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RAPID HEADSPACE GAS CHROMATOGRAPHIC METHOD FOR THE DETERMINATION OF LIQUID/GAS PARTITION COEFFICIENTS

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SUMMARY

A rapid, efficient and low-cost headspace technique useful for the determination of liquid/gas partition coefficients of gases and volatile substances of low and intermediate solubility is described. The equilibration step is carried out at constant pressure using glass syringes, with a ratio of liquid/gas phase volumes of ca. 1:3; after 30 min at the desired temperature, the headspace is recovered by transfer into another syringe and analyzed by gas chromatography. A study of the partition coefficients in water at 37°C of 27 volatile compounds demonstrated that the method is fully applicable for all gases, with exception of those with a partition coefficient higher than 300.

INTRODUCTION

Headspace gas chromatographic (HS-GC) analysis is one of the most powerful tools for the detection and quantitation of high-vapour-pressure substances present in liquids or solids [1,2]. This methodology is not restricted to trace analysis, but also provides a valuable means of obtaining physicochemically relevant data [3–5].

One of the most interesting applications of HS-GC in the field of physicochemical studies is the determination of the liquid/gas partition coefficient (K). Measurement of K, which is numerically the same as the Ostwald solubility coefficient (λ), is important from the chemical point of view and also for biomedical sciences, because the blood/gas partition coefficient is one of the main factors that regulates gas exchange in the lungs [6–8]. Thus, in the study of kinetics and behaviour of gases in the living organism a knowledge of K

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values is required, and hence methods that allow its accurate determination are important.

Although such methods exist [9–13], many of them are time-consuming or require special instrumentation not easily available in many laboratories. Wagner et al. published in 1974 [14] a general static HS-GC procedure that facilitates the determination of K using very simple material. The method we present here is based on this procedure, but with some important modifications that allow the calculation of K with a reduced volume of liquid, and with fewer gas–liquid phase equilibration steps. The accuracy is also improved by the use of correction factors for dilutions during the headspace recovery process.

EXPERIMENTAL

Gases and volatile liquids

All products used in this study were high-performance liquid chromatography (HPLC), analytical or pure grade. The liquid solvents were purchased from Scharlau-Ferosa (Barcelona, Spain), E. Merck (Darmstadt, F.R.G.) or Carlo Erba (Milan, Italy), except enflurane (Ethrane®) and halothane (Fluothane®), which were obtained from Abbott Labs. (Madrid, Spain) and ICI-Farma (Pontevedra, Spain), respectively. Gaseous substances were obtained in pressurized cylinders from Abelló-Oxigeno Linde (Barcelona, Spain), except methane, propane and *n*-butane, which were purchased from E. Merck.

For analysis, the thirty substances investigated were distributed in six groups, A–F, as shown in Table I. This distribution was based on their chromatographic separation (see analytical conditions below).

Syringes

All the studies were carried out using 10-ml matched barrel/plunger glass syringes, with a Luer-type stainless-steel cone (Becton-Dickinson, Rutherford, NJ, U.S.A.). They were coupled with polyethylene and polycarbonate three-way stopcocks (B. Braun, Melsungen, F.R.G., and Bexen, Guipúzcoa, Spain). Each syringe used was first carefully checked for leaks using gases of group B (see general method below), and those that showed the largest leaks (more than 7% per hour for acetone or more than 4% per hour for enflurane) were discarded. Only 4% of the syringes were discarded for this reason.

A leak test for gases of groups B, C, E and F in analytically acceptable syringes was carried out as follows: an appropriate mixture of gases and vapours of each group, all of them at concentrations within the linearity range for detectors, was prepared in a 100-ml syringe. This pooled gas mixture was distributed (10 ml) in ten different syringes, maintained at room temperature. The content of each syringe was injected three times at 90-min intervals. Calculations were done in terms of the percentage variation of peak height (h) with

