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LIQUID-SOLID CHROMATOGRAPHY ON AMBERLITE XAD-2 AND OTHER STYRENE-DIVINYLBENZENE ADSORBENTS

I. DEVELOPMENT OF A SOLVENT ELUOTROPIC SCALE*

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SUMMARY

Equilibrium and chromatographic distribution constants of selected polycyclic aromatic hydrocarbons and *n*-hexadecane have been measured on Amberlite XAD-2, Amberlite XAD-4, and Bio-Beads SM-2 in a wide variety of organic solvents. The solvent polarity range examined included reversed-phase, normal-phase and size-exclusion modes of separation. The adsorption reversibility and linear capacity of the above solutes on XAD-2 was investigated to establish linear elution conditions. A semi-quantitative eluotropic scale is presented which shows some similarities to the relative solvent strengths given in eluotropic scales for carbon black and chemically bonded octadecyl reversed-phase packings.

INTRODUCTION

Non-polar macroporous polymeric adsorbents such as copolymers of styrene and divinylbenzene have been applied to a wide variety of separation problems in recent years. One adsorbent, Amberlite XAD-2, has been used to separate benzodiazepine drugs and their metabolites from urine¹, eliminate interferents prior to the analysis of *p*-hydroxybenzoate esters in pharmaceutical syrups², isolate halogenated organics from wastewater³, and neutral organics from potable water⁴. A similar adsorbent, Amberlite XAD-4, has been used to separate nucleic acid hydrolysis products and related substances from nucleotide mixtures⁵.

The adsorption of polycyclic aromatic hydrocarbons (PAHs) by styrene-divinylbenzene (SVB) adsorbents from methanol was observed in 1970⁶. Since then, the overall recovery efficiency of eight PAHs and a large number of other organics has been reported⁷. The retention index of substituted PAHs has been reported for a

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styrene-ethylenedimethacrylate copolymer using the eluents water-methanol-diethyl ether (11:18:71), methanol-water (7:3 and 3:2), and acetonitrile-water (7:3 and 3:2)^{8,9,24}. (The polystyrene gel used was a cross-linked styrene-ethylenedimethacrylate copolymer, available commercially as Separon SE (previously Spheron SE))²⁴. In addition, linear relationships between the logarithm of the retention index and the number of carbon atoms per molecule were observed for each of several homologous series such as unsubstituted PAHs, *n*-alkylbenzenes, isoalkyl benzenes, and the *n*- and isoalkyl PAH series of naphthalene and phenanthrene. Retention data on XAD-2 using ethanol-water and ethanol-heptane mixtures has been reported for the three PAHs, benzene, naphthalene, and anthracene^{10,11}.

Systematic investigations of the elution behavior of SVB adsorbents have been conducted for a number of solute-eluent systems. Distribution coefficients for several nitro- and chloro-phenols and organic bases have been reported as a function of water-ethanol concentration and pH in 10% ethanol^{10,12}. Equations based on ionization equilibria were derived to express the distribution ratio, K_D , as a function of pH and related elution conditions. More recently, retention data for amino acids, and benzoic acid, phenoxyacetic acid, and benzenesulfonic acid derivatives have been reported on XAD-2, XAD-4, and XAD-7 as a function of water-organic solvent ratio, pH effect, and presence of electrolyte in the eluent^{11,13}. Diprotic acids and bases, polyprotic acids and bases, and ampholytes have been the subject of similar investigations¹⁴. It was observed that retention of acids and bases was high for the neutral form and low for the dissociated form. Ampholytes had a retention that passed through a maximum or minimum as a function of pH. Amino acids, selected derivatives, and peptides have been investigated as a function of pH in ethanol-water and acetonitrile-water mixtures¹⁵. Pertinent equations were developed to describe the capacity factor as a function of pH. The emphasis of the preceding references toward systematization has been to consider solution equilibria of ionizable solutes and utilize such behavior to conduct separations as a function of pH.

The retention of organic acids, esters, and aldehydes likely to be found in pharmaceuticals have been investigated on XAD-2, using methanol-water solutions as the eluent. A linear equation was used to express the logarithm of the distribution coefficient as a function of the number of methylene groups in the *n*-alkyl chain of the ester². In addition, 29 drug compounds related to pharmaceutical syrups have been chromatographed in acidic, neutral, and basic solutions of varying composition of methanol and water¹⁶. The data presented was not expressed in the form of mathematical equations.

Other SVB adsorbents (Shodex HP-255 and A801) have been used to investigate the retention of phthalate esters, aliphatic ketones, and eight neutral aromatic compounds¹⁷. Eluents were methanol, ethanol, chloroform, cyclohexane, and *n*-hexane. An equation was derived that expressed the distribution coefficient as a function of solubility parameters of the solvent, gel, and solute. It was suggested that the SVB copolymers are able to function in one or more of three different separation modes (adsorption, partition, or molecular size exclusion) dependent upon the chosen solvent. A more recent publication has demonstrated separations by a combination of size-exclusion and normal-phase partition modes of chromatography¹⁸.

The retention of neutral aromatic and polycyclic aromatic hydrocarbons, both parent and substituted compounds, has been the subject of an investigation^{9,24}. The

logarithm of the retention index was observed to be linearly related to the number of carbon atoms in the solute. Moreover, substituted PAHs were shown to deviate from the homologous parent PAHs; however, each homologous substituted PAH series was linearly related to the number of carbon atoms in each molecule. Slopes and intercepts for each series were reported. In a related work, the retention behavior of substituted benzenes was used to calculate the contribution of different functional groups to the experimental retention index⁸. It remains to be demonstrated that these group contributions have utility in calculating the retention index of species not previously studied.

Alkylbenzenes and alkyl benzoates have been investigated on Hitachi Gel 3010, using methanol-water mixtures (100:0, 95:5, 90:10) as the eluent over the temperature range of 10–50° (ref. 19). Partial molar enthalpies of the compounds were obtained and it was also demonstrated that the logarithm of the capacity factor was an approximate linear function of carbon number. Other investigations on the above gel with aqueous methanol eluents of various pH values have produced capacity factors as a function of pH for a number of *meta*-substituted anilines and *ortho*-substituted phenols²⁰. The capacity factor was expressed as a function of pH similar to a previously-reported approach^{10,12}. An earlier study on this gel revealed that aromatic esters, various phenols, and substituted amines could be separated to varying degrees with eluents such as methanol, methanol-water (95:5 and 90:10), and methanol-hexane (95:5); however, the role of the solvent in the separation was not established in a quantitative fashion²¹.

A similar SVB adsorbent, Hitachi Gel 3011, has been utilized for the separation of aliphatic fatty acids, *trans*- α,β -unsaturated fatty acids, dicarboxylic acids and benzoate esters²². Capacity factors, k' , were obtained at 50° with methanol-water eluent ranging in composition from 40–100% methanol. It was shown that k' values were linear functions of the number of methylene groups in aliphatic chains. Another investigation with the same adsorbent demonstrated that members of homologous series of fatty acid mono- and diethanolamides having C₁₀–C₁₈ alkyl groups could be separated using water-methanol (3:97) eluent²³. The k' values were also linear functions of alkyl chain length.

