It will be interesting to see whether a suitable electron-microscopic technique can be developed for confirming that, at the oil/water interface, these and other films also exist in the micellar form, their cross-sectional areas being between 200 and 400 Å based on the number of molecules per micelle.

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BIOPHYSICS

Diffusion Constants of Proteins determined by a Multi-unit Analytical Method

WHEN optical methods are not available, reliable diffusion constants may be obtained by techniques depending on the quantitative determination of the solute concentration in the diffusate such as the porous disk method of Northrop and Anson¹ (see also ref. 2). For these techniques a substance of known diffusion constant, established independently, is needed for calibration, which is a disadvantage.

An apparatus which requires no calibration and is easy to operate was described by Polson^{3,4}. This is referred to as the multi-unit method and enables one to make observations at different time-intervals on the same substance, or different substances may be examined under identical conditions.

The apparatus consists of six independent units, each consisting of four stainless magnetic steel disks; a bottom fixed disk, a 2-cm, 1-cm and 0.5-cm section, the last acting as a lid. Each disk has six cylindrical holes arranged in a circular fashion which may be exactly aligned by proper rotation.

The sample to be examined is introduced into three alternate holes of the bottom disk, filling these completely, the holes in all four sections being in alignment. After filling these, the top three sections are rotated as a unit so that their cavities are shut off from the sample-containing disk. The dispersion medium is introduced into three alternate holes of the top unit filling the two central sections completely. After proper rotation of the lid to seal the units, the apparatus is submerged in a thermostatically controlled water bath to reach temperature equilibrium. The samples and dispersion medium may then be opposed by rotating the top three sections, including the lid, as a unit. The experiment is terminated by similar rotation of the sections at exactly timed intervals. The contents of the top section are withdrawn after adequate mixing and the concentration of the diffused substance determined by suitable means. In the case of proteins, absorption at 280 mμ and a modification of Weichselbaum's biuret procedure⁵ have been found satisfactory.

The diffusion constant is calculated from the equation:

$$D = \left(\frac{C_t}{C_0} H \right)^2 \frac{\pi}{t}$$

(for its derivation see Polson³).

In this equation C_t/C_0 is the ratio between the concentration of the substance in the diffusate and in the original solution. H is the height of the column containing the diffusate and t is the duration of the experiment in seconds.

By plotting C_t against $\sqrt{t}$ a straight line passing through the origin is obtained. Fig. 1 represents the results obtained in an experiment on γ -globulin prepared by the polyethylene glycol procedure⁶.

Fig. 1. Diffusion experiment on human γ -globulin. The concentration of protein in the diffusate is plotted against the square root of the time of diffusion $C_0 = 0.963$ g/100 ml. Saline $T = 23.5^\circ \text{C}$

In Table 1 the diffusion constants of several proteins as determined by the multi-unit technique and the well-known Lamm-scale method are compared. There is good agreement between the two sets of values although the latter method gave slightly higher figures because these were derived by extrapolation to infinite dilution.

Table 1. DIFFUSION CONSTANTS OF SOME PROTEINS AS DETERMINED BY THE MULTI-UNIT APPARATUS AND LAMM-SCALE METHOD

Protein	Concentrations in g/100 ml.	Multi-unit apparatus $D \times 10^7$ cm ² /sec	Lamm-scale method $D \times 10^7$ cm ² /sec	Ref.
Ovalbumin	0.8	7.60	7.63	8
Human haemoglobin	1.0	6.60	6.90	8
γ -globulin	0.963	4.80	4.81	7
<i>Jasus lalandii</i> haemocyanin	1.4	3.39	3.40	9

The discrepancy between the results obtained on γ -globulin and the figure of 4.2×10^{-7} cm²/sec usually adopted for this protein may be explained by the absence of macroglobulins in our preparations, as shown by ultracentrifugation^{6,7}.

This method has been used successfully in determinations of diffusion constants on unpurified samples of biologically active material such as the soluble antigens of viruses¹⁰⁻¹².

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