BBA 3943

# SOME PHYSICAL INVESTIGATIONS OF THE BEHAVIOUR OF BACTERIAL SURFACES

## VII. THE RELATIONSHIP BETWEEN ZETA POTENTIAL AND SURFACE CHARGE AS INDICATED BY MICROELECTROPHORESIS AND SURFACE-CONDUCTANCE MEASUREMENTS

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SUMMARY

[illegible] of normal [illegible] treated cells of *Aerobacter aerogenes* [illegible] been determined [illegible] results demonstrate the importance of gegenion concentration.

2. The variation of surface conductance of these cells has been determined at various ionic strengths.

3. The surface-conductance correction to the ζ-potential of cells is very important for ionogenic surfaces, particularly at low ionic strengths. In contrast the surface-conductance correction is negligible for non-ionogenic surfaces.

4. The surface charge of *A. aerogenes* is unaffected by ion adsorption onto the basic matrix in contrast to the marked ion adsorption onto the unionized amino surface of the ethyleneimine-treated cells (pH 10.3).

## INTRODUCTION AND THEORY

It has been suggested[1,2] that the inclusion of a correction for surface conductance in the equation for ζ-potential would remove the maxima in the ζ-potential-concentration curves reported for many organisms[3]. This work was undertaken to investigate the significance of surface conductance for cells of *A. aerogenes*. Surface-conductance data on small particles is at present largely restricted to suspensions of inorganic particles[2,4,5].

Two equations have been derived for the evaluation of surface conductance, $K_s$(ohm$^{-1}$), from measurements on the conductivity of suspensions of particles; the first (Eqn. 1) due to FRICKE AND CURTIS[6] describes the surface conductance of spheres in terms of the conductivity of the suspension, $K_1$(ohm$^{-1}\cdot$cm$^{-1}$), the con-

Abbreviations: DAM, diazomethane; EI, ethyleneimine.

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ductivity of the suspension medium, $K_0$(ohm$^{-1}$·cm$^{-1}$), the volume concentration of the particles, $G$, and their radius, $a$.

$$K_s = \frac{\left(\frac{K_1}{K_0} - 1\right) + \frac{G}{2}\left(\frac{K_1}{K_0} + 2\right)}{G\left(\frac{K_1}{K_0} + 2\right) - \left(\frac{K_1}{K_0} - 1\right)} K_0 a \qquad (1)$$

The second equation (Eqn. 2), due to STREET[5], was derived after work on kaolinite particles had shown that Eqn. 1 gave unreliable results at low suspension concentrations.

$$K_s = (FK_1 - K_0)\frac{P}{S} \qquad (2)$$

$F$, "the formation factor", depends upon the number and shape of particles present, $P = 1 - G$ and $S$ is the surface area of the particles in 1 ml of suspension.

FRICKE[6] has calculated values of $F$ for a number of different shaped particles. The value for a cylinder has not been calculated, but since $F$ is a constant for a given shape and in the present work the bacteria are short cylinders then $F$ was calculated assuming the particles to be spherical when:

$$F = \frac{3 - P}{2P} \qquad (3)$$

Both Eqns. 1 and 2 ignore the contribution to the conductivity of the suspension arising from the charge carried by the electrophoretically moving spheres, $K_p$-(ohm$^{-1}$·cm$^{-1}$). This can be calculated[7] from:

$$K_p = 2D^2\zeta^2 G f(\mathrm{K}a)\frac{1 + \mathrm{K}a}{16\pi^2 a^2 \eta} \qquad (4)$$

where $D$ is the bulk dielectric constant, $\zeta$ the $\zeta$-potential, $f$(K$a$) the HENRY factor[8], K the reciprocal thickness of the electrical double layer[9] and $\eta$ the bulk viscosity.

The surface conductance can also be calculated from the relationship:

$$K_s = \frac{\sigma L}{\mathrm{F}} \qquad (5)$$

where F is the Faraday (e.s.u.·g-equiv$^{-1}$), $L$ the ionic conductance of the counterions in the double layer and $\sigma$ the charge density in the double layer. This total charge ($\sigma$) is normally divided into two parts, that in the diffuse or Gouy layer, $\sigma_G$, and that in the fixed or Stern layer, $\sigma_S$ (ref. 2), and is calculated using the usual equations for these[9]:

$$\sigma_G = \sqrt{\frac{NDkT}{2000\pi}\left[\Sigma c_i\left\{\exp\left(\frac{-z_i e\zeta}{kT}\right) - 1\right\} \quad \left\{\Sigma c_j\left(\exp\frac{+z_j e\zeta}{kT}\right) - 1\right\}\right]} \qquad (6)$$

$$\sigma_S = \frac{n_s z e}{1 + \frac{N}{Mc_i}\exp\left(\frac{-z_i e\zeta + \phi_i}{kT}\right)} \qquad (7)$$

where $c_i$ and $c_j$ are the concentrations and $z_i$ and $z_j$ the valencies of the cations and anions, respectively, $n_s$ the number of possible adsorption "sites" per cm², $e$ the electronic charge, $N$ the Avogadro number, $k$ the Boltzmann constant, $M$ the molecular weight of the solvent and $\phi_i$ the specific chemical adsorption potential. In this work since $n_s$ and $\phi_i$ are unknown $\sigma_S$ cannot be evaluated. The value of $L$ to be used with $\sigma_G$ is the limiting ionic conductance of the counterions ($l_+$ for a negative surface) while that to be used in the Stern layer is open to some conjecture. Since the Stern-layer charges are considered to be fixed the normal limiting ionic conductance cannot be used. In the present work and in others in the literature[20] the actual surface conductance is much greater than that calculated from Eqn. 5 and would suggest that the conductance in the Stern layer is much greater than that predicted even using $l_+$. This may be due to a Grotthuss type of conductivity or even electrical conductivity similar to that in metals[19]. STREET[5] obtained good agreement between the experimental value of surface conductance and that calculated using Eqns 5 and 6 without resource to Eqn. 7. The use of $\zeta$ instead of the actual potential at the boundary between the Stern and Gouy layers can only be justified in the absence of any better approximation to that potential.

