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Dielectric Behavior of Yeast Cell Suspensions: Effects of Some Chemical Agents and Physical Treatments on the Plasma Membranes and the Cytoplasms

Koji ASAMI*

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Dielectric measurements were made on suspensions of yeast cells treated with various chemical agents such as ionic and nonionic detergents, sodium tetraphenylborate, KI and gramicidins, and with various physical means such as heating, freezing-thawing and irradiation of UV light. Two different types of changes in the dielectric dispersion were observed by these treatments. For the treatments with nonionic detergents and sodium tetraphenylborate, the characteristic frequency of the dielectric dispersion was found to shift to lower frequencies without changing the limiting dielectric constant and conductivity at low frequencies, indicating a decrease in conductivity of the cytoplasm without remarkable changes in the conductivity and capacitance of cytoplasmic membranes. On the other hand, the dielectric dispersion was reduced by the treatments with ionic detergents and physical means. Since the reduction was accompanied by a decrease in packed volume of the cells and by a leakage of the intracellular compounds, it is considered that the reduction is caused by remarkable increase in the conductivity of cytoplasmic membrane and decrease in the cell volume.

I. INTRODUCTION

It is known from many examples[1,2] that suspensions of biological cells show marked dielectric dispersions due to the Maxwell-Wagner mechanism, because the cells are covered with poorly conducting surface membranes. With a view to carrying out closer analysis of the dielectric bahavior of biological cell suspensions, Pauly and Schwan[3] developed a dielectric theory for a suspension of spherical particles covered with a shell. A general equation of their theory for the complex dielectric constant ε^* of such a system with volume fraction P is expressed as

$$\frac{\varepsilon_a^*-\varepsilon^*}{2\varepsilon_a^*+\varepsilon^*}=\frac{(\varepsilon_a^*-\varepsilon_m^*)(2\varepsilon_m^*+\varepsilon_i^*)+(\varepsilon_a^*+2\varepsilon_m^*)(\varepsilon_m^*-\varepsilon_i^*)(1+2d/D)^{-3}}{(2\varepsilon_a^*+\varepsilon_m^*)(2\varepsilon_m^*+\varepsilon_i^*)+2(\varepsilon_a^*-\varepsilon_m^*)(\varepsilon_m^*-\varepsilon_i^*)(1+2d/D)^{-3}}P. \quad (1)$$

where the definition for symbols and subscripts are given in List of Symbols.

It is predicted from the numerical calculation of Eq. (1) that an increase in electrical conductivity of cell membranes gives rise to a reduction of the dielectric dispersion as shown in Fig. 1, so that the dielectric approach is useful to detect the change in membrane conductivity or destruction of membranes caused by treatment with detergents, organic solvents, heating, freezing and thawing and so on. Furthermore, even when an increase in membrane conductivity is not large enough to reduce di-

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

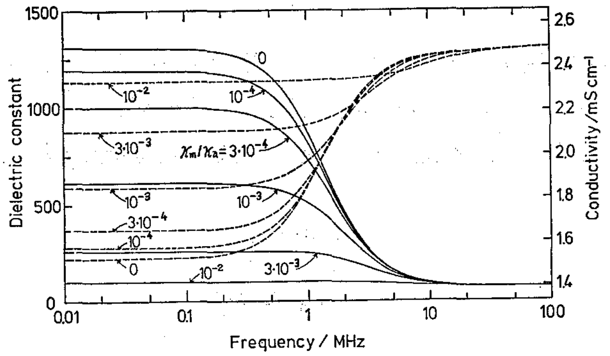


Fig. 1. Effect of varying κ_m on frequency dependence of dielectric constant and conductivity. The curves are numerically calculated from Eq. (1) with phase parameters as: $\varepsilon_a=80$; $\kappa_a=2.5$ mS/cm; $\varepsilon_m=6.5$; $\varepsilon_i=50$; $\kappa_i=2.5$ mS/cm; $d=50$ Å; $D=3.8$ μm; $P=0.3$. The solid lines and the broken those indicate dielectric constant and conductivity, respectively. The numbers beside the curves indicate the ratio of κ_m to κ_a

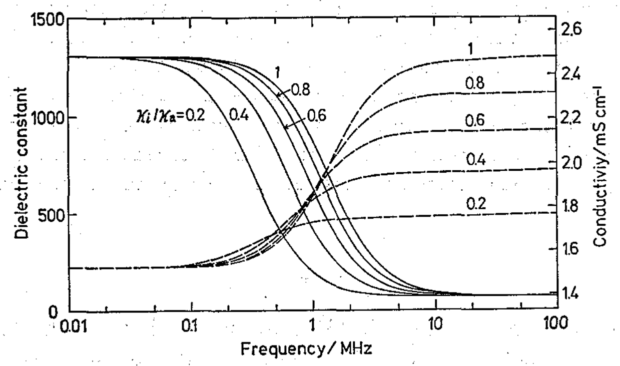


Fig. 2. Effect of varying κ_i on frequency dependence of dielectric constant and conductivity. The calculation is carried out by Eq. (1) with the same parameters as used in Fig. 1 except $\kappa_m=0$ mS/cm. The solid lines and the broken those indicate dielectric constant and conductivity, respectively. The numbers beside the curves indicate the ratio of κ_i to κ_a

electric dispersion, an increase in ionic permeability in plasma membranes can be inferred from a decrease in characteristic frequency of dielectric dispersion f_p, because, as shown in Fig. 2, the value of f_p is shifted to lower frequencies by the decrease in κ_i resulted from a decrease in ionic concentration in cytoplasm. However, little work has so far been reported on the relationship between the ionic permeability of plasma membrane and the dielectric dispersion of cell suspensions. The dielectric approach is particularly effective for cells covered with cell walls, since changes in dielectric properties of plasma membranes and cytoplasms can readily be examined without removing cell walls owing to little contribution of the cell walls to the dielectric dispersion of cell suspensions.[4)]

In a previous paper,[4)] we demonstrated that a dielectric approach is suitable for studies of electrical properties of yeast cells. The purpose of this paper is to examine a change in permeability induced in yeast plasma membranes by various chemical agents and physical treatments. Dielectric measurements were carried out for suspensions of the cells treated with various chemical agents: ionic and nonionic detergents, sodium tetraphenylborate, potassium iodide and gramicidins, and by various physical means: heating, freezing-thawing and irradiation of UV light. The two types of changes in dielectric dispersions, predicted from Pauly–Schwan's theory, were observed by these treatments. One is reduction in dielectric dispersion which is caused by increase in membrane conductivity or destruction of membrane. The other is decrease in f_p without reduction of dielectric dispersion, resulting from decrease in conductivity of inner phase of the cells. The treatments with ionic detergents, heating and freezing-thawing were included in the former type, the treatments with nonionic detergent and sodium tetraphenylborate in the latter one.

II. LIST OF SYMBOLS

- ε dielectric constant
- κ electrical conductivity
- ϵ_0 dielectric constant of free space
- ε^* complex dielectric constant given by $\varepsilon^*=\varepsilon-j\frac{\kappa}{2\pi f\epsilon_0}$
- f measuring frequency
- f_p characteristic frequency of the P-dispersion
- D inner diameter of cell
- d thickness of cell membrane
- C_M specific membrane capacitance
- P volume fraction of the spheres consisting of the cell membrane and its interior

Subscripts

- a outer phase
- i inner phase of cell
- m membrane phase
- l limiting value at low frequencies
- h limiting value at high frequencies

III. MATERIALS AND METHODS

A) Preparation of Intact Yeast Cells

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 harvested at an early stationary phase and then washed twice with distilled water. Yeast cells observed under an optical microscope were of a spheroid ranging from 1 to 5 μm in minor diameter and from 2 to 6 μm in major diameter. The mean diameter of the cells was calculated to be 4.3 μm by assuming a sphere of the same volume as that of the spheroid. The mean diameter inside the cell wall was assumed to be 3.8 μm by using a value of 0.25 μm for the thickness of the cell wall reported by Agar and Douglas.[5)]

Table I. Physicochemical Properties of Ionic Detergents

Sodium alkylsulfonates		Dimethylbenzylalkylammonium chlorides		
Alkyl chain	CMC[a] mM	Alkyl chain	Abundance[c] %	CMC[b] mM
C_8H_{17}	140–150 (25°C)	C_8H_{17}	98.2	
$C_{10}H_{21}$	37–39 (25°C)	$C_{10}H_{21}$	98.6	6.1
$C_{12}H_{25}$	8.8–9.5 (33.5°C)	$C_{12}H_{25}$	98.9	2.8
$C_{14}H_{29}$	2.35–2.6 (42.5°C)	$C_{14}H_{29}$	97.5	0.37
		$C_{16}H_{33}$	88.4	0.044
		$C_{18}H_{37}$	93.7	0.0071

a. measured by Klevens[28)]
b. measured by Ross *et al.*[29)]
c. The percentage by mole of the abundance of the alkyl chain among total alkyl chain of the detergent.

