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DRUG LIQUID–LIQUID DISTRIBUTION BASED ON THE FUNDAMENTALS OF SEGMENTED FLOW

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SUMMARY

A method for the rapid determination of drug liquid–liquid distribution properties, (partition coefficients, ion-pair extraction constants, etc.), is described. The approach is based on fast solute phase equilibration in oil/water segmented flow through helically coiled tubes, with phase splitting based on dissimilar phase wetting of hydrophobic and hydrophilic surfaces. The method has a capacity for up to 45 determinations per hour with a precision error of less than 2%, and is shown to be suitable for either single 'one-off' determinations of K_d etc., or for more detailed studies examining various constitutional and environmental factors, (e.g. pH, ionic strength, temperature), which can affect distribution. The rapidity of determination makes the method appropriate for examining the distributive properties of unstable drugs.

INTRODUCTION

There is considerable interest in the determination of solute oil–water distribution properties, whether these are rationalized in terms of solute liquid–liquid distribution coefficients, K_d, or ion-pair extraction constants, K_{ex}, etc. Such data are utilized in, amongst others, preformulation, analysis and design of drugs, and in solvent extraction chemistry. Data are determined largely by batch extraction which is tedious, inefficient and, for high partitioning values, often inaccurate (Seiler, 1974). Additionally, such a method is regarded (Davis et al., 1976) as inappropriate for examining the effect of a large number of experimental variables on distribution.

A number of papers have reported the use of more sophisticated approaches. Saha et al. (1963) have shown that counter-current distribution methods are unsuitable for the

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examination of large numbers of samples. Further, the AKUFVE apparatus (Davis and Elson, 1974) – where phase separation after equilibration is achieved by centrifugation – or a related spectrophotometric titration method (Cantwell and Mohammed, 1979, Mohammed and Cantwell, 1980), have both been described as suitable for rapid determination of distributive properties. Although the AKUFVE system is extremely useful, (Davis et al., 1976), it is costly and cannot be used efficiently for single determinations of K_d, etc. With the advent of high performance liquid chromatography a number of studies, (e.g. Huber et al., 1972; McCall, J.M., 1975; Mirrless et al., 1976; Yamana et al., 1977; Riley et al., 1979; Veith et al., 1979), have attempted to relate reliable liquid chromatographic data to bulk-phase liquid–liquid distribution data with varying degrees of success. Although these data are found generally to be more precise than earlier approaches which used reversed-phase thin-layer chromatographic methods (Tomlinson, 1975), if properly controlled, thin-layer techniques can have a use (Renberg and Sundström, 1979). Other approaches have been based on potentiometric two-phase titration (Kaufman et al., 1975; Johansson, 1977; Li Wan Po and Irwin, 1980) but require test solutes to be acidic or basic.

Continuous extraction systems, both with, (Eriksson and Nyberg, 1967; Robertson et al., 1972; Gfeller and Frey, 1978; Gfeller et al., 1978), and without, (Kalberg et al., 1979; Nyberg, 1970; Kina et al., 1978; Tsuji, 1978; Kawase et al., 1979; Lawrence et al., 1979), air segmentation, have been constructed for effecting ion-pair extraction of charged solutes, and a recent study, (Johansson et al., 1980) has described the determination of ion-pair extraction constants using a segmented flow method.

The prime requirements for K_d, (or K_{ex}), measurement using equilibrium approaches are equilibration followed by phase separation. In this present paper the possibilities of using segmented flow coupled with an on-line phase-splitting device for rapid measurement of drug liquid–liquid distribution properties is presented and discussed. The design and construction of this combined system is given and its applicability for examining various constitutional and experimental variables on model systems is shown. A preliminary report on this approach has been made previously (Tomlinson, 1979).

MATERIALS AND METHODS

Apparatus and procedures

The segmented flow/phase-splitting system is shown in Fig. 1, and is comprised of 5 principal parts: reservoirs and pumping devices, injector, segmentor, mixing coil and splitting device. Aqueous and oil phases (presaturated with the other phase) were pumped separately using, for the aqueous phase, a multi-channel peristaltic pump, (Gilson, miniplus 2), having Tygon pump tubes, (Technicon, Tarrytown, U.S.A. and Rubber, Weesp, The Netherlands); and for the oil phase a modified double-reciprocating high pressure liquid chromatographic pump (Waters 6000). Segmented flow was achieved by pumping both phases through a T-piece constructed of polytetrafluoroethylene, ptfe, and with the dimensions of this junction being kept constant, (internal diameter 0.7 mm; Fig. 1, detail 1), segment size was varied by changing the differential flow between the two phases. Segments were then pumped through a helical coiled ptfe tube, Waranko Industrial Plastics, Bristol, U.K., and Rubber, Weesp, The Netherlands) of variable length, (see Results

