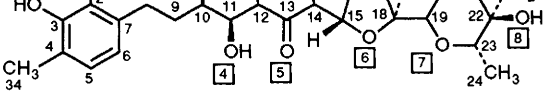
Evidence for Self-aggregation of the Ionophore Lasalocid Free Anion in Aqueous Solution

Rachid Lyazghi, Yvon Pointud, Gérard Dauphin and Jean Juillard

Laboratoires de Chimie Physique des Solutions et de Chimie Organique Biologique, URA CNRS 434 et 485, Université Blaise Pascal, 63177 Aubière Cedex, France

Surface tension, conductivity and NMR data for tetraalkylammonium salts of lasalocid in water show that they behave as surfactants; their high solubility thus involves lasalocid anion self-aggregation.

Lasalocid **1** is one of a growing class of naturally occurring carboxylic polyether ionophores, isolated from strains of *Streptomyces*.[1] These substances, acting in membranes, are very soluble in organic solvents, as also are their metal cation and tetraalkylammonium salts. In methanol, unlike the monovalent metal salts which are strongly associated, the tetraalkylammonium salts are highly dissociated electrolytes. This is clear from analysis of the titration curves of these ionophores with tetraalkylammonium methoxides,[2–4] and from conductivity data for the salt solutions.[5] Monovalent metal complexes of the ionophores are formed by coordination of the metal by the oxygens along the chain and formation of a pseudo-ring through head-to-tail buttoning; the metal cation is enclosed in the cavity thus formed. Tetraalkylammonium cations are not able to enter this cavity both because of their size and because of the unfavourable interactions between the oxygens and the outer alkyl groups of the cation. Even in chloroform, ion pairs or triplets are formed, not complexes.[6,7]



In water, although carboxylic natural ionophores are practically insoluble and their monovalent metal salts only sparingly soluble,[8,9] their tetraalkylammonium salts were shown, first by Cox *et al.*,[10] to be highly soluble. What then is the state of carboxylic ionophore anion solutions in water? Some answers to this question are offered here.

Measurements of the surface tension of aqueous solutions of lasalocid tetramethyl- and tetraethyl-ammonium salts as a function of their concentration results in the usual $\gamma = f(\log c)$ surfactant graph (Fig. 1); the aqueous solution surface tension decreases linearly then stabilizes. Critical micellisation concentration (c.m.c.) values thereby obtained are about 1.1×10^{-2} and 6×10^{-3} mol dm^{-3} respectively for the tetramethyl- and the tetraethyl-ammonium salts at 25 °C. A smooth increase of the c.m.c. with temperature was observed from 10 to 50 °C. Using a Gibbs absorption isotherm for 1 : 1 electrolytes, the excess surface concentration and the surface area of a single lasalocid anion were estimated; this area is about 200 Å^2 in the temperature range investigated, suggesting that lasalocid anion lies flat on the surface, most probably with all the oxygens in contact with the water and the alkyl groups pointing away from the surface.

Solubilities were found to be very high even at very low temperatures; Kraft points could not be reached.

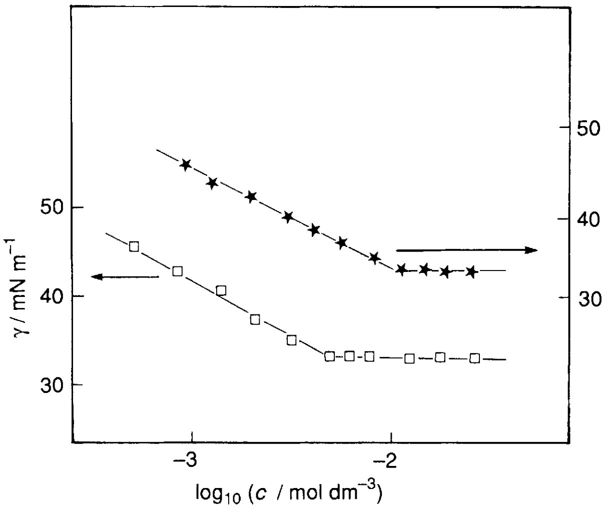


Fig. 1 Surface tension γ of aqueous solutions of Me_4N^+ ★ and Et_4N^+ □ lasalocid salts in water at 25.0 °C as a function of their concentration, *c*

