

AN EXPERIMENTAL DETERMINATION OF THE INTER-NITROGEN DISTANCE IN SOME BIS-QUATERNARY AMMONIUM GANGLIONIC AND NEUROMUSCULAR BLOCKING AGENTS

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The conductances in water of hexamethonium bromide and iodide, decamethonium bromide and iodide, hexadecamethonium iodide, and decaethonium iodide have been measured. The values of the limiting cationic conductances have been interpreted in terms of molecular shape, and the internitrogen distance for the above compounds calculated, using hydrodynamic relationships. The internitrogen distances found were 6.3 Å for hexamethonium, 9.5 Å for decamethonium, 13.5 Å for hexadecamethonium, and 10.2–13.5 Å for decaethonium. The effect of the hydrocarbon/water interfacial energy on the extended lengths of these molecules is discussed.

WHEN organic ions having aliphatic hydrocarbon chains as part of their structure are in aqueous solution, there is a considerable interfacial energy between the hydrocarbon chain and the solvent. The organic part of the molecule will contract as much as possible to minimise the interfacial area and thus the interfacial energy. The amount of contraction for mono- and di-carboxylate ions, trimethylalkylammonium ions, and dodecylamine and dodecyl sulphate ions has recently been calculated (Elworthy, 1963), and it has been shown that the amount of contraction depends on the length of the aliphatic chain, and on the number of ionised groups present in the molecule. Many neuromuscular and ganglionic blocking agents contain aliphatic hydrocarbon chains, and would be expected to show such contraction. As the internitrogen distance is one of the important quantities in developing theories of the mode of action of this type of drug, then clearly it is important to determine the distance for the molecule as it exists in aqueous solution, rather than in the dry state. Calculations of the inter-nitrogen distance have been made by Gill (1959) for ganglionic blocking agents. In this paper an experimental method for determining this distance is reported.

Theoretically, transport properties can be interpreted in terms of molecular shape by applying Perrin's (1934, 1936) relationships between the frictional resistance and the shape of the molecule. This is an approach familiar in macromolecular chemistry. For ions with a radius below 5 Å, difficulties in interpretation are encountered as Stokes' Law fails in this region of molecular size. Recently however, Perrin's relationships have been shown to apply to small molecules (Elworthy, 1962), and following a method based on that suggested by Stokes and Robinson (1959a), deviations from Stokes' law have been assessed, a procedure involving the determination of volumes and hydrations of ions (Elworthy, 1963). From the limiting equivalent ionic conductance λ^0 , the molecular shape can be obtained.

The balance between the driving force on an ion, and its frictional resistance leads to the following equation (Stokes and Robinson, 1959b)

$$\lambda^{\circ} = |z| F^2/Nf \quad \dots \quad (1)$$

$|z|$ being the valency, f the frictional coefficient, N is Avogadro's number and F the Faraday. For spherical ions, Stokes' Law gives f in terms of the ionic radius, r , and the viscosity of the medium, η . Asymmetric ions are represented by models, usually ellipsoids, and for such a model the mean frictional coefficient is

$$f_{\circ}^{\circ} = 12\pi \eta/S \quad \dots \quad (2)$$

giving
$$\lambda_{\circ}^{\circ} = |z| F^2.S/(12\pi.\eta N) \quad \dots \quad (3)$$

S is a function of the three semi-axes of the ellipsoid, a , b , and c . Prolate ($a > b = c$) and oblate ($b > a = c$) ellipsoids of revolution are usually chosen to represent molecules, avoiding the indeterminacy of interpreting an experimental quantity in terms of three unknowns. The molecules studied here fit best into the prolate model for which:

$$S = (2/\sqrt{a^2-b^2}) \ln. [(a + \sqrt{a^2-b^2})/b] \quad \dots \quad (4)$$

knowing the volume of the ellipsoid, $V = 4\pi ab^2/3$, a and b can be obtained. The above equations can also be used in terms of the ratio $f_{\circ}^{\circ}/f_{\circ}^{\circ}$, where f_{\circ}° is the frictional coefficient of a sphere of the same volume as the ellipsoid. The ratio a/b can be found from the value of $f_{\circ}^{\circ}/f_{\circ}^{\circ}$, either using Perrin's equation (4) or from tables (Svedburg and Pedersen, 1940).

A method for assessing the volumes of organic ions for this type of hydrodynamic calculation has been developed, being a procedure roughly similar to the determination of the closest distance of approach of water molecules to the ion (Elworthy, 1963). Stokes and Robinson (1959c) suggest that quaternary ammonium ions are generally unhydrated in solution.