Table II. Physicochemical Properties of a Homologous Series of POEnonylphenols

n[a]	HLB[b]	Cloud point[c] °C	CMC[d] μM
6.0	10.9		67
8.2	12.4	40±2	76
10.8	13.7	74±1	88
14.6	14.9	97±1	109
15.6	15.1		115

a. average number of ethylene oxide units.
b. obtained from the technical literature provided by Kao Soap Co., Ltd.
c. measured with 2% aqueous solution of detergents
d. calculated from the relation for nonylphenol derivatives proposed by Hsiao *et al.* [30]. The equation is $\ln C_0 = 3.87 + 0.056n$ where C_0 is the cmc in μM and n is average number of ethylene oxide units.

B) Treatments of Cells

1. *Chemical agents*

The intact yeast cells were suspended in various chemical agents, care being taken to adjust the cell concentration to $5–6 \times 10^8$ cells/ml. The suspensions were incubated for 30 min at 30°C, then centrifuged. The precipitated cells were washed twice with distilled water and resuspended in a 20 mM KCl solution except for the treatment with KI. The suspensions were then stored at 4°C to protect the treated cells from autolysis. The series of sodium alkyl (C_8, C_{10}, C_{12}, C_{14}) sulfonates were obtained from Tokyo Kasei Co., Ltd. The series of alkyl (C_8, C_{10}, C_{12}, C_{14}, C_{16}, C_{18}) benzyl dimethyl ammonium chlorides and the homologous series of polyoxyethylene(POE) nonylphenol were kindly supplied by Mr. Tuji at Kao Soap Co.,Ltd. The physicochemical properties of these detergents are shown in Tables I and II. Sodium tetraphenylborate and potassium iodide were obtained from Wako Pure Chemical Industries, Ltd. and gramicidin D (Dubos) which is a mixture of gramicidin A, B and C was obtained from P–L Biochemicals, Inc. Milwaukee.

2. *Heating*

Intact yeast cells suspended in distilled water were incubated for 10 min in water bath at temperatures ranging from 30°C to 70°C. The treated cells were washed with 20 mM KCl and resuspended in 20 mM KCl. The suspensions were stored at 4°C.

3. *Repetition of freezing and thawing*

Intact cells were suspended in 20 mM KCl, being adjusted to a cell concentration appropriate for dielectric measurements. The suspension placed in test tubes was immersed in acetone bath cooled by dry ice and transferred into water bath at 30°C. For each freezing and thawing, a small amount was taken out of the suspension for dielectric measurements.

4. *Irradiation of UV light*

The sufficiently diluted suspension of intact cells were spread over the bottom of a dish in a 1 mm thick layer and irradiated for 10 min with a 10 watt UV lamp from a hight of 15 cm. During irradiation, the dish was shaken in order to equally irradiate all the cells. The treated cells were washed with 20 mM KCl and then resuspended in 20 mM KCl.

C) Dielectric Measurements

1. *Samples*

Before the measurements, the treated cells stored at 4°C were washed again with a 20 mM KCl solution which was kept at measuring temperature, and then resuspended in a 20 mM KCl solution containig 0.1% agar. The addition of agar prevents the cells from sedimentation during dielectric measurements, giving no effect on the dielectric constant and conductivity of the medium. In each set of the dielectric measurements, cell concentrations of specimens were kept constant.

2. *Measuring cell*

The measuring cell was a parallel plate condenser consisting of two platinized platinum plates and a lucite spacer, the cell constant being about 0.03 pF. 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. Correction for the residual inductance was made following Schwan's method.[6] The residual inductance was estimated by Schwan's method[6] and a new method[7] to be 2.8×10^{-8}H.

3. *Bridge*

Measurements of the capacitance and the conductance were carried out with a TR–1C Transformer Ratio-Arm Bridge of Ando Electric Co., Ltd. and with a 250 A

RX-Meter of Boonton Radio Corporation over a frequency range of 10 KHz to 3 MHz and of 1 MHz to 100 MHz respectively.

D) Measurements of Released Intracellular Compounds and Packed Volume of Cells

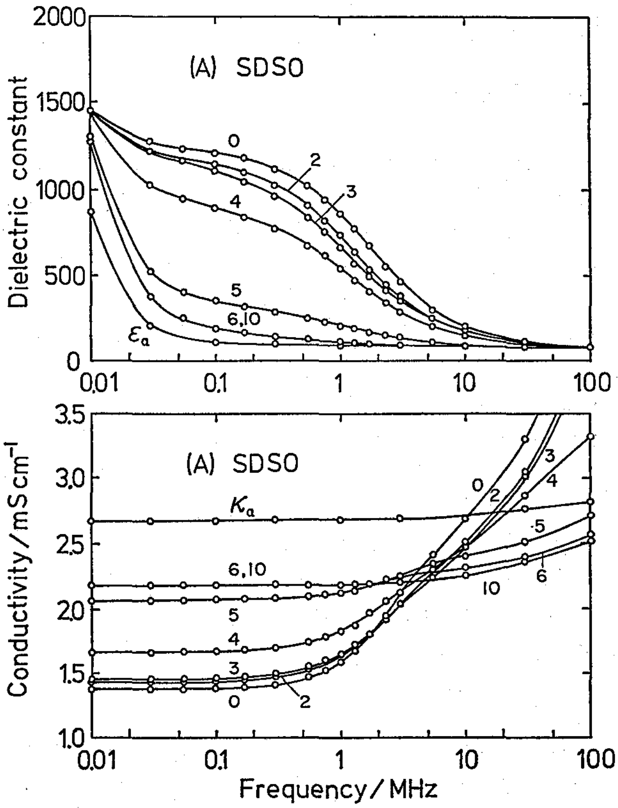
Compounds flowed out of the cells were measured with a spectrophotometer. Volume of the cells packed by centrifugation (1000×g for 10 min) were measured with a hematocrit.

IV. RESULTS

A) Effect of Ionic Detergents[8)]

1. *Dielectric behavior for suspensions of yeast cells treated with ionic detergents in various concentrations*

Figure 3 shows frequency dependence of dielectric constant ε and conductivity κ for the suspension of yeast cells treated with sodium dodecyl sulfonate (SDSO) and dodecyl benzyl dimethyl ammonium chloride (DBDAC). Each specimen was adjusted to the same cell concentration. The treatments with both kinds of ionic detergents reduced the dielectric dispersion observed for the intact yeast cell suspension. The value of limiting dielectric constant ε_l at low frequencies decreased gradually



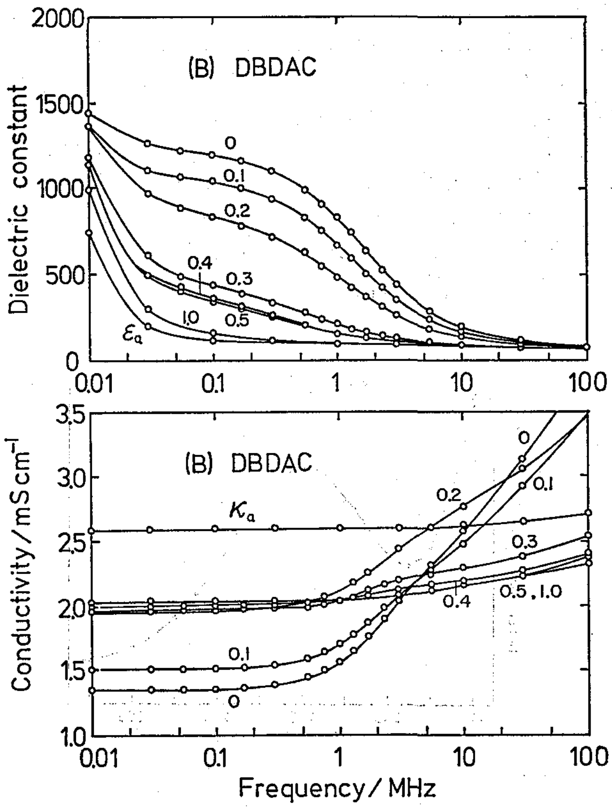


Fig. 3. Frequency dependence of dielectric constant and conductivity for suspensions of yeast cells treated with (A) sodium dodecyl sulfonate (SDSO) and (B) dodecyl benzyl dimethyl ammonium chloride (DBDAC) solutions in various concentrations. Numbers beside the curves indicate the concentration of SDSO and DBDAC in mM. The cell concentrations of specimens are kept constant in each series. The final suspending medium is 20 mM KCl solution. Dielectric measurements were made at (A) 23°C and (B) 20°C.

with the increase in concentration of the detergents below 4 mM and 0.2 mM for SDSO and DBDAC respectively and were remarkably diminished by 5 mM of SDSO and 0.3 mM of DBDAC, while the values of limiting dielectric constant ε_h at high frequencies were almost unchanged. The values of limiting conductivity κ_l at low frequencies increased with increasing concentration of the detergents but remained lower than that of suspending medium at higher concentrations of the detergents. Finally dielectric dispersions disappeared completely at about 10 and 1 mM for SDSO and DBDAC respectively. Since the reduction effects of DBDAC and SDSO on the dielectric dispersion were observed below their critical micelle concentration, the detergents in a monomer form may affect the membrane structure, resulting in the increase in membrane conductivity. A steep rise in dielectric constant at frequencies below 0.1 MHz is due to electrode polarization.

