DIFFUSION OSMOTIC EQUILIBRIUM

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SUMMARY

A new method for measuring diffusion constants is described. In this method an equilibrium is established in a cell between upward diffusion and downward migration on to a semi-permeable membrane across which is established a strong osmotic gradient.

The diffusion constant of bovine serum albumin determined by this method agrees well with the value obtained with the boundary spreading method. The method can also be used for testing the homogeneity of colloidally dispersed substances.

INTRODUCTION

The method most commonly used for the determination of the diffusion constant of a substance is to establish a sharp interface between the solution of the substance and its solvent, and then to measure the spreading rate of the boundary. These measurements are made by optical means such as light absorption, light refraction or interferometry. Apart from the determination of diffusion constants, the boundary spreading method has also been used for testing the homogeneity of dispersions qualitatively. This was not always successful especially when mixtures of substances whose diffusion constants did not differ greatly, were examined. The diffusion constant of a substance is proportional to the square of the average distance traversed by a molecule from a given point in a given time interval. It follows, therefore, that although diffusion constants of the components in a mixture of two substances may differ markedly, the average distances traversed by the molecules of the individual components will necessarily differ less.

In this report a new approach is made to the determination of the diffusion constant whereby the quadratic distance term is resolved into two factors, a velocity and a linear distance component.

By this means it is possible to indicate inhomogeneity in colloidally dispersed systems when the conventional boundary spreading techniques give less satisfactory answers. The method is based on the establishment of equilibrium between downward migration of a substance on to a semi-permeable membrane and upward migration through the process of diffusion.

Abbreviations: P.E.G., polyethylene glycol; D.O.E., diffusion osmosis equilibrium; B.S.A., bovine serum albumin.

Theoretical

Consider a solution of a substance in a vertical cell, one end of which is closed by a semi-permeable membrane. Let the solution be in ionic equilibrium with an electrolyte solution above it and below the semi-permeable membrane. Further, let the substance migrate on to the membrane at a constant rate of V cm/sec and diffuse outwards at a rate of D cm²/sec. Let C be the concentration at any level x cm removed from the membrane surface. When equilibrium is reached between outward diffusion and downward migration, the amount of substance which passes downwards through a definite level, equals that amount which passes upwards through that level. The amount of material which passes downwards through a section of unit area in time dt equals $Vcdt$, and the amount which diffuses outwards equals

$$-D\frac{dc}{dx}\cdot dt$$

at equilibrium

$$Vcdt + D\frac{dc}{dx}\cdot dt = 0$$

Rearranging and integrating between the limits x_1 and x_2 we have:

$$V(x_1 - x_2) = D \ln \frac{c_2}{c_1}$$

from which follows

$$D = \frac{V(x_1 - x_2)}{\ln \frac{c_2}{c_1}} \qquad (1)$$

By establishing the concentration distribution in the column after the equilibrium state had been reached, the diffusion constant may be calculated.

Methods and apparatus

In Fig. 1 is a sketch of the apparatus used in the present work. A is the central section of the Tiselius electrophoresis cell, B is a container provided with two slots which correspond to the openings in the limbs of the electrophoresis cell. A cellophane membrane m is interposed between A and B. D is a levelling section provided with four slots, each of which has approximately three times the capacity of a rectangular cavity in A. Cavities 1 and 1′ are joined on to glass capillaries of 2 mm bore. Cavities 2 and 2′ are used for introducing the material into the cavities in A. The surfaces between D and A, and A and B are well greased and the cellophane membrane is held tightly between A and B by metal spring blades. The sections D and B are made of perspex.

Several methods were tried to produce a constant rate of migration of a colloid suspension on to a semi-permeable membrane, but without success. A positive pressure on the column caused leaks to occur in the apparatus. Movement of the dispersed phase by the application of an electrical potential gradient across the column resulted in the accumulation of electrolyte on the membrane, an effect observed previously during electrodecantation of proteins[1]. Strong osmotic gradients that were created across the semi-permeable membrane at the bottom of the cell caused the desired migration of the fluid on to the membrane. By the use of suitable substances such as P.E.G., any desired osmotic gradient may be obtained.

