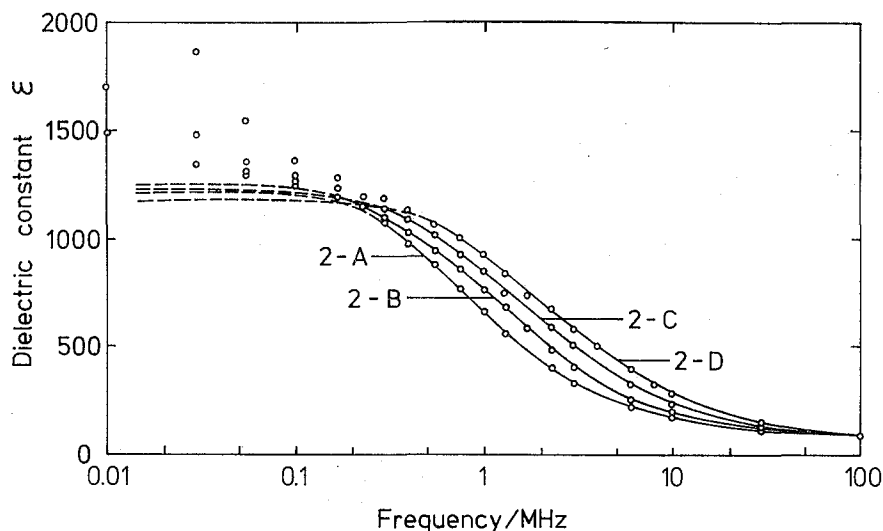


Fig. 11. Frequency dependence of dielectric constant ϵ of yeast cells suspended in various KCl concentrations. The KCl concentration of the specimens: Specimen 2-A, 10 mM; 2-B, 20 mM; 2-C, 40 mM; 2-D, 80 mM. Osmolarity of the suspending medium were kept constant (150 m osM) by addition of sorbitol. Volume fractions are in the range of 0.26 to 0.31.

Table II. Dielectric Parameters of Yeast Suspensions in Varying KCl Concentrations of Suspending Medium and Estimated Phase Parameters of Yeast Cells

Specimen	KCl concentration mM	Dielectric parameters					Phase parameters				
		κ_a mS/cm	κ_l mS/cm	ϵ_l	ϵ_h	f_p MHz	P	C_M $\mu\text{F}/\text{cm}^2$	ϵ_m	ϵ_i	κ_i mS/cm
2-A	10	1.5	0.98	1237	74	1.0	0.26	1.22	6.9	49	2.2
2-B	20	2.9	1.8	1251	72	1.4	0.28	1.14	6.4	54	2.5
2-C	40	5.6	3.3	1240	77	1.9	0.31	1.07	6.0	52	2.9
2-D	80	11.0	6.5	1230	67	2.5	0.31	1.04	5.9	42	3.5

Osmolarity of suspending medium = 150 mosM, $\epsilon_a = 80$, $d = 50 \text{ \AA}$, $R = 3.8 \text{ }\mu\text{m}$,
measuring temperature = 28°C .

addition of sorbitol, which is hardly metabolized by the strain. The dielectric parameters obtained are listed in Table II. The values of ϵ_l and ϵ_h are almost independent of KCl concentration of the suspending medium, while f_p shifts to higher frequencies markedly with increasing conductivity of the suspending medium. A steep rise in dielectric constant at frequencies below 0.1 MHz is due to electrode polarization, shifting to higher frequencies with the increase in the specimen conductivity. The phase parameters obtained by the analysis of the dielectric data for each specimen are listed in Table II. For the variation of salt concentration in the outer medium, κ_l shows a tendency to increase slightly with increasing conductivity of the medium, while C_M , ϵ_m , and ϵ_i remain unchanged.