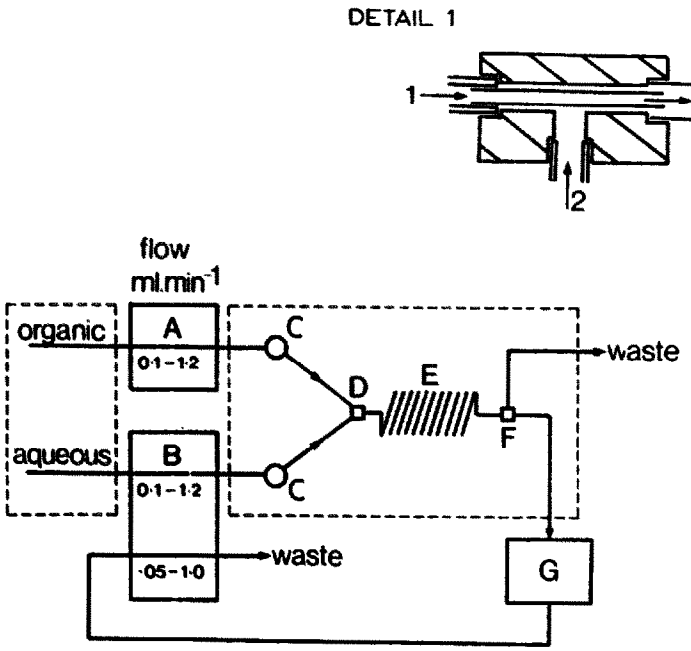


Fig. 1. Construction of the segmented flow/phase-splitter apparatus for determination of distribution properties. (A) double reciprocating pump; (B) peristaltic pump; (C) injection valve; (D) ptfe T-piece, see Detail 1 – where flows 1, 2 and 3 refer to probed phase, second phase and segmented phases respectively; (E) extraction coil; (F) phase-splitter; and (G) flow cell. The apparatus enclosed by the dashed line is thermostatted.

and Discussion), having between 0.3 and 1.4 mm internal diameter. Fig. 1 indicates how the phase segments inhabit the ptfe tubing, with the water segments remaining discrete and the oil phase wetting the coil walls. Wall-drag and the coiled aspect induces turbulent flow in the extraction coil (Snyder, 1976, and Snyder and Adler, 1976), and rapid solute (or ion-pair) distribution occurs between the two phases, with smaller segments resulting in faster equilibrium due to greater turbulence.

After equilibration, phases were separated in a phase-splitter device which acts on the basis of dissimilar wetting of hydrophilic and hydrophobic surfaces, and was a modification of that designed by Copsey (1977). The splitter (Fig. 3) consisted of two surfaces, ptfe and silica glass, channelled to provide a chamber of approximate volume of 28 μl

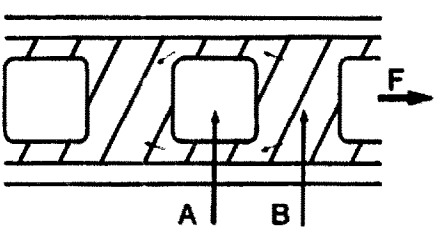


Fig. 2. Segmentation of aqueous (A) and organic (B) phases in the ptfe tubing showing overall flow direction (F), and sample movement. (After Snyder, 1976).

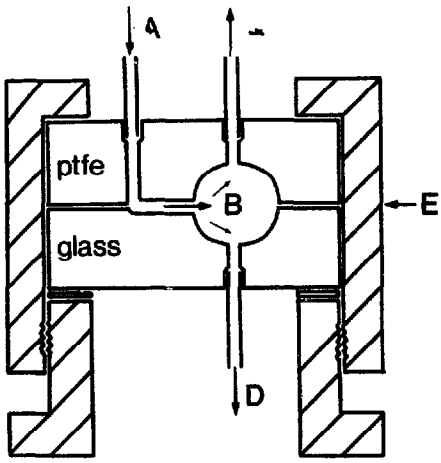


Fig. 3. Construction of phase-splitter showing segmented flow inlet (A); splitting chamber (B); oil phase (C) and aqueous phase (D) outlets and stainless steel jacket (E).

having entrance and exit ports, and cut as discs clamped together by a steel jacket so as to avoid leakage.