The material to be investigated was dialysed overnight against a suitable concentration of P.E.G. dissolved in saline, before dilution in saline to the required concentration. The container B was filled with the P.E.G. solution and all the air trapped in the slots was removed by inversion of the apparatus. The solution to be

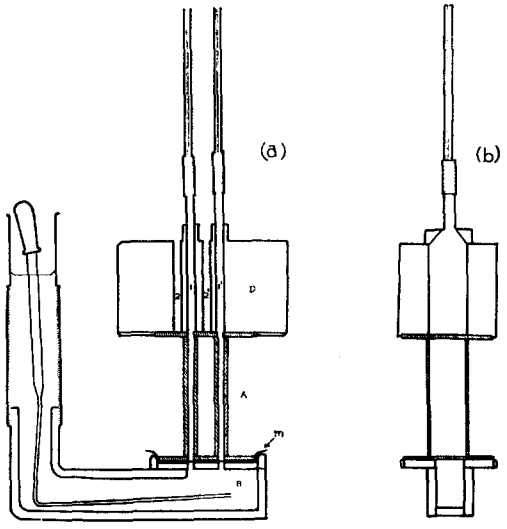

Fig. 1. (a) Sectional elevation of apparatus in which D.O.E. experiments were performed. A, central chamber of Tiselius electrophoresis apparatus. B, P.E.G. container provided with a Pasteur pipette and plastic tube to agitate the fluid at intervals. D, levelling section with 4 slots, 1, 1′, 2 and 2′. 1 and 1′ are joined on to capillary tubes. Fig. 1. (b) Side view of apparatus.

investigated was introduced into the two limbs of A *via* cavities 2 and 2′. The cavities 1 and 1′ were filled with saline. Section D was moved across so that 1 and 1′ were in apposition with the cavities in A. The interfaces which formed between solution and solvent were lowered to approx. 1 cm from the membrane's surfaces with the aid of a finely drawn pipette, following the technique of POLSON[2] and KAHN AND POLSON[3]. To ensure against leakages, cavities 2 and 2′ were filled with molten vacuum grease, and the glass capillaries were attached to 1 and 1′ and filled with saline. The apparatus was attached to a frame and lowered into the electrophoresis bath which was regulated at room temperature*. After temperature equilibrium had been obtained, the heights of the menisci were carefully noted.

The concentration gradients in the cells were recorded daily with the LAMM scale method[4], and the lowering of the saline menisci in the capillaries noted. After each observation the capillaries were filled to their original levels. Photographic recordings of the concentration gradients in the cells were made daily until the state of equilibrium had been reached.

To convert the dimensions on the photograph, as plotted on the graph paper, to those in the diffusion cell, the abscissae were multiplied by a conversion factor—

$$K = \frac{l - b}{l} \cdot \frac{1}{G}$$

* No disturbance sufficiently large to influence the course of the experiment was noticed when this procedure was followed.

where l and b are the optical distances from the scale to the camera lens and from the scale to the centre of the diffusion cell respectively. G is the photographic enlargement factor[4]. Equation (1) now becomes:

$$D = \frac{V\,(x_1 - x_2)}{\ln \frac{c_2}{c_1}} \cdot K$$

In the present work $K = 1.111$.

When equilibrium is reached $\frac{c_2}{c_1} = \frac{dc_2/dx}{dc_1/dx}$ where $\frac{dc_2}{dx}$ and $\frac{dc^1}{dx}$ are the concentration gradients at x_2 and x_1 respectively, (SVEDBERG AND PEDERSEN[5]). The concentration gradients are recorded as refractive index gradients.

RESULTS

Several D.O.E. experiments were performed on B.S.A. (Armour) and rabbit serum. The latter substance was selected for investigation on account of its inhomogeneous character. In Figs. 2 and 3 are given typical concentration gradient curves obtained during the course of D.O.E. experiments on the two substances respectively. In Figs. 4 and 5 are given the static D.O.E. curves from similar experiments on B.S.A. and rabbit serum. The data from these curves, together with the average V values of Table III

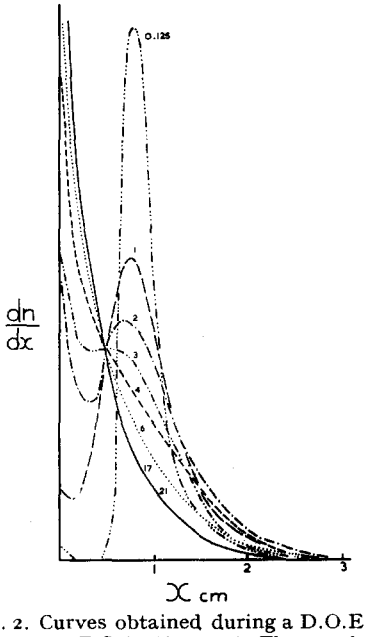

Fig. 2. Curves obtained during a D.O.E. experiment on B.S.A. (Armour). The numbers denote the time interval in days from the start of the run. Equilibrium reached in 13 to 17 days. The ordinates are the refractive index gradients. ———, equilibrium curve.