EXPERIMENTAL

Reagents and solvents

Reagents were analytical grade or purest available. All ¹⁴C-labeled compounds were obtained from Amersham Corporation, Arlington Heights, Ill., U.S.A. except for anthracene which was obtained from California Bio-Nuclear (Sun Valley, Calif., U.S.A.). Solvents were obtained from Burdick and Jackson Labs. (Muskegon, Mich., U.S.A.) when possible; otherwise, they were obtained from Fisher Scientific (Pittsburgh, Pa., U.S.A.). Some of the heptane and methanol was purified by distillation through a 20-plate Oldershaw column. Amberlite XAD-2 and XAD-4 were obtained from Rohm and Haas (Philadelphia, Pa., U.S.A.) and Scientific Products (Grand Prairie, Texas, U.S.A.). Bio-Beads SM-2 was obtained from Bio-Rad Labs. (Richmond, Calif., U.S.A.). The adsorbents used in equilibrium distribution experiments were initially cleaned by washing with distilled water and methanol. The air-dried adsorbent was Soxhlet extracted for 24 h per washing for a total of eight times with fresh por-

tions of methanol. The adsorbent was air-dried and finally dried at 100–105° for 6–7 h under vacuum in an oven. The adsorbents were stored in a stoppered Pyrex bottle in a desiccator over Drierite until used. Most batches were tested for purity by soaking 1 g of adsorbent in 25 ml of ethanol overnight. The ultraviolet (UV) spectrum of the ethanol showed an adsorbance of 0.10 or less near 260 nm. Five samples of the adsorbents were sent to Micromeritics Instrument Corporation (Norcross, Ga., U.S.A.) for specific surface area analysis by the standard multipoint BET technique with the Model 2100 analyzer.

Apparatus

One high-performance liquid chromatographic (HPLC) system was assembled from a Milton-Roy mini pump, Precision Sampling Model 4201 liquid chromatograph inlet, pressure gauge, tube pulse dampener, Perkin-Elmer Model 250 ultraviolet absorption detector with detection at 254 nm, and a Beckman Model 1005 potentiometric recorder. Another system was assembled from an Altex Model 110 pump, Rheodyne Model 70-10 valve, Perkin-Elmer Model LC-55 variable-wavelength detector, and Beckman recorder. Columns were dry-packed with XAD-2 of size 45–53 μm , obtained by grinding and dry sieving the commercially available adsorbent.

The column packing procedure consisted of tapping the end of the column on a bench between addition of small portions of dry adsorbent. Columns were constructed from lengths of $\frac{1}{4}$ in. stainless-steel tubing. The outlet end was fitted with a zero-dead volume reducing union and stainless-steel frit (Mott Metallurgical).

Equilibrium distribution experiments

Solutions were prepared by adding 50 ml of the selected solvent to 0.5–1.0 g of vacuum-dried adsorbent in a glass-stoppered 60-ml Pyrex bottle or 2-ounce French bottle fitted with a cap having a polyethylene cone liner. When radioactive tracers were used, 100 μl (approximately 440,000 dpm) of appropriate tracer was added to the bottle. The specific activity of different batches of ^{14}C -benzo[α]pyrene ranged from 73.8 to 233 $\mu\text{Ci}/\text{mg}$, which means that 0.86 to 2.7 μg of benzo[α]pyrene was used, giving a solute/adsorbent ratio of $5.4 \cdot 10^{-6}$ or less. In experiments with unlabeled PAHs, the solute was added to give a final solution that would be $(2\text{--}44) \cdot 10^{-6}$ molar in the PAH. Solutions were equilibrated in a constant temperature water bath at $25 \pm 0.1^\circ$ for 4–6 days, and were shaken several times daily.

Radiotracer solutions were assayed by adding 2.00 ml to a low-potassium scintillation vial, and 13.00 ml of Beckman Ready-solve EP or HP, or other commercial scintillation solution. The cocktails were counted on a Beckman Model LS-100C liquid scintillation spectrometer. Counting was performed to 0.5% error or less; both the external standards channels ratio (ESCR) and the channels ratio (CR) methods were used in initial experiments to correct for quench and calculate dpm. When it was observed that both methods gave the same results, the ESCR method was used in later work. In order to estimate the effect of solvent quench, an initial experiment was conducted in which 2.00 ml of solvent, 16,760 dpm of ^{14}C -benzo[α]pyrene, and 13.00 ml of scintillation solution were added to a vial. All solvents, except some halogenated hydrocarbons and nitromethane could be corrected for quench with a calibration curve prepared from the standard Beckman and Amersham quench sets.

The concentration of solute in non-radiotracer solutions was measured spec-

trophotometrically at an appropriate wavelength with a Beckman DB-G spectrophotometer. A calibration curve was made at each wavelength using the appropriate solvent.

Values for K_D were calculated with the equation:

$$K_D = \frac{\text{g of solute on the resin/g of resin}}{\text{g of solute in solution/ml of solution}}$$

In the case of radiotracer experiments, the dpm of solute in each phase was substituted for weight of solute.

Chromatographic elution K_D values were calculated using the equation: $K_D = V_R - V_0/W$ where V_R is the uncorrected retention volume, measured from the point (time) of injection of a sample to the peak maximum. The weight of adsorbent (W) in the column was determined by two methods: (1) weighing the empty and filled column, and (2) weighing the amount of air-dried adsorbent used to fill the column. The column void volume (V_0) was obtained by measuring the retention volume of non-adsorbed solutes such as sodium acetate, formic acid, or sodium nitrate.