2. *Change in packed volume of cells and leakage of compounds*

Intracellular metabolites such as amino acids, purines, pyrimidines and pentose are known to be released from certain bacteria treated with anionic and cationic detergents as a results of breakdown of permeability barrier.[9] In the present study, it was found that the yeast cells treated with SDSO and DBDAC released some compounds showing absorption maximum at about 260 nm, probably a mixture of purines, pyrimidines, nucleoside and mononucleotides. The absorption spectrum of the released compounds is shown in Fig. 4. In Fig. 5 are shown changes in optical density at 260 nm (OD_{260}) of suspending medium with which the cells were treated, together with changes in packed volume of the treated cells and changes in relative magnitude of the dielectric dispersion $\Delta\varepsilon/\Delta\varepsilon_{intact}$, where $\Delta\varepsilon$ is difference in dielectric constant at 0.1 MHz and 100 MHz.

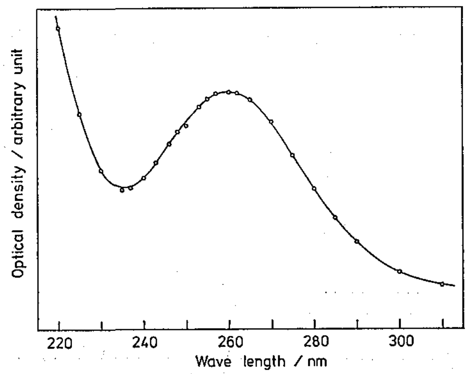


Fig. 4. Example of absorption spectrum of intracellular compounds released from the cells by treatments with ionic detergents.

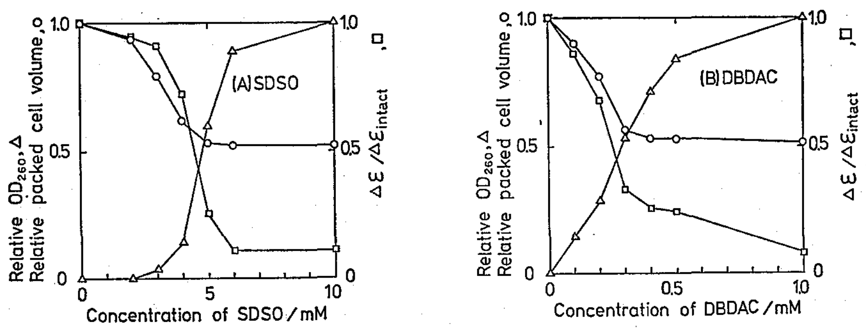


Fig. 5. Effects of (A) SDSO and (B) DBDAC on packed volume of cells, leakage of compounds showing absorption maximum at about 260 nm and relative magnitude of dielectric dispersion.

In the case of SDSO-treated cells as seen in Fig. 5, the reduction of dielectric dispersion accompanied only by the decrease in packed volumes of the cells was observed at concentrations lower than 3 mM where no appreciable increase was found for the OD_{260} value. At concentrations higher than 5 mM, the dielectric dispersion continued to be reduced with the concomitant increase in the OD_{260} value and without further decrease in packed volume.

In contrast with the behavior of SDSO-treated cells, the dielectric dispersion for DBDAC-treated cells (Fig. 5) showed a remarkable decrease at concentration as low as 0.1 mM with both changes in packed volume and in OD_{260}. At concentrations higher than 0.1 mM, the dielectric dispersion continued to be reduced with further increase in OD_{260}, whereas the packed volume of the cells remaind at its lower limiting value.

3. *Effect of alkyl chain length of the detergents on the yeast cells*

In general, bactericidal activity and hemolysis are known to be dependent upon alkyl chain length of the homologous series of ionic detergents. A similar effect of the chain length of detergents on reduction in dielectric dispersions were observed

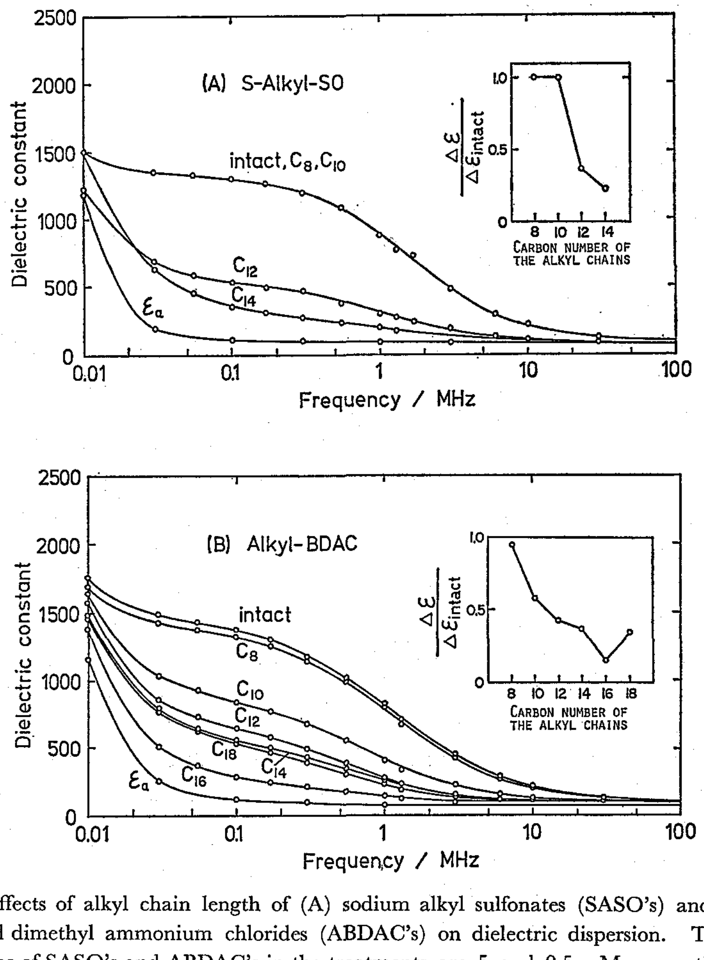


Fig. 6. Effects of alkyl chain length of (A) sodium alkyl sulfonates (SASO's) and (B) alkyl benzyl dimethyl ammonium chlorides (ABDAC's) on dielectric dispersion. The concentrations of SASO's and ABDAC's in the treatments are 5 and 0.5 mM, respectively. The cell concentrations of specimens are kept constant in each series. The suspending medium is 20 mM KCl solution. Dielectric measurements were made at (A) 20°C and (B) 28°C. Figures, C_n's beside the curves indicate alkyl chain of the detergents used. The relative magnitude $\Delta\varepsilon/\Delta\varepsilon_{intact}$ of the dielectric dispersion versus carbon number of the alkyl chains is also depicted in the Figures.

for the present series of detergents, sodium alkyl sulfonates (SASO) and alkyl benzyl dimethyl ammonium chlorides (ABDAC). Figures 6A and 6B show the frequency dependence of dielectric constant of the yeast cell suspensions treated with the solution of these detergents. As readily seen in the Figures, the magnitude of the dielectric dispersion of the suspensions was markedly dependent on the alkyl chain length of the detergents. The effect of the detergents on the reduction of dielectric dispersions was in order of $C_{14}>C_{12}>C_{10}$, C_8 for SASO's and $C_{16}>C_{18}$, $C_{14}>C_{12}>C_{10}>C_8$ for ABDAC's. The higher members of SASO's such as C_{16} and C_{18} were not examined owing to their low solubility in water. The reverse order observed for C_{16} and C_{18} in ABDAC's might be due to the low values of effective concentrations of monomer form of C_{18} compared with C_{16}.

B) Effect of Nonionic Detergents

Polyoxyethylene derivatives show an effect of increase in ion permeability of mitochondrial membranes at sublytic concentrations.[10] This effect has been supported by the study in bilayer lipid membranes.[11] However, little is known about the effect of these detergents on permeability in plasma membranes of microorganisms such as bacteria and yeasts, though it has so far been reported that bactericidal and lytic activity of nonionic detergents is much lower than that of ionic detergents[12,13] and that plasma membranes isolated from bacteria are solubilized by some nonionic detergents.[14]

1. *Dielectric behavior for suspensions of yeast cells treated with POE(8.2)nonylphenol*

In Fig. 7 are shown frequency dependence of dielectric constant ε and conductivity κ for suspensions of the cells treated with POE(average number of ethylene oxide chain n=8.2)nonylphenol over the concentration range from 0.01 mM to 100 mM without adjusting tonicity. Some dielectric parameters evaluated are listed in Table III. Each specimen was adjusted to the same cell concentration (1.25×10^{10} per ml). The value of f_p shifted remarkably to a lower frequencies with the increase in concentration of the detergent, whereas the values of ε_l and κ_l were almost unchanged except for the treament with an extremely high concentration of the detergent (100 mM). This dielectric behavior differs markedly from that of suspension of cells treated with sodium alkyl sulfonate and alkyl benzyl dimethyl ammonium chlorides, which reduced remarkably the values of ε_l and κ_l. Figure 8 shows the complex plane plots of Specimen A, a suspension of intact cells, and Specimen D, a suspension of the detergent-treated cells. It was found that the suspension of the detergent–treated cells shows more depressed circular arcs as compared with that of the intact cells without alteration in ε_h and ε_l. The phase angles termed by Cole[2] for the circular arcs of the specimens shown in Fig. 7 are listed in Table III. The phase angles, which indicate the degree of depression of circular arcs corresponding to broadening of relaxation curves, decreased with the increase in detergent concentration, attaining a limiting value at higher concentrations. It is expected from the numerical calculations of Eq. (1) that the values of ε_l and κ_l remain constant, when the ratio of conductivity of membrane to that of suspending medium, κ_m/κ_a, is less than 10^{-5} under constant cell volume. This feature will be shown in Discussion. The present