$\zeta$-potentials were calculated from the mobility:

$$\bar{v} = \frac{D\zeta}{6\pi\eta} f(\mathrm{K}a) \tag{8}$$

using the values of $f(\mathrm{K}a)$ tabulated by HENRY[8]. The $\zeta$-potentials can be corrected for the surface conductance by the factor deduced by HENRY[7]:

$$\zeta_{\mathrm{corr.}} = \zeta\left(\frac{K_0 + K_s/a}{K_0}\right) \tag{9}$$

In work on the relationship between the $\zeta$-potential of particles and their surface charge it would be advantageous to have an independent method of evaluating this charge. The method must be selective for the surface charge since it is likely that, particularly with biological cells, ionogenic groups would be present throughout the the depth. The determination of the charge-reversal-concentration by electrophoresis in the presence of a large cation, *e.g.* hexol nitrate, might well lead to such an evaluation[10], although even with the hexol ion some diffusion and combination with internal groups might take place.

## EXPERIMENTAL

*Aerobacter aerogenes* (N.C.T.C. 418) used throughout this work, was grown and harvested at 24 h, and treated with EI, when necessary, as described in the previous paper[11]. Hexol nitrate was prepared from cobalt nitrate and ethylenediamine[12] and used as freshly prepared solutions. All suspensions for electrophoresis[11] or surface-conductance measurements were prepared by washing the cells at least three times in the required suspension medium. Suspensions in hexol nitrate solution made by mixing known concentrations of cells in distilled water with hexol nitrate, were used as such. Barbiturate buffer solutions[11] and all salt solutions were made from analytical-grade reagents dissolved in glass-distilled water. The pH of these solutions

was always > 5, *i.e.* on the plateau of the pH–mobility curve[11], unless otherwise stated.

Bacterial counts were made in a haemocytometer and size determinations by direct measurement of Indian-ink preparations at 800 × magnification with a calibrated eye-piece graticule.

Conductivity measurements were made in a bottle-type conductivity cell (cell constant 0.1732 $cm^{-1}$), with grey platinum electrodes. The conductance of the cell was measured using a Wayne Kerr Universal Bridge (B221) operating at a frequency of 1500 cycles/sec. Measurements at 25.00° ± 0.02° were recorded when a constant reading was attained; the suspension ($1–4 \cdot 10^9$ cells/ml) was well shaken before taking a reading.

## RESULTS

The dimensions of the cells, including the capsule, used throughout were: length $3.83 \cdot 10^{-4}$ cm and radius $1.025 \cdot 10^{-4}$ cm. The volume of a cell is thus $12.62 \cdot 10^{-12}$ $cm^3$ and the surface area $31.24 \cdot 10^{-8}$ $cm^2$ (calculated assuming the cell is a cylinder with flat ends). The size of the cells was unchanged by chemical modification with EI, microscopical examination showed that the internal structure of the cell was disorganized after suspension in dioxan, and also after EI-treatment.

### *The surface conductance ($K_s$)*

The correction to the specific conductivity of the suspension ($K_1$) for the electrophoretically moving particle ($K_p$) (Eqn. 4), $< 6 \cdot 10^{-7}$ $ohm^{-1} \cdot cm^{-1}$ at high ionic strengths and $< 1 \cdot 10^{-7}$ $ohm^{-1} \cdot cm^{-1}$ at low ionic strengths, was insignificant and was therefore ignored.

(*a*) *Normal cells.* Measurements were made in barbiturate buffer solutions and HCl ($I$ 0.05) in the pH range 1.5 to 10. There is general agreement between the results (Fig. 1) calculated using Eqns. 1 and 2. The increase in $K_s$ with decrease in pH is probably a measure of the mobility of the hydrogen ion in the double layer.

The conductivities of suspensions were determined at various ionic strengths of NaCl at pH 5.0 (Table I). Constant conductance readings could not be obtained at $I < 0.001$; even after several washings and resuspensions in the solutions the conductance continued to rise for 24 h. This was attributed either to leaching out of

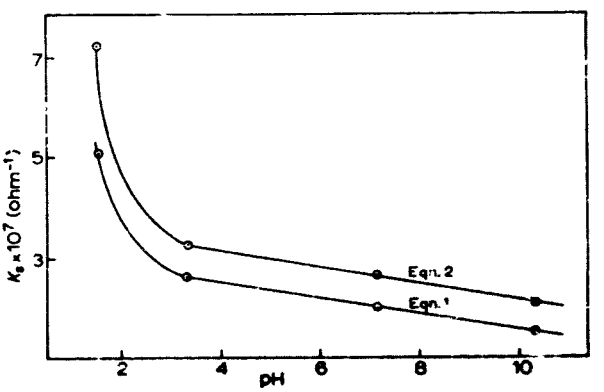



**Fig. 1. The variation of the surface conductance of normal cells of *A. aerogenes* with pH at $I$ 0.05.**

ions from the cells or gradual solution of macromolecular material. Similar effects have been reported for glass spheres[4] and kaolinite particles[5].