EXPERIMENTAL

Materials. A sample of hexamethonium bromide was converted to the iodide by releasing the free base with a strong anion exchanger, and neutralisation with A.R. hydriodic acid. Decamethonium iodide and bromide were gifts of Allen & Hanburys, Ltd., while decaethonium iodide was supplied by Professor Stenlake. Hexadecamethonium iodide was prepared by treating 1,16-diiodohexadecane with trimethylamine in ethanol. All samples were recrystallised at least three times from suitable solvents, and assayed by converting a weighed amount into free base or free acid by passage over the relevant strong ion-exchanger, and titrating the effluent with standard acid or alkali.

Sodium and potassium chloride A.R. were recrystallised twice from conductance water, and dried at 250°. Water twice distilled, had a specific conductance $\sim 1 \times 10^{-6}$ mhos.

Measurements. The conductance bridge was an LKB 3216B high precision model, used in conjunction with a Cambridge low inductance six decade box as a measuring resistance. The conductance of the water

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was determined on the same day as the solutions made from it, using a cell with cell constant, $k = 0.102_5$. The solutions were made up by weight, and measured in a cell, $k = 0.952_2$. The cells were extensively calibrated with solutions of potassium chloride, and checked by measuring the conductances of sodium chloride solutions. Agreement to within ± 0.1 per cent of the literature figures for the latter solutions was obtained. During measurement, the cells were placed in an oil thermostat controlled to $25 \pm 0.003^\circ$.

RESULTS AND DISCUSSION

The conductance results are shown in Figs. 1 and 2 as plots of equivalent conductance (Λ) against (normality) $^{\frac{1}{2}}$. Preliminary Λ^0 values were found by extrapolating the phoreograms to zero concentration.

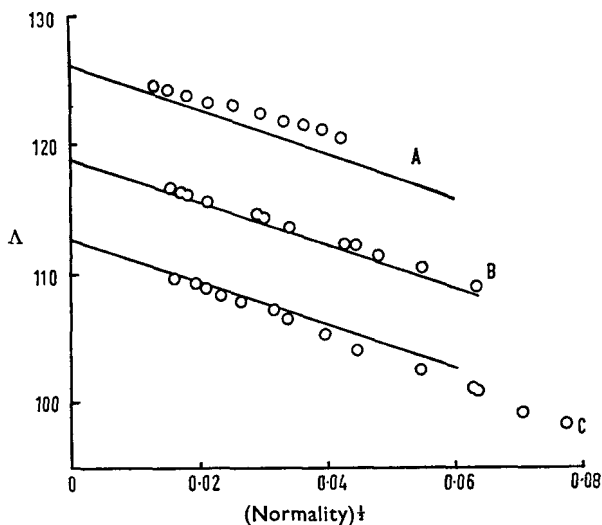


FIG. 1. Plots of equivalent conductance (Λ) against (normality) $^{\frac{1}{2}}$ for A, hexamethonium iodide. B, decamethonium iodide. C, hexadecamethonium iodide.

The hexa- and decamethonium salts approach the Onsager limiting tangent from above, which is the general criterion for a non associated electrolyte. The results were fitted using the Fuoss-Onsager (1957) equation.

$$\Lambda = \Lambda^0 - Sc^{\frac{1}{2}} + Ec \log c + Jc \dots \dots \dots (5)$$

where c is the concentration in equivalents per litre, E and J are constants.

The Onsager limiting tangent is shown in the Figures as a full line. Hexadecamethonium and decaethonium iodide both approached the limiting tangent from below, thus showing some measure of association in solution. As the precision of the results was only ± 0.1 per cent, the full scale analysis based on the extended law was not undertaken. The method of Fuoss and Shedlovsky gave a reasonable means of determining Λ^0 (see Fuoss and Accascina, 1959).

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Using 78.1 for λ^0 (Br^-) and 76.8 for λ^0 (I^-), the λ^0 values for the cations were:

		λ^0 from bromide	λ^0 from iodide
Hexamethonium	49.3	49.4
Decamethonium	42.0	42.1
Decaethonium	—	35.5
Hexadecamethonium	—	35.9

We are concerned only with the interpretation of the limiting ionic conductance in terms of molecular shape, using hydrodynamic methods. The other method of approach is that of Rice (1958) which represents the chain as a set of spheres joined by links, imposing restrictions due to hindered rotation about bonds, interactions between charged groups, etc. In Rice's method, as in Gill's calculations, the effect of the hydrocarbon/water interfacial energy has been neglected whereas in the present method, the model used takes this effect into account.