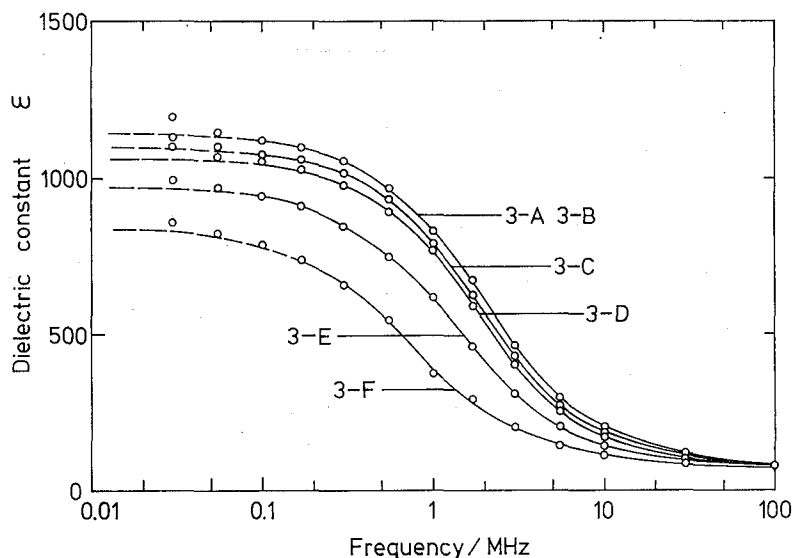


Fig. 12. Frequency dependence of dielectric constant ϵ of yeast cells suspended in various osmolarity. The KCl concentration of the suspending medium was 20 mM. Osmolarities of suspending medium were varied by addition of sorbitol as: Specimen 3-A, 0 M sorbitol; 3-B, 0.2 M; 3-C, 0.4 M; 3-D, 0.6 M; 3-E, 1.0 M; 3-F, 1.6 M.

Table III. Dielectric Parameters of Yeast Suspensions in Varying Osmolarity of Suspending Medium and Estimated Phase Parameters of Yeast Cells

Specimen	Sorbitol concentration M	Dielectric parameters					Phase parameters							
		κ_a mS/cm	κ_l mS/cm	ε_l	ε_h	f_p MHz	P	P/P_A	R^a μm	ε_i	κ_i mS/cm	ε_m	C_M $\mu\text{F}/\text{cm}^2$	$C_{M \text{ cor.}}^b$ $\mu\text{F}/\text{cm}^2$
3-A	0	2.9	1.7	1149	76	2.0	0.33	1	3.80	73	2.9	5.3	0.94	0.94
3-B	0.2	2.7	1.5	1149	76	2.0	0.33	1.0	3.80	73	2.9	5.3	0.94	0.94
3-C	0.4	2.5	1.5	1104	73	1.9	0.31	0.95	3.73	63	2.9	5.4	0.95	0.92
3-D	0.6	2.4	1.4	1069	73	1.8	0.28	0.85	3.59	59	3.0	5.8	1.03	0.92
3-E	1.0	2.0	1.3	976	73	1.4	0.24	0.71	3.39	56	2.5	6.4	1.13	0.90
3-F	1.6	1.5	1.1	840	73	0.8	0.18	0.55	3.12	49	1.3	7.2	1.27	0.86

a. $R=R_A (P/P_A)^{1/3}$, b. $C_{M \text{ cor.}}=C_M(P/P_A)^{2/3}$, $d=50\text{\AA}$, measuring temperature= 26°C , $\varepsilon_a=80$

Variation of Osmolarity in Outer Medium

Figure 12 shows frequency dependence of dielectric constant of the yeast suspensions in varying osmolarity of the suspending medium containing 20 mM KCl by addition of sorbitol. Their dielectric parameters are summarized in Table III. The value of ϵ_i remarkably decreases at higher osmolarity, while the value of ϵ_h is almost unchanged. The decrease in κ_a accompanied by addition of sorbitol results in the decrease in the values of κ_i and f_p . The volume fraction P , which is calculated from the values of κ_a and κ_i by Eq. (6), decreases with the increase in osmolarity of the suspending medium as shown in Table III. Since each specimen is prepared in the same cell concentration, the volume fraction may change in proportion to the volume of protoplast. Thus the protoplast volume relative to that of Specimen 3-A, V/V_A , is equal to the relative volume fraction of specimen, P/P_A . In order to analyze the dielectric data, it is assumed that the diameters of the protoplasts for each specimen are given by