Table I again illustrates the similarity between values of $K_s$ calculated from the two equations. As the values of $K_s$ were to be used later for the correction of ζ-potentials it was necessary to know the values of $K_s$ in solutions of $I < 0.001$, the region inaccessible to experimental measurement. Whatever technique is employed for extrapolation the values of $K_s$ at $I < 0.001$ obtained will depend upon the method used. Only if the experimental results obeyed a known physical law could a reliable

TABLE I

THE VARIATION OF $K_s$ OF NORMAL CELLS IN NaCl SOLUTION WITH IONIC STRENGTH (pH 5.0)

| $I$ | $K_s \times 10^7$ ($ohm^{-1} \cdot cm^{-1}$) | $K_1 \times 10^7$ ($ohm^{-1} \cdot cm^{-1}$) | cells/ml $\times 10^{-6}$ | $K_s \times 10^7$ ($ohm^{-1}$) | |
|---|---|---|---|---|---|
| | | | | Eqn. 1 | Eqn. 2 |
| 0.001 | 1 307 | 1 387 | 5 000 | 0.161 | 0.1325 |
| 0.002 | 2 603 | 2 797 | 6 250 | 0.311 | 0.260 |
| 0.003 | 3 850 | 4 001 | 3 250 | 0.468 | 0.385 |
| 0.004 | 5 085 | 5 101 | 2 000 | 0.341 | 0.413 |
| 0.005 | 6 334 | 6 291 | 2 120 | 0.285 | 0.324 |
| 0.005 | 6 252 | 6 124 | 5 500 | 0.242 | 0.302 |
| 0.006 | 7 527 | 7 435 | 2 120 | 0.233 | 0.323 |
| 0.008 | 9 959 | 9 661 | 4 310 | 0.313 | 0.382 |
| 0.01 | 12 020 | 11 730 | 5 400 | 0.532 | 0.553 |
| 0.01 | 12 304 | 12 229 | 2 810 | 0.528 | 0.672 |
| 0.05 | 56 381 | 54 750 | 4 600 | 2.31 | 1.96 |
| 0.10 | 107 160 | 101 880 | 11 250 | 4.76 | 4.86 |
| 0.50 | 455 430 | 460 300 | 8 600 | 22.78 | 29.20 |

TABLE II

THE VARIATION OF $K_s$ OF EI-TREATED CELLS IN BARBITURATE BUFFER SOLUTION WITH IONIC STRENGTH AT pH 4.2 AND 10.3

| $I$ | $K_s \times 10^7$ ($ohm^{-1} \cdot cm^{-1}$) | $K_1 \times 10^7$ ($ohm^{-1} \cdot cm^{-1}$) | cells/ml $\times 10^{-6}$ | $K_s \times 10^7$ ($ohm^{-1}$) | |
|---|---|---|---|---|---|
| | | | | Eqn. 1 | Eqn. 2 |
| *pH 4.2* | | | | | |
| 0.001 | 1 408 | 4 846 | 3 500 | −0.168 | 3.30 |
| 0.001 | 1 410 | 3 138 | 1 690 | −0.162 | 3.38 |
| 0.005 | 6 407 | 9 345 | 2 390 | −0.995 | 3.97 |
| 0.0075 | 9 259 | 10 730 | 2 000 | −2.380 | 2.995 |
| 0.01 | 12 180 | 14 604 | 2 380 | −2.99 | 4.06 |
| 0.025 | 29 060 | 31 140 | 3 660 | 6.05 | 3.63 |
| 0.05 | 55 150 | 56 380 | 1 910 | 7.40 | 5.51 |
| 0.10 | 102 170 | 103 500 | 1 780 | 15.47 | 8.36 |
| *pH 10.3* | | | | | |
| 0.001 | 1 262 | 1 181 | 4 700 | 0.178 | 0.0200 |
| 0.005 | 5 213 | 5 106 | 5 800 | 0.351 | 0.0253 |
| 0.0075 | 7 546 | 7 427 | 7 500 | 0.456 | 0.0399 |
| 0.01 | 9 973 | 8 372 | 10 000 | 1.75 | 0.0556 |
| 0.025 | 23 745 | 20 500 | 8 500 | 4.15 | 0.128 |
| 0.05 | 44 670 | 40 300 | 6 400 | 7.10 | 0.233 |
| 0.10 | 82 860 | 81 680 | 5 400 | 5.20 | 4.33 |

estimation of the behaviour of the line in the unknown region be made. On the basis of the results for the EI-treated cells (see later) 4 possible extrapolations were drawn (Fig. 2, *a–d*); these are discussed later. Extrapolations *b* and *c* were deduced from the nearly linear plot of loglog $I$ against loglog $K_s$.

(*b*) *EI-treated cells.* The surface conductance of these cells was measured in barbiturate buffer solutions at pH 4.2 ± 0.2 and pH 10.3 ± 0.1 of various ionic strengths. These pH values correspond to points on the plateaux of the pH–mobility curve of these cells[11]. The mobility of the cells was constant at the value characteristic of EI-treated cells throughout measurements.

The results (Table II) indicate discrepancies, similar to those observed by STREET[5], between the values of $K_s$ calculated from Eqns. 1 and 2; the use of Eqn. 1 gives rise to some negative values of $K_s$.