RESULTS AND DISCUSSION

Equilibration time required in equilibrium distribution experiments

The rate of adsorption of benzo[α]pyrene by XAD-2 in equilibrium distribution experiments (EDT) using ^{14}C -tracers was examined using three different methods of agitation. In one case, the solution was stirred with a magnetic stirrer and was sampled periodically. In another trial an ultrasonic bath was used to agitate the solution. In the first two cases, temperature control could not be accurately maintained. In a third case, the solution temperature was controlled at 25° while being stirred with a magnetic stirrer. The data in Fig. 1 (third case) shows that 58.5% of the initial benzo[α]pyrene spike is adsorbed by 1.00 gram of XAD-2 in about 2 h, which is 85.8% of the amount that is adsorbed at equilibrium. After 2 h, adsorption is significantly slower in approaching equilibrium. Comparative data is presented for Amborsorb XE-340, in which a solution was swirled periodically, but not continually after spiking with benzo[α]pyrene. An adsorption plateau is again reached in about 2 h where 95% of the benzo[α]pyrene has been adsorbed. After this point, adsorption is slower in approaching equilibrium. Additional experiments with XAD-2 in binary aqueous solutions (organic solvents: methanol, acetonitrile, tetrahydrofuran) have borne out the observation that 80–90% of maximum attainable adsorption is attained in approximately 2–3 h, after which the equilibrium state is approached at a slower rate. Presumably, this is due to slower diffusion into micropores of the adsorbent. As a comparison, an analogous study of the adsorption of ferroin type chelates of iron(II) revealed that a plateau was approached in 50–60 min by continuously agitating the solutions, interrupting only to measure concentration²⁵; however, rate study data was not reported beyond 50 min, after which a slight increase in adsorption would be expected if the iron and benzo[α]pyrene systems were closely similar. Pseudo-first-order rate constants of $-(0.9 \text{ to } 2.3) \cdot 10^{-3} \text{ sec}^{-1}$ for the iron chelate study are comparable with values of $-9.2 \cdot 10^{-4}$ and $-1.6 \cdot 10^{-3} \text{ sec}^{-1}$ for Amborsorb XE-340 and

TABLE I
COMPARISON OF XAD-2 CLEANING PROCEDURES

| Procedure | Lot | Absorbance Tests** | | | | | K_D Values of Benzo(a)pyrene/XAD-2 | | | |
|---|------|--------------------|----------|------------|------------|-------------|--------------------------------------|--|--|--|
| | | 245 nm | 265 nm | Heptane | Ethanol | Methanol | 2,2,4-Trimethylpentane | | | |
| 1 Dried as received for 4 h at 105°; stored in desiccator over Drierite | WENA | 0.68 | 1.01 (P) | 17.6 ± 0.6 | 98 ± 1 | 168 ± 1 | 200 ± 4 | | | |
| 2 Washed* with distilled water, then dried and stored as in Procedure 1 | WENA | 0.65 | 1.01 (P) | 14.6 ± 0.5 | 85.2 ± 0.4 | 156 ± 5 | 178 ± 8 | | | |
| 3 Washed* with methanol and water, then treated as in Procedure 1 | WENA | 0.54 | 0.95 (P) | 18.5 ± 0.5 | 109 ± 2 | 189 ± 1 | 236 ± 10 | | | |
| 4 Washed* with methanol and water, then vacuum dried for 6 h at 104°; stored as usual | WENA | 0.54 | 0.92 (P) | 18.2 ± 0.2 | 112 ± 0 | 190 ± 1 | 247 ± 4 | | | |
| 5 Washed* with water, Soxhlet extracted 8 times for 24 h each with methanol; vacuum dried for 8 h at 105° | WENA | 0.03 | 0.06 | 22.2 ± 0.1 | 147 ± 0.5 | 229 ± 10 | 364 ± 4 | | | |
| 6 EPA Procedure: Soxhlet extracted with water, methanol, ethyl ether, and pentane; vacuum dried as above | WELJ | 0.06 (P) | 0.03 | 20 | 129 | 214 ± 1 | 340 ± 16 | | | |
| 7 Methanol Procedure No. 5 above, except extracted only 6 times | WELJ | 0.25 (P) | 0.06 | — | — | 209.6 ± 0.1 | 253 ± 2 | | | |
| | WELJ | 0.21 (P) | 0.11 | — | — | 209.4 ± 0.1 | 263.5 ± 0.1 | | | |

* 20 g were washed with 250 ml of each solvent.

** P denotes measurement at an absorbance peak.

XAD-2, respectively, in Fig. 1. Composite data for four other experiments with XAD-2 gave a rate constant of $-6.5 \cdot 10^{-4} \text{ sec}^{-1}$ using data for the first 25 min of ~~adsorption~~.

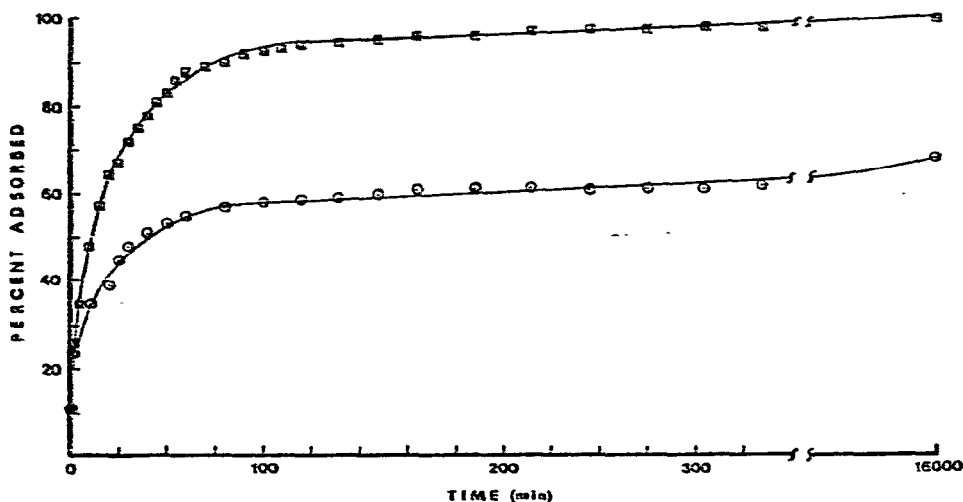


Fig. 1. Rate of adsorption of benzo[α]pyrene from methanol by XAD-2 (○) and Ambersorb XE-340 (□).

Comparison of various XAD-2 cleaning procedures

Five portions of a batch of XAD-2, Lot WENA, were subjected to different cleaning procedures, denoted as Procedures 1–5 in Table I. The cleaned portions were then evaluated in EDT experiments with ^{14}C -benzo[α]pyrene by measuring the K_D of benzo[α]pyrene for each portion in four different solvents. The solvents heptane, ethanol, methanol, and 2,2,4-trimethylpentane were selected to give a reasonable range in K_D values. It was observed that cleaning Procedure 5 which used eight successive methanol extractions of the XAD-2 gave the largest and most consistent K_D values in all four solvents. Other data in the table allow a comparison between two different lots of XAD-2 (see Procedure 5), showing that the two lots were reasonably consistent. Cleaning Procedure 5 and an EPA Procedure²⁶ listed as Procedure 6 in the table are compared, and it was observed that the latter procedure was not as effective in cleaning the XAD-2 for use in EDT experiments; however, it appears that the EPA procedure is about as effective as six successive washings with methanol (see Procedure 7).