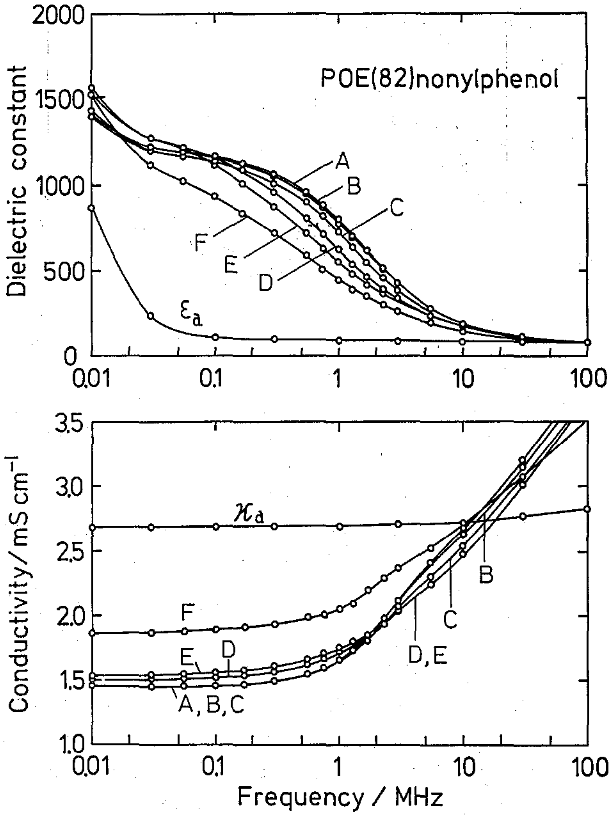


Fig. 7. Frequency dependence of dielectric constant and conductivity for suspensions of yeast cells treated with POE(8.2)nonylphenol solution. The suspending medium of the treated cells is 20 mM KCl solution. Dielectric measurements were made at 23°C. The concentration of the detergent: Specimen A, 0 mM; B, 0.01 mM; C, 0.1 mM; D, 1 mM; E, 10 mM; F, 100 mM.

Table III. Dielectric Parameters of the Suspensions of Yeast Cells Treated with POE(8.2)-nonylphenol and the Estimated Phase Parameters of the Cells

Specimen	Detergent concentration	Dielectric parameters					Phase parameters				
		κ_l	ε_l	ε_h	f_p	phase angle	P	C_M	ε_m	ε_i	κ_i
	mM	mS/cm			MHz	degree		μF/cm^2			mS/cm
A	0	1.45	1180	67	1.66	74	0.36	0.91	5.1	49	2.3
B	0.01	1.46	1180	67	1.60	72	0.36	0.91	5.1	49	2.2
C	0.1	1.46	1170	67	1.40	71	0.36	0.90	5.1	50	1.8
D	1	1.51	1200	65	0.95	66	0.34	0.97	5.5	42	1.3
E	10	1.55	1170	63	0.80	66	0.33	0.97	5.5	35	1.1
F	100	1.88	940	63	0.82	68	—	—	—	—	—

κ_a=2.68 mS/cm, ε_a=79, d=50 Å, D=3.8 μm, measuring temperature=23°C

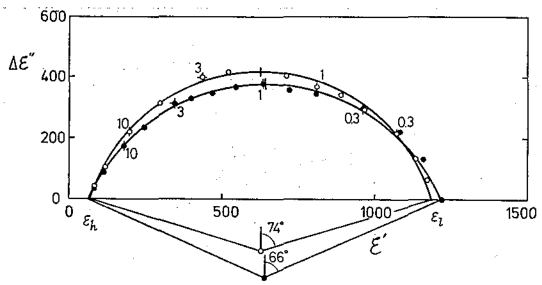


Fig. 8. Complex plane plots of complex dielectric constant for the dielectric data of Specimens A (○) and D (●) shown in Fig. 1. ε' is dielectric constant. ε'' is loss factor. Numbers beside the measured points are frequencies in MHz.

results showed that the values of ε_l and κ_l in Specimens B to E are nearly the same as those of Specimen A (intact cell suspension). This suggests that the membrane conductivity remains sufficiently low and that the cell volume is almost unchanged for the cells treated with the detergent in a concentration range from 0.01 to 10mM. Furthermore, this suggestion is supported by the fact that neither the release of any compound showing absorption maximum at about 260 nm from the cells nor the change in packed volume of cells were observed on the treatment of the detergent.

2. *Phase parameters of yeast cells treated with POE(8.2)nonylphenol*

The dielectric dispersions of intact yeast cell suspensions were analysed by the procedure which was proposed by Hanai *et al.*[15] based on a general expression (Eq. (1)) of Pauly-Schwan's theory,[3] because the cytoplasmic membranes of intact yeast cells have sufficiently low conductivity. Since membrane conductivity of yeast cells treated with POE(8.2)nonyphenol in a concentration range from 0.01 to 10 mM may be regarded to be sufficiently low, the dielectric data shown in Fig. 7 can be analysed by the same procedure as used in the case of intact yeast cells. The evaluated phase parameters are listed in Table III. It is noted that the values of κ_i are remarkably affected by the treatment with the detergent, whereas the values of C_M and ε_m remain unchanged.

3. *Effect of osmotic pressure*

In the preceding section, the tonicity of the detergent solution was not adjusted in the preparation of the detergent-treated cells, being lower than that of the cytoplasm. In this section, the effects of tonicity of the detergent solution on dielectric dispersion were examined. Figure 9 shows frequency dependence of dielectric constant for suspensions of the yeast cells treated with 1 mM POE(8.2)nonylphenol solution at varied tonicity over an osmolarity range from 0 to 1 osM adjusted by sorbitol and KCl. It was found that the dielectric dispersion for the suspension of the detergent-treated cells was reduced depending on the concentration of sorbitol and KCl added to the detergent solution. It was confirmed that the dielectric dispersion of intact yeast cell suspensions were not affected by the osmotic treatment in the absence of the de-

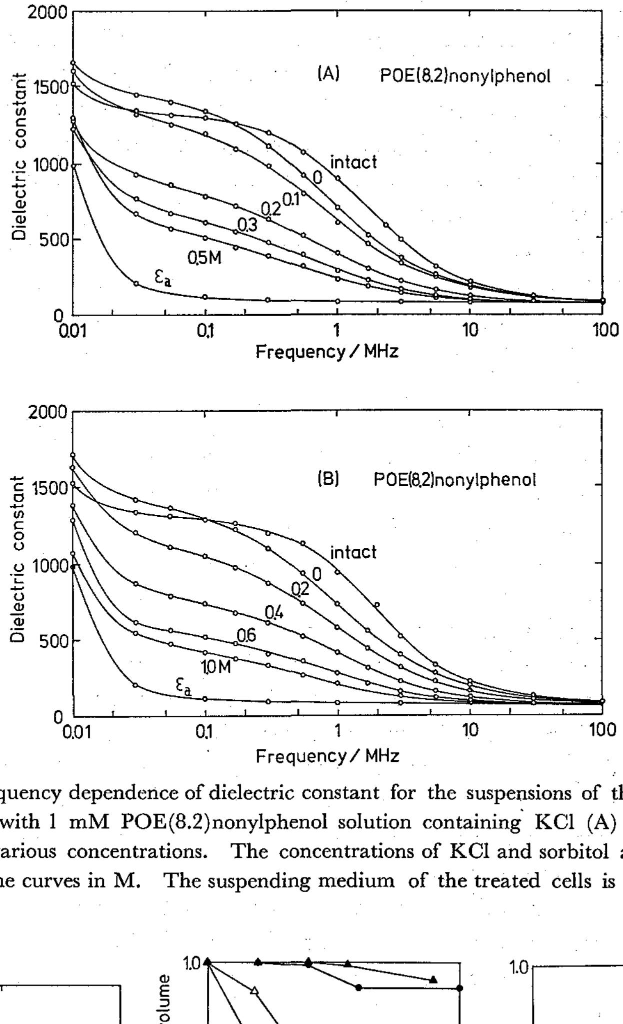


Fig. 9. Frequency dependence of dielectric constant for the suspensions of the yeast cells treated with 1 mM POE(8.2)nonylphenol solution containing KCl (A) and sorbitol (B) at various concentrations. The concentrations of KCl and sorbitol are indicated beside the curves in M. The suspending medium of the treated cells is 20 mM KCl solution.