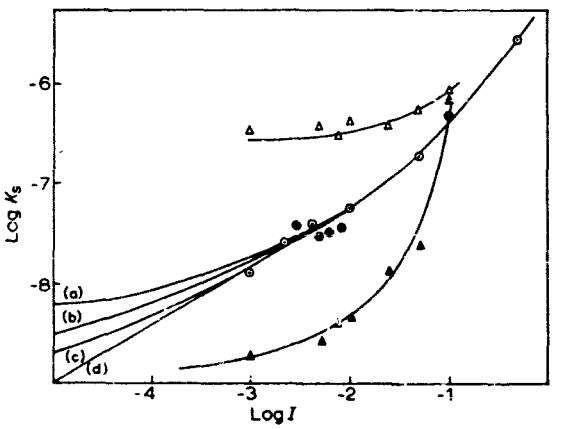



Fig. 2. The variation of the surface conductance, plotted as log $K_s$ (calculated using Eqn. 2), of normal and EI-treated cells with ionic strength. ⊙ and ● (different experiments), normal cells at pH 5; △—△, EI-treated cells at pH 4.2; ▲—▲, EI-treated cells at pH 10.3.

The results of the variation of $K_s$ with $I$ at both pH values calculated using Eqn. 2 are plotted in Fig. 2. The large difference between the surface conductances of EI-treated and normal cells at pH 4.2 was not due to the disruption of the cells by prolonged treatment in dioxan. The surface conductance of cells after suspension in dioxan, when measured at pH 4.2, was the same as that for normal cells.

The surface-conductance values used in the remainder of the paper are those calculated using Eqn. 2.

### *The mobility and ζ-potential*

ζ-potentials of the cells were calculated from the measured mobility[13] using Eqn. 8. The values of $D$ and $\eta$ used were those of the bulk solutions, these only differ significantly from water at high ionic strengths.

(*a*) *Normal cells.* The electrophoretic mobilities of cells were determined in NaCl, $Na_2SO_4$, sodium barbiturate buffer and $BaCl_2$ solutions. The mobility values (Fig. 3) are plotted as a function of the gegenion concentration ($G$) of the solution, *i.e.* the equivalent concentration of positive ions in the solution. The superimposition of the curves for NaCl, $Na_2SO_4$ and sodium barbiturate buffer solutions demonstrates

the relative importance of the cation over that of the anion in determining the mobility. The corresponding results in $BaCl_2$ (Fig. 3) show that, even when charge and concentration are taken into account, the curve is significantly different from that for univalent ions. This is due to the higher specific adsorption potential ($\phi_1$) of these ions.

Fig. 4 shows the variation of the $\zeta$-potential of normal cells with ionic strength in NaCl solution at pH 5.

(*b*) ***EI-treated cells.*** The variation of the $\zeta$-potential of these cells in barbiturate buffer solutions of various ionic strengths at pH 4.2 – 0.2 and pH 10.3 ± 0.1 is

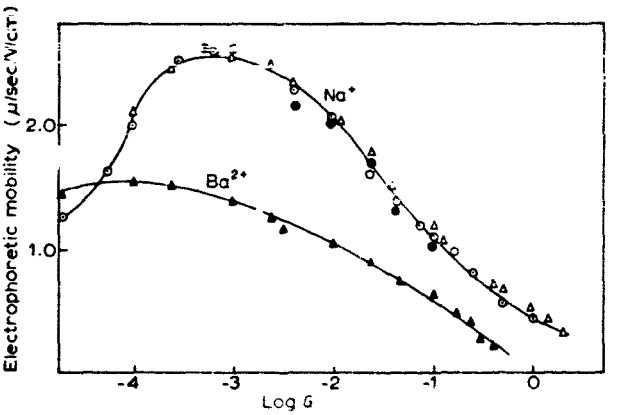
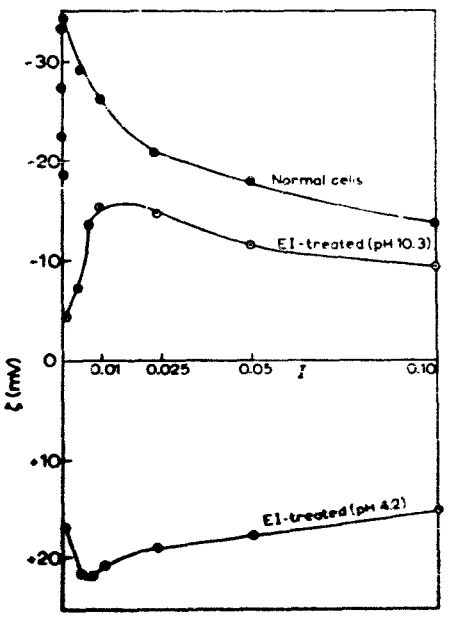



Fig. 3. The variation of the mobility of normal cells with gegenion concentration, $G$ (mole/l), in NaCl, $Na_2SO_4$, sodium barbiturate and $BaCl_2$ solutions. ○—○, NaCl; ●—●, sodium barbiturate buffer; △—△, $Na_2SO_4$; ▲—▲, $BaCl_2$ solutions.



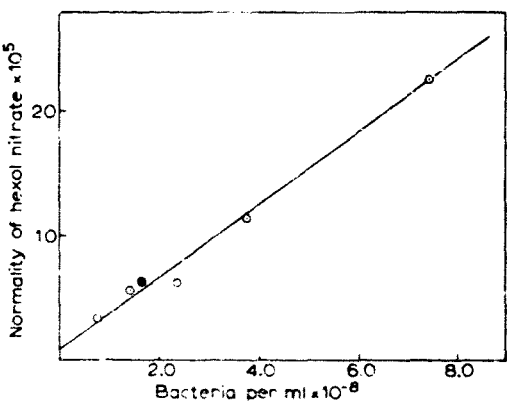




Fig. 5. The variation of the normality of hexol nitrate solution required for zero mobility, with bacterial concentration. ○, after 1.5 h; ●, after 30 sec.

