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Diffusion coefficient determination using a filter-paper diaphragm cell technique

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While many diffusion coefficients have been measured and collected (Tuwiner 1962), the permutations of solute and solvent are almost limitless and it is therefore desirable to have a technique which is able, quickly and reproducibly, to determine the required value. The method reported in this paper uses a filter-paper diaphragm cell similar to that described by Kreevoy & Wewerka (1967).

It is a steady-state method based on the equation of Fick's 1st Law of Diffusion (Robinson & Stokes 1965). Diffusion cells described by previous workers (cf. Stokes 1950a) have a relatively thick glass sintered diaphragm and the measurement of a diffusion coefficient requires one to three days. The use of filter-paper diaphragms introduced by Gage (1948) allows the time-scale of the experiment to be considerably reduced.

The filter-paper diaphragm diffusion cell used in our studies is an adaptation of that described by Kreevoy & Wewerka (1967) (Figure 1). The diaphragm of the cell is a 0·45 μm pore size Millipore filter about 150 μm thick and cut from a larger (47 mm) disc, held firmly in position when the lower threaded cup is screwed into the upper part of the cell. Different Millipore discs are used in each run. The solutions in the two compartments are stirred magnetically: the 'flea' in the lower compartment is quite large (relative to the size of the compartment itself) while the smaller flea in the upper compartment sits directly on the filter. This arrangement is designed to preclude the possibility of stagnant diffusion layers of unknown thickness forming on either side of the filter.

The lower compartment containing a stirrer bar is filled with a previously thermostatted (25 °C) diffusant solution of known concentration until the surface tension holds the meniscus slightly above the level of the inner compartment: no air bubbles should adhere to the cell walls or to the stirrer bar. The filter is then positioned gently on the solution avoiding air bubbles; excess solution around the edges of the filter is removed with a clean tissue and the two halves of the cell are screwed firmly together and a smaller stirrer bar is placed on the filter. An appropriate volume of thermostatted (25 °C) pure solvent is added to the upper chamber, the cell is then thermostatted at 25 ± 0·02 °C and stirred.

After 30 min, complete thermal equilibration is achieved and a steady state concentration profile is produced in the diaphragm (with a much thicker glass sinter as the diaphragm, between 2 and 3 h are typically required). The upper compartment solution is then sampled, a stop-clock being started when approximately half the volume has been removed (time = 0). A further sample is withdrawn after another 25–60 minutes (time = t) and the concentration of solute in the two samples determined. Using the volumes of the two compartments (V_L and V_U), we may determine the concentration of solute in the lower and upper compartments at zero time and t, respectively c_1 and c_2, c_3 and c_4.

The results are analysed using the conventional diaphragm cell procedure (Robinson & Stokes 1965), the diffusion coefficient D being calculated from equation (1)

$$D = \frac{1}{\beta t} \ln \left\{ \frac{c_1 - c_2}{c_3 - c_4} \right\} \quad \ldots \quad \ldots \quad \ldots \quad (1)$$

The calibration constant β is defined by equation (2)

$$\beta = \frac{A}{d}(V_L^{-1} + V_U^{-1}) \quad \ldots \quad \ldots \quad \ldots \quad (2)$$

where A is the total effective cross-sectional area of the diaphragm pores, the effective average length of which along the diffusion path is d.

The diaphragm cell has been used to measure solute diffusion coefficients in water and isopropyl myristate (IPM), the latter chosen because of its frequent use in drug absorption and distribution studies as a model for various membrane lipids (e.g. Poulsen et al 1968; Albery & Hadgraft 1979; Armstrong et al 1979). Although equation (2) predicts that the calibration constant of the cell is independent of the system studied, we have calibrated the cell for both water and IPM as

* Correspondence.

Table 1. Diffusion cell calibration.

