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DETERMINATION OF THE ADSORPTION COEFFICIENT (K_{oc}) OF SOME AROMATICS FOR SOIL BY RP-HPLC ON TWO IMMOBILIZED HUMIC ACID PHASES

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ABSTRACT

By determination of High Performance Liquid Chromatography (HPLC) capacity factors (k'), a comparison has been made of two new HPLC phases - physically and chemically immobilized humic acid - of their potential for determining soil adsorption coefficients (K_{oc}) for organic pollutants. Log K_{oc} values were estimated from log K_{oc} versus log k' calibration curves and were determined by using k' and the humic acid content of the HPLC phases. These calculated log K_{oc} values were compared with log K_{oc} values taken from the literature.

INTRODUCTION

An understanding of the partitioning of hydrophobic organic contaminants within the environment is of fundamental importance in determining their fate and transport. A number of studies have examined the adsorption of organic pollutants on to sediment or on to soil and have shown that the affinity for association of a contaminant with solid particulates is well correlated with both the hydrophobicity of the contaminant and with the organic content of the soil or sediment (1,2).

The sediment/water partition coefficient, or soil/water partition coefficient, (K_p), quantifies the equilibrium partitioning of a liquid or "supercooled" organic solute between two phases

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$$K_p = a_1/a_2 = c_1/c_2 \qquad \text{[eqn. 1]}$$

where *a* is the activity and *c* is the concentration of a solute in phases 1 and 2. Due to the problematic nature of defining activities of the wide variety of nonionic compounds, the experimental partition coefficients are defined as the ratio between the concentrations. Because of the complex nature of soil and sediments it is difficult to obtain a useful parameter which can be used to model the transport of organic pollutants. However, if it is assumed that the sole adsorbent phase of soil is the soil organic carbon, it is posible to model the fate of organic pollutants by using the "soil independent" adsortion coefficient, K_{oc}. From equation 2, K_p can be calculated.

$$K_{oc} = Kp \times 100/\%OC \qquad \text{[eqn. 2]}$$

where %OC is the percentage soil organic carbon.

Both the Organisation for Economic Co-operation and Development (3) and the US Environmental Protection Agency (4) guidelines for testing of chemicals describe tests for measuring K_{oc} using natural soils. There are many disadvantages of using a test involving soil, for example, an erroneous K_{oc} value may be obtained due to loss of the chemicals by biological degradation, volatilization or adsorption other than on the soil. In addition prolonged analytical techniques involving difficult separation steps may be necessary. Some workers (5,6) have resorted to model adsorbents such as cellulose to overcome separation and inhomogenity problems associated with soil, but these are also susceptible to chemical losses and may still require lengthy chemical analyses.

Although direct methods of measuring K_{oc} are to be preferred, its estimation from other physical properties such as water solubility (S) and the n-octanol/water partition coefficient (K_{ow}) can also be used. Unfortunately such data are often unavailable. As values for K_{ow} and K_{oc} are difficult to determine, alternative methods for their determination have been considered. Foremost amongst these alternative methods has been their determination by Reverse-Phase High Performance Liquid Chromatography (RP-HPLC) (7-9). Recently some researchers (10,11) have used RP-HPLC with a cyanopropylsilica column and a mobile phase of high water content to measure sediment/water adsorption coefficients for alkyl benzenes, polyaromatic hydrocarbons and some pesticides. They showed, under these conditions that the relationship between HPLC capacity factors (k') and K_p or K_{oc} could be satisfactorily described by a simple correlation equation, whereas the octadecylsilica (ODS) column required the use of three correlation equations to describe the relationship between these properties. Szabo et al (12,13) have shown that

ethylsilica and chemically immobilized humic acid phases are also suitable phases for predicting the soil/water partition coefficient.
Vowels and Mantoura (10) generated an order:

organic matter on the soil > cyanopropylsilica > n-octanol > ODS

while Szabo et al (12) have suggested the order:

organic matter on the soil > ethylsilica > n-octanol > phenylsilica > ODS

In a subsequent study Szabo et al (13) have suggested an order:

organic matter on the soil > immobilized humic acid > ethylsilica > n-octanol

This is approximately the order which might be expected for the polarity of the sorbent phase. It is easily seen that there is some uncertainty for determination of K_{oc} or K_p using different types of chromatographic materials as these materials have polarities which might not reflect the polarity of the organic material in soil.