Conductivity experiments also showed specific conductivity–concentration (*K vs. c*) and molar conductivity–square root of concentration (Λ *vs.* $\sqrt{c}$) plots typical of surfactant solutions. The c.m.c values estimated from the first type of plot, corresponding to the intercepts of two straight lines obtained at low and high concentrations, were somewhat higher than those obtained from surface tension experiments: 1.4×10^{-2} and 9×10^{-3} mol dm^{-3} respectively for lasalocid tetramethyl- and tetraethyl-ammonium salts. The second type of plot (Fig. 2) showed that below the apparent c.m.c., Λ varied linearly with $\sqrt{c}$, the slope being akin to the theoretical Onsager slope calculated using an initially extrapolated value of Λ^0. More sophisticated processing using the conductivity eqn. (1) limited to *c* terms with theoretical expression for *E* and *S* parameters[11] afforded better Λ^0 values: Λ^0 (Las^- Et_4N^+) = 48.9 and Λ^0 (Las^- Me_4N^+) = 62.8 which leads to a mean value of $\lambda^0 = 17.5\ \Omega^{-1}$ cm^2 mol^{-1} for the lasalocid monomeric anion in water; given the charge and size of this species, this is within the range of expected values. Evidently then, lasalocid tetraalkylammonium salts in dilute aqueous solution are strong electrolytes, highly dissociated to tetraalkylammonium cation and lasalocid monomeric anion. Processing of the whole *K vs. c* data using the Evans procedure [12] provided the ratio of the number of fixed counter-ions to the number of lasalocid anions in the aggregates; this is, for the tetraethylammonium salts, of the order of 0.65 assuming agregation numbers ranging from 20 to 80.

$$\Lambda = \Lambda^0 - S\sqrt{c} + Ec\log c + Jc \qquad (1)$$

The ^{13}C and ^1H NMR spectra of lasalocid tetramethylammonium salt in D_2O were expected to provide some information on the structure of both monomers and aggregates. Proton, carbon broad-band and carbon *J*-modulated spectra were recorded at various concentrations using a Bruker MSL 300 NMR spectrometer. Well resolved spectra were obtained under the c.m.c. Above the c.m.c., a broadening of the signals was observed. In the proton spectra, no reliable ^1H–^1H or ^1H–^{13}C correlation experiments could be done. Assignments could therefore only be made by comparison with spectra in methanol for the same species.[6] By this means, complete assignments were achieved for ^{13}C resonances, but not for ^1H resonances for which only some signals could be reliably identified. Spectra at various concentrations gave data on both monomeric lasalocid anion and the anion in aggregates. In methanol, it was shown[6] that lasalocid anion is monomeric and has a rather open structure, *i.e.* with relative freedom of its tail (from C-12 to C-23), the main hindrance affecting the salicylic head, probably through an $O_2 \cdots HO_4$ hydrogen bond. ^{13}C chemical shifts for the monomeric anion species in water, observed at low concentrations, can be compared with values reported in methanol.[6] With allowance being made for systematic variations due to differences in standardisation in the two solvents, the main effects, corresponding to shifts towards high fields, were observed for C-7, C-8, C-9 and C-14, C-30. This corresponds to conformation changes in these regions, probably related to complete unfolding of the anion molecule by cleavage of the $O_2 \cdots HO_4$ ligation, this being favoured by strong solvation of all the oxygens of this species in water.