Two means of sample introduction have been used in this present study. First, solute was dissolved in the aqueous reservoir, and after extraction and phase-splitting, was assayed off-line (i.e. step input). Second, solute was introduced into either the polar or non-polar stream as an aqueous or oily solution respectively (i.e. pulse input). This was accomplished using an HPLC valve (Rheodyne, 2600) equipped with a 175 μl sample loop, positioned just prior to the T-piece junction (Fig. 1). For this latter method, after leaving the phase-splitter, a fraction of the phase to be assayed was pumped through a flow-cell (80 μl vol.) of a spectrophotometer (Pye-Unicam, SP8-100). Except where indicated in the text all tubing was made of ptfe; the distances between the injection site and the T-piece, and between the phase-splitter and the flow-cell were kept as small as possible to avoid peak dispersion effects. For the temperature studies the components of the apparatus enclosed in the dashed line of Fig. 1 were thermostatted by immersion in a temperature-controlled water-bath (±0.1°C).

Materials

Water was double-distilled from an all glass still, all other solvents were at least of spectroscopic grade and were obtained from Merck (Amsterdam, The Netherlands), and were used as received except for chloroform which was shaken with water a number of times to remove ethanol. Benzoate esters were obtained from Merck; anilines were from Eastman-Kodak, (ex. Tramedico, Weesp, The Netherlands) sodium cromoglycate and alkylbenzyldimethylammonium chlorides, ABDACs, were as described previously, (Tomlinson and Davis, 1978); benzoic acid and benzaldehyde were obtained from Merck, and were of analytical grade.

RESULTS AND DISCUSSION

Copsey (1977) and Kalberg and Thelander (1978) have shown that continuous extraction of material using segmented flow can be achieved without air-segmentation, and this has been applied recently to the analysis of pharmaceuticals (Kalberg et al., 1979), and the determination of picrate–drug ion-pair extraction constants (Johansson et al., 1980). To assess the performance and utility of the present system the liquid–liquid distribution of drugs and related compounds under a variety of conditions have been studied. The important variables that can influence the K_d (or the K_{ex} of an ion-pair) are given in Table 1. Each of these has been examined in turn and where possible results compared to either measured shake-flask or literature values. The ability of the system to efficiently separate phases and the effect of the various system components on both peak dispersion and apparent distribution constants have also been examined.

(i) Phase-splitting

Solvent extraction chemistry employs a wide variety of oil phases in solute distribution studies, ranging in nature from polar (e.g. nitrobenzene) to non-polar (e.g. cyclohexane). Table 2 indicates how well the present arrangement can separate various binary and ternary mixtures. The separation of binary water/oil phases can be related to the mole fraction of water dissolved in the oil phase at equilibrium (Davis, 1975), with segment separation becoming inefficient when this value is above approximately 0.7. Table 2 indicates that differences in phase density are not the reason for phase-splitting and these two observations have indicated a theoretical model for describing the optimal conditions for phase-splitting based on solvent–solvent, and solvent–material works of adhesion and cohesion, and will be the subject of a future communication from this laboratory. Although not all ternary systems were well separated by the splitter, interestingly it was observed that the emulsified layers of such systems were broken down on immediate contact with ptfe tubing, which has implications for drug emulsion stability.

TABLE 1

THE IMPORTANT VARIABLES AFFECTING SOLUTE DISTRIBUTION EXAMINED IN SCREENING OF THE SEGMENTED FLOW SYSTEM

Solute	Molecular structure
	Complexation (e.g. ion-pairing)
	Ionization
	Concentration
	Stability
Phase sytem	Nature of extracting phase
	Phase volume ratio
	Ionic strength
	Temperature
	pH