In order to model the adsorptive surface properties of the soil we have prepared two HPLC packing materials bearing immobilized humic acid and studied the effect of the column type on the correlation between log k' and log K_{oc}. So that we might evaluate these new phases we have constructed a calibration curve using the log K_{oc} values, listed in Table I, and experimentally determined values for the theoretical HPLC capacity factor, k'_w. By using this calibration curve we have re-determined log K_{oc} values for the 10 chemicals listed in Table I. In addition we have calculated log K_{oc} values for 4 other chemicals. By way of a comparison we have also calculated log K_{oc} by using k' and the humic acid carbon content of our new HPLC phases.

EXPERIMENTAL

APPARATUS and MATERIALS. Chromatographic retention data were measured with an LKB 2150 solvent delivery system and LKB Wavescan Diode-array detector. Sample introduction was via a Rheodyne 7125 injection valve fitted with 20 µl loop. Chromatographic data were recorded on an Olivetti M24 personal computer using commercially available software *(LKB Wavescan)*. The immobilized humic materials - their preparation is described below - were packed in 250 x 4.6 mm stainless steel tubes by Jones Chromatography, Hengoed, Wales. HPLC grade silica (Hypersil WP 300 5 µm) was obtained from Shandon, Cheshire; 3-aminopropyltriethoxysilane (Fluka Chemicals Ltd) was used as received. Humic acid was purchased from Aldrich Chemical Co. All other chemicals obtained from commercial sources were used without purification.

CHROMATOGRAPHY. Mobile phases, HPLC grade methanol and water, were mixed volume/volume and helium aspiration used to remove dissolved air. The test solutes were dissolved in methanol at a concentration of 0.1 mg/ml. Flow rates were 0.8 ml/min as usual. A laboratory temperature of 20-23 °C was used for all HPLC measurements. The mobile phase methanol content was changed from 50% to 30% by 5% steps for the physically immobilized humic acid phase and from 60% to 40% by 5% steps for the chemically immobilized humic acid phase. Methanol was used for the measurement of the retention time (t_0) of an unretained compound. The relationship:

$$k' = t_r - t_0 / t_0$$

was used to calculate the capacity factor, k', from the retention time (t_r) of each compound. All capacity factors reported are the mean of at least three measurements.

ELEMENTAL ANALYSIS. The stationary phases were analysed by the Micro Analytical Laboratory at the University of Manchester.

PREPARATION OF CHEMICALLY IMMOBILIZED HUMIC ACID SILICA GEL (CIHAC). The preparation of the chemically immobilized humic acid packing material has been described elsewhere (13). Basically, dried silica gel (10g) was refluxed with 5% 3-aminopropyltriethoxysilane in anhydrous toluene (14) and the resulting aminopropyl silica gel removed by filtration, washed with toluene, methanol and water, washed again with methanol and dried. The reaction product was activated with 10 volumes of 5% aqueous glutaraldehyde for 5 h to produce an activated gel which on isolation was washed with 15 volumes of distilled water. This purified gel was in turn reacted with 100 ml of 1% aqueous solution of humic acid, pH 7.5, for 8 h at ambient temperature. After the immobilized humic acid gel had been washed with 10 volumes of 0.5M phosphate buffer and distilled water it was treated with 0.1M buffered ethanolamine, pH 7.5, for 3 h. The reaction product was washed with a large excess of distilled water and dried to yield a dark-brown product.

PREPARATION OF PHYSICALLY IMMOBILIZED HUMIC ACID SILICA PHASE(PIHAC). The preparation of this packing material was based on the procedure of Marko-Varga et al (15), whose study established that the humic acid adsorption on an amino silica gel is very strong, and cannot be destroyed by using water or methanol. Although we have termed this physical immobilization, because of the potential for ionic bond formation between immobilized amines and carboxylic acids, it can not be excluded that some Schiff bond formation between amines and carbonyl groups might also occur in which case the immobilization might be better termed covalent and ionic

bond immobilization. Dried amino silica gel (10 g) was treated with 100 ml of 1% aqueous solution of humic acid pH 7.5, for 4 h, at ambient temperature. The resulting silica gel was filtered off, washed with 10 volumes of 0.5M phosphate buffer, copious amounts of distilled water and methanol. Finally, the gel was dried to yield a light brown product.