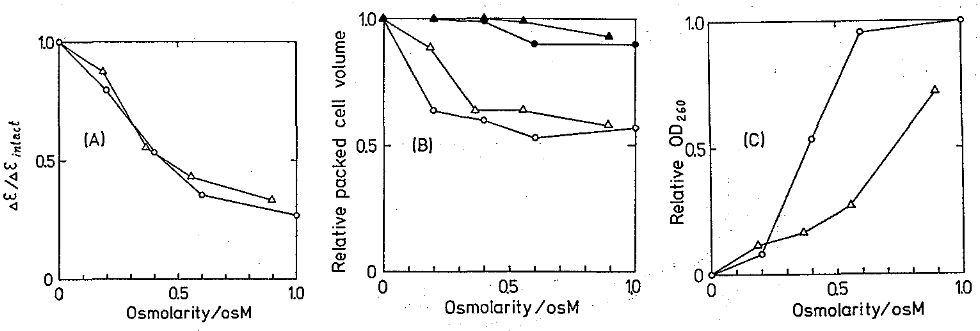


Fig. 10. Effects osmolarity of 1 mM POE(8.2)nonylphenol solution adjusted by KCl (△) and sorbitol (○) on relative magnitude of dielectric dispersion (A), packed volume of cells (B) and leakage of compounds showing absorption maximum at about 260 nm (C). The relative packed volume of intact cells treated osmotically with various concentrations of KCl (▲) and sorbitol (●) was measured in 20 mM KCl solution.

tergents. In order to make a comparison between the effect of KCl and that of sorbitol regarding the dielectric dispersion shown in Fig. 9, $\Delta\varepsilon/\Delta\varepsilon_{\mathrm{intact}}$ are plotted against osmolarity in Fig. 10A, where $\Delta\varepsilon$ denotes difference of dielectric constant at 0.1 MHz and 100 MHz. Almost no significant difference was observed between sorbitol and KCl. As observed on the treatment of yeast cells with ionic detergents,[8] this reduction of dielectric dispersion was accompanied by release of some compounds showing absorption maximum at about 260 nm from the cells and by decrease in packed volume of the cells. In Figs. 10B and 10C are shown the changes in packed volume of the cells and in optical density at 260 nm of suspending medium with which the yeast cells are treated. The increase in osmolarity of detergent solution by addition of sorbitol and KCl induced the decrease in packed volume of the cells and the increase in the release of intracellular compounds, though sorbitol was more effective in this effect of osmolarity compared with KCl. In the absence of the detergent, neither decrease on packed volume nor release of intracellular compounds was observed by the osmotic treatment.

In order to make a comparison with the results obtained under hypotonic condition shown in Fig. 7, an effect of the concentration of POE(8.2)nonylphenol in the presence of 1 M sorbitol is shown in Fig. 11. The dielectric dispersion was reduced at concetration more than 0.01 mM and disappeared at the concentrations of 1 mM. The reduction of dielectric dispersion was caused by the detergent in the same concentration range where the decrease in the value of f_p was induced by the detergent in hypotonic medium as shown in Fig. 7.

Figure 12 shows dielectric dispersions of specimens prepared by various processes which are indicated in Table IV. Specimens I and L were prepared by the same way as Specimen D in Fig. 7 and the specimen for 1 M sorbitol in Fig. 9B, respectively, except for 2nd Step. The dielectric behavior of the cell suspensions was affected markedly by the order of the treatments with the detergent and with 1 M sorbitol. When the cells were treated with the detergent after the osmotic treatment (Specimen J), the dielectric curve was closed to that of Specimen I which was treated with the detergent alone. On the other hand, the reduction in dielectric dispersion is observed in Specimen K which was treated with the detergent before the osmotic treatment, being smaller than that of Specimen L prepared by detergent-treatment under hypertonic condition.

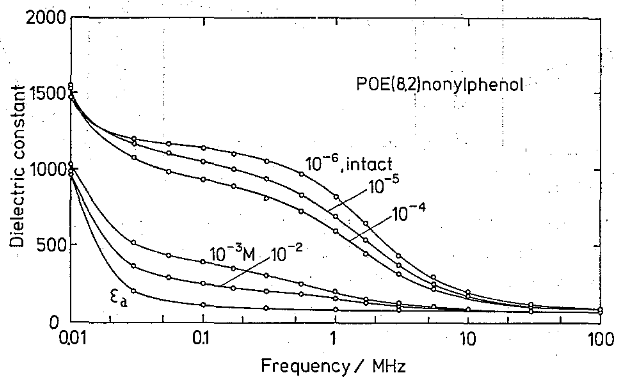


Fig. 11. Effect of concentration of POE(8.2)nonylphenol in the presence of 1 M sorbitol on the dielectric dispersion. The suspending medium of the treated cells is 20 mM solution.

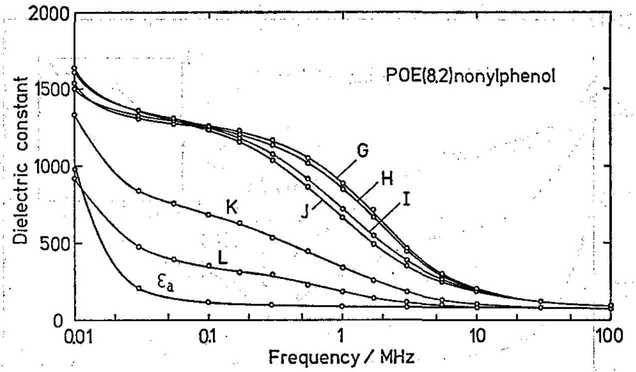


Fig. 12. Frequency dependence of dielectric constant for the suspensions of yeast cells prepared by various processes which are indicated in Table II.

Table IV. Suspending Media Used for Treatments of Yeast Cells

Specimen	1st Step	washing	2nd Step	washing
G	D. W.	D. W.	D. W.	D. W.
H	1 M sorbitol	D. W.	D. W.	D. W.
I	1 mM detergent	D. W.	D. W.	D. W.
J	1 M sorbitol	D. W.	1 mM detergent	D. W.
K	1 mM detergent	D. W.	1 M sorbitol	D. W.
L	1 mM detergent 1 M sorbitol	D. W.	D. W.	D. W.

In each step, the suspensions of cells are incubated for 30 min at 30°C. The detergent used for these treatments is POE(8.2)nonylphenol. The final suspending medium of the treated cells is 20 mM KCl. Distilled water is abbreviated as D. W.

4. *Effect of POE chain length*

Examinations of effect of POE chain length on yeast cells were made both in the absence and in the presence of 1 M sorbitol in the detergent solution used for treatment of the cells. The POEnonylphenol homologs used were varied in average number n of ethylene oxide units per molecule from 6.0 to 15.6.

Figure 13A shows frequency dependence of dielectric constants for the suspensions of yeast cells treated with 1 mM POEnonylphenol homologs in the absence of sorbitol. The dielectric dispersions were not affected at n=15.6 and the value of f_p decreased gradually with the increase in the n-value. POEnonylphenol with n=8.2 was most effective for the decrease in f_p without the decrease in ε_l. Since the decrease in f_p is caused by that of κ_i as shown in Table III, the sequence of decrease in κ_i is 8.2> 10.8> 14.6> 15.6 in the n-value of ethylene oxide units. On the other hand, reduction in dielectric dipersion was observed by treatment with POE(6.0)nonylphenol solution which was turbid because of its low cloud point. The members lower than n=6.0 were not examined owing to their low solubility.

When the yeast cells were treated with 0.5 mM POEnonylphenol homologs in the presence of 1 M sorbitol, the effect of POE chain length on reduction of dielectric

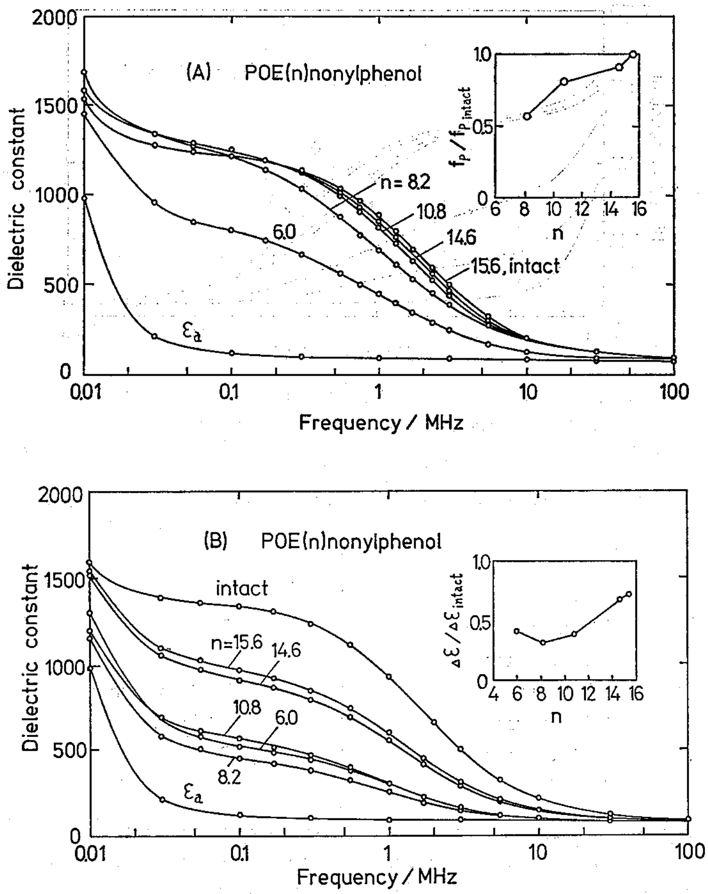


Fig. 13. Effect of POE chain length n on dielectric dispersion in the absence (A) and in the presence of 1 M sorbitol (B). The concentrations of the detergents are 1 mM (A) and 0.5 mM (B). The relative characteristic frequency $f_p/f_{p_{intact}}$ (A) and the relative magnitude $\Delta\varepsilon/\Delta\varepsilon_{intact}$ (B) of dielectric dispersion are depicted in the figures.

dispersion were shown in Fig. 13B. The magnitude of the dielectric dispersion of the suspension was also markedly dependent on the POE chain length of the detergents. The effect of the detergents on the reduction of dielectric dispersions was in order of $8.2 > 6.0 \approx 10.8 > 14.6 > 15.6$ in the n-value of ethylene oxide units.