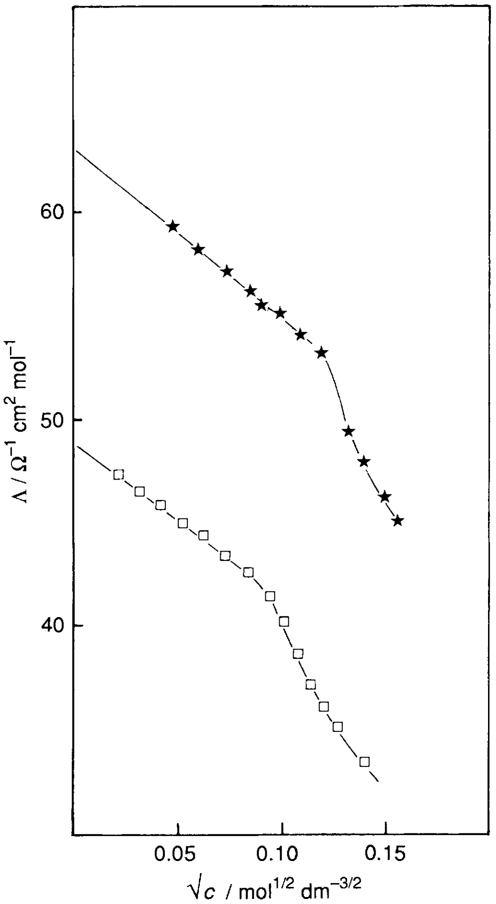


Fig. 2 Molar conductivity Λ of Me_4N^+ ★ and Et_4N □ salts of lasalocid in water at 25.0 °C as a function of the square root of their concentration, *c*

Above the c.m.c., the broadening of the signals may be mainly ascribed to restricted internal motion in the aggregates[13] and slow exchange between monomers and aggregates. ^{13}C and observable ^1H chemical shifts for concentrations below and above the c.m.c. (8×10^{-3} and 10^{-1} mol dm^{-3}), show some significant differences; ^{13}C chemical shift variations from monomers to aggregates are all positive for methyl and ethyl groups, the greater variations being observed for C-26 (0.4 ppm), C-27 (0.6), C-28 (1.0), C-31 (0.8) and C-34 (0.6). This would correspond to the location of these groups in a more hydrophobic environment in the nucleus of the aggregate. Other changes concern the salicylate group and its ligation to the rest of the molecule: 5-H (−0.18 ppm) and 6-H (−0.13), and C-1 (−0.9 ppm), C-6 (−0.9), C-7 (1.3) and C-8 (1.7), and are probably related to perturbations resulting from partial interactions of the carboxylate group with the counter-ions; C-11 which bears an OH group is also affected (−0.8 ppm); small conformation changes occur in the tetrahydrofuran ring, more marked ones in the tetrahydropyran ring. Thus the aggregation involves some conformational and internal changes in the anion molecule.

The size, shape and structure of the aggregates will be investigated using various methods. In addition, experiments involving other carboxylic ionophores show that aggregation of their anions also occurs in water. Further research is in progress to investigate these effects more fully.

Received, 27th November 1991; Com. 1/06032E

References

1 See, for example, *Polyether Antibiotics, Naturally Occurring Acid Ionophores*, ed. J. W. Westley, Dekker, New York, 1982.
2 Y. Pointud and J. Juillard, *J. Chem. Soc., Faraday Trans. 1*, 1988, **84**, 959.
3 Y. Pointud, C. Pierzo, C. Tissier and J. Juillard, *J. Chim. Phys.*, 1985, **82**, 891.
4 A. M. Albrecht-Gary, S. Blanc-Parasote, D. W. Boyd, G. Dauphin, G. Jeminet, J. Juillard, M. Prudhomme and C. Tissier, *J. Am. Chem. Soc.*, 1989, **111**, 8598.
5 P. Gachon, G. Chaput, G. Jeminet, J. Juillard and J.-P. Morel, *J. Chem. Soc., Perkin Trans. 2*, 1975, 906.
6 R. Lyazghy, A. Cuer, G. Dauphin and J. Juillard, *J. Chem. Soc., Perkin Trans. 2*, 1992, 35.
7 F. Vaufrey-Mary, Thesis, Université Blaise Pascal, Clermont, France, 1990.
8 R. Lyazghy, Thesis, Université Blaise Pascal, Clermont, France, 1991; R. Lyazghy, Y. Pointud and J. Juillard, *J. Chem. Soc., Faraday Trans.*, in the press.
9 M. Hebrant, Y. Pointud and J. Juillard, *J. Phys. Chem.*, 1991, **95**, 3653.
10 B. G. Cox, N. V. Truong, J. Rzezotarzka and H. Schneider, *J. Am. Chem. Soc.*, 1984, **106**, 5965.
11 R. M. Fuoss and F. Accascina, *Electrolytic Conductance*, Interscience, New York–London, 1959.
12 H. C. Evans, *J. Chem. Soc.*, 1956, 579.
13 H. Okabayashi and K. Matsushita, in *Surfactants in Solution*, ed. K. L. Mittal and B. Lindman, Plenum, New York, 1985, vol. 1, p. 517.