$$R = R_A \left(\frac{V}{V_A} \right)^{1/3} = R_A \left(\frac{P}{P_A} \right)^{1/3}, \quad (8)$$

where R_A is the mean diameter of the protoplasts in Specimen A ($R_A = 3.8 \mu\text{m}$). The phase parameters, ϵ_i , κ_i , ϵ_m , and C_M obtained by the analysis are summarized in Table III. The value of ϵ_i decreases with the decrease in cell volume, while the value of κ_i remains constant except for Specimen 3-F. The values of ϵ_m and C_M , though essentially independent of protoplast volume, increase with the decrease in protoplast volume. This is because the surface area of the membrane is altered in this analysis owing to the use of the diameters given by Eq. (8), while the shrinkage of cells actually causes no change in the specific surface area of cytoplasmic membrane. It is necessary, therefore, to correct the estimated value of C_M by reducing the surface area of the membrane to the original one. The correction of C_M is carried out by the following equation.

$$C_{M \text{ cor.}} = C_M \left(\frac{R}{R_A} \right)^2 = C_M \left(\frac{P}{P_A} \right)^{2/3}. \quad (9)$$

The value of the corrected membrane capacitance $C_{M \text{ cor.}}$ is listed in Table III, remaining constant except for Specimen 3-F in the case of extreme shrinkage.

DISCUSSION

Conductivity of Cell Walls

The change in the conductivity of the cell wall against salt concentration of the suspending medium was similar to that reported by Carstensen, Cox, Mercer, and Natale¹⁵⁾ for intact cells of *E. coli* and *M. lysodeikticus*, and by Carstensen and Marquis¹⁶⁾ for isolated cell walls of *M. lysodeikticus*. They suggested that κ_w showed the behavior similar to that of an ion exchange resin membranes owing to ionized components contained in cell walls. We will discuss the present results along the same line as laid down by Carstensen *et al.*¹⁵⁾ If the wall region with fixed charges is in equilibrium with the outer solution of a uni, uni-valent electrolyte, the electro-

chemical potentials of each species of ions in the both regions are equal to each other. By assuming that the activity coefficients of all the ions in the both regions are unity, we can derive the following equation:

$$C_w^+ \cdot C_w^- = C_a^2, \quad (10)$$

where C_w^+ and C_w^- are concentrations of the co- and the counter-ion within the wall respectively, and C_a is the concentration of the electrolyte in the outer medium. The condition of electroneutrality in the wall is given by

$$C_w^+ - C_w^- - C_w^f = 0 \quad (11)$$

where C_w^f is the concentration of the fixed charge within the cell wall. The C_w^+ and C_w^- can be solved from Eqs. (10) and (11) as

$$C_w^+ = \frac{1}{2} C_w^f \left[1 + \sqrt{1 + \left(\frac{2C_a}{C_w^f} \right)^2} \right], \quad (12)$$

$$C_w^- = \frac{1}{2} C_w^f \left[-1 + \sqrt{1 + \left(\frac{2C_a}{C_w^f} \right)^2} \right]. \quad (13)$$

The conductivity of the cell wall is the sum of products of concentrations of ions and the ionic equivalent conductivities in the wall region, λ_w^+ and λ_w^- , being expressed as

$$\begin{aligned} \kappa_w &= \lambda_w^+ C_w^+ + \lambda_w^- C_w^- \\ &= \frac{1}{2} C_w^f \left[(\lambda_w^+ - \lambda_w^-) + (\lambda_w^+ + \lambda_w^-) \sqrt{1 + \left(\frac{2C_a}{C_w^f} \right)^2} \right]. \end{aligned} \quad (14)$$