An alternate method we have used for evaluating cleaned adsorbent is to place 1.00 gram in 25 ml of ethanol, and obtain an UV spectrum of the ethanol after the adsorbent has soaked for 24 h. If the absorbance at 245 and 265 nm where wavelength maxima occur is less than 0.06 above the baseline, the adsorbent is considered to be adequately cleaned. Two wavelengths were used to report the UV background of the ethanol test solution since the wavelength peak (denoted P) shifted from 265 nm in Procedures 1–4 to 245 nm in Procedures 5–7. The results in Table I show that the much higher UV absorption obtained in Procedures 1–4 is consistent with lower K_D

values for the XAD-2 cleaned by these procedures relative to the low UV absorption of the ethanol and high K_D values obtained from the XAD-2 in Procedure 5. The exact nature of the UV absorbing species that is removed from the XAD-2 in the cleaning process has not been ascertained; however, we believe it is an aromatic substance such as a polymerization component or polymerization solvent.

Reversibility of adsorption

A desirable feature of an adsorbent is that the adsorption process be reversible in nature. Otherwise, sample species cannot easily be recovered after the adsorption step. The results of an adsorption reversibility test for XAD-2 are shown in Table II. After sampling five bottles from an EDT experiment, the solvent was removed by suction and was concentrated to a small volume for counting in those cases where benzo[α]pyrene was the solute. When *n*-hexadecane was the solute the solutions could not be concentrated by evaporation because of its volatility. The ^{14}C -solute on the adsorbent was desorbed by washing the XAD-2 with four portions of toluene or scintillation solution. The total dpms of tracer recovered were compared with the dpms of the initial spike to calculate the percent activity recovered. From the percentage recovery it can be concluded that the adsorption of PAHs (specifically benzo[α]pyrene) onto XAD-2 is a highly reversible process. The percentage recovery values reported here agree very favorably with a value of 97% reported elsewhere⁴ for the recovery of naphthalene from XAD-2 columns in which the solute was initially adsorbed from water. Other percentage recoveries listed in the reference ranged from 94 to 102% for four other representative solutes having amine, carboxylic acid, and phenolic functional groups. To date, the only types of species we have encountered which are difficult to desorb from XAD-2 are some of the dark pigments from cigarette smoke condensate, and pigment residues from water-soluble writing ink. The latter are eventually removed by continued elution with an appropriate solvent. The accumulation of small quantities of these pigments on the adsorbents do not adversely affect their performance in the chromatographic fractionation of cigarette smoke condensate by step-wise gradient elution techniques²⁷.

TABLE II
RECOVERY OF ADSORBED COMPOUNDS FROM XAD-2

| <i>Solute</i> | <i>Solvent</i> | K_D | <i>Activity recovered (%)</i> |
|-------------------------|------------------------------|-------|-------------------------------|
| Benzo[α]pyrene | Ethanol | 155 | 99.06 |
| Benzo[α]pyrene | 50% Ethanol/trimethylpentane | 83.7 | 99.97 |
| Benzo[α]pyrene | 2,2,4-Trimethylpentane | 383 | 98.62 |
| Benzo[α]pyrene | Methanol | 235 | 97.92 |
| <i>n</i> -Hexadecane | Methanol | 40 | 101.8 |

Linear capacity of XAD-2

The linear capacity of an adsorbent has been defined as the weight of sample per gram of adsorbent, W_s , which causes a 10% reduction of the specific retention volume (or K_D) of some standard compound relative to the constant retention volume observed at lower W_s values²⁸. The value of the linear capacity of an adsorbent is that it establishes the maximum amount of sample that can be introduced into the column

without causing overloading and operation under non-linear elution conditions. Another consequence of sample load is that the column efficiency (number of theoretical plates, N) begins to decrease rapidly when the maximum linear loading capacity is reached²⁹. In the case of equilibrium experiments operation within the linear capacity region of the adsorbent will give the maximum attainable K_D values at the specified temperature. Figs. 2-4 show the effect of sample size on K_D values for three different solutes. The data in Fig. 2 are reasonably consistent considering that it represents more than one lot of XAD-2, and was obtained with both EDT and EDS (equilibrium distribution using spectrophotometric measurement) methods. The linear extrapolated K_D value of 209 for benzo[α]pyrene gives a maximum linear capacity, $\theta_{0.1}$, of about $1 \cdot 10^{-5}$ g benzo[α]pyrene/g adsorbent. *n*-Hexadecane, which has a lower K_D value than benzo[α]pyrene, gave a larger linear capacity of $5.8 \cdot 10^{-5}$ in methanol, as shown in Fig. 2. The trend of larger linear capacity for solutes with smaller K_D values continues for benzene (see Fig. 3, $K_D = 2.4$) where the maximum linear capacity is about $2.7 \cdot 10^{-4}$. It has not been determined if this observed trend between linear capacity and K_D has general validity. The $\theta_{0.1}$ values reported here for XAD-2 are comparable with a value of $4.4 \cdot 10^{-4}$ estimated for Corasil II, but are 10-100 times lower than a value of $1.4 \cdot 10^{-3}$ estimated for 5-10 μm porous silica³⁰, and are in the vicinity of a value of $5 \cdot 10^{-4}$ estimated for another type of silica³¹. From these comparisons we would conclude that XAD-2 has about the same linear capacity as other adsorbents used in chromatography.

Comparison of different methods to measure K_D values

Since both equilibrium and chromatographic elution (CE) methods were used in this work to characterize the behavior of XAD-2, the K_D values of several PAHs were measured at least in triplicate by three different methods in order to provide data for a comparison of the methods. The data in Table III compares the EDT, EDS and CE methods. In general, the EDT and EDS methods provide comparable results as the K_D values obtained by these methods usually differ by no more

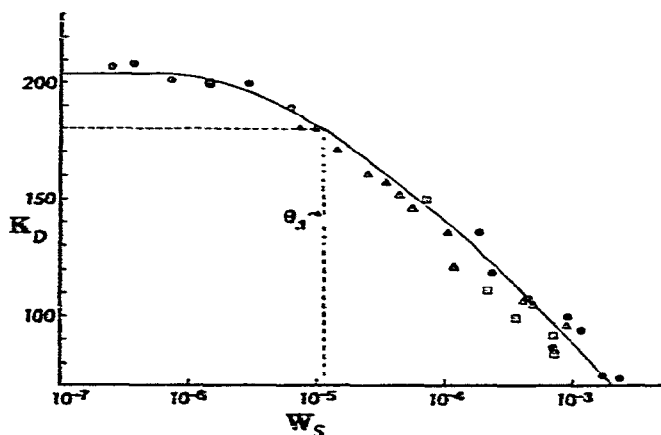


Fig. 2. Linear capacity of XAD-2 measured by adsorption of benzo[α]pyrene from methanol. \odot = EDT data on lot WCRE; \oplus , Δ = EDT data on unknown lot; \square = EDS data on unknown lot. W_s = weight of sample/weight of adsorbent.