C Effect of Sodium Tetraphenylborate

Tetraphenylborate (TPB) is well known as a good precipitant for potassium ions-owing to poor solubility of TPB potassium salt in water (1.5–1.8^{-4} mole/l[16,17]), while sodium TPB is freely soluble to water. Further, Liberman *et al.*[18] reported that the increase in conductance of bilayer lipid membrane was induced by TPB ions readily penetrating across membrane. Figure 14 shows the frequency dependence of dielectric constant and conductivity for suspensions of the cells treated with sodium TPB in various concentrations. The dielectric dispersion curve for 1 mM TPB showed no difference from that for intact cells. At 10 mM of sodium TPB, the characteristic frequency of dielectric dispersion f_p shifted to lower frequencies without change in the values of ε_l and κ_l from those of the intact cell suspension. The reduction of dielectric dispersion was observed by the treatment with 60 mM TPB. Finally dielectric dispersion disappeared completely at 100 mM of sodium TPB.

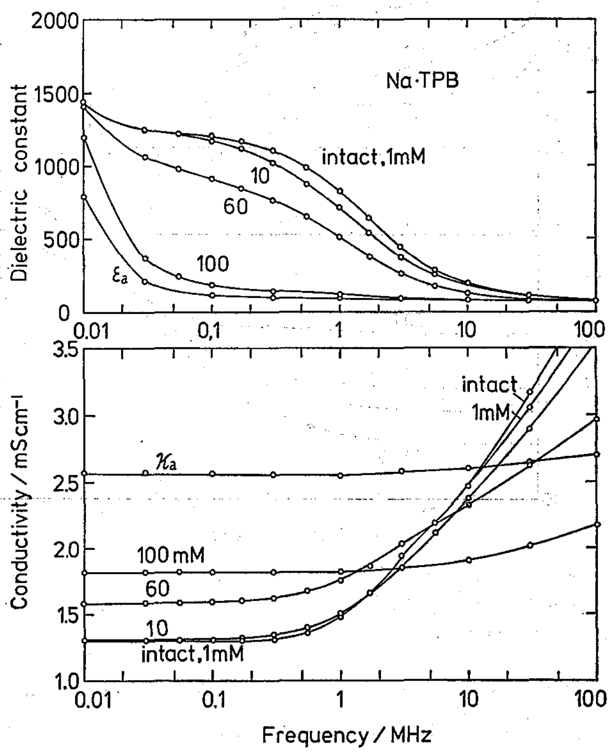


Fig. 14. Frequency dependence of dielectric constant and conductivity for the suspension of NaTPB-treated cells. Figures beside the curves are concentration of NaTPB in mM used for treatment of cells.

D) Effect of Potassium Iodide and Gramicidins

As well as TPB ions, iodides in the presence of small amount of iodine and gramicidins are known as good reagents to increase conductance of bilayer lipid membranes (BLM).[19,20] As KI salt contains a small amount of iodine, the effect is usually observed without addition of iodine. Suspensions of yeast cells treated with a solution of 20 mM KI and with a gramicidin D solution of about 0.6 mg/ml showed no difference in dielectric dispersion from intact cell suspensions.

E) Effect of Heating

In Fig. 15 are shown frequency dependence of dielectric constant and conductivity for the suspensions of yeast cells treated at various temperatures for 10 min. No difference was observed in the curves of dielectric constant and conductivity between 30°C and 40°C. At temperatures higher than 50°C, dielectric dispersion was reduced

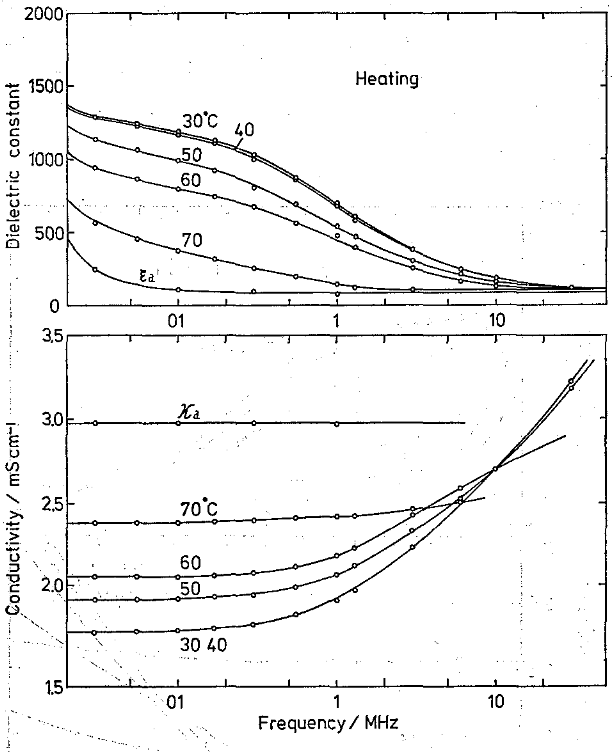


Fig. 15. Frequency dependence of dielectric constant and conductivity for suspensions of the cells treated by heating. Cells are incubated for 10 min at the temperatures which is indicated beside the curves.

remarkably with the increase in temperature. Figure 16 shows the values of ε_l and κ_l of the data in Fig. 15 plotted against temperature. The change in conductance of the medium of suspensions treated by heating are shown in Fig. 17. The increase in conductance of suspending medium indicates ion leakage from the cell interior to the outer medium due to the increase in ion permeability in the membranes.

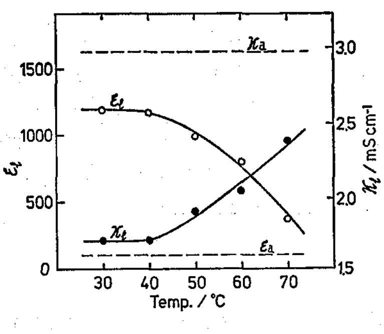


Fig. 16. Plot of the value of ε_l and of κ_l the data in Fig. 15 for temperature.

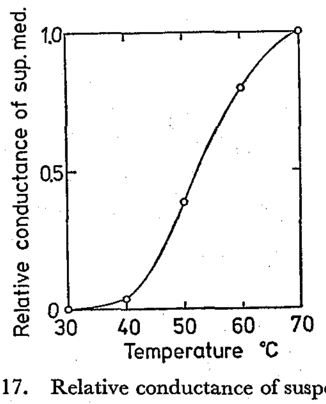


Fig. 17. Relative conductance of suspending medium after the yeast cell suspensions incubated at various temperatures for 10 min.

The comparison between Figs. 16 and 17 suggests that the changes in ε_l and κ_l are accompanied by ion leakage from the cell interior.

F) Effect of Freezing and Thawing

The frequency dependence of dielectric constant and conductivity for the suspensions subjected to freezing and thawing are shown in Fig. 18. In the first freezing and thawing, the remarkable reduction was observed and in the further repetition, dielectric dispersion was gradually reduced. The values of κ_l of the suspensions subjected to freezing and thawing became higher than the value of the conductivity of suspending medium (20 mM KCl) for the intact cells because of release of ions from the cell interior to the outer medium.

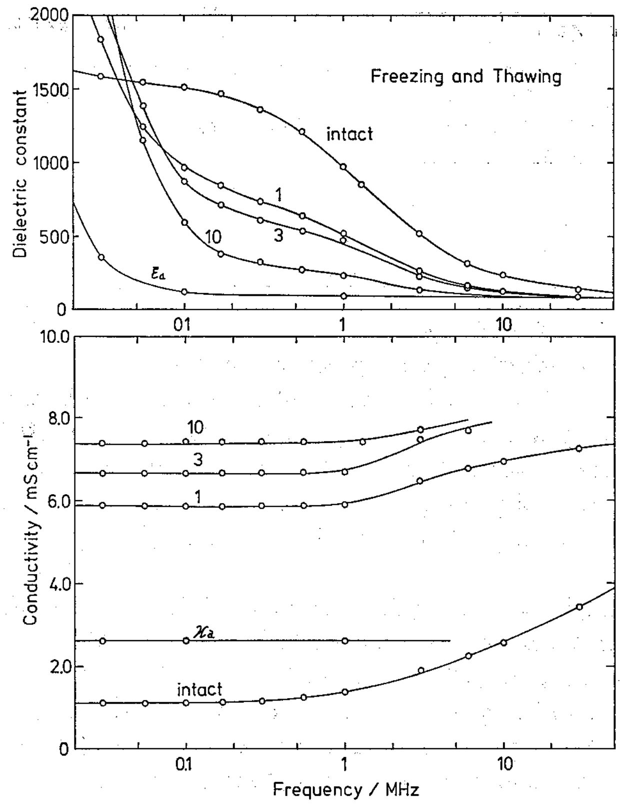


Fig. 18. Effect of repetition of freezing and thawing of cell suspension on dielectric dispersion. The numbers of repetition are indicated beside the curves.