If we assume that

$$\lambda_w^+ = \lambda_w^- = \frac{1}{2} A_w,$$

then Eq. (14) is reduced to

$$\kappa_w = \frac{1}{2} C_w^f A_w \sqrt{1 + \left(\frac{2C_a}{C_w^f} \right)^2}. \quad (15)$$

Equation (15) is simplified, for the case where $C_a \gg C_w^f$, as

$$\kappa_w = A_w C_a, \quad (16)$$

and, for the case where $C_a \ll C_w^f$, as

$$\kappa_w = \frac{1}{2} C_w^f A_w. \quad (17)$$

The two straight lines of Eqs. (16) and (17) intersect at a point given by

$$C_w^f = 2C_a. \quad (18)$$

In Fig. 8, the straight lines corresponding to Eqs. (16) and (17) are shown by the broken lines. At the point of intersection of these two lines, C_w^f is estimated to be 0.011 equiv/l. The solid line in the figure is calculated from Eq. (15) with the

estimated values of C_w^f and A_w . It is readily seen that the theoretical curve fits in well with the observed points. The present value of C_w^f for the yeast cells is fairly low compared with *E. coli* (0.07 equiv/l) and *M. lysodeikticus* (0.2 equiv/l).¹⁵⁾ Among components of the yeast cell wall, the ionized components may be phosphate-bridging mannan and proteins.

Osmotic Behavior of Yeast Cells

Since yeast cells carry cell walls, it is necessary to take account of the turgor pressure due to the cell wall P_T , in addition to the osmotic pressure of the protoplasm π_i , and that of the outer medium π_a . When the cell interior is in equilibrium with the outer medium, the turgor pressure is given by

$$P_T = \pi_i - \pi_a. \quad (19)$$

By assuming no leakage of solutes from the protoplasm, one obtains the Van't Hoff-Boyle relationship:

$$\pi_i(V-b) = a, \quad (20)$$

where V is the volume of the protoplast, b is the osmotic dead space and a is a constant. From Eqs. (19) and (20), the following equation is obtained.

$$V = \frac{a}{P_T + \pi_a} + b. \quad (21)$$

When π_a is higher than the critical osmotic pressure of plasmolysis π_c , the turgor pressure is assumed to be zero. Thus one obtains

$$V = \frac{a}{\pi_a} + b. \quad (22)$$

It was reported by many investigators^{17,18,19)} that this equation expressed successfully the osmotic behavior of intracellular organelles and biological cells without cell walls. For the case of $P_T \simeq \pi_i \gg \pi_a$, Eq. (21) is reduced to

$$V = \frac{a}{P_T} + b. \quad (23)$$

If V is plotted against the reciprocal of π_a , the following is predicted from Eq. (21). At osmotic pressures higher than π_c , V increases in proportion to $1/\pi_a$ as given by Eq. (22). At osmotic pressures lower than π_c , the increment of V becomes small with the increase in $1/\pi_a$, V eventually being unchanged and independent of $1/\pi_a$. From the plots of V against $1/\pi_a$, the turgor pressure may be estimated as follow. When $\pi_a = \pi_1$, the cell volume V_1 is given by

$$V_1 = \frac{a}{P_T + \pi_1} + b. \quad (24)$$

The value of π_2 is estimated by extrapolation of the linear portion of the plots of V versus $1/\pi_a$ at osmotic pressures higher than π_c to the cell volume V_1 .

$$V_1 = \frac{a}{\pi_2} + b. \quad (25)$$

The value of π_2 is equal to the osmotic pressure of the protoplasm. The turgor pressure is then obtained from Eqs. (24) and (25) as

$$P_T = \pi_2 - \pi_1.$$

In Fig. 13, the relative volume V/V_A ($=P/P_A$) shown in Table III is plotted against the reciprocal of π_a and that of osmolarity of the suspending medium. The broken line is drawn from Eq. (22). The threshold pressure of plasmolysis π_c , is found to be about 1 osM. The value of b/V_A is estimated by extrapolation of the broken line to be approximately 0.2–0.3. The turgor pressure is obtained by the method described above, being listed in Table IV together with the osmotic pressures of the protoplasm and the outer medium. Marquis and Carstensen²⁰⁾ reported a similar osmotic behavior for *S. faecalis* cells and found the value of π_c to be 1.0–1.1 osmol/kg.