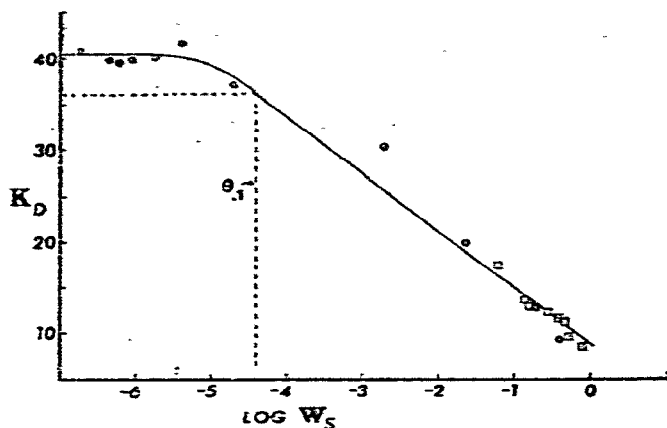


Fig. 3. Linear capacity of XAD-2 measured by adsorption of *n*-hexadecane from methanol. O, □ = EDT data on different lots of XAD-2.

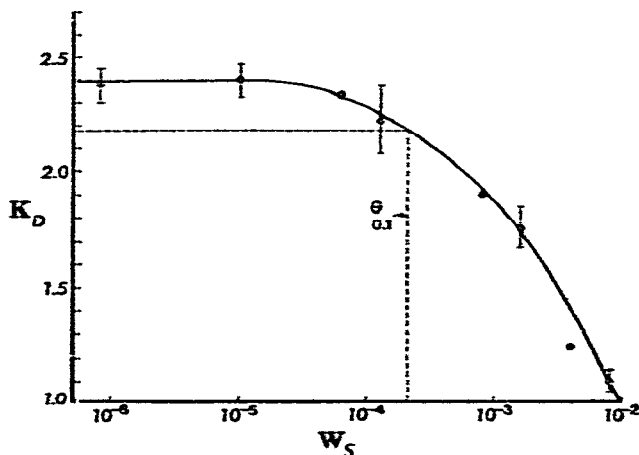


Fig. 4. Linear capacity of XAD-2 measured by chromatographic elution of benzene. Eluent = methanol.

than 10–20%; however, EDT results are consistently larger, probably because EDT measurements are normally made at significantly lower W_s values ($7 \cdot 10^{-6}$ vs. $1 \cdot 10^{-4}$, respectively). The EDT method is convenient to use, is not plagued by the problem of UV background absorbance, and is a very powerful technique for measuring extremely low solute concentrations in systems having large K_D values. All three methods are comparable in the lower K_D region; however, at higher K_D values the equilibrium and chromatographic methods differ by 50% or more. At present, we are not able to account for this gradual divergence shown by the CE and ED methods other than to suggest that use of only a fraction of the adsorbent in a column at any instant by the solute, non-equilibrium conditions in a column, and non-gaussian peaks (especially when K_D is large) cause these differences to be magnified at larger K_D values. Some of the observed scatter can be attributed to the use of different batches of XAD-2; however, it is estimated this would not account for more

than 10–20% of the difference. Previously, some K_D differences have been observed between equilibrium and chromatographic elution methods of measuring K_D values³². In one case chromatographic data for naphthalene whose K_D was 7.8 was about 7% lower than equilibrium data. Other aromatic hydrocarbons whose CE K_D values ranged from 4.8 to 27.5 were 9 to 21% lower than ED values, respectively.

TABLE III

COMPARISON OF K_D VALUES MEASURED BY DIFFERENT METHODS

Average deviations are reported for the K_D values shown in the table.

| Compound | A_s | Equilibrium tracer | Equilibrium spectral | Chromatographic elution ^{***} | Solvent |
|----------------------------|-------|----------------------|-------------------------|--|-------------------|
| Benzene | 6.0 | 4.3 ± 1.4 | — | 2.41 ± 0.08 | Methanol |
| Naphthalene | 8.1 | 9.0 ± 2.4* | 8 ± 2 | 8.27 ± 0.02 | Methanol |
| Phenanthrene | 10.2 | 41 [†] | 34 ± 1 | 24.8 ± 0.8 | Methanol |
| Anthracene | 10.2 | 31 ± 2 ^{**} | — | 28.4 ± 0.6 | Methanol |
| Pyrene | 10.7 | — | 62 ± 2 ^{***} | 26.6 ± 1.4 | Methanol |
| Benz[<i>a</i>]anthracene | 12.3 | 124 ± 1 | 103 ± 15 ^{***} | 54.8 ± 4 | Methanol |
| Perylene | 12.8 | — | 142 ± 4 ^{††} | — | Methanol |
| Benzo[<i>a</i>]pyrene | 12.8 | 230 ± 1 [*] | 187 ± 15 | 93 ± 4 [†] | Methanol |
| Benzo[<i>a</i>]pyrene | 12.8 | 20 ± 2 | 15 ± 1 | — | <i>n</i> -Heptane |
| Benzo[<i>a</i>]pyrene | 12.8 | 8 ± 3 | 11 | — | Acetone |
| Benzo[<i>a</i>]pyrene | 12.8 | 26 ± 2 | 26 ± 4 ^{***} | — | Acetonitrile |

* XAD-2 lot WCRE.

** $W_s = 4.2 \cdot 10^{-5}$.

*** Fluorescence methods used to measure solute concentration.

† $W_s = 2.6 \cdot 10^{-6}$.

†† Another experiment using fluorescence detection gave 148 ± 5 .

††† 24.5 cm × 5.33 mm I.D. column filled with 2.12 g XAD-2, 45–53 μm; $V_0 = 3.27$ ml, obtained with NaNO_3 .

† $W_s = 1.0 \cdot 10^{-5}$ for benzo[*a*]pyrene; W_s ranged up to $7 \cdot 10^{-5}$ for chromatographic elution measurements in order to obtain detectable peaks.

The data in Table III was examined in greater detail to determine if it showed trends that was characteristic of homologous series. Table IV shows the results of fitting each data set to a linear equation, $\log K_D = \log V_a + mA_s$, where $\log V_a$ (the intercept) and m (the slope) were obtained by linear regression analysis. The A_s value of each PAH represents the area covered on the adsorbent surface by an adsorbed sample molecule. The A_s value of unsubstituted hydrocarbons, C_hH_h , can be calculated by the formula $A_s = 6 + 0.8(h - 6) + 0.25(c - h)$; V_a is the volume occupied by an adsorbed monolayer of sample completely covering the surface of the adsorbent and is related to the specific surface area (SA, m^2/g) of the adsorbent by the equation, $V_a = 0.00035 \times \text{SA}$ ³³.

The slope of the line obtained by ED methods is slightly greater than that obtained by the CE method, e.g., the slope windows, defined as $m \pm \sqrt{vs}$, do not quite overlap with one another. One noticeable feature of the data is that the intercepts all overlap, and give calculated SA values that vary from 187 to 350 m^2/g . These calculated values compare favorably with the five values that range from 300–366 m^2/g in Table V.