A: KCl in water, $D = 1{\cdot}841 \times 10^{-5}$ cm^{2}s^{-1}

c_1/M	c_2/M	c_3/M	c_4/M	t/s	β/cm^{-2}
0·8613	0·0606	0·4745	0·2734	1800	41·69
0·7781	0·0924	0·4871	0·2525	1500	38·84
0·8373	0·0716	0·4831	0·2664	1800	38·08
0·8689	0·0577	0·4966	0·2624	1800	37·48
0·8621	0·0606	0·4808	0·2704	1800	40·34
0·8165	0·0755	0·4821	0·2594	1800	36·28
				Mean =	38·79
				s.d.	1·97

B: Methyl nicotinate in IPM, $D = 0{\cdot}410 \times 10^{-5}$ cm^{2}s^{-1}

c_1/mM	c_2/mM	c_3/mM	c_4/mM	t/s	β/cm^{-2}
81·46	8·332	63·68	18·11	3300	34·97
83·75	7·324	66·86	16·62	3000	34·11
82·50	7·824	65·95	16·98	3000	34·30
				Mean =	34·46
				s.d.	0·45

the solvent. This is desirable because a particular solvent, for example, may cause the membrane to swell (and thereby alter the effective values of A and d), or a change in the viscosity of the solvent might affect β especially if a stagnant diffusion layer is able to form in the lower compartment. The calibration procedure involves performing an experiment with a solute whose diffusion coefficient D is known; β may then be calculated by rearrangement of equation (1). The aqueous calibration of the cell is carried out using KCl as the diffusant. The value of D substituted into equation (1) is calculated from the observed values of c_1, c_2, c_3 and c_4 using the method of Stokes (1950b, 1951) and the data of Gosting (1950) and Harned & Nuttall (1947, 1949). For IPM as solvent, the diffusant chosen for calibration is methyl nicotinate, the diffusion coefficient of which we have measured independently using a high precision Goüy interferometer. The samples taken at

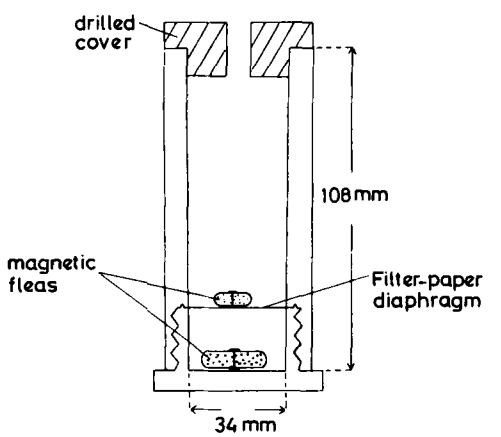

FIG. 1. Cross-sectional diagram of the filter-paper diaphragm diffusion cell used in this study.

Table 2. Results from diffusion experiments.

Solute	10^5D(H_2O)/ cm^{2}s^{-1}	10^6D(IPM)/ cm^{2}s^{-1}
Methyl nicotinate	0·81 ± 0·04	—
Salicylic acid	0·96 ± 0·03	4·33 ± 0·04
p-Hydroxybenzoic acid	1·05 ± 0·05	1·48 ± 0·03
p-Hydroxyphenylacetic acid	1·04 ± 0·05	2·30 ± 0·11

$t = 0$ and $t = t$ are shaken at constant temperature with an equivalent volume of water for 48 h after which time the aqueous layers are analysed spectrophotometrically. From a knowledge of the water–IPM partition coefficient the concentrations c_1 — c_4 can then be calculated. The calibration results are collected in Table 1.

We observe a small but nevertheless significant difference between the values of β for the two solvents. This leads us to the conclusion that, while the results presented below suggest that the technique can provide reliable values for the diffusion coefficients of various solutes in both solvents, it is preferable to perform an effective calibration of the cell for each solvent used. We draw no firm conclusions as to why the calibration constants differ but we suggest that the two possibilities proposed above are among the likeliest reasons.

The results of diffusion experiments carried out after calibration of the cell are summarized in Table 2. Each value of D quoted is the mean of at least three determinations, the variation being represented by the standard deviation.

The aqueous diffusion coefficient of methyl nicotinate is in excellent agreement with the separate determination made using the Goüy technique ($D = 0{\cdot}817 \times 10^{-5}$ cm^{2}s^{-1}) and compares reasonably with a previously quoted value ($0{\cdot}88 \times 10^{-5}$ cm^{2}s^{-1}) determined using a glass sinter diaphragm diffusion cell (Albery et al 1976). The value of D for salicylic acid in water is consistent with that found by Blasinski et al (1968) at 20 °C, $D = 0{\cdot}96 \times 10^{-5}$ cm^{2}s^{-1}. The results in IPM reflect the considerable difference in physical properties of the two solvents. Particularly striking is the difference between the values of D(IPM) for salicylic acid and *p*-hydroxybenzoic acid. The favourable intramolecular attraction between the -OH and -COOH groups in salicylic acid is lost as the hydroxy group is moved to the *para*-position and diffusion through the apolar solvent is consequently hindered by the increased adverse solute-solvent interaction.