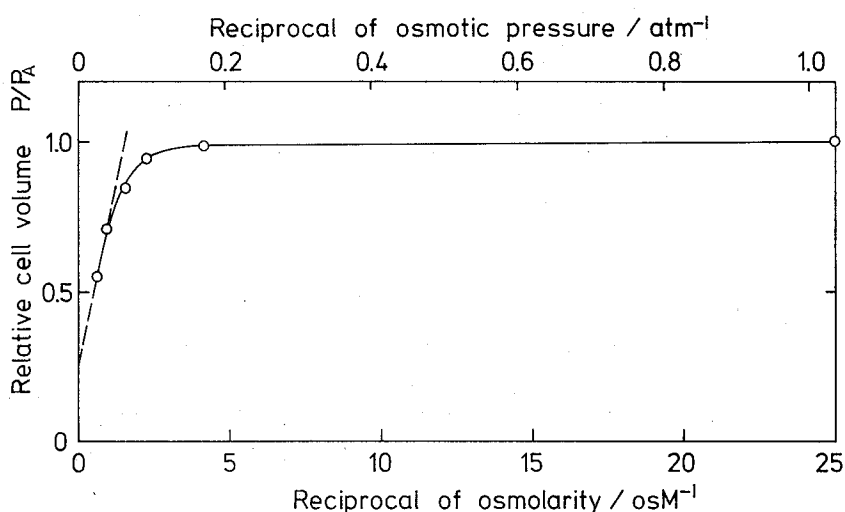


Fig. 13. Plots of relative cell volume P/P_A against reciprocal of osmotic pressure and osmolarity of the suspending medium. The broken line shows the curve for Eq. (22).

Table IV. The Estimated Turgor Pressures and Osmotic Pressures of Protoplasm

Specimen	Osmotic pressure of suspending medium (atm)	Osmotic pressure of protoplasm (atm)	Turgor pressure (atm)
3-A	0.9	15.5	14.6
3-B	5.8	15.9	10.1
3-C	10.6	16.8	6.2
3-D	15.5	19.5	4.0
3-E	25.1	25.1	0
3-F	39.6	39.6	0

Capacitance and Dielectric Constant of the Yeast Plasma Membranes

A mean value of $1.1 \mu\text{F}/\text{cm}^2$ estimated for the specific membrane capacitance of yeast cells is consistent with those of most of biological cells so far reported with values of about $1 \mu\text{F}/\text{cm}^2$. Only available values of membrane thickness of yeast cells are obtained by electron microscopic studies. According to Vitols *et al.*²¹⁾ cytoplasmic membranes of yeast cells are composed of two dense layers of 20 to 25 Å thick separated by a less dense layer of the same thickness. Probably the three layers may be regarded as a hydrophilic-hydrophobic-hydrophilic sandwich structure of the membranes. Provided that the thickness of the hydrophobic layer corresponds to dielectric thickness of the membrane, dielectric constants of the membranes are estimated to be 2.6 to 3.2 by using the values of 20 to 25 Å for the less dense layer in the membrane of yeast cells. This value of dielectric constant is consistent with dielectric constant 2–3 estimated for hydrocarbon.

Fettiplace, Andrews, and Haydon²²⁾ discussed the difference between the capacitances of biological cell membranes ($1 \mu\text{F}/\text{cm}^2$) and those of bilayer lipid membranes (BLM) ($0.4\text{--}0.6 \mu\text{F}/\text{cm}^2$). Provided that dielectric constant of biological membrane is the same value as BLM, the dielectric thickness of biological membrane is considered to be thinner than that of BLM. In order to explain the thin dielectric thickness of biological membranes, they proposed a model of the stretched reaflet, in which hydrocarbon chains interact with non-polar residues of protein. Another possible explanation for the difference is the increase in effective dielectric constant by the presence of proteins immersed in the hydrocarbon region of the membrane.