TABLE IV
RESULTS FROM FITTING K_D DATA TO A LINEAR EQUATION

| | Equilibrium tracer | | Equilibrium spectral | Chromatographic elution |
|--------------------------------------|----------------------|----------------------|----------------------|-------------------------|
| Number data points used | 6 | 25 | 6 | 7 |
| Slope = m | 0.253 | 0.252 | 0.266 | 0.223 |
| Intercept = $\log V_s$ | -0.993 | -1.021 | -1.185 | -0.9114 |
| V_s | 0.102 | 0.0953 | 0.0653 | 0.123 |
| Surface area, calculated, m^2/g | 290 | 272 | 187 | 350 |
| Variance of slope = vs | $2.32 \cdot 10^{-4}$ | $1.83 \cdot 10^{-4}$ | $3.21 \cdot 10^{-4}$ | $1.01 \cdot 10^{-4}$ |
| Variance of intercept = vi | 0.0241 | 0.0148 | 0.0408 | 0.0106 |
| Correlation coefficient | 0.989 | 0.966 | 0.987 | 0.993 |
| Std. dev. of y values | 0.656 | 0.579 | 0.499 | 0.528 |
| Std. dev. of slope = \sqrt{vs} | 0.015 | 0.0135 | 0.018 | 0.010 |
| Std. dev. of intercept = \sqrt{vi} | 0.155 | 0.122 | 0.202 | 0.103 |
| Slope window = $m \pm \sqrt{vs}$ | 0.25 ± 0.02 | 0.25 ± 0.01 | 0.27 ± 0.02 | 0.22 ± 0.01 |
| Intercept window = $i \pm \sqrt{vi}$ | -0.99 ± 0.2 | -1.0 ± 0.1 | -1.2 ± 0.2 | -0.9 ± 0.1 |

TABLE V
PROPERTIES OF XAD-2 AND XAD-4
na = not available.

| Adsorbent | Lot batch I.D. | Surface area m^2/g | Source of data |
|-----------|----------------|----------------------|----------------|
| XAD-2 | na | 300 | Ref. 34 |
| XAD-2 | na | 330 | Ref. 35 |
| XAD-2 | WCRE | 333 | * |
| XAD-2 | 77A | 356 | * |
| XAD-2 | 77C | 366 | * |
| XAD-4 | 77B | 847 | * |
| XAD-4 | na | 750 | Ref. 35 |
| XAD-4 | na | 780 | Ref. 34 |

* BET values obtained as described in Experimental section.

Equilibrium distribution studies in various solvents

Since SVB adsorbents have not been as thoroughly characterized as traditional polar adsorbents (silica and alumina), an extensive EDT investigation was conducted on selected PAHs and *n*-hexadecane, a model aliphatic hydrocarbon, in a wide variety of pure organic solvents. The data in Table VI show the distribution behavior of benz[α]anthracene, benzo[α]pyrene and *n*-hexadecane on three SVB adsorbents, Bio-Beads SM-2 (SM-2), XAD-2, and XAD-4. The general trend for K_D values of benzo[α]pyrene on the three adsorbents is that K_D on SM-2 < K_D on XAD-2 < K_D on XAD-4, in agreement with the surface areas of XAD-2 and XAD-4 (the SA value for SM-2 was not available for comparison). The table reveals some subtle features of aliphatic hydrocarbon (ALH) solvents that are more pronounced with the benzo[α]pyrene/XAD-4 system. Branched hydrocarbons such as 2,2,4-trimethylpentane and isohexanes, and one cyclic hydrocarbon (cyclohexane), give significantly higher K_D values than *n*-hydrocarbons, indicating that branched hydrocarbons are weaker solvents for the elution of PAHs. This trend is more evident upon comparing alcohol

solvents in Table VII, where it is observed that *iso*-, *sec*-, and *tert*-alcohols give significantly larger K_D values than the normal isomer. Aromatic hydrocarbons such as benzene, toluene, and *o*-xylene are the strongest hydrocarbon solvents, which is a manifestation of their ability to strongly interact with SVB copolymers. It has been suggested that the π -electron systems are involved in such interactions between aromatic solvents and the SVB copolymer matrix³⁵. Qualitatively the hydrocarbon solvents would be ranked as follows in the order of increasing solvent strength: 2,2,4-trimethylpentane < cyclohexane < isohexanes < *n*-aliphatic hydrocarbons < aromatic hydrocarbons. This ranking is consistent with the trend from a normal-phase mode of separation to size-exclusion chromatography since the solubility parameters (δ) of hydrocarbon solvents (δ range = 6.85–8.2 for above solvents) are less than SVB copolymers (δ = 8.5, 9.1), which are about the same as aromatic solvents (δ range = 8.8–9.2 for above aromatics)³⁷. Alcohols and other water-miscible solvents (acetonitrile, dimethyl sulfoxide, *N,N*-dimethylformamide, tetrahydrofuran and acetone) that are discussed later have δ values greater than SVB copolymers. These solvents when used either neat, or as components in binary aqueous solutions, extend the chromatographic separation mode of XAD-2 to the reversed-phase category, which has been exploited to accomplish many interesting separations (see references in introductory section). The application of SVB copolymers to various combinations of normal-phase, size-exclusion, and reversed-phase modes of separation has been illustrated recently in two publications^{17,18}.

TABLE VI
DISTRIBUTION CONSTANTS IN HYDROCARBON SOLVENTS

| Solvent | K_D values | | | | |
|------------------------|------------------------------|---------------------------------|------------------------------|------------------------------|---------------------------|
| | Adsorbent | | | | |
| | SM-2 | XAD-2 | XAD-2 | XAD-4 | XAD-2 |
| | Solute | | | | |
| | Benzo[α]- pyrene | Benz[α]- anthracene | Benzo[α]- pyrene | Benzo[α]- pyrene | <i>n</i> -Hexa- decane |
| <i>n</i> -Pentane | 13 | 16 | 26 | 84 | 0 |
| Cyclopentane | 8 | 5 | 13 | 68 | 0 |
| Hexanes | 13 | 14 | 20 | 57 | 0 |
| Isohexanes | 21 | 20 | 37 | 117 | 0 |
| Cyclohexane | 13 | 18 | 40 | 250 | 0 |
| <i>n</i> -Heptane | 14 | 15 | 20 | 67 | 0 |
| <i>n</i> -Nonane | 12 | — | 20 | 59 | 0 |
| 2,2,4-Trimethylpentane | 71 | 200 | 364 | 1380 | 0 |
| Light petroleum | 18 | 13 | 27 | 84 | 0 |
| Benzene | 0 | 0 | 0 | 0 | 0 |
| Toluene | 0 | 0 | 0 | 0 | 0 |
| <i>o</i> -Xylene | 0 | 0 | 0 | 0 | 0 |

The adsorption behavior shown by *n*-hexadecane in Tables VI–VIII provides an interesting comparison of the differences between aliphatic hydrocarbon (ALH) and PAH solutes. The K_D value of 0 for *n*-hexadecane in all solvents shown in the Table VI indicates that *n*-hexadecane does not distinguish any differences among the