TABLE 2

EFFICIENCY OF SEGMENT FORMATION AND SUBSEQUENT PHASE-SPLITTING FOR BINARY AND TERNARY SYSTEMS

Binary two-phase systems		Ternary two-phase systems
System [a]	Mole fraction water in oil phase at equilibrium [b]	System [d]
water/tetrachloromethane		
water/dichloroethane	0.008	10% and 20% (v/v) methanol in 1 : 1
water/chloroform	0.005	water/alcohol (butan-1-ol;
water/hexane	0.005	pentan-1-ol; hexan-1-ol; octan-1-ol).
water/cyclohexane	0.000	
water/isooctane	0.004	
water/nitrobenzene	0.012	
water/nitrotoluene	0.007	
water/butan-1-ol	0.513	methanol/water/cyclohexane mixtures
water/butan-2-ol [c]	0.765	(v/v):
water/iso-butanol	0.456	(20/20/40) [e]
water/butan-2-one	0.308	(30/10/40)
water/pentan-1-ol	0.283	(35/ 5/40)
water/pentan-2-ol	0.396	(40/ 1/40) [c]
water/iso-pentanol		
water/tert-pentanol		
water/pentan-2-one	0.113	
water/hexanol		
water/cyclohexanol		
water/octan-1-ol	0.268	
water/ethyl acetate	0.131	
methanol/iso-octane	–	
methanol/cyclohexane [c]	–	

[a] 40 ml of each phase were pre-equilibrated with one another then pumped separately to effect segmentation and phase-splitting.
[b] Davis, 1975.
[c] Segmentation achieved but splitting efficiency poor.
[d] Solvents mixed and resulting phases pumped separately to effect segmentation and phase-splitting.
[e] Initially 3-phase system, with emulsified phase separating upon contact with ptfe tubing.

(ii) Peak dispersion

Extraction reactor coils coupled with phase-splitters have been employed in drug analysis for some time; for example in the Technicon autoanalyser, and as post-HPLC column reactors (Lawrence et al., 1979; Tsuji, 1978). The potential use of the present system in HPLC, and the necessity to determine whether peak area or peak height is the preferred measured parameter for indicating distribution in the pulsed sample input mode (see Methods), has prompted us to determine the effects of various component and operational factors on the dispersion of an injected sample. Defining total sample dispersion

TABLE 3

EFFECT OF ASSEMBLY COMPONENTS ON SAMPLE (PULSE INPUT) DISPERSION

System component					D_t
Injector	T-piece	Coil (3 m X 0.7 mm i.d.)	Splitter	Cuvette (including 5 cm X 0.3 mm entry tubing)	
+				+	1.6
+		+		+	2.7
+	+	+		+	2.5
+			+	+	2.6
+	+	+	+	+	2.7

+ Present in system.

as D_t, then, (Růžička and Hansen, 1978):

$$D_t = D_1 \cdot D_2 \cdot D_3 \cdot ...D_{n+1} = V_d \cdot V_i^{-1} \quad (1)$$

where the subscript integers refer to individual components of the system, (e.g. coil, etc.), and where V_d and V_i are the total volumes of phase containing the sample at the detector and injector respectively. Table 2 gives D values for the various components of the system using benzoic acid in water/isooctane as a model solute. Although here D_t is 2.7, approxi-

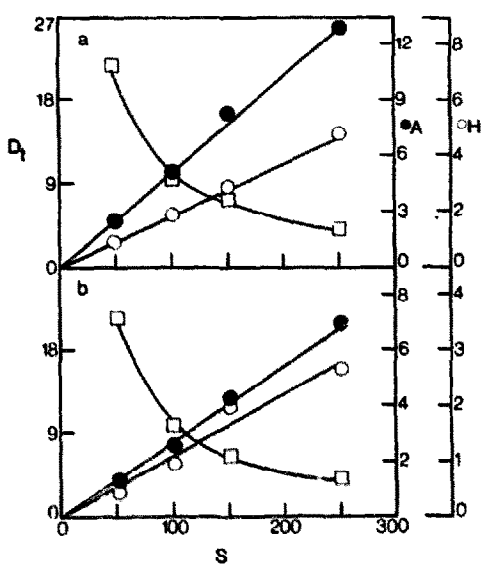


Fig. 4. Effect of sample volume, S, (μl), on peak dispersion, D_t, (Eqn. 1), peak height, H, (mm X 10^{-1}), and peak area, A, (mm^2), for unsegmented (a) and segmented (b), systems. Solute: para-chloroaniline; phases: water/isooctane, (r = 1.05).

mately 50% of this total dispersion (Eqn. 1) occurs within the cuvette, and the injection mixing area. All remaining dispersion occurs within the coil (Table 3), in contradiction to earlier assumptions that the coil has zero effect on dispersion (Karlberg and Thelander, 1979; Johansson et al., 1980). Further, under these conditions (segment volume approximately 0.25 X splitter chamber volume) the phase-splitter did not contribute to the dispersion.