Electrical Conductivity and Dielectric Constant of the Protoplasm

Electrical conductivity of an aqueous phase in the protoplasm can not be discussed directly from κ_i , because yeast cells contain intracellular organelles. For the specimens listed in Table II, a remarkable increase in conductivity of the suspending medium was observed by treating the yeast cells with heat, detergents and repetition of freezing and thawing. This increase apparently resulted from ion leakage from the interior of cells to the outer medium. Hence the ionic concentration of the aqueous phase in the protoplasm is considered to be higher than that of the suspending medium. The measured κ_i is nevertheless lower than κ_a except for Specimen 2-A in Table II. These results imply that ionic mobility in the aqueous phase in the protoplasm is considerably low as compared with that of the outer medium as suggested by Pauly and Schwan,²³⁾ Marquis and Carstensen,²⁰⁾ and/or that the cells contain some regions which are not available to the ionic movement.

No change in κ_i is observed with the variation of salt concentration of the outer medium. The ionic concentration in the aqueous phase in the protoplasm, therefore, seems to be kept unchanged throughout this range of the experimental condition. Similar results were reported for living cells such as pleuropneumonia-like organisms,²⁴⁾ chlorella²⁵⁾, and lymphoma cells.²⁶⁾ On the other hand, for isolated subcellular organelles such as mitochondria,^{27,28)} and synaptosomes,²⁹⁾ κ_i varied in proportion to κ_a .

When the cell volume is changed by varying the osmotic pressure of the sus-

pending medium, the value of κ_i is found to remain unchanged, except for the cells suspended in a medium of extremely high osmolarity (Specimen 3-F). The decrease in cell volume results in the increase in concentration of ions, and lowers the mobility of ions in the protoplasm owing to the increase in concentration of intracellular compounds. Probably because the decrease in mobility of ions in the protoplasm offsets the increase in conductivity due to increase in concentration of ions, the value of κ_i may remain unchanged.

The dielectric constant of protoplasm of the yeast cells is always lower than the suspending medium and decreases with shrinkage of the cells. This fact implies that the parts having low dielectric constant is included in the protoplasm and that their volume fraction increases with the decrease in cell volume. These parts are probably membranous organelles, lipid granules and proteins.

A Problem on the Analysis of Dielectric Data

The analysis of the observed data was carried out by fitting the observed data to the theory with respect to the four dielectric parameters ϵ_i , κ_i , ϵ_h , and f_p . Making a comparison between the observed points and the theoretical curves in Figs. 4 and 5, the observed points were found to deviate appreciably from the theoretical curve. The measured dielectric dispersion against the frequency was broader than the theoretical prediction and the measured conductivity increased markedly beyond the theoretical values at higher frequencies. The complex plane plots of the observed data showed a circular arc, while the theoretical curve showed a semicircle. This deviation, which was formally explained by distribution of relaxation times, remains for future investigation. Irimajiri *et al.*²⁰⁾ presumed from their numerical examination that the broadening observed for dielectric dispersion curve of synaptosome suspensions is attributed to some distribution of internal conductivity of synaptosomes rather than to size heterogeneity of the suspended particles. This effect must be considered in the case of yeast cells, because yeast cells contained in suspension are in various growth stages. In addition, it is necessary to examine an effect of cell shape on dielectric dispersion, since yeast cells is regarded as non-spherical but prolate spheroidal. A dielectric dispersion due to membranous organelles in cytoplasm may also partly cause the broadening and the increase in conductivity at higher frequencies.

ACKNOWLEDGMENT

The author wishes to express his gratitude to Professor N. Koizumi and Assistant Professor T. Hanai for many valuable discussion and suggestions during the course of this study. He thanks Dr. A. Irimajiri, Dept. of Physiology, Kyoto University, for helpful suggestions and Mr. K. Awano for technical assistance in cultivation of yeast cells. Thanks are also due to Professor T. Yamamoto for active interest in this study. This work was supported in part by a research grant from the Ministry of Education, Japan.