G) Effect of Irradiation of UV Light

In Fig. 19 is shown the time-course of the value of κ_l for the suspension after the irradiation for 10 min by UV light. The value of κ_l rapidly increased in the initial stage, and gradually increased at and after about 20 min beyond the value of initial suspending medium of the cells. Figure 20 shows frequency dependence of dielectric constant for the suspension irradiated by UV light measured at the time when the change in κ_l was no longer detectable. A marked reduction in dielectric dispersion is observed.

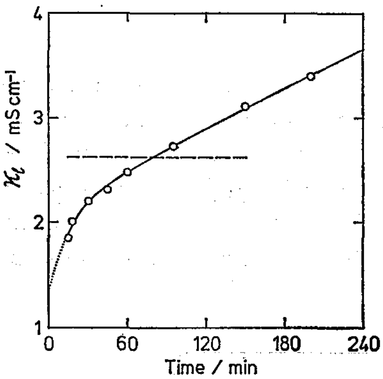


Fig. 19. Time course of κ_l for the suspension after cells are irradiated for 10 min. The broken line indicates the conductivity of initial suspending medium of cells.

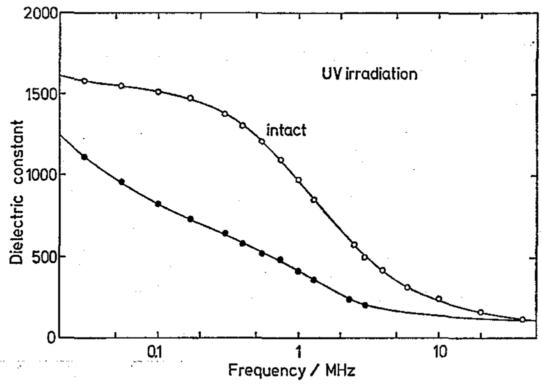


Fig. 20. Frequency dependence of dielectric constant for the suspension of UV light irradiated-cells (●) and intact cells (○).

V. DISCUSSION

A) Effect of Ionic Detergents

1. *Reduction of dielectric dispersion by ionic detergents*

The dielectric behavior of biological cell suspensions can be discussed on terms of a dielectric theory which was developed by Pauly and Schwan[3] for a suspension of spherical particles covered with a shell. It is expected from the theory that the reduction of dielectric dispersion results from the decrease in volume fraction of the cells in suspensions and/or the increase in conductivity of cytoplasmic membranes κ_m.

In view of the present result that the packed volume of the cells in suspensions was decreased markedly by treatment with the detergents, the reduction in dielectric dispersion of the yeast cell suspensions is attributable to the decrease in volume fraction due to the shrinkage of the cells. The values of ε_l and κ_l of dielectric dispersion in varying cell diameter were calculated from Eq. (1) by using the phase parameters which are close to the estimated phase parameters for intact yeast cells,[4] the results being shown in Fig. 21. As seen in the figure, the decrease in cell diameter corresponding to the shrinkage causes the decrease in ε_l and the increase in κ_l, leading to the reduction in dielectric dispersion.

Provided that the relative packed volume corresponds roughly to the relative cell volume, it is expected from the results shown in Fig. 5 that the relative cell volume after the shrinkage is about 0.5. At such a relative cell volume, a certain dielectric dispersion still remains as shown in Fig. 21. To understand the complete disappearance of the dielectric dispersions, we have to take into consideration the increase in the membrane conductivity κ_m in addition to the shrinkage of the cells. Numerical estimation of ε_l and κ_l for varying κ_m were made with Eq. (1) by use of the same values

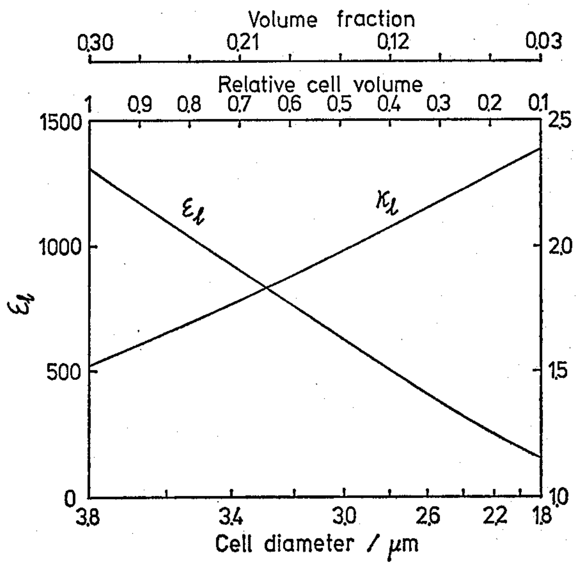


Fig. 21. Effect of change in cell diameter on ε_l and κ_l. The curves are numerically calculated from Pauly-Schwan's equation with phase parameters as: $\varepsilon_a=80$; $\kappa_a=2.5$ mS/cm; $\kappa_m=0$ mS/cm; $\varepsilon_i=50$; $\kappa_i=2.5$ mS/cm; $d=50$ Å. The cell diameter is varied from 3.8 to 1.8 μm. The volume fraction is calculated from the cell diameter under a constant cell number. Relative cell volume is the volume of a cell relative to that for 3.8 μm.

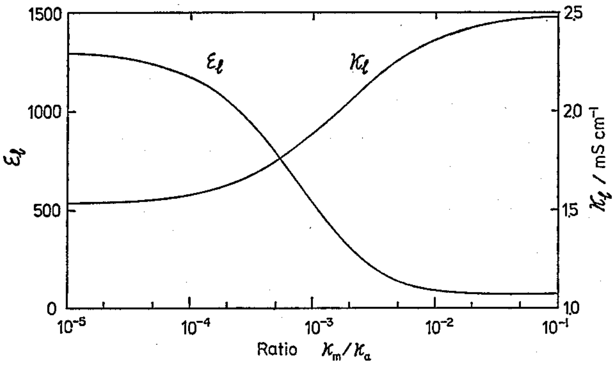


Fig. 22. Effect of change in κ_m on ε_l and κ_l. The calculation is carried out by Pauly–Schwan's equation. Volume fraction and diameter of cells are 0.3 and 3.8 μm, respectively, through the calculation. Other parameters are the same as those in Fig. 21.

of phase parameters of intact cells as used in Fig. 21, the results being shown in Fig. 22, where the dielectric dispersion begins to reduce at $\kappa_m/\kappa_a \approx 10^{-5}$, and almost disappears at $\kappa_m/\kappa_a \gtrsim 10^{-2}$.

Since the changes in packed volume of cells and in the OD_{260} value reflect qualitatively changes in the cell volume and the membrane permeability, the results shown in Fig. 5 suggest that reduction of dielectric dispersion in question is attributable to two different processes: the shrinkage of cells and the increase in conductivity of cytoplasmic membranes. In this instance, the decrease in cell volume caused mainly the reduction in dielectric dispersion at relatively low concentration of the detergents, while the increase in membrane conductivity was dominant at the higher concentrations as obviously shown in the case of SDSO (Fig. 5A). When the dielectric dispersion completely disappear, the value of κ_l remains always lower than the conductivity of suspending medium κ_a. This result implies that the interior of the cells keeps lower conductivity compared with κ_a, even after the destruction of the cytoplasmic membrane. The low conductivity of the inner phase of the cells may be attributed to the presence of intracellular organella, suggesting that the membranes of the intracellular organella probably are not appreciably damaged in contrast to the cytoplasmic membranes.

2. *Comparison of reduction of dielectric dispersion of yeast cell suspensions with the effect of detergents on bacteria and red blood cells*

Salton[9] reported the difference between anionic and cationic detergents in lytic action on protoplast of *B. megaterium* by the observation of the lysed protoplast with phase contrast microscope. Protoplast membranes were completely dissolved by sodium dodecyl sulfate, while lysis of protoplast by cetyl trimethyl ammonium bromide was not accompanied by such a kind of dissolution of the protoplast membranes. This difference, however, was not observed by the present measurements, because this method can make no distinction between the presence of membranes with higher conductivity ($\kappa_m/\kappa_a \gtrapprox 10^{-2}$) and the absence of membranes.

According to the present results, cationic detergent (DBDAC) is more effective for reducing the dielectric dispersion of yeast cell suspensions compared with anionic detergent (SDSO). Gilby and Few[21] reported the similar sequence for lytic action on protoplast of *M. lysodeicticus* and the bactericidal activity as $-NH_3^+ > -N^+(CH_3)_3 > -SO_4^- > SO_3^-$. In contrast, the opposite order was obtained on hemolytic activity by Ross and Silverstein,[22] that is $-SO_4^- > -C_6H_5CH_2N^+(CH_3)_2 > -N^+(CH_3)_3$.