For unsegmented flow injection analysis systems it has been shown (Růžička and Hansen, 1978) that 'medium' dispersion is necessary to ensure, for example, rapid complexation effects. For segmented flow in which solute is interacting with a second phase such medium dispersion intuitively appears to be also needed. Since dispersion in unsegmented flow systems is related to initial sample volume, it is useful to examine how in the present system sample volume affects dispersion. Fig. 4 illustrates how peak height, area and dispersion (Eqn. 1) are related to sample volume, S. Since dispersion is not constant over the range shown, and peak area, ($r = 0.99$), rather than peak height, ($r = 0.86$) is better correlated with sample volume, it is concluded that the former is the better parameter for indicating distribution effects. Růžička and Hansen (1978) have argued that if only one mixing stage is involved in the injection process then the concentration of solute, C, in the sample volume at the detector can be given as:

$$C = C^0(1 - e^{-kS}) \tag{2}$$

for the ascending portion of the response peak, and for the descending portion as:

$$C = C^0 \cdot e^{-kS} \tag{3}$$

where C^0 is the initial sample concentration at injection, and k is a constant for the system. If similar arguments can be made for segmented flow a plot of log $(1 - D_t^{-1})$ vs S

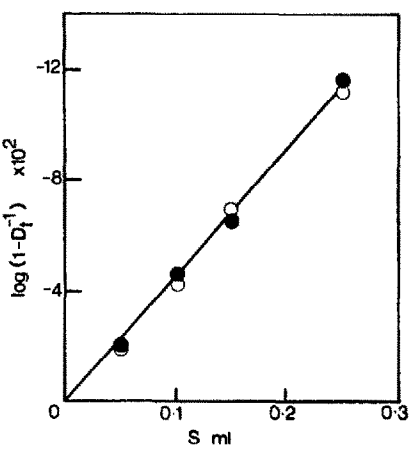


Fig. 5. Plot of log $(1 - D_t^{-1})$ vs sample volume, S, (Eqns. 2 and 3) for the unsegmented, (closed data points), and segmented, (open data points) systems described in Fig. 4a and b. The slope coefficient gives, (Eqn. 4), an $S_{0.5}$ value of 0.175 ml.

should be linear, (as C/C^0 is proportional to D_t); since, from Eqns. 2 and 3

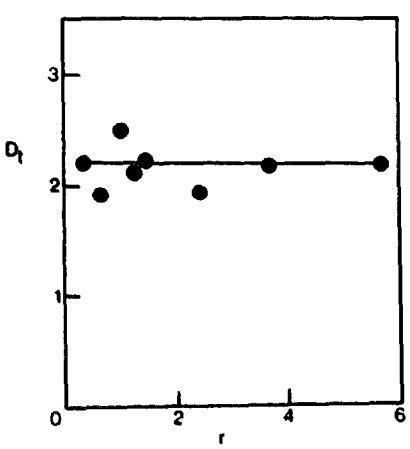
$$S_{0.5} = 0.693 \cdot k^{-1} \quad (4)$$

where $S_{0.5}$ is the sample volume required to give a peak response 50% of that if no dispersion had taken place, then such a plot, if linear, will give $S_{0.5}$ from the slope. Fig. 5 shows that transformation of the experimental data given in Fig. 4 for both segmented and unsegmented systems into the form described above, does indeed give linear relationships, with an $S_{0.5}$ value of 175 μl for both unsegmented and segmented systems. As an indicator of 'medium' dispersion we have taken the $S_{0.5}$ value for any single arrangement as the sample volume to be injected. Hence all studies performed to determine distribution have been carried out using a 175 μl sample volume. Dispersion has been found to be slightly dependent on phase volume ratio (Fig. 6), which is due to the influence of the changing flow velocity (Růžička and Hansen, 1978).

Using step input it has been found that constant solute K_d values are obtained above a coil (0.7 mm i.d.) of length 1.75 m; and hence with regard to the above findings the following system characteristics have been used in all further K_d determinations, i.e. injection volume 175 μl, (i.e. equal to $S_{0.5}$); T-piece dimensions 1.4 mm i.d.; coil 3 m × 0.7 mm i.d.; splitter volume ~28 μl; and splitter to detector cell tubing 0.7 mm (lower internal diameter resulting in air bubbles in the detector cell).