TABLE I

SOME PHYSICOCHEMICAL AND CHROMATOGRAPHIC CHARACTERISTICS OF THE GASES AND VOLATILE COMPOUNDS ANALYSED

	Molecular mass	Boiling point (°C)	Retention time (min)	Chromatographic group
Methane	16.0	−161.4	0.567 ± 0.013	A
Propane	44.1	−42.1	0.853 ± 0.003	A
n-Butane	58.1	−0.5	1.229 ± 0.011	A
n-Heptane	100.2	98.4	5.388 ± 0.012	A
Ethane	30.1	−88.7	0.667 ± 0.004	B
Cyclopropane	42.1	−32.9	1.025 ± 0.004	B
Sulphur hexafluoride	146.1	−63.8	1.661 ± 0.014	B
Diethyl ether	74.1	34.6	2.446 ± 0.043	B
Acetone	58.1	56.5	4.060 ± 0.051	B
Enflurane	184.5	56.5	5.610 ± 0.116	B
Dichlorodifluoromethane[a]	120.9	−29.8	0.934 ± 0.004	C
Ethylene oxyde[a]	44.1	10.7	1.917 ± 0.006	C
n-Hexane	86.2	68.7	3.195 ± 0.019	C
2-Propanol	60.1	82.5	4.555 ± 0.035	C
Isooctane	114.2	99.3	6.286 ± 0.056	C
2-Butanol[a]	74.1	99.5	8.603 ± 0.065	C
Acetaldehyde	44.1	20.2	1.956 ± 0.003	D
Ethanol	46.1	78.5	3.365 ± 0.021	D
Cyclohexane	84.2	80.7	4.304 ± 0.035	D
1,1,1-Trichloroethane	133.4	74.1	6.126 ± 0.026	D
Benzene	78.1	80.1	7.323 ± 0.009	D
Methanol	32.0	64.7	2.149 ± 0.058	E
Dichloromethane	84.9	39.8	3.727 ± 0.126	E
Acetonitrile	41.1	81.6	5.095 ± 0.174	E
Ethyl acetate	88.1	77.1	6.202 ± 0.194	E
1,2-Dichloroethane	99.0	84.0	9.397 ± 0.315	E
Isopropyl ether	102.2	68.5	4.201 ± 0.015	F
Halothane	197.4	50.2	5.395 ± 0.033	F
Chloroform	119.4	61.3	6.428 ± 0.032	F
Trichloroethylene	131.4	86.7	7.690 ± 0.034	F

[a]Used only for leak studies.

respect to that at time 0 min, and normalizing for Δh per hour, using for each syringe the mean of the two values.

Chromatographic analysis

Analyses were carried out using a Hewlett-Packard (Waldbronn, F.R.G.)

Model 5880A, equipped for flame ionization detection (FID) and electron-capture detection (ECD). The original injectors were replaced by two constant-volume inlet sample loop injectors [15], similar to those used in HPLC apparatus, connected in series. The loop size for FID was 1.5 ml and for ECD 0.8 ml. The total volume needed to load both injectors simultaneously was 3 ml. Injectors were thermostatted and maintained at 50°C.

For FID analyses a stainless-steel Porapak T column (Supelco, Bellefonte, PA, U.S.A.), 1800 mm × 3 mm I.D. and 80–100 mesh was used. The oven temperature was maintained at 160°C. The flow-rate of nitrogen carrier gas was 16.9 ml/min and those of hydrogen and air were 35 and 420 ml/min, respectively. The detector temperature was 200°C.

ECD analyses were carried out using three columns coupled in series, two Porapak T and one Porapak Q, of the same characteristics as mentioned above for FID analyses. The nitrogen carrier gas flow-rate was 42.2 ml/min, the oven temperature 160°C and the detector temperature 200°C.

Although many of the gases listed in Table I responded to both detectors, FID was preferred because of its greater range of linearity. For this reason, only sulphur hexafluoride, which gives no signal on FID, was analysed with ECD.

Sample manipulation

The general procedure for manipulation of syringes was essentially the same as previously described [14–16], with the exceptions of the volumes liquid and gas used, and the introduction of a new dilution correction factor. Briefly, the exact volume of liquid (V_{L1} and V_{L2}) in the syringe was determined by weight, with the specific gravity known, and that of gas (V_{G0} at room temperature and V_{G1} and V_{G2} at equilibration temperature) by reading the volumetric marks of the syringe. Equilibration was carried out in a thermostatted water-bath under continuous shaking for 30 min. Recovery of the gaseous headspace equilibrated with liquid was made by transfer into a clean, dry and ungreased receptor syringe. The volume of transferred gas was rapidly and exactly measured (V_{GT1} and V_{GT2}) in order to apply subsequent corrections to the measured chromatographic peak heights. Care was taken in all gas phase measurements to consider the dead volume of syringes plus stopcocks (0.23 ± 0.01 ml). Receptor syringes were stored at 40°C in a small oven for 20 min before injection and analysis by GC, to avoid water vapour condensation on their inner walls. Fig. 1 shows schematically the equilibration and transfer steps.