SELECTION OF LOG K_{oc} AND LOG K_{ow} VALUES. Only a limited number of compounds with reliable log K_{oc} values can be found in the literature. In Table I we have compiled log K_{oc} and log K_{ow} values for 10 compounds used in this study. To compile this table we have used the well established criteria originally used by Brooke et al (16) for selection of reliable log K_{oc} values.

TABLE I. Reference chemicals with reliable log K_{oc} and log K_{ow} values obtained from the literature

Chemicals	Log K_{oc}	Literature	Log K_{ow}	Literature
Benzene	1.91	1	2.11	1
Toluene	2.18	16	2.65	18
Ethylbenzene	2.41*	10	3.13	18
Propylbenzene	2.86*	10	3.69	18
Butylbenzene	3.40*	10	4.28	18
o-Xylene	2.34*	10	3.13	18
Naphthalene	3.11	1	3.36	1
Phenanthrene	4.28*	10	4.57	1
Anthracene	4.41	1	4.54	1
Pyrene	4.83	17	5.18	18

*NOTE:converted from literature log K_p value using equation [2].

All capacity factors reported are the mean of at least three measurements. The correlation analysis for all compounds was made by linear regression analysis of log k' or log K_{ow} versus log K_{oc} using commercially available software (Graph pad^{tm}, Institute for Scientific Information). A least squares fit routine used for curve fitting.

RESULTS AND DISCUSSION

Elemental analysis data of the CIHAC and PIHAC phases are shown in Table II.

TABLE II. Elemental analysis data of chemically and physically immobilized humic acid phases.

	Total elemental analyses			Elemental content from the bonded humic acid		
	C %	H %	N %	C %	H %	N %
CIHAC	4.7	0.7	0.5	1.8	0.3	0.3
PIHAC	1.7	0.4	0.4	0.3	0.1	0.3

From Table II, it can be seen that we have prepared two different types of immobilized humic acid phase where the main difference is the carbon content of the gel.

Examination of Fig. 1 shows the correlation between log K_{oc} and log K_{ow} values, using the data available from Table I, and gives the relationship:

$$\log K_{oc} = 1.023 \log K_{ow} - 0.578 \qquad r^2 = 0.922 \quad n = 10 \qquad \text{[eqn. 3]}$$

The slope parameter (1.023) compares well with values reported elsewhere by Karickoff (1,17) and Means (2) who reported a value 1.0 for various substrates and solutes.

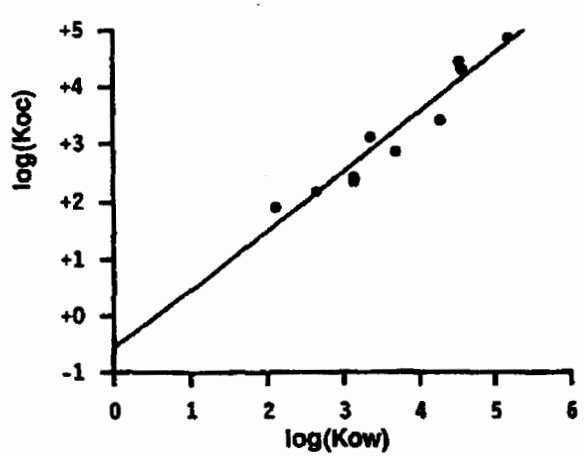

Fig. 1. Relationship between soil/water partition coefficients and n-octanol/water partition coefficient.

In order to eliminate selective solute-solvent interactions (18,19), we have used log k'_w, the capacity factor obtained by extrapolation of retention data

from binary eluents to 100% water instead of using log k', the capacity factor obtained from binary eluents. Snyder et al (20) showed linear equation [4]

$$\log k' = \log k'_w + S\phi \qquad \text{[eqn. 4]}$$

can be used to describe the relationship between log k' and log k'_w where ϕ is the volume fraction of organic solvent in the water-organic solvent mixture, k'_w represents the capacity factor of a solute with pure water as the mobile phase and S is a constant for a given solute-eluent combination. The log k'_w of 10 organic compounds calculated from equation [4] using data arising from the immobilized humic acid phases are presented in Table III; the values of log K_{oc} for these chemicals are also tabulated.

TABLE III. Log K_{oc} and log k'_w values obtained from equation [4], on different HPLC stationary phases.