The analysis of the results reported here differs completely from that adopted by Kreevoy & Wewerka (1967) in their diaphragm cell studies. They diffused the solute of unknown D together with an internal standard, the diffusion coefficient of which had been previously and independently determined. Comparison of the rates of appearence of the standard and the solute in the upper compartment then provided

information which allowed D for the solute to be found. While this procedure cancels out any variation in the properties of the filter papers used as diaphragms, it makes no allowance for the enhancement or retardation of solute flux caused by interaction with the flux of the standard. We believe that the reproducibility of our results, in particular those obtained for the calibration of the cell, indicate that the Millipore discs employed are of an acceptably consistent standard. Having calibrated the cell for a particular solvent, therefore, the straightforward technique and analysis described in this paper provides a fast, reliable and reproducible means for diffusion coefficient determination.

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Isolation and identification of probenecid acyl glucuronide

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Purification of isolated and synthesized glucuronides has often proved difficult and has frequently resulted in impure gums. We have isolated probenecid acyl glucuronide (PAG), a glucuronide not previously isolated in the pure form or fully characterized (Perel et al 1971), using reversed phase high performance liquid chromatography and ^{13}C nuclear magnetic resonance. Separations were performed with a Waters Associates ALC/GPC 244 liquid chromatograph with a μBondapak-C_{18} column, and methanol–water–acetic acid (40:59:1) as solvent. ^{13}C n.m.r. spectra were obtained using a Varian F T–80A instrument, with DMSO-d_6 as solvent.

A single 2·0 g oral dose of probenecid was administered to a volunteer and urine was collected for 52 h. A sample of each voiding was filtered through a 0·45 μm membrane filter and 10 μl analysed by h.p.l.c. to assess the quantity of probenecid metabolites present. Fig. 1 shows the results for a voiding after 6·5 h. The metabolites (excluding PAG) were synthesized (Guarino et al 1969; Conway & Melethil 1974) and peak A was shown to be *p*-(propylsulphamoyl) benzoic acid, while peak B was a mixture of *p*-(*N*-propyl *N*-2-hydroxypropylsulphamoyl) benzoic acid and *p*-(*N*-propyl N-2-carboxyethylsulphamoyl) benzoic acid. (A μBondapak-CN column using as solvent methanol–water–acetic acid (35:63:1) resolved all the known metabolites but this column did not have sufficient resolution for preparative loading and collection of the group of compounds labelled C and peak D).

Peaks C and D were collected and evaporated. Hydrolysis of either (5M HCl, reflux 1 h) yielded probenecid (identified by h.p.l.c.) whereas reduction of either using diborane yielded glucose (p.c. and g.l.c.).

A urine sample (250 ml) was acidified (25 ml of 5M HCl) and extracted with ethyl acetate (3 × 250 ml). After evaporation, the organic phase was partitioned between chloroform and water (250 ml of each). The aqueous phase was extracted with ethyl acetate (3 × 250 ml) and the organic layer evaporated. The residue was taken up in methanol (1 ml) and 200 μl injected into the h.p.l.c. Peaks C and D were collected and the mobile phase evaporated. After reinjection of the collected fractions, using as solvent methanol–aqueous phosphoric acid pH 3 (40:60), C was shown to be a mixture of at least two compounds whereas fraction D was a single compound. This solvent system gave better resolution than the acetic acid system but acetic acid was preferred for preparative chromatography as it is easily removed from collected fractions.

Fraction D was amorphous to X-rays and had m.p. 93–95 °C and optical rotation $[\alpha]_D^{20}$ −4·1 (c = 0·32 in methanol). Its elemental composition of $C_{19}H_{27}NO_{10}S$ (found: C, 49·05; H, 6·11; N, 2·94; S, 7·38 and calc: C, 49·45; H, 5·90; N, 3·04; S, 6·95%) is consistent with the structure of D being a glucuronic acid conjugate of

* Correspondence.