**Fig. 4. The variation of the $\zeta$-potential with ionic strength of normal cells in NaCl solution at pH 5, and EI-treated cells in barbiturate buffer solutions at pH 4.2 and 10.3.**

shown in Fig. 4. The range of ionic strengths used was more restricted than for normal cells because of the necessity for strict control of pH of the suspensions, *i.e.* on the plateaux of the pH–mobility curve[11].

*Determination of the charge on normal cells using hexol nitrate*

The variation of the charge reversal-concentration[10] in hexol nitrate solutions with bacterial concentration on 3 separate samples is shown in Fig. 5. Bacteria suspended in 0.001 N hexol nitrate solution flocculated and had a low positive mobility. When these cells were washed three times and suspended in barbiturate solution (pH 7.0, $I$ 0.05), however, they had the normal control mobility value indicating that hexol-complexing was completely reversible.

$8 \cdot 10^8$ bacteria per ml, which require $24.0 \cdot 10^{-5}$ N hexol nitrate for zero mobility, are equivalent to $24.0 \cdot 10^{-8}$ g-equiv of –COOH. Combining this with the surface area of the cells gives the surface-charge density as $2.78 \cdot 10^5$ e.s.u./cm$^2$. This value is high due to the dissociation of the hexol complex[10], the corrected value, $2.34 \cdot 10^5$ e.s.u. per cm$^2$ is about 100 times greater than that predicted by Eqn. 6 from mobility measurements in buffer solution pH 7.0, $I$ 0.05, *viz.* $2.78 \cdot 10^3$ e.s.u./cm$^2$.

This large discrepancy was originally attributed to the penetration of the large hexol ions into the capsular layer. To test this the hexol nitrate solution was mixed with the bacterial suspension and the mobility of the cells measured within 30 sec *i.e.* before the hexol ions had time to penetrate to and complex with the underlying groups. The value obtained did not differ significantly from a control left for 1.5 h (Fig. 5). This indicates that either diffusion is a very rapid process, which is unlikely considering the large size of the cation and the pore size in the capsule, or that the calculated value of the surface charge ($2.34 \cdot 10^5$ e.s.u./cm$^2$) is in fact correct.

## DISCUSSION

The correction to the $\zeta$-potential for surface conductance (Eqn. 9) has usually been ignored in the electrophoretic study of small particles. This is due to the lack of experimental data and also the fact that the theoretical calculation of the surface conductance generally introduces a negligible correction[14]. The observed values of the surface conductance of both normal and EI-treated cells of *A. aerogenes* are greater than those calculated from Eqn. 5 using values of $\sigma$ from Eqn. 6 (Table III). The values of $l_+$ or $l_-$, the limiting ionic conductances, used are for the ions indicated and a correction was made for the electroosmotic effect ($+38$ ohm$^{-1}$·cm$^2$·equiv$^{-1}$ at 25° for water). The $\zeta$-potentials used in the calculation of the charge densities were obtained from Fig. 4 and the data of Fig. 3.

If correction for the surface conductivity of the Stern layer was available this would increase the calculated value of $K_s$. The actual experimental value is not only larger than that calculated in every case, but there is also a more marked increase in $K_s$ at higher ionic strengths than that predicted by the calculated values. At higher ionic strengths the experimental values of $K_s$ for all surfaces tend towards a similar value, $300–800 \cdot 10^{-9}$ ohm$^{-1}$ (see also Fig. 2). This suggests that, at these ionic strengths, the major contribution to surface conductance comes from the large concentration of ions to be found in the electrical double layer, which is then exceedingly thin. In contrast, at low ionic strengths, the major contribution to surface con-

TABLE III

THE OBSERVED AND CALCULATED VALUES OF THE SURFACE CONDUCTANCE

| Cells | pH | $I$ | $l_\pm$ for | $K_s \cdot 10^9$ (ohm$^{-1}$) | |
|---|---|---|---|---|---|
| | | | | Observed | Calculated |
| Normal cells | 1.5 | 0.05 | $H^+$ | 733 | 0 |
| | 7.0 | 0.05 | $Na^+$ | 270 | 0.798 |
| | 10.0 | 0.05 | $Na^+$ | 220 | 0.798 |
| | 5.0 | 0.001 | $Na^+$ | 13.2 | 0.245 |
| | 5.0 | 0.01 | $Na^+$ | 55.3 | 0.571 |
| | 5.0 | 0.10 | $Na^+$ | 486 | 0.914 |
| EI-treated cells | 4.2 | 0.001 | $H^+$ | 330 | 0.504 |
| | 4.2 | 0.01 | $H^+$ | 406 | 1.04 |
| | 4.2 | 0.10 | $H^+$ | 836 | 4.54 |
| | 10.3 | 0.001 | $Cl^-$ | 2.00 | 0.0386 |
| | 10.3 | 0.01 | $Cl^-$ | 5.56 | 0.414 |
| | 10.3 | 0.10 | $Cl^-$ | 133 | 0.797 |