REFERENCES

- (1) H. P. Schwan, "Advances in Biological and Medical Physics," Vol. V, J. H. Lawrence and

- C. A. Tobias, Ed., Academic Press, New York, (1957), p. 147.
- (2) K. S. Cole, "Membranes, Ions and Impulses," University of California Press, Berkeley and Los-Angeles, California, 1968.
- (3) H. Fricke and H. J. Curtis, *Nature (London)*, **134**, 102 (1934).
- (4) Y. Sugiura, S. Koga, and H. Akabori, *J. Gen. Appl. Microbiol.*, **10**, 163 (1964).
- (5) J. C. Maxwell, "A Treatise on Electricity and Magnetism," Clarendon Press, (1891), p. 435.
- (6) K. W. Wagner, *Arch. Electrotech. (Berlin)*, **2**, 371 (1914).
- (7) H. Pauly and H. P. Schwan, *Z. Naturforsch.*, **14b**, 125 (1959).
- (8) H. P. Schwan, "Physical Techniques in Biological Research," Vol. VI, Part B, W. L. Nastuk, Ed., Academic Press, New York and London, (1963), p. 323.
- (9) K. Asami, A. Irimajiri, T. Hanai, and N. Koizumi, *Bull. Inst. Chem. Res., Kyoto Univ.*, **51**, 231 (1973).
- (10) H. D. Agar and H. C. Douglas, *J. Bacteriol.*, **70**, 427 (1955).
- (11) T. Hanai, N. Koizumi, and A. Irimajiri, *Biophys. Struct. Mechanism*, **1**, 285 (1975).
- (12) Y. Sugiura and S. Koga, *Biophys. J.*, **5**, 439 (1965).
- (13) K. Asami, T. Hanai, and N. Koizumi, *J. Membrane Biol.*, **34**, 145 (1977).
- (14) P. Gerhart and J. A. Judge, *J. Bacteriol.*, **87**, 945 (1964).
- (15) E. L. Carstensen, H. A. Cox, W. B. Mercer, and L. A. Natale, *Biophysical J.*, **5**, 289 (1965).
- (16) E. L. Carstensen and R. E. Marquis, *Biophysical J.*, **8**, 536 (1968).
- (17) A. R. Gilby and A. V. Few, *J. Gen. Microbiol.*, **20**, 321 (1959).
- (18) P. G. Le Fevre, *J. Gen. Physiol.*, **47**, 585 (1964).
- (19) H. Tedeschi and D. L. Harris, *Arch. Biochem. Biophys.*, **58**, 52 (1955).
- (20) R. E. Marquis and E. L. Carstensen, *J. Bacteriol.*, **113**, 1198 (1973).
- (21) E. Vitols, R. J. North, and A. Linnane, *J. Biophys. Biochem. Cytol.*, **9**, 689 (1961).
- (22) R. Fettiplace, D. M. Andrews, and D. A. Haydon, *J. Membrane Biol.*, **5**, 277 (1971).
- (23) H. Pauly and H. P. Schwan, *Biophysical J.*, **6**, 621 (1966).
- (24) H. P. Schwan and H. J. Morowitz, *ibid.*, **2**, 395 (1962).
- (25) A. B. Hope, *Aust. J. Biol. Sci.*, **19**, 53 (1956).
- (26) A. Irimajiri, Y. Doida, T. Hanai, and A. Inouye, *J. Membrane Biol.*, in press. (1977).
- (27) H. Pauly and L. Packer, *J. Biophys. Biochem. Cytol.*, **7**, 603 (1960).
- (28) H. Pauly, L. Packer, and H. P. Schwan, *ibid.*, **7**, 589 (1960).
- (29) A. Irimajiri, T. Hanai, and A. Inouye, *Biophys. Struct. Mechanism.*, **1**, 273 (1975).