The effect of alkyl chain length of cationic and anionic detergents on the reduction of dielectric dispersion is consistent with the trend of hemolytic and bactericidal activity. The maximum action of hemolysis occurs at C_{14} for sodium alkyl sulfonate and C_{16} for alkyl benzyl dimethyl ammonium chloride.[22] The most effective alkyl chain length on bactericidal activity is in the vicinity of C_{16}, depending upon the test organisms and detergents. The optimum alkyl chain length reported are C_{18} on *E. coli* and C_{16} on *M. aureus* for alkyl trimethyl ammonium chloride [23] and C_{14} on *M. pyogenes var. aureus* and *S. typhosa* for alkyl benzyl dimethyl ammonium chloride.[24]

B) Effect of Nonionic Detergents

This series of experiments demonstrates that the dielectric dispersion curves of the detergent-treated cells show changes depending on the tonicity of the detergent solution used for treatment of the cells.

1. *Under hypotonic condition*

In the case of low osmolarity as shown in Fig. 7, the decrease in f_p of the dielectric dispersion is observed without changing the value of ε_l and κ_l, suggesting the decrease in κ_i without change in the cell volume and the value of C_M or ε_m. Since the decrease in κ_i may correspond to the decrease in salt concentration inside the cells, it is inferred that POEnonylphenols promote ion leakage from interior of the cell without remarkable increase in the membrane conductivity ($\kappa_m/\kappa_a \lesseqgtr 10^{-5}$) or without lysis of the cells. However, no appreciable increase in conductance of detergent solution after treatment of the cells due to the release of ions from cells was detected because of a small amount of ions released from the cells and contamination by ions in the detergents.

The increase in the passive permeability of the membrane to ions inducd by POE derivatives had been reported by Brierley *et al.*[10] and Van Zutphen *et al.*[11] on mitochondrial membranes and bilayer lipid membranes respectively. Brierley *et al.* elucidated that POE derivatives activated the respiration-dependent accumulation of K^+ salts by mitochondria at sublytic concentrations due to increase in the passive permeability of the membrane to K^+. This ionophore activity, resembles the effect of the macrocyclic antibiotic valinomycin, was supported by Van Zutphen *et al.* in the study on bilayer lipid membrane. With respect to the effect of POE chain length, the ability of decreasing the value of f_p shown in Fig. 13A is generally in good agreement with the increase in ion permeability of mitochondrial membranes and of bilayer lipid membrane reported by Brierley *et al.* and Van Zutphen *et al.* respectively. Further, the effective chain length of detergents for increasing membrane permeability is in the range of HLB number between 12.4 and 13.5 where nonionic detergents show the maximum solubilization of membrane compounds as reported by Umbreit *et al.*[26] A requisite for the two effect of detergents, namely the increase in membrane permeability and the membrane solubilization, may be the uptake of detergents into the membranes. Therefore, it is suggested that detergents in the range of HLB number are most appropriate to the uptake into membranes.

In addition, it is found that the dispersion curve for the detergent-treated cells are broader than that for the intact yeast cells. Since Irimajiri *et al.*[25] discussed that the broadening may be attributed to increase in distribution of internal conductivity of the cells rather than that of cell size, the increase in distribution of internal conductivity of cells may be caused by the treatment of cells with the detergent, resulting from various sensitivities of yeast cells which are in various growth stages.

2. *Under hypertonic condition*

Dielectric dispersion was reduced by increasing the tonicity of detergent solutions. This reduction was accompanied by the shrinkage of cell volume and the release of intracellular compounds, being caused by breakdown of plasma membrane acting as a permeability barrier. It is thus suggested that the detergent lyses the yeast cells under high osmotic condition, while no significant lysis of the cells takes place when the cells are treated with the detergent under low osmotic condition. The reduction of the dielectric dispersion, however, arose not only from treatment of the cell with the detergent under high osmotic conditions but from treatment of the cells in hypertonic medium after the detergent-treatment on hypotonic medium as shown in Fig. 12. This results imply that destruction of the plasma membrane is not directly caused by solubilization of the membranes by the detergent but by mechanical action produced by osmotic pressure and further that interaction of the detergents with the membranes may not be affected by the tonicity of the detergent. The latter suggestion may also be supported by the fact that effective concentrations and chain length of the detergents in the decrease in f_p observed under low osmotic condition is consistent with that in the reduction of dielectric dispersion in high osmotic conditions. The detergent penetrating into the plasma membranes may modify their structure, probably resulting in the change in membrane permeability. The modified membrane structure may further be destroyed by cell-shrinkage resulted from osmotic pressure and/or by separation of the plasma membrane from the cell wall which reinforces the membrane structure.

C) Effect of Sodium TPB

The changes in dielectric dispersion curves of the yeast cell suspensions induced by treatment with sodium TPB are classified into two types: one is the decrease in the value of f_p without changes in ε_l and κ_l observed at lower concentration of TPB and the other is the reduction of dielectric dispersion at higher concentrations.

The change observed at lower concentrations of sodium TPB (10 mM) is similar to the change observed for the treatment with POEnonylphenols under hypotonic conditions, suggesting decrease in κ_i without changes in C_M or ε_m. In view of characteristic properties of TPB ions which are a good precipitant for K^+ and high permeability in bilayer lipid membrane, the process of decreasing κ_i for TPB ions substantially is different from that for POEnonylphenols which promote ion leakage from interior of cells. The TPB ions penetrate plasma membranes and enter cytoplasms where potassium ions are usually kept at a high concentration by active transport of the cells. Then the TPB ions introduced into cytoplasms readily form potassium salts which are insoluble in water and electrically neutral. The decrease in conductivity of cytoplasm is consequently caused by the decrease in free potassium ions in cytoplasm.

At higer concentrations, the dielectric dispersion is found to be remarkably reduced. This result suggests that the TPB ions in a high concentration increase the membrane conductivity or lyse yeast cells. Little, however, has been known about the mode of action of TPB ions in lysis of yeast cells. It may be of some use to propose a possibility about the question. TPB ions have high permeability in plasma membranes and high hydrophobicity, so that asymmetry of concentration of TPB ions across plasma membranes by addition of TPB ions to the outer medium caused a large diffusion and adsorption potential across the membranes. The large membrane potential may consequently induce electrical breakdown of plasma membranes, resulting in the lysis of the yeast cells.

D) Effects of Sodium Iodide and Gramicidin D

Sodium iodide and gramicidin D showed no reducing effect on dielectric dispersion. This result suggests that the conductivity of plasma membranes of the treated–cells remains lower values than $\kappa_a \times 10^{-5}$ (see Fig. 22), even if the increase in conductivity of plasma membranes is induced by KI and gramicidins.

It is reported by several authors[27] that gramicidin A causes increase in permeability of alkali metal cations in the biological membranes as well as BLM. However, the permeability change in plasma membranes of yeast cells is not suggested from the present result that gramicidin D induced no decrease in characteristic frequency of dielectric dispersion of yeast cell suspensions.

E) Effects of Physical Treatments

Physical treatments examined in this experiment is found to reduce dielectric dispersion of intact yeast cell suspensions. For the treatments of heating and freezing–thawing, the reduction effect on dielectric dispersions occurs immediately after the treatments, whereas ample time is necessary for the reduction of dielectric dispersion by UV irradiation. This observation may be explained from the difference in the killing mechanism between the treatments. The treatments of heating and freezing–thawing directly destroy the plasma membranes. On the other hand, the cells are killed by UV light without serious damage to plasma membranes, which is gradually destroyed by autolysis.

VI. SUMMARY

The results of the present study are summarized in Table V. The changes in dielectric dispersion induced by various treatments are classified into two types. Type 1 is reduction of dielectric dispersion which is caused by the large increase in the conductivity of cytoplasmic membrane and the decrease in the cell volume due to release of intracellular compounds from the cell interior. Type 2 is the decrease in characteristic frequencies of dielectric dispersions without changes in the limiting dielectric constant and conductivity at low frequencies, indicating that decrease in conductivity of cytoplasm without remarkable increase in conductivity of cytoplasmic membrane. Therefore, effects of many chemical agents and physical treatments on plasma membranes and cytoplasms may be readily elucidated from the type of changes in dielectric dispersions observed for the suspensions of the treated cells.

Table V. Changes in Dielectric Dispersions for Various Treatments

Treatments	Change in dielectric dispersion
DBDAC at concentrations more than 0.1 mM	Type 1
SDSO at concentrations more than 1 mM	Type 1
POE(8.2)nonylphenol at concentrations more than 0.01 mM under hypotonic condition	Type 2
POE(8.2)nonylphenol at concentrations more than 0.01 mM under hypertonic condition	Type 1
TPB at the concentration of 10 mM	Type 2
TPB at concentrations more than 60 mM	Type 1
Sodium iodide at the concentration of 20 mM	No change
Gramicidin D at the concentration of about 0.6 mg/100 ml	No change
Heating at temperatures more than 50°C	Type 1
Freezing and thawing	Type 1
Irradiation of UV light for 10 min	Type 1

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