Determination of the liquid/gas partition coefficient

The method was divided in two parts. The first was used for gases with a K greater than 3 and the second for the remaining gases. Obviously, for gases of K close to 3, both methods gave very similar results [17]. The first step consisted of taking 1 ml of liquid in a syringe and verifying that no interfering

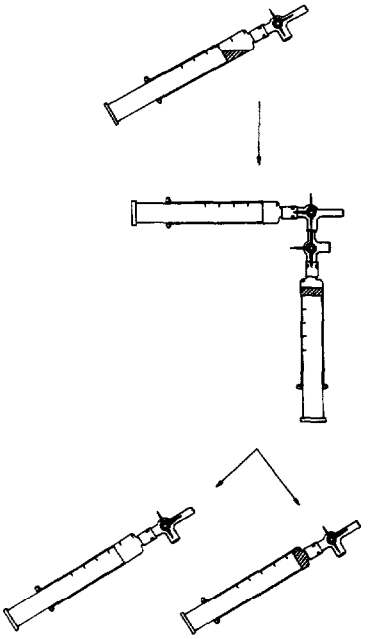

Fig. 1. Equilibration of the gas–liquid phases in a syringe, and transfer of the equilibrated headspace to a receptor-injection syringe.

gases were present. This was done by analysing 3 ml of pure nitrogen equilibrated with the liquid phase. Simultaneously, a mixture (10 ml) of the gases to be analysed (groups A–F) was prepared in nitrogen in another syringe, taking care that each gas represented less than 1% in volume (the initial concentration used was always between 0.002 an 0.5%). Approximately 3 ml of this mixture was then analysed by GC, by direct injection or with a previous dilution with nitrogen if the initial concentration was outside the linear range of the detector. A further 3 ml of the mixture were introduced in the syringe, and the first equilibration was carried out. The whole headspace was recovered and analysed by GC. The equilibration syringe was again loaded with 3 ml of pure nitrogen. After a second equilibration, the headspace was again recovered and analysed. Fig. 2 represents schematically the complete process.

Calculations were done applying the following equations:

$$K=\frac{V_{G1}}{V_{L1}}\left(\frac{m_0}{m_2}-1\right) \tag{1}$$

$$K=\frac{V_{G2}}{V_{L2}}\left(\frac{1}{\frac{m_2}{m_4}-1}\right) \tag{2}$$

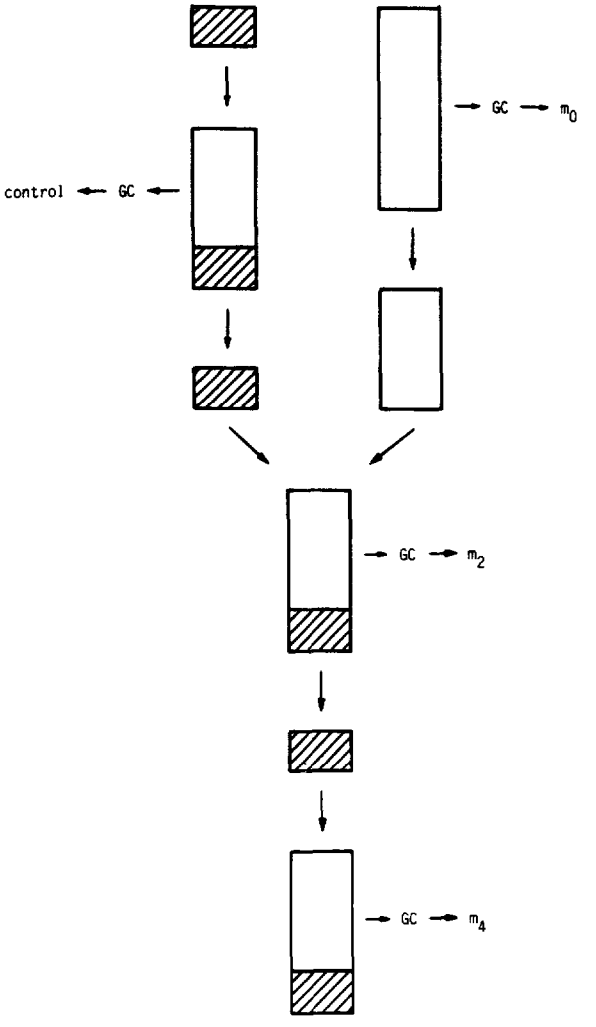

Fig. 2. General procedure for the determination of K.

where subscripts 1 and 2 in volumes represent volumes in the first and in the second equilibration, and m is the mass of solute in gaseous phase before (m_0) and after the first (m_2) equilibration, and after the second equilibration (m_4). Eqn. 1 was used for gases of $K > 3$ and eqn. 2 was used for gases of $K < 3$. Both equations were derived by application of mass balance principles and the same mathematical definition of K. Eqn. 2 is essentially that derived and used by Wagner and co-workers [14,15], but substituting pressures for masses.