TABLE VII
DISTRIBUTION CONSTANTS IN ALCOHOL SOLVENTS

| Solvent | K_D values | | | | |
|-----------------------|--------------------------|------------------------|--------------------------|--------------------------|----------------------|
| | Adsorbent | | | | |
| | SM-2 | XAD-2 | XAD-2 | XAD-4 | XAD-2 |
| | Solute | | | | |
| | Benzo[α]-pyrene | Benz[a]-anthracene | Benzo[α]-pyrene | Benzo[α]-pyrene | <i>n</i> -Hexadecane |
| Methanol | 102 | 123 | 217 | 420 | 42 |
| Ethanol | 57 | 85 | 138 | 330* | 8.0 |
| <i>n</i> -Propanol | 59 | 81 | 138 | 340* | 2.4 |
| Isopropanol | 100 | 202 | 330 | 1200 | 4.5 |
| <i>n</i> -Butanol | 36 | 60 | 77 | 199 | 1.8 |
| <i>sec.</i> -Butanol | 59 | 95 | 156 | 393 | 2.6 |
| Isobutanol | 110 | 209 | 310 | 1230 | 4.3 |
| <i>tert.</i> -Butanol | — | — | 1790 | — | — |

* Average deviations are ± 20 and ± 5 for the two alcohols.

various ALH and aromatic solvents, and leads to the conclusion that PAH and ALH mixtures could be separated by normal-phase chromatography on SVB adsorbents as PAHs would but ALHs would not be retained and are expected to emerge within the column void volume, V_0 . If the column exclusion limits were appropriate, the PAHs could be subsequently fractionated by size-exclusion (gel permeation) techniques, provided the particular SVB packing is sufficiently rigid to be able to tolerate a switch from an aliphatic eluent such as 2,2,4-trimethylpentane to an aromatic eluent such as benzene. Recent work in our laboratory has demonstrated that PAHs and ALHs can be isolated as one fraction from cigarette smoke condensate, then be separated from one another on an XAD-2 column (30×2.0 cm I.D.) using sequential modes of separation that transverse from reversed-phase to normal-phase chromatography; subsequently, the PAHs are eluted from the column with tetrahydrofuran and benzene²⁷. It has not been established to what extent, if any, that size-exclusion separation is involved in fractionating the PAHs in the final step.

Table VII shows K_D data for several common alcohol solvents. Upon comparing the *n*-alkyl alcohols for a given adsorbent and solute it is observed that K_D generally decreases from methanol to butanol, indicating a relative eluotropic strength of: methanol < ethanol < 1-propanol < 1-butanol. This strength gradually increases in the benzo[α]pyrene/XAD-2 system for other alcohols (1-pentanol, $K_D = 59$; 1-heptanol, $K_D = 31$; and 1-octanol, $K_D = 26$) with 1-octanol having a K_D value that is close to that of *n*-aliphatic hydrocarbons. This would suggest that the role of the hydroxyl group in determining solvent strength has been over-shadowed by larger aliphatic chains. A puzzling aspect of the solvent trend in *n*-alkyl alcohols is the near-equivalence in solvent strengths for ethanol and 1-propanol in some solute/adsorbent systems (benzo[α]pyrene/SVB adsorbents) whereas phenanthrene/XAD-2 (methanol, $K_D = 41$; ethanol, $K_D = 35$; 1-propanol, $K_D = 29$), benz[a]anthracene/XAD-2, and *n*-hexadecane/XAD-2 systems show that ethanol is the stronger solvent. Gas chroma-

TABLE VIII
DISTRIBUTION CONSTANTS IN OTHER SOLVENTS

| Solvent | K_D values | | | | |
|---------------------------------------|--------------------------|------------------------------|--------------------------|--------------------------|----------------------|
| | Adsorbent | | | | |
| | SM-2 | XAD-2 | XAD-2 | XAD-4 | XAD-2 |
| | Solute | | | | |
| | Benzo[<i>a</i>]-pyrene | Benzo[<i>a</i>]-anthracene | Benzo[<i>a</i>]-pyrene | Benzo[<i>a</i>]-pyrene | <i>n</i> -Hexadecane |
| Methyl chloride | — | — | 0 | 0 | 0 |
| Chloroform | 0 | 0 | 0 | 1.8 | 0 |
| Carbon tetrachloride | — | — | 10 | 86 | 0 |
| 1,2-Dichloroethane | — | — | 0 | 4.4 | 0 |
| 1,1,1-Trichloroethane | — | — | 4.5 | 42 | 0 |
| 1,1,2-Trichloro-1,2,2-trifluoroethane | — | — | 237 | 1460 | — |
| Tetrahydrofuran | 0 | 0 | 0 | 0 | 0 |
| 1,2-Dimethoxyethane | — | — | 0 | 0 | 0 |
| Diethyl ether | 1.2 | 0 | 2.1 | 6 | 0 |
| Isopropyl ether | 8.4 | 9.5 | 16 | 58 | 0 |
| Acetone | 4 | 6 | 8 | 16 | 2.5 |
| Methyl ethyl ketone | 6 | — | 2.6 | 8 | 1.7 |
| 2-Pentanone | — | — | 2.6 | 5.2 | 0.7 |
| Methyl isobutyl ketone | — | — | 6.0 | 19 | 1.6 |
| Ethyl acetate | 0 | 0 | 0 | 0 | 0 |
| N,N-Dimethylformamide | 1.2 | 2 | 0.82 | 4.3 | 0 |
| Dimethyl sulfoxide | 10 | 9 | 11 | 42 | 89 |
| Acetonitrile | 21 | 15 | 26 | 74 | 9.5 |
| Propylene carbonate | 17 | 30 | 46 | 157 | 94 |

tography analysis of the *n*-alcohol solvents used in benzo[*a*]pyrene/XAD experiments for traces of water revealed approximately 0.08% water present in the alcohols; hence differing amounts of water in the alcohols was not responsible for the nearly equivalent K_D values. Qualitatively the overall solvent strength ranking of the alcohols is: *tert*.-butanol < isopropanol \approx isobutanol < methanol < *sec*.-butanol < ethanol \approx *n*-propanol < *n*-butanol.

