Non-Coincidence of 'Gas Hold-Up' and Retention of Air in Gas-Liquid Chromatography

In order to determine true relative retentions or partition coefficients in gas-liquid chromatography the 'gas hold-up'[1] of the apparatus must be subtracted from all retention data measured from the point of injection. It has been recommended[1] that the time or gas volume between the injection point and the emergence of the air peak should be taken as the gas hold-up. Moreover, when using detectors which do not respond to atmospheric gases the use of methane has been suggested[2].

The small, but measurable, solubility of these gases in organic solvents and the adsorptive effects of solid powders such as are used for supports have prompted me to check the validity of these assertions.

It has in consequence been found that more or less serious errors (depending on the column used) are introduced by these assumptions. Thus on a column (15 ft. × 4 mm.) of 5 per cent squalane on 'Celite' (60–72 mesh acid-washed) at 20° C. oxygen and nitrogen had small, but statistically significant, different retentions which, although insufficient to resolve the peaks, imply that at least one of these gases is retained sufficiently to introduce serious errors in the retention of volatile materials. Methane eluted about 1 ml. later than oxygen on this column.

The retention of these permanent gases can be due to adsorption or solution and therefore varies from apparatus to apparatus, but this result shows that for precise work the 'air peak' is not invariably the correct point from which to measure relative retention data.

A solution to this problem can be derived from the linearity of log (corrected retention) versus carbon number for the *n*-paraffins.

Provided all retentions are measured from the true gas hold-up point on the chromatogram log (correct retention) is a linear function of carbon number even as low in the series as methane. Consequently this linearity for the rapidly eluted *n*-paraffins is confirmation that the true gas hold-up correction has been made.

At first sight it could be argued that the lower members of the *n*-paraffins might not display a linear relation between the logs of their partition coefficients and their carbon numbers. However, by working at higher temperatures on the same column these early members are not used. Nevertheless, the same gas hold-up for the particular column is invariably obtained. In consequence the linearity of the above relation from methane onwards can be confidently assumed.

In order to 'linearize' the log plot I have developed a simple calculation procedure which obviates the 'trial and error' approach and which will be published shortly.

In the R_{x_9} [3,4] system of presenting retention data an *n*-paraffin calibration is part of the existing procedure and consequently no extra experimental time is required to locate the true gas hold-up point on the chromatogram by the above method.

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BIOPHYSICS

Monolayer Film of Rhodopsin at the Air/Water Interface

Many reports have been made of chemical and physical properties of rhodopsin[1,2]. A role of rhodopsin in visual excitation has been fully discussed by Wald[3,4]. However, most of the work so far has been carried out using aqueous solutions of rhodopsin extracted with digitonin, resulting in a digitonin complex. Accordingly, there is a risk of observing reactions of rhodopsin different from those primarily found *in vivo*. Recently, electron microscopic findings on the ultra-fine structure of visual cells[5,6] suggest that rhodopsin may be located between the double layers of the disk-like units of rod outer-segment which forms a monolayer. It seems useful to pursue the physico-chemical properties of rhodopsin monolayer *in vitro* in order to postulate models for the molecular function of rhodopsin. Weitzel *et al.*[7] reported properties of the monolayer of vitamin A and its derivatives spread on aqueous solution. Many workers since Devaux[8] have investigated the properties of protein films spread on water. We have succeeded in preparing the monolayer film of rhodopsin—vitamin A_1 aldehyde combined with protein—at the air/water interface, and have measured the changes of surface pressure following illumination. An extracted rhodopsin solution was dropped on a clean aqueous surface with a micropipette as was the case with spreading a monolayer of protein. The substrate surmounted with rhodopsin consisted of a phosphate buffer solution having *p*H of 6·8. The surface pressure exerted on the film was measured with a modified method of Sasaki[9] under dim red light of wave-length longer than 750 mμ. To plot the film pressure against the area of the spread films, a barrier placed at a distance of 40 cm. from the other fixed barrier was slid on a trough at a constant speed with certain intervals until the distance was reduced to about 25 cm. Thus the plotting of the

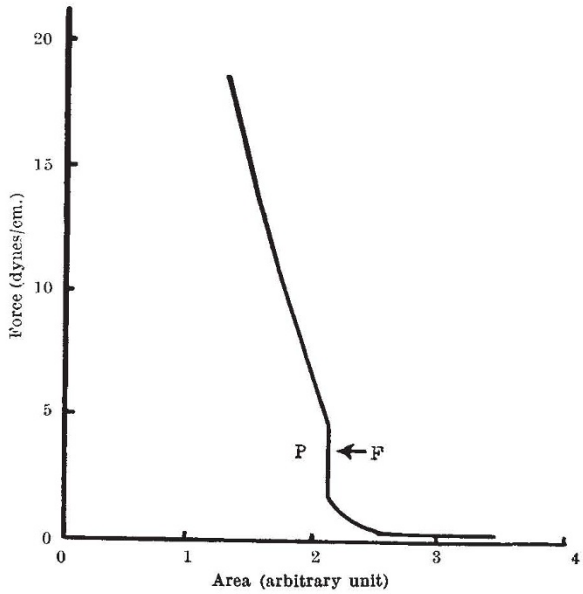

Fig. 1. Force-area curve of a monolayer film of rhodopsin extracted with diluted ethanol (8 per cent ethanol solution with distilled water). The arrow (*F*) indicates the exposure to flash light while the barrier was kept unslid (*P*) to keep area constant