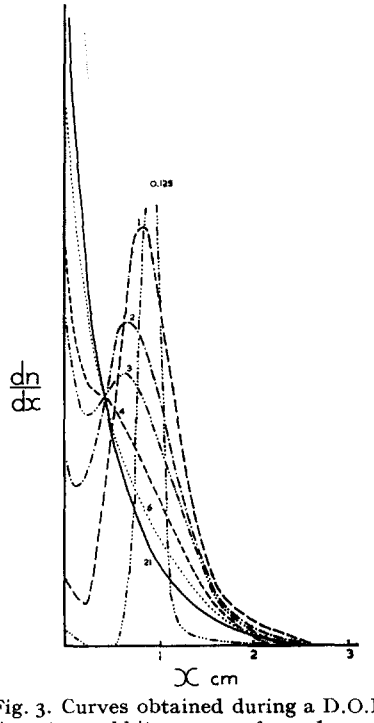

Fig. 3. Curves obtained during a D.O.E. experiment on rabbit serum performed concurrently with the B.S.A. run (Fig. 2). The numbers denote the time intervals in days. Equilibrium was reached after approx. 21 days. The ordinates are the refractive index gradients. ———, equilibrium curve.

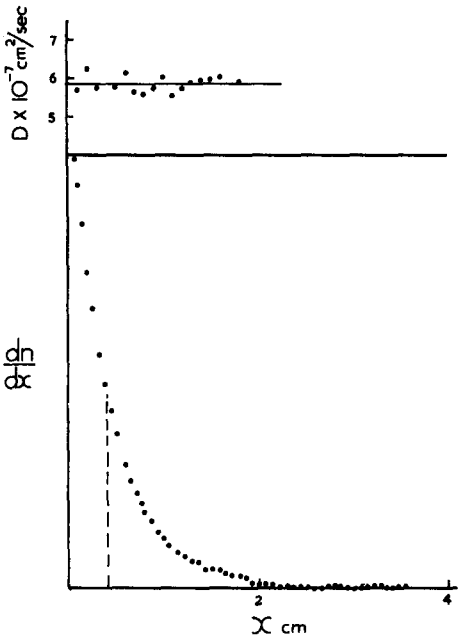

Fig. 4. Equilibrium curve obtained during a D.O.E. experiment on B.S.A. The dashed line is the ordinate at the final point of intersection of the gradient curves used as reference ordinate C_2. The straight line at top of diagram shows the variation of D with x.

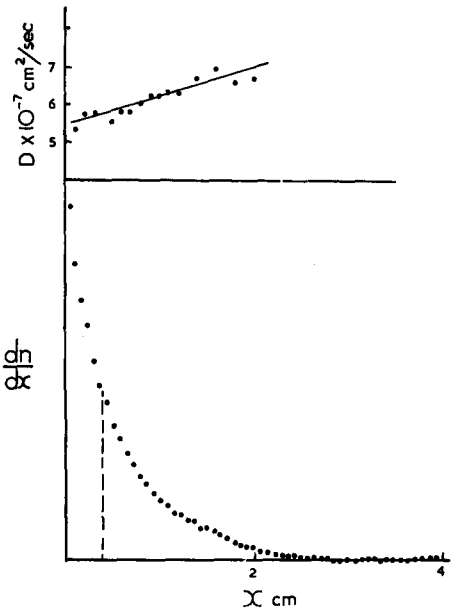

Fig. 5. Equilibrium curve obtained during a D.O.E. experiment on rabbit serum. The dashed line is the ordinate at the final point of intersection of the gradient curves used as reference ordinate C_2. Straight line at top of diagram shows the variation of D with x.

TABLE I

DIFFUSION CONSTANT OF B.S.A. CALCULATED FROM THE EQUILIBRIUM CURVE FIG. (2)

Temperature, 20°; V, $1.15 \cdot 10^{-6}$ cm/sec. Initial protein concentration 0.5 g/100 ml. P.E.G. (mol. wt. 6000) concn. 8.0%. Dispersion medium: saline. Diffusion constants calculated at intervals (in mm) from the reference ordinate along the curve. Distances to be multiplied by conversion factor $K = 1.111$.