(iii) K_d determination

(a) Step input. As described earlier (Methods), K_d values have been determined using either sample step input or pulse input. Fig. 7 and Table 4 show how there exists good correlation between drug liquid–liquid distribution obtained using step input and seg-



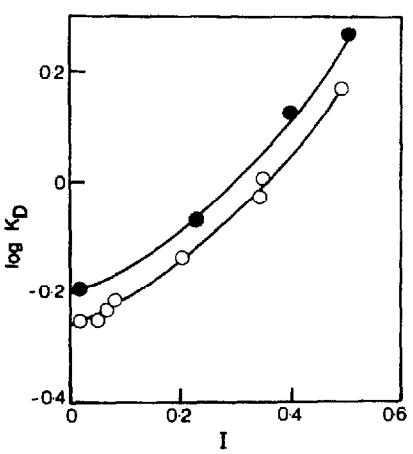


Fig. 6. Effect of phase volume ratio, r, on total dispersion, D_t, (Eqn. 1). Solute: benzoic acid; phases: 0.01 N H_2SO_4/isooctane; injection volume: 175 μl.

Fig. 7. Effect of ionic strength, I, (mol · dm^{-3}), on the distribution of para-methylphenol between water and cyclohexane, as measured using segmented flow, (closed data points), and shake-flask, (open data points), methods.

TABLE 4

COMPARISON BETWEEN SHAKE-FLASK AND SEGMENTED FLOW METHODS FOR DETERMINATION OF K_d VALUES, USING BOTH STEP AND PULSE SAMPLE INPUT

Solute	Liquid-liquid distribution coefficient							
	log K_d + S.D.		n	r	log K_d + S.D.		n	r
	(i) Segmented flow – step input [a]				*(i) Literature values – shake-flask* [b]			
meta-nitroaniline	−0.34	0.01	5	1.0	−0.42			
para-methoxyaniline	−0.43	0.01	5	1.0	−0.41			
para-chloroaniline	0.64	0.01	5	1.0	0.69			
meta-chloroaniline	0.86	0.01	5	1.0	0.89			
	(ii) Segemted flow – pulse input [c]				*(ii) This study – shake-flask*			
ortho-chloroaniline	0.974	0.008	3	0.9–1.2	0.983	0.012	3	1.25
para-chloroaniline	0.462	0.016	9	0.3–4.0	0.462	0.032	12	0.2–5.0
methylbenzoate	1.778	0.049	4	0.2–1.0	1.769	0.046	4	0.2–1.0

S.D. n and r, are the standard deviation number of determinations and phase volume ratio respectively.
[a] Water/cyclohexane.
[b] Leo et al., 1971.
[c] Water/isooctane, 25°C, with injection and probing in the aqueous phase except for para-chloroaniline.

mented flow and those obtained by shake-flask or from the literature. Although step input has a use it is somewhat tedious and not amenable to repeated determinations, and hence pulse input of sample, (Methods), has been examined.

(b) Pulse input. Previous discussion has shown that it is preferable to use peak area to quantify distribution. Since experimentally the phase into which the sample was injected was probed it follows that if this was the water stream then:

$$K_d = [(A_u^w - A_s^w) \cdot (A_s^w)^{-1}] \cdot r^{-1} \quad (5)$$

where A^w is the area of the sample peak determined in the water phase, subscripts s and u refer to segmented and unsegmented flow, and r is the oil/water phase volume ratio, such that $r = f^0/f^w$, where f is the stream flow rate. For oil phase probing K_d is obtained from:

$$K_d = [(A_s^0) \cdot (A_u^0 - A_s^0)^{-1}] \cdot r^{-1} \quad (6)$$

Table 4 gives the K_d values for a number of solutes determined using both the present method and a conventional shake-flask procedure. The agreement between both methods is excellent, even though for the segmented flow system phase volume ratios between 0.2 and 6 were necessary. Fig. 8 gives the effects of differential phase flow rates on the determined K_d values for *p*-chloroaniline and methylbenzoate. These plots show that based on shake-flask experiments there is excellent agreement between measured and predicted K_d values. The range of K_d values that can be measured in the present system depends on the