For practical purposes, and because K has no unit, m can be defined in terms of arbitrary units as a function of the chromatographic peak height (h):

$$m_0 = h_0 V'_{G0} \tag{3}$$

$$m_2 = \frac{h_2 V_{G1}}{1 - V_d/V_{GT1}} \tag{4}$$

$$m_4 = \frac{h_4 V_{G2}}{1 - V_d/V_{GT2}} \tag{5}$$

where V'_{G0} is the initial volume V_{G0} at equilibration temperature and V_d the dead volume of transfer, which corresponds to the dead volume of the receptor syringe plus the dead volume of interconnection between the two stopcocks. Because the receptor syringe and the connections were purged before the transfer, the diluent gas was always pure nitrogen.

Finally, it should be noted that all these calculations are easily programmable in a microcomputer.

RESULTS AND DISCUSSION

Table I lists the thirty gases used in this study, distributed in six different groups, each of four (groups A and F) to six (groups B and C) components. Note the diversity of chemical groups included, and the wide range of molecular masses and boiling points of the selected compounds. Retention times are mean ± S.D. of ten analyses over a six-month period.

From a methodological point of view, syringes are not hermetic vessels and losses due to diffusion may be expected (Table II). The results obtained from gases of groups B, C, D and E agree quite well with Graham's law; leaks per hour are linearly correlated ($r = 0.64$) with the inverse of the square root of the molecular mass. However, some individual values suggest the involvement of other factors, such as a slight adsorption on stopcocks. It has been also determined that these leaks remain unchanged for at least the first 5 h, and that many of them are greater at 40°C than at room temperature [17]. These results contribute new information to this aspect of HS-GC analysis, which, as pointed out by Drozd and Novák [1], has not received enough attention.

By using syringes, the complete process of manipulation and analysis of samples can be verified without contact with ambient air. This is important in order to avoid contamination from airborne sources, which has been found in HS-GC carried out in closed vessels [18].

With respect to K, Table III shows the results obtained from the gases studied. Data from the literature were not available in all cases, but it should be noted that, when present, the agreement is good. The mean value obtained for benzene ($K = 5.059$) is similar to the values at 40°C reported by Drozd and Novák [20] (Table III), but both are higher than the 2.78 at 37°C found by Sato and Nakajima [12]; the reason for this difference remains unclear.

TABLE II

PERCENTAGE VARIATION OF PEAK HEIGHT PER HOUR FOR GASES OF GROUPS B, C, D AND E IN SYRINGES AT ROOM TEMPERATURE

Results are mean ± S.D. ($n = 10$).

	Δh/h (%)		Δh/h (%)
Ethane	−4.1 ± 0.8	Acetaldehyde	−5.2 ± 0.2
Cyclopropane	−3.1 ± 0.4	Ethanol	−4.4 ± 0.5
SF_6	−3.4 ± 0.7	Cyclohexane	−2.3 ± 0.3
Diethyl ether	−2.8 ± 0.9	1,1,1-Trichloroethane	−2.2 ± 0.3
Acetone	−4.1 ± 1.1	Benzene	−2.9 ± 0.4
Enflurane	−1.6 ± 0.6		
		Dichlorodifluoromethane	−2.5 ± 0.4
Methanol	−6.1 ± 0.5	Ethylene oxyde	−3.4 ± 0.6
Dichloromethane	−4.1 ± 0.3	*n*-Hexane	−2.1 ± 0.7
Acetonitrile	−9.3 ± 1.0	2-Propanol	−3.2 ± 0.4
Ethyl acetate	−3.2 ± 0.6	Isooctane	−1.7 ± 0.7
1,2-Dichloroethane	−4.1 ± 0.4	2-Butanol	−3.0 ± 0.7

The reproducibility of the method, expressed in terms of coefficients of variation (C.V.), is in general satisfactory and similar to other HS-GC methods [11,14]. The high C.V. for isooctane can be explained by the difficult measurement of the height of its peak, which is not well resolved from that of 2-propanol.