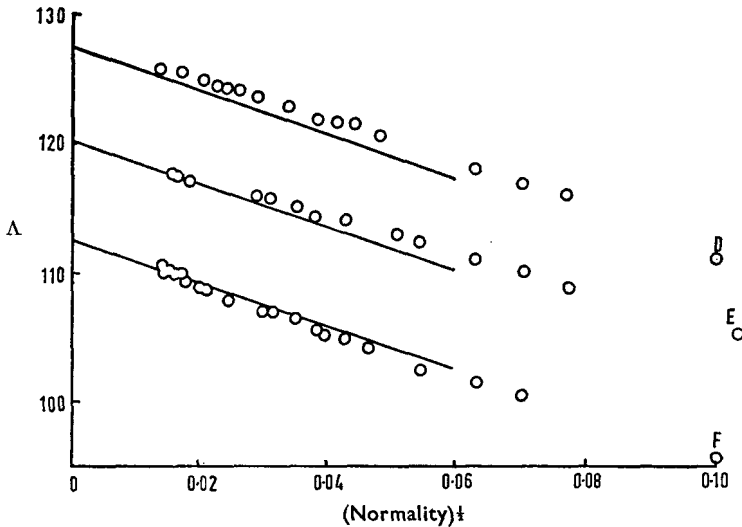


FIG. 2. Plots of equivalent conductance (Λ) against $(\text{normality})^{1/2}$ for D, hexamethonium bromide. E, decamethonium bromide. F, decaethonium iodide.

Using the observed λ^0 values, and the correction procedure for Stokes' Law previously described (Elworthy, 1963), a and b for prolate ellipsoids representing the molecules were found. The model chosen to fit the molecule is an ellipsoid equivalent to it, and the positions of the centres of the nitrogen atoms in this equivalent ellipsoid were estimated by the following procedure.

Integration to find the volume of half the ellipsoid leads to

$$\frac{1}{2}V = \frac{\pi b^2}{a^2} \left[a^2 x - \frac{1}{3} x^3 \right]_0^x \quad \dots \quad (6)$$

x being a distance along the major axis to the chosen point. Knowing a, b, and the volume equivalents of each group in the molecule, the values

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of x corresponding to the volume of the molecule inside and outside the nitrogen atom can be found from (6). Hence the distance between the centres of the nitrogen atoms can be found.

	Hexa- methonium	Deca- methonium	Hexadeca- methonium	Deca- ethonium
N ⁺ -N ⁺ distance (Å)	6.3	9.5	13.5	10.2-13.1

For decaethonium there are several possible arrangements of the ethyl groups on the nitrogen atoms. These groups can lie alongside the main part of the hydrocarbon chain, or point outwards in the x direction. To minimise the total hydrocarbon/water interfacial energy, it would be expected that as many groups as possible would lie in the first position, giving 13.1 Å for the internitrogen distance. With two ethyl groups in close proximity to the main hydrocarbon chain, and one directed outwards, 11.5 Å is obtained; while with one group close to the chain, and two directed outwards 10.2 Å is found. The remaining arrangement does not seem possible, as molecular models show that there is insufficient space on one side of a nitrogen atom to accommodate all three ethyl groups. The internitrogen distances found are larger than that of decamethonium. The results reflect that a measure of protection against the contracting effect of the hydrocarbon/water interfacial energy is given to the hydrocarbon chain by introducing large substituents on the nitrogen atom.

In previous work the degree of extension of molecules containing the same hydrocarbon chain was, dicarboxylic acids > monoalkyl trimethylammonium compounds > monocarboxylic acids. The present series of bistrimethylammonium compounds gives a slightly greater degree of extension than the dicarboxylic acids; due to the repulsive forces between the polar groups, which oppose the contracting effect of the interfacial energy, and possibly due to the "protecting" effect of the paraffinoid groups attached to the quaternary nitrogen atoms. This effect was clearly shown in the greater extension in solution of monoalkyltrimethylammonium compounds as compared with monocarboxylic acids.

The internitrogen distance found for hexamethonium (6.3 Å) is slightly shorter than the mean internitrogen distance calculated by Gill (6.9 Å) but falls within the range of his probability calculations, e.g. between 6 and 7.8 Å; it is about 3 Å shorter than that measured on a Catalin model arranged in a regular configuration. The additional reduction found here is due to the interfacial energy effect discussed above. At the moment no attempt at detailed discussion of potential attachment points on receptor surfaces will be made, as conductance measurements on other ganglionic and neuromuscular blocking agents are required.

For decamethonium, the N⁺-N⁺ distance of 9.5 Å is some 4 Å shorter than the Catalin distance, but is in accord with previous suggestions of 9-10 Å (Carey, Edwards, Lewis, and Stenlake, 1959). It is interesting to note that substitution of ethyl for methyl groups on the nitrogen atom increases the internitrogen distance (though this effect will only occur if the chain linking the nitrogen atoms is a flexible one). Peak activity in the bistrimethylammonium series occurs in the tridecyl compound (Barlow and Ing, 1948), which would have an internitrogen distance of between

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12 and 15 Å, a value roughly interpolated from a graph of N⁺-N⁺ distance against the number of carbon atoms joining the nitrogen atoms. This value greatly exceeds that of 9.5 Å found for decamethonium, which has peak neuromuscular blocking activity in the methonium series. Although internitrogen distance is an important factor in relation to activity, many others have to be considered.

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