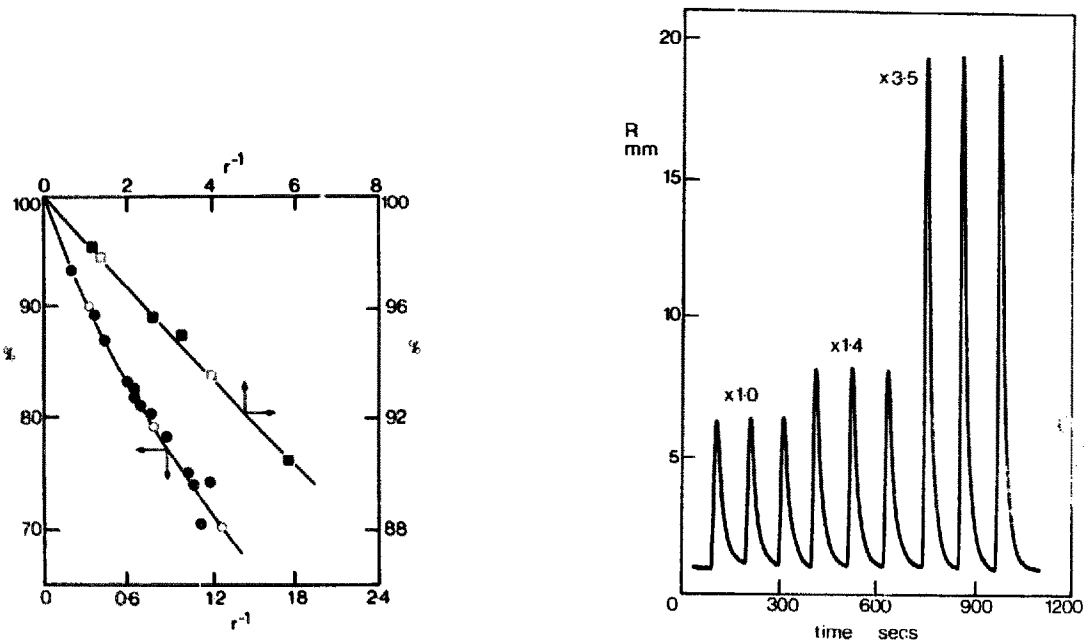
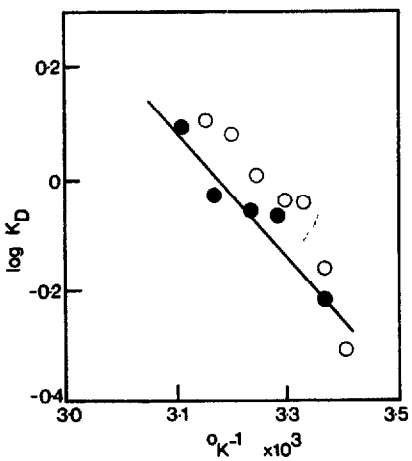


Fig. 8. Agreement between segmented flow, (closed data points), and shake-flask, (open data points), methods, as given by the effect of phase volume ratio, r, on the per cent of solute extracted into the oil phase. Drawn lines are theoretical extraction curves based on shake-flask K_d values. Solutes: para-chloroaniline, (○), and methylbenzoate, (□); phases: water/isooctane, at 25°C.

Fig. 9. Effect of solute concentration on detector response, R, at a constant sample volume of 175 μl. Solute: para-chloroaniline; phases: water/isooctane at 25°C; phase volume ratio, 2.1. Numbers indicate multiples of a 5×10^{-3}% w/v concentration injected.

phase volume ratio and the maximum time for determination considered to make this method appropriate. For example, setting the difference in peak area between an unextracted and extracted solute as either 95% or 5%, Eqns. 5 and 6 show that log K_d values between −2.5 and +2.5 can be determined using r values between 20 and 0.05 respectively. Using the present system, which has a maximum throughput of approximately 45 injections per hour, to extend the K_d range further would require much longer coils, resulting in longer residence times coupled with the problems encountered in maintaining precise flow rates using peristaltic pumping and tubing. The system responds to changes in sample concentration (Fig. 9), and is appropriate therefore for examining concentration-dependent distribution properties.