TABLE IV

THE VARIATION OF THE $\zeta$-POTENTIAL OF NORMAL CELLS, CORRECTED FOR $K_s$, WITH $I$ OF NaCl SOLUTION (pH 5.0)

| $I$ | $\zeta$ (mV) | $\zeta_{corr.}$ (mV) | | | |
|---|---|---|---|---|---|
| | | (a) | (b) | (c) | (d) |
| 0.000 01 | −18.5 | −252 | −120 | − 92.1 | −55.8 |
| 0.000 05 | −22.5 | −159 | −124 | − 89.1 | −70.8 |
| 0.000 1 | −27.4 | −194 | −127 | −104.1 | −84.6 |
| 0.000 5 | −34.3 | −103 | − 93.2 | − 84.4 | −84.4 |
| 0.001 | −34.4 | − 79.8 | − 64.3 | − 70.0 | −70.8 |
| 0.005 | −29.3 | | −45.7 | | |
| 0.01 | −26.2 | | −38.2 | | |
| 0.05 | −17.7 | | −23.7 | | |
| 0.1 | −13.7 | | −18.6 | | |
| 0.5 | − 7.6 | | −12.3 | | |
| 1.0 | − 5.1 | | −10.7 | | |

ductance is probably provided by the ionogenic groups present on the surface, since there will be few electrolyte ions present in the double layer.

The $\zeta$-potentials corrected for $K_s$ for normal cells in NaCl solutions are given in Table IV; the values of $K_s$ in solutions of $I < 0.001$ used were obtained from the four extrapolations *a–d* of Fig. 2.

Similar calculations were made for the EI-treated cells at pH 4.2 and 10.3 (Table V).

The correction $F_p$ to $K_1$ recalculated using the new values of $\zeta$ still makes an insignificant contribution for all the surfaces studied.

While the surface conductance varies significantly with the change in pH of the suspension (Fig. 1) the surface-conductance correction has no effect on the general shape of the pH–$\zeta$ curve at $I$ 0.05 although the absolute value of $\zeta$ is increased (*e.g.* at pH 2.5, $\zeta_{corr.} = 7.6$, $\zeta = 5.15$ mV; and at pH 7, $\zeta_{corr.} = 23.4$, $\zeta = 16.7$ mV).

TABLE V

THE VARIATION OF THE ζ-POTENTIAL OF EI-TREATED CELLS, CORRECTED FOR $K_s$, WITH $I$, at pH 4.2 AND 10.3 IN BARBITURATE BUFFER SOLUTIONS

| $I$ | $\zeta$ (mV) | $\zeta_{corr.}$ (mV) |
|---|---|---|
| *pH 4.2* | | |
| 0.001 | +17.0 | +325 |
| 0.005 | +21.6 | +119 |
| 0.0075 | +21.8 | + 93.0 |
| 0.01 | +20.6 | + 75.4 |
| 0.025 | +19.1 | + 44.6 |
| 0.05 | +17.9 | + 34.5 |
| 0.10 | +15.4 | + 27.9 |
| *pH 10.3* | | |
| 0.001 | − 4.5 | − 5.1 |
| 0.005 | − 6.6 | − 7.0 |
| 0.0075 | −13.4 | −14.1 |
| 0.01 | −15.1 | −15.7 |
| 0.025 | −14.6 | −15.2 |
| 0.05 | −11.5 | −12.3 |
| 0.10 | − 9.3 | −13.9 |

The correction to the ζ-potential for surface conductance is negligible for EI-treated cells at pH 10.3 in contrast to the large effect at pH 4.2 (Table V). The absence of any effect at pH 10.3 is of significance in the general elucidation of the origin of surface conductance. If the charge on EI-treated cells at pH 10.3 is due to adsorption of anions (*i.e.* non-ionogenic surface) as is believed[11] then a different situation to that encountered with an ionogenic surface obtains. The experimental surface conductance, of the EI-treated cells at pH 10.3, is much lower than that of any other surface, even allowing for the high pH of the suspensions. This brings the value nearer the calculated value of $K_s$ and also accounts for the small correction to $\zeta$ ($K_s/a$ is small compared with $K_0$). This indicates that the abnormal $K_s$ of normal and EI-treated cells at pH 4.2 is due, in part at least, to the ionogenic groups on these surfaces. The large value of the correction to ζ at low ionic strengths further supports the hypothesis that in this region it is the ionogenic groups, or some factor closely associated with them, on the surface which contribute the major part of $K_s$.

The results obtained support the statement that the maximum in the ζ–concentration curves can be accounted for by a surface-conductance correction[2], provided, however, that the surface is ionogenic. The statement is incorrect for non-ionogenic surfaces. It would have been most instructive to measure the value of $K_s$ of DAM-treated cells[11] where there is apparently no adsorption and no ionogenic groups; unfortunately these suspensions were not stable enough to study.

There is a gradual decrease of $\zeta_{corr.}$ for EI-treated cells at pH 4.2 as the ionic strength of the suspension increases (Table V), similar to that obtained using the surface conductance from extrapolation (*a*) of Fig. 2 for the correction to ζ of normal cells. This decrease can be explained by postulating a direct adsorption of anions onto the positive groups of the EI-treated cells and cations onto normal cells. The results for the EI-treated cells at pH 10.3 can be explained in terms of the generally accepted theory. The maximum in the ζ–ionic strength curve is interpreted in terms

of an increase in the surface-charge density as the ionic strength increases, due to simple adsorption of ions, accompanied by a decrease in the thickness of the double layer; the latter is the more important factor in solutions of high ionic strength. If either of the extrapolations (*b*) or (*c*) of Fig. 2 are accepted as correct, then $\zeta$ tends to a constant value as the ionic strength decreases. This could again be explained on the basis of direct adsorption of cations onto the negative surface. One of the extrapolations (*a*), (*b*) or (*c*) on the basis of these considerations would thus seem most probable and this would allow a fairly comprehensive theory to be proposed.