Chemical	log K_{oc}	log k'_w on CIHAC phase	log k'_w on PIHAC phase
Benzene	1.91	0.096	-0.640
Toluene	2.18	0.514	-0.357
Ethylbenzene	2.41	0.782	0.031
Propylbenzene	2.86	1.107	0.579
Butylbenzene	3.40	1.407	0.917
o-Xylene	2.34	0.627	-0.030
Naphthalene	3.11	1.452	0.754
Phenanthrene	4.28	2.569	1.916
Anthracene	4.41	2.907	2.055
Pyrene	4.83	3.212	2.416

A plot of log K_{oc} versus log k'_w for the chemicals listed in Table III yields the calibration curve depicted in Fig. 2 for the CIHAC phase. Analysis of this calibration curve yields:

$$\log K_{oc} = 0.948 \log k'_w + 1.781 \qquad r^2 = 0.986 \quad n=10 \qquad \text{[eqn. 5]}$$

A similar treatment for the data for the PIHAC phase (Fig. 3) yields

$$\log K_{oc} = 0.963 \log k'_w + 2.436 \qquad r^2 = 0.994 \quad n=10 \qquad \text{[eqn. 6]}$$

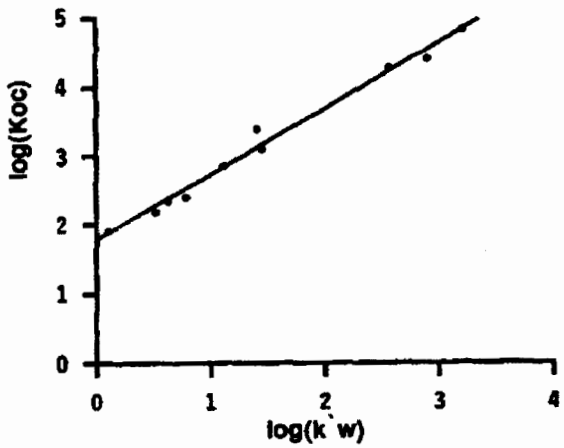

Fig. 2. Relationship between soil/water partition coefficient and the theoretical capacity factor for the CIHAC phase.

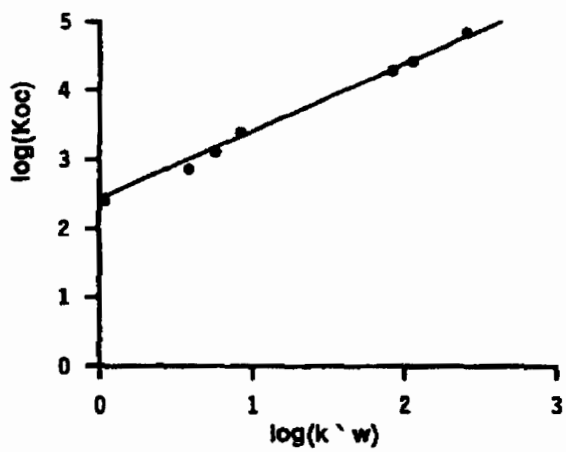

Fig. 3. Relationship between soil/water partition coefficient and the theoretical capacity factor for the PIHAC phase.

From a comparison of the equations [5] and [6] it is evident that the immobilized humic acid phases give very similar correlation between log K_{oc} and log k'_w. On the basis of correlations of K_{oc} versus K_{ow} and log K_{oc} versus log k'_w, it is more accurate to estimate log K_{oc} from k'_w determined humic acid phase columns than via a single relationship between log K_{oc} and log K_{ow}. The divergences for the 10 chemicals listed in Table I are small for both humic acid phases, suggesting that their adsorptive properties are similar to those of sediment and soil organic matter. Such similarity might be expected as humic acids represent a large proportion of the organic matter

in soil. From the Table III it can be seen that higher values of log k'_w are obtained for the CIHAC phase than for the PIHAC phase. From a comparison of equations [5] and [6] it is evident that the physically immobilized humic acid phase is slightly superior to the chemically bonded humic acid. Perhaps this superiority reflects the nature of the association of humic acids with the mineral phase in the environment.

By using equations [5] and [6], log K_{oc} values have been calculated and these values are shown in Table IV, together with the divergence from literature values. In addition we have calculated the values of log K_{oc} for acenaphthylene, acenaphthene, fluorene and fluoranthene for which there appear to be no log K_{oc} values in the literature.

TABLE IV. Log K_{oc} values obtained by using immobilized humic-acid gels and differences from reported values.