The effects of temperature and pH on K_d have been examined using the segmented flow technique. Fig. 10 gives the van 't Hoff plot for *p*-cresol. Agreement between these data and those obtained using an AKUFVE instrument (Davis et al., 1976) is good, indicating a further use of this method for rapid determination of the thermodynamics of drug partitioning. Fig. 11 gives the effect of pH on the K_d of a stable solute, and shows how drug pK_a measurements can be rapidly made with this system. The K_d of unstable species can be determined also, provided the sample residence time does not approach its



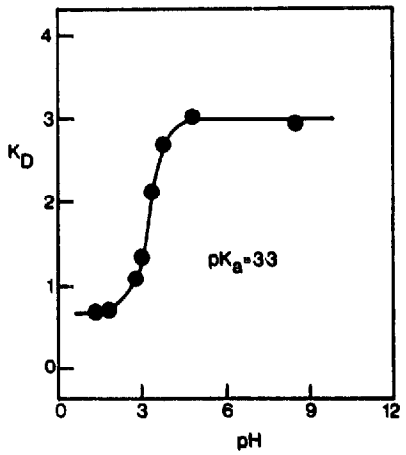


Fig. 10. van 't Hoff plot for the distribution of para-cresol between water and cyclohexane. Closed circles – this study; open circles from Davis et al., (1976), determined using an AKUFVE apparatus.

Fig. 11. Determination of ionization constant of para-chloroaniline by following liquid–liquid distribution constant, K_d, with change in aqueous phase pH. Phases: water/isooctane at 25°C; phase volume ratio, 1.0. Second derivative of this plot gives a pK_a of 3.3.

half-life. For example we have determined the K_d of a development compound as 2.4 at pH 1.0, and 25°C although at pH 1.5 the compound's half-life, (at 30°C), is 14 min, (Ritter, 1980). Distribution studies on unstable drugs have already been described using either an AKUFVE apparatus (Davis et al., 1976), or a kinetic method (Byron et al., 1980; Tomlinson et al., 1980), and it is suggested that segmented flow techniques are an alternate approach for such measurements.

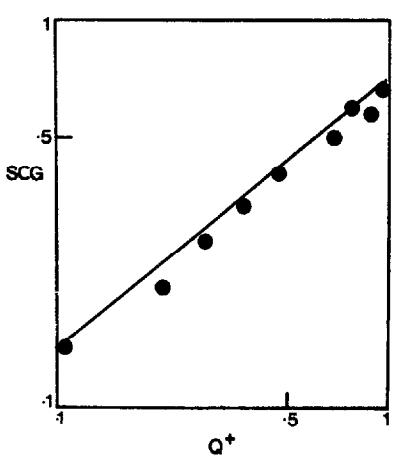


Fig. 12. Extraction of sodium cromoglycate, SCG, (mol · dm^{-3} X 10^4), into chloroform from water as ion-pairs with varying concentrations of hexadecylbenzyldimethylammonium chloride, Q^+, (mol · dm^{-3} X 10^4). Initial aqueous concentration of SCG, 1 X 10^{-4} mol · dm^{-3}. Datum points are from segmented flow measurements and the drawn line is the regression slope for data obtained by a shake-flask method.

(iv) K_{ex} determination

Both step input (Fig. 12) and pulse input have been used to examine the extraction of ion-pairs of alkylbenzyldimethylammonium chlorides and sodium cromoglycate between water and chloroform.

Since, for ion-pair extraction

$$D = K_{ex}[Q^+] \tag{7}$$

where D and $[Q^+]$ are the ratio of solute ion in both phases at equilibrium and the concentration of pairing ion respectively, then for step input D is obtained directly, and for pulse input the extraction constant, (for oil phase probing), is given by:

$$K_{ex} = [(A_s^0) \cdot (A_u^0 - A_s^0)^{-1}] \cdot r^{-1} \cdot [Q^+]^{-1} \tag{8}$$

Fig. 12 describes the loss of cromoglycate ion from an aqueous environment into chloroform as an ion-pair with hexadecylbenzyldimethylammonium chloride at 25°C. Both methods permit K_{ex} to be determined and for this present study a value of 0.6 is obtained which is indistinguishable from that obtained by a shake-flask method.

CONCLUSIONS

Liquid–liquid distribution (partition) coefficients and ion-pair extraction constants can be determined rapidly (sample throughout ~45 h^{-1}) using the described segmented flow and phase-splitter apparatus. The apparatus is convenient for examining both single constants and for investigations into the effect of environment, (ionic strength, temperature, pH, etc.), on distribution. The speed of determination suggests that the method can be used to measure drug ionization constants and K_d values of unstable compounds. The present system is appropriate for log K_d values in the −2.5 to +2.5 range; improved pumping methods, and a greater control of peak dispension due to management of hydraulic and mixing effects should lead to an extension of this range.

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