The $\zeta$-potential depends mainly on the following:

(1) the adsorption of anions or cations from the solution onto the non-ionogenic areas of the surface or onto ion pairs formed from (2);

(2) the neutralisation of the charge on the surface due to the ionogenic groups by the association of these with ions of the opposite sign (gegenion association), as the ionic strength increases;

(3) decrease in the thickness of the electrical double layer as the ionic strength increases;

(4) the alteration of the position of the shearing plane due to the changes of viscosity in the double layer associated with the increase in field strengths at high ionic strengths, *i.e.* thin double layer.

The following evidence obtained using *A. aerogenes* supports these ideas. When the carboxyl groups on the surface are esterified the mobility is zero and independent of ionic strength[11]. Thus adsorption only occurs onto or in association with, the ionogenic groups. Gegenion concentration is an important factor (Fig. 3) in the electrolyte solution. Ion adsorption, onto the uncharged surface or non-ionogenic areas on the charged surface of *A. aerogenes*, is probably inhibited by the presence of a hydrated layer on the polysaccharide surface. This forms a barrier to the adsorption of ions from solution unless they have a high specific adsorption energy. The non-ionogenic surfaces of some hydrocarbons[15,16] possess a negative charge at neutral pH values. These surfaces are not hydrated because of the absence of slightly polar bonds, *e.g.* hydroxyl groups, over the greater proportion of their surface onto which the water

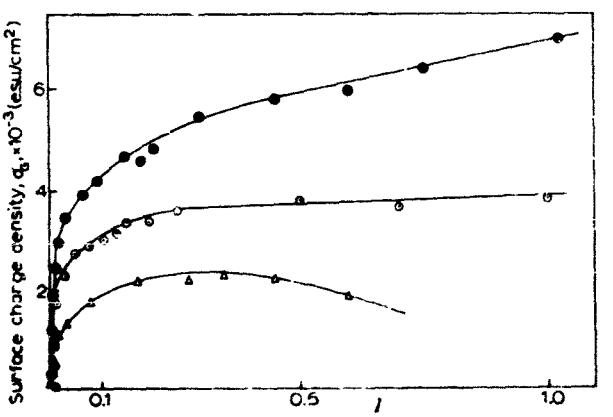



**Fig. 6. The variation of surface-charge density ($\sigma_G$) of normal cells (pH 5.0) with ionic strength for various salt solutions. ⊙—⊙, NaCl; ●—●, $Na_2SO_4$; △—△, $BaCl_2$ solutions.**

molecules may bond. There is thus no hydrated layer to form a barrier to the adsorption of ions.

The calculation of the surface-charge density ($\sigma_G$) of normal and EI-treated cells of *A. aerogenes* can be made using Eqn. 6. The results of such calculations using $\zeta$-potentials calculated from Eqn. 8 are summarized in Figs. 6 and 7 and Tables VI and VII. These charge densities are, of course, the algebraic sum of the surface charge and that in the Stern layer (inside the shearing plane when using $\zeta$ instead

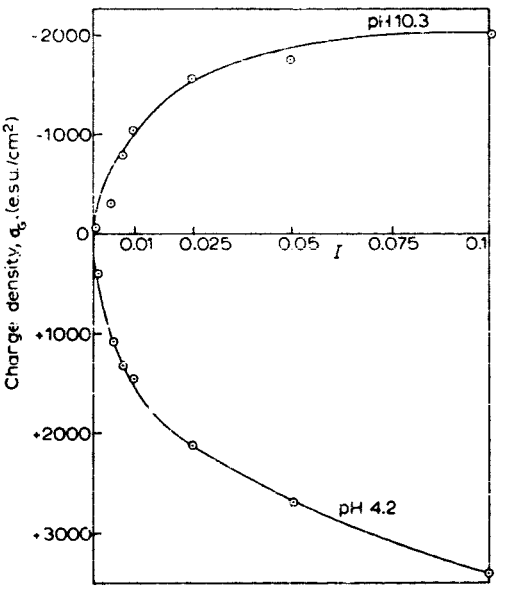



Fig. 7. The variation of surface-charge density ($\sigma_G$) of EI-treated cells with ionic strength of barbiturate buffer solution at pH 4.2 and 10.3.

of $\psi_d$). The charge density calculated for suspensions in solutions containing the sodium ion as the gegenion increase as the ionic strength increases while in $BaCl_2$ solutions it passes through a maximum (Fig. 6). The results are in complete accord with those of HAYDON[17] for *E. coli*. He explained the curves in terms of considerable non-ionogenic areas on the surface interspersed with a few negative groups. The sodium salt curves are due to preferential desorption of the sodium ion leaving the anion nearer the surface thereby increasing the net negative charge; surface ionogenic groups are unaffected. This process occurs for divalent ions, *e.g.* barium which also absorbs specifically onto the negative acidic groups. This theory adequately explains all the results except those for the zero-mobility DAM-treated cells[11].