Distance from reference ordinate in mm	$D \times 10^7$ cm²/sec
—3.0	5.70
—2.0	6.28
—1.0	5.74
1.0	5.80
2.0	6.16
3.0	5.67
4.0	5.6
5.0	5.74
6.0	6.05
7.0	5.54
8.0	5.72
9.0	5.91
10.0	5.98
11.0	6.00
12.0	6.04
13.0	
14.0	5.91
	Average 5.87
	Corrected = 5.96

were used for the calculation of the diffusion results given in Tables I and II. The flow rates V of Table III were calculated from the dimensions of the capillaries, the cross-sectional areas of the rectangular cavities in A and the displacement of the saline in the capillaries after definite time intervals. From inspection of the diagrams depicted in Figs. 2 and 3 it is interesting to note that the gradient curves tend to intersect at a

TABLE II

DIFFUSION CONSTANT OF RABBIT SERUM CALCULATED FROM THE EQUILIBRIUM CURVE FIG. 3

Temperature, 20°; V, $0.972 \cdot 10^6$ cm/sec. Initial protein concn., 0.5 g/100 ml. P.E.G. (mol. wt., 6000) concn. 8.0 %. Dispersion medium: saline. Diffusion constants calculated at intervals (in mm) from the reference ordinate along the curve. Distances to be multiplied by conversion factor $K = 1.111$.

Distance from reference ordinate in mm	$D \times 10^7$ *cm²/sec*
—3.0	5.35
—2.0	5.75
—1.0	5.87
1.0	5.55
2.0	5.82
3.0	5.79
4.0	6.06
5.0	6.26
6.0	6.27
7.0	6.37
8.0	6.33
9.0	
10.0	6.71
11.0	
12.0	6.95
13.0	
14.0	6.58
15.0	
16.0	6.69

TABLE III

FLOW RATES OF THE SALINE THROUGH THE CELLOPHANE MEMBRANE FOR THE LIMBS CONTAINING B.S.A. AND RABBIT SERUM RESPECTIVELY

Capillary diameter 0.2037 cm. Cross. area of cells 0.790 cm².

Time interval in hours	*V in B.S.A. cell*	*V in serum cell*
47.75	$1.201 \cdot 10^{-6}$ cm/sec	$1.006 \cdot 10^{-6}$ cm/sec
28.00	$1.147 \cdot 10^{-6}$ cm/sec	$0.961 \cdot 10^{-6}$ cm/sec
24.5	$1.111 \cdot 10^{-6}$ cm/sec	$0.932 \cdot 10^{-6}$ cm/sec
23.5	$1.167 \cdot 10^{-6}$ cm/sec	$0.966 \cdot 10^{-6}$ cm/sec
25.41	$1.206 \cdot 10^{-6}$ cm/sec	$0.978 \cdot 10^{-6}$ cm/sec
20.00	$1.147 \cdot 10^{-6}$ cm/sec	$1.023 \cdot 10^{-6}$ cm/sec
22.66	$1.162 \cdot 10^{-6}$ cm/sec	$0.978 \cdot 10^{-6}$ cm/sec
28.66	$1.158 \cdot 10^{-6}$ cm/sec	$0.961 \cdot 10^{-6}$ cm/sec
26.00	$1.090 \cdot 10^{-6}$ cm/sec	$0.921 \cdot 10^{-6}$ cm/sec
24.5	$1.114 \cdot 10^{-6}$ cm/sec	$0.932 \cdot 10^{-6}$ cm/sec
Average	1.150	Average $0.972 \cdot 10^{-6}$ cm/sec

point. It is also significant to note that the final position of intersection is reached early in the course of the process and that the curves of both homogeneous and inhomogeneous substances (B.S.A. and Serum) behave similarly in this respect.

DISCUSSION

A new method for the determination of diffusion constants is decribed. The method is based on the establishment of a state of equilibrium between downward migration of the material on to a semi-permeable membrane and outward migration through diffusion. The method is analogous to the sedimentation equilibrium method, (SVEDBERG AND PEDERSEN[5]), except that an osmotic gradient is substituted for the centrifugal field.

While the experiments were conducted in the central section of the Tiselius electrophoresis cell of which the lower ends communicated with polyethylene glycol through a cellophane membrane, better apparatus more suited for such measurements could undoubtedly be devised.

In diffusion experiments on 0.5 % B.S.A. solution in saline and rabbit serum diluted to contain 0.5 % protein, it was found that the transport rate of the solvent through the membrane was constant over the period of 15 to 21 days, the period required to reach equilibrium. This constancy of flow rate enabled the determination of a reliable diffusion constant. The diffusion constant of B.S.A. determined from the equilibrium curve, *i.e.* $5.95 \cdot 10^{-7}$ cm²/sec is in good agreement with the value $6.15 \cdot 10^{-7}$ cm²/sec obtained for human serum albumin using the boundary spreading technique combined with the LAMM scale method[6]. The diffusion constants calculated from the equilibrium curve of rabbit serum showed an increase with distance from the membrane surface. This is to be expected, as serum is known to be a mixture of protein components of different molecular weights.

The fluctuations in the values of the diffusion constants can be ascribed to inaccuracies in the scale line measurements. These errors could, most probably, be ruled out if the more accurate interferometric method of registering the concentration gradient be used.

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