Chemicals	log K_{oc} from HPLC on CIHAC	Difference from reported value	log K_{oc} from HPLC on PIHAC	Difference from reported value
Benzene	1.87	0.04	1.82	0.09
Toluene	2.26	-0.08	2.10	0.08
Ethylbenzene	2.52	-0.11	2.47	-0.06
Propylbenzene	2.83	0.03	2.98	-0.12
Butylbenzene	3.15	0.25	3.32	-0.08
o-Xylene	2.37	-0.03	2.40	-0.06
Naphthalene	3.15	-0.04	3.16	-0.05
Acenaphthene	3.79		3.59	
Acenaphthylene	3.83		3.75	
Fluorene	4.15		4.21	
Phenanthrene	4.22	0.06	4.28	0.00
Anthracene	4.53	-0.12	4.42	-0.01
Fluoranthene	4.74		4.62	
Pyrene	4.82	0.01	4.77	-0.06

The degree of partitioning in reverse phase chromatography is quantified in terms of a solute distribution coefficient, K_{RP}, characterizing the equilibrium ratio of solute concentration in the stationary and mobile phases

$$K_{RP} = C_1/C_2 \qquad \text{[eqn. 7]}$$

where C is the concentration of the solute in phases 1 and 2. This constant is analogous to the partition coefficient defined in equation [1], especially in this case where the stationary phases are very similar to the natural soil and sediment. The volume of a solvent required to elute a solute associated with the stationary phase in a RP-HPLC column, V_t, is related to K_{RP}. Indeed this volume, corrected for the volume, V_r, retained in the pore spaces of the column, is directly equal to K_{RP} times the volume of the solid phase, V_s (21). The most common way of expressing the retention capacity of a column is through the use of a capacity factor k', which is the equilibrium ratio of the number of moles of solute in the stationary and mobile phases

$$K_{RP} = C_1/C_2 = k' \times V_v/V_s \qquad \text{[eqn. 8]}$$

Because k' is directly proportional to K_{RP} or in this particular case to K_p, it is possible to calculate the K_{oc} values from log k'_w values on immobilized humic acid phases by using equation [2].

By using equation [2] and k'_w data obtained on CIHAC (A) and PIHAC (B) phases, instead of K_p values, log K_{oc} values have been calculated. These log K values are shown in Table V, together with the difference from each other and the average of these calculated values. In addition, we have calculated the values of log K_{oc} for acenaphthylene, acenaphthene, fluorene and fluoranthene which have not found in the literature. In the equation [2] we have used the carbon content obtained from only the humic acid on the phases instead of the total carbon content of these phases because it was noticed that calculation using total carbon content resulted in values of log K_{oc} which were too low.

TABLE V. Log K_{oc} values derived using equation [2] and k'_w values on chemically (A) and physically (B) immobilized humic acid phases.

Chemicals	A	B	A-B	A+B/2	literature values
Benzene	1.84	1.88	-0.04	1.86	1.91
Toluene	2.25	2.17	0.08	2.21	2.18
Ethylbenzene	2.53	2.55	-0.02	2.54	2.41
Propylbenzene	2.86	3.10	-0.24	2.98	2.86
Butylbenzene	3.17	3.43	-0.26	3.30	3.40
o-Xylene	2.38	2.49	-0.11	2.43	2.34
Naphthalene	3.21	3.27	-0.06	3.24	3.11
Acenaphthene	3.87	3.72	0.15	3.79	
Acenaphthylene	3.90	3.88	0.02	3.89	
Fluorene	4.24	4.11	0.13	4.17	
Phenanthrene	4.31	4.43	-0.12	4.37	4.28
Anthracene	4.65	4.58	0.07	4.61	4.41
Fluoranthene	4.86	4.79	0.07	4.82	
Pyrene	4.95	4.94	0.01	4.94	4.83

From Table V, it is evident that any humic acid phases are useful to determine K_{oc} values without using calibration curves if we know the humic acid content of the HPLC phase.

CONCLUSIONS

The results of this work show that provided it is accepted that organic carbon is the most important component for soil adsorption for aromatic hydrocarbons, then the log K_{oc} value may be determined from HPLC capacity factors measured on immobilized humic acid columns using reference chemicals for calibration. We suggest the best way to model the soil/water sorption of aromatics in the environment is to use log k'_w, the capacity factor obtained by extrapolation of retention data from binary eluents to 100% water. For the different humic acid phases the correlation between log K_{oc} and log k'_w has been found to be very similar and to be better than the correlations between log K_{oc} and log K_{ow}. The best prediction of log K_{oc} arises using k'_w instead of log k'.

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