Table III shows only values for gases with a K below 300, except for acetone which is slightly higher than this limit value. Gases such as acetonitrile and the alcohols give values of K in water lower than expected for these kinds of compound, and with inadequate reproducibility. For example, with ethanol the value of K found is 342 ± 166, with a C.V. of 48.5%. This is because when the ratio m_0/m_2 is high, as for very soluble gases, the curve of the plot m_0/m_2 versus K is clearly asymptotic. In this zone, small changes in the mass ratio introduce great changes in K. Acetone is on the borderline, but the value of K obtained of 308 ± 56 at 37 °C has an acceptable reproducibility of 18.0%. Previously published values of K for acetone are 395 ± 49 [12] at the same temperature and 611 ± 31, probably at 25 °C [11].

This is the only limitation of applicability of the method, and this limit can be situated around a K value of 300. In any case, this gives an important range of application from very small (with no limit) to large (less than 300) values of K.

The method can be applied for the simultaneous determination of K of several gases. Experimentally it was determined that neither the presence of other gases in the mixture nor the change in initial concentration of gases (between restricted limits) has important consequences, at least for gases of group B

TABLE III

WATER/GAS PARTITION COEFFICIENTS AT 37°C FOR THE GASES AND VOLATILE COMPOUNDS ANALYSED

$n = 8$.

	This study		Literature data[b]		
	K found[a] (mean ± S.D.)	C.V. (%)	K (mean)	Temperature (°C)	Ref.
Sulphur hexafluoride	0.0037 ± 0.0001	2.7	0.0051	30	19
Isooctane	0.0119 ± 0.0048	40.3	N.A.[b]	–	–
n-Heptane	0.0155 ± 0.0014	9.0	N.A.	–	–
n-Hexane	0.0172 ± 0.0020	11.6	N.A.	–	–
n-Butane	0.0206 ± 0.0004	1.9	0.0256	30	19
Propane	0.0250 ± 0.0006	2.4	0.032	30	19
Methane	0.0302 ± 0.0008	2.6	0.029	40	19
Ethane	0.0331 ± 0.0007	2.1	0.0349	40	19
Cyclohexane	0.0871 ± 0.0082	9.4	N.A.	–	–
Cyclopropane	0.2018 ± 0.0032	1.6	0.204	37-38	6
Enflurane	0.6949 ± 0.0172	2.5	N.A.	–	–
Halothane	0.7270 ± 0.0181	2.5	0.88	37	9
1,1,1-Trichloroethane	0.8241 ± 0.0359	4.5	N.A.	–	–
Trichloroethylene	1.420 ± 0.140	9.9	1.51	37	9
Benzene	5.059 ± 0.654	12.9	3.8-4.8	40	20
Chloroform	5.505 ± 0.339	6.2	4.6	37-38	6
Isopropyl ether	7.293 ± 0.477	6.5	N.A.	–	–
Dichloromethane	8.705 ± 0.500	5.7	N.A.	–	–
Diethyl ether	16.36 ± 0.92	5.6	15.5	37-38	6
1,2-Dichloroethane	19.68 ± 1.36	6.9	N.A.	–	–
Ethyl acetate	111.7 ± 6.4	5.7	N.A.	–	–
Acetaldehyde	184.2 ± 29.4	16.0	N.A.	–	–
Acetone	308. ± 56	18.0	395.	37	12
Ethanol	> 300	–	N.A.	–	–
2-Propanol	> 300	–	N.A.	–	–
Methanol	> 300	–	N.A.	–	–
Acetonitrile	> 300	–	N.A.	–	–

[a] See text for values of K greater than 300.
[b] N.A. = not available.

[17]. This could be explained by the fact that with the low initial concentrations used, there were no significant changes in the activity coefficients [21].

CONCLUSIONS

The present method is suitable for the accurate determination of liquid/gas partition coefficients of a wide variety of gases, except those extremely soluble in the liquid matrix. It needs no special instrumentation and does not require calibration, because determination of K is based on ratios and arbitrary units

are sufficient. The minimum volume of liquid needed is only 1 ml. This is important for certain samples and in special situations.

The method can be applied for the determination of K of one or several volatile components at the same time.

Calculation of K for soluble gases ($K > 3$) needs only on equilibration and two chromatographic analyses, whereas for less soluble gases two equilibrations and chromatographic analyses are required. With the aid of a suitable computer program, less than 1 h is needed to accomplish the calculation of K for soluble gases and less than 1.5 h for insoluble gases. If both types of gas are present, the whole process takes less than 2 h.

The general preparative procedure can be applied to any detection system, other than FID or ECD. Particularly, the use of a thermal conductivity detector may allow the application to many inorganic gases. The method can also be applied to liquids other than water.

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