Charge-density curves have been used in the past as a means of differentiating surfaces where ion-adsorption plays a significant or major part, from those where ionized groups predominate. Ideally the charge density of an ionogenic surface should decrease as the ionic strength increases due to specific adsorption at the ionized groups, thus reducing the apparent surface charge. The surface-charge density for a non-ionogenic surface should increase due to ion adsorption or desorption or remain constant at zero (*cf.* DAM-treated cells[11]) if this does not occur. Thus a com-

TABLE VI

THE VARIATION OF THE SURFACE-CHARGE DENSITY ($\sigma_G$) OF NORMAL CELLS IN NaCl SOLUTIONS BEFORE AND AFTER CORRECTING THE $\zeta$-POTENTIALS FOR SURFACE CONDUCTANCE

| $I$ | $\sigma_G$ (e.s.u./cm²) | | | | |
|---|---|---|---|---|---|
| | uncorrected | corrected for $K_s$ | | | |
| | | (a) | (b) | (c) | (d) |
| 0.000 01 | − 58 | −7540 | − 646 | 324 | − 147 |
| 0.000 05 | − 127 | −2760 | −1250 | − 680 | − 459 |
| 0.000 1 | − 212 | −7710 | −2060 | −1300 | − 873 |
| 0.000 5 | − 566 | −2860 | −2340 | −1900 | −1960 |
| 0.001 | − 804 | −2530 | −1780 | −2070 | −2040 |
| 0.005 | −1503 | | −2520 | | |
| 0.01 | −1877 | | −2870 | | |
| 0.05 | −2780 | | −3760 | | |
| 0.10 | −3000 | | −4120 | | |
| 0.50 | −3710 | | −6040 | | |
| 1.00 | −3830 | | −7380 | | |

TABLE VII

THE VARIATION OF THE SURFACE-CHARGE DENSITY ($\sigma_G$) OF EI-TREATED CELLS IN BARBITURATE BUFFER SOLUTIONS, AT pH 4.2 AND 10.3, BEFORE AND AFTER CORRECTING THE $\zeta$-POTENTIALS FOR SURFACE CONDUCTANCE

| $I$ | $\sigma_G$ (e.s.u./cm²) | | | |
|---|---|---|---|---|
| | pH 4.2 | | pH 10.3 | |
| | Uncorrected | Corrected | Uncorrected | Corrected |
| 0.001 | + 375 | +310 000 | − 98.1 | − 111 |
| 0.005 | +1080 | + 12 600 | − 319 | − 339 |
| 0.0075 | +1330 | + 9 060 | − 804 | − 845 |
| 0.01 | +1450 | + 7 250 | −1050 | −1090 |
| 0.025 | +2120 | + 5 460 | −1090 | −1070 |
| 0.05 | +2675 | + 5 720 | −1780 | −1900 |
| 0.10 | +3380 | + 6 160 | −2020 | −2060 |

promise may exist as HAYDON indicated for *E. coli*. The results for DAM-treated cells, indicating that no ion adsorption occurs on the *A. aerogenes* surface, are in accord with the conclusion of DOUGLAS AND SHAW[18] that ionogenic and non-ionogenic surfaces are not necessarily differentiated in this manner.

Values of $\zeta_{corr.}$ from Tables IV and V were also used for the calculation of the surface-charge density from Eqn. 6 (Tables VI and VII). The values for EI-treated cells at pH 10.3 (Table VII) further indicate the resemblance of this surface to a non-ionogenic surface with simple ion adsorption giving rise to the charge. The corrected charge densities for EI-treated cells at pH 4.2 (Table VII) completely alter the shape of the charge density–ionic strength curve. They show that if the surface-conductance correction is applied the surface charge density now decreases with increase in ionic strength at least up to $I$ 0.05. The increase above this ionic strength might be attributed to adsorption of cations onto the dipoles formed by gegenion association.

The results for normal cells are less conclusive (Table VI) since only surface-conductance values from hypothetical extrapolations are available. All the extra-

polations except (*a*) give rise to the normal variation of $\sigma_G$ with ionic strength. Extrapolation (*a*), the one most like the curve for EI-treated cells at pH 4.2 (Fig. 2), gives rise to an increase in charge density at low ionic strengths, although not comparable with that for EI-treated cells at pH 4.2. It is probable that, due to the inaccuracy of the surface-conductance determination (at least ± 10 %), the EI-treated cells have been overcorrected, and normal cells under-corrected since results are not available in the most critical region.

These preliminary observations on the effect of surface conductance on the $\zeta$-potential and surface-charge density of biological surfaces emphasise the need for further measurements (the subject of current investigations), and caution in the interpretation of existing data. Surface conductance plays a more important role in electrokinetic measurements than was previously imagined, particularly at low ionic strength. The major proportion of the observed surface conductance appears to arise in the Stern layer or the region inside the shearing plane since the calculated value of $K_s$ even using $\sigma_G$ corrected for $K_s$ (Eqn. 5) is still much lower than the observed value. The charge on the organism obtained from measurements with hexol nitrate and DAM[11] may not be altogether incorrect as a measure of purely surface charge. The values $2.15 \cdot 10^5$ and $2.34 \cdot 10^5$ e.s.u./cm$^2$, respectively, agree exceptionally well and compare well with the charge densities obtained after surface-conductance correction, *e.g.* $3.16 \cdot 10^5$ e.s.u./cm$^2$ for EI-treated cells at $I$ 0.001. The latter value of the charge density means that one charged group would occupy an area of 15.2 Å$^2$, on the surface. Since the area of a carboxyl group is approx. 20 Å$^2$, for surface-charge densities of this order to be possible successive layers of charge must contribute to electrokinetic measurements as suggested by HAYDON[16]. The action of some of the compounds used for specific chemical modifications confirm this[11].

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