Table VIII shows K_D data in ethers, ketones, halogenated hydrocarbons, and several other solvents. It is observed that ethers such as tetrahydrofuran and 1,2-dimethoxyethane (also known as Glyme) are very strong solvents for PAHs, whereas isopropyl ether, having hydrocarbon branching, is significantly weaker as a solvent. The four ketones (acetone, methyl ethyl ketone, 2-pentanone, and methyl isobutyl ketone) are also weaker solvents than tetrahydrofuran or 1,2-dimethoxyethane. The ketones, like previous homologous series, show an increase in solvent strength with increasing hydrocarbon chain length, and also show a reduction in solvent strength with increased branching in the hydrocarbon chain. Halogenated hydrocarbon solvents exhibit a wide range in solvent strength with methylene chloride and chloroform being stronger solvents and 1,1,2-trichloro-1,2,2-trifluoroethane being the weakest solvent for PAHs. The K_D values in the halo-methanes and halo-ethanes suggest that increasing halogenation decreases solvent strength within each series. Thus the solvent

strength would increase as: 1,1,2-trichloro-1,2,2-trifluoroethane < carbon tetrachloride < 1,1,1-trichloroethane < 1,2-dichloroethane < chloroform \approx methylene chloride. The last five solvents in Table VIII show a wide variation in solvent strength, with propylene carbonate being the weakest and ethyl acetate the strongest. Acetonitrile, a common solvent for reversed-phase chromatography on chemically bonded phases such as C_{18} -silica packings, is a much stronger solvent than methanol on SVB adsorbents; however, other water-miscible solvents such as *N,N*-dimethylformamide and dimethyl sulfoxide are even stronger than acetonitrile. *n*-Hexadecane shows the same relative trends in solvent strength within the various series in Table VIII, and in most cases is weakly adsorbed by XAD-2; however, two solvents in which this is an exception are dimethyl sulfoxide and propylene carbonate. In both solvents *n*-hexadecane is more highly adsorbed by XAD-2 than benzo[*a*]pyrene, giving rise to a possible scheme where PAHs could possibly be eluted before ALHs. Since both dimethyl sulfoxide and propylene carbonate are good solvents for the preferential extraction of PAHs relative to ALHs from ALH solvents^{38,39}, a suggested explanation for the lower K_D values of PAHs relative to ALHs on XAD-2 is strong solute-solvent attractions between the π -electrons of a PAH solute and the carbonyl carbon in propylene carbonate or the sulfur atom in dimethyl sulfoxide.

The K_D values of benzo[*a*]pyrene on XAD-2 and XAD-4 can be examined in greater detail to check for internal consistency, to identify possible anomalous behavior, and to test the general applicability of a theory of liquid-solid chromatography (LSC) by utilizing the linear elution adsorption chromatography (LEAC) theory developed by Snyder³³. Initially LEAC theory was applied to the characterization of polar adsorbents such as alumina, silica, and florisil. More recently LEAC theory has been modified⁴⁰ and applied to LSC on modified carbon black, a non-polar, non-specific adsorbent⁴¹. Adopting the terminology of Snyder, the general LEAC equation is:

$$\log K_D = \log V_s + \alpha S^0 - \alpha A_s \epsilon^0 + \Delta_{ess} \quad (1)$$

where α is activity of the adsorbent, S^0 is solute adsorption energy, ϵ^0 is the solvent eluotropic value, a measure of solvent strength, Δ_{ess} is the secondary effect factor which Eon⁴⁰ has related to activity coefficients of species in the system, and other terms in the equation have been defined in a previous section. To compare the K_D values of a solute on two batches of adsorbent that differ only in surface area the general LEAC equation can be written for each adsorbent, and then the two LEAC equations can be subtracted to give:

$$\log K_{D,XAD-2} - \log K_{D,XAD-4} = \log V_{s,XAD-2} - \log V_{s,XAD-4} \quad (2)$$

Substituting the surface area equation from a previous section we have

$$\log K_{D,XAD-2} = \log (SA_{XAD-2}/SA_{XAD-4}) + \log K_{D,XAD-4} \quad (3)$$

The result is that the ratio of K_D values of the solute (when compared in the same solvent) is equal to the SA ratio (SAR) of the two batches of adsorbent. When K_D value pairs in several solvents are to be compared the preceding equation is linear

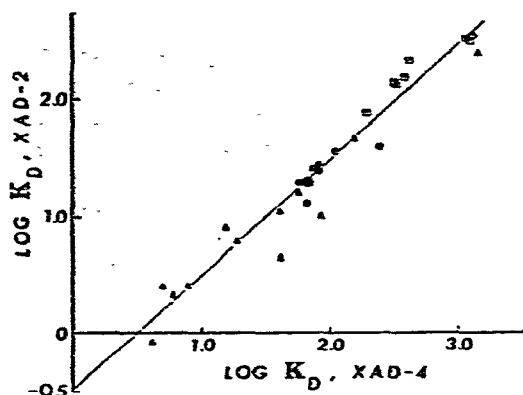


Fig. 5. K_D values of benzo[*a*]pyrene/XAD-2 versus K_D values of benzo[*a*]pyrene/XAD-4 on log-log scale for 29 solvents. ○ = Hydrocarbons (Table VII); □ = alcohols (Table VII); △ = other solvents (Table VIII).

where the slope is unity and intercept is log SAR. The K_D data for the adsorption of benzo[*a*]pyrene by XAD-2 and XAD-4 in 29 of the solvents listed in Tables VI–VIII is shown in Fig. 5. The least squares slope and standard deviation is 0.98 ± 0.04 , which is remarkably close to the theoretical value. The intercept of -0.49 ± 0.09 gives a SAR value of 0.32 ± 0.07 which is close to the SAR range of 0.354 to 0.488 calculated from appropriate combinations of the values given in Table V. The data in Table IX summarizes the results of fitting the equation to the data of each table separately. Only the alcohols (Table VII) show diverging behavior, which is due to fitting both the slope and intercept. When each SAR is calculated individually for the data in Table VII, and then averaged (see last line in Table IX) a more reasonable ratio is obtained.

TABLE IX

APPLICATION OF K_D DATA TO CALCULATE SURFACE AREA RATIO FOR XAD-2 AND XAD-4 AS A TEST OF LEAC THEORY

| | All solvents* (Table VI–VIII) | Hydrocarbon (Table VI) | Alcohols (Table VII) | Others (Table VIII) |
|----------------------------------|----------------------------------|---------------------------|-------------------------|------------------------|
| No. of Points | 29 | 9 | 7 | 13 |
| Slope | 0.98 | 0.91 | 0.71 | 0.87 |
| Standard deviation | ± 0.04 | ± 0.08 | ± 0.09 | ± 0.07 |
| Intercept*** | -0.494 | -0.372 | 0.34 | -0.38 |
| Standard deviation | ± 0.09 | ± 0.18 | ± 0.23 | ± 0.12 |
| Correlation coefficient | 0.97 | 0.96 | 0.95 | 0.96 |
| SAR***, linear regression | 0.320 | 0.425 | 2.16 | 0.417 |
| SAR***, averaging | 0.310 | 0.288 | 0.379 | 0.289 |
| Averaging***, standard deviation | ± 0.105 | ± 0.060 | ± 0.090 | ± 0.125 |

* Solvents whose $K_D = 0$ were not included.

** Calculated by obtaining the SAR for benzo[*a*]pyrene K_D values on XAD-2 and XAD-4 in each solvent, then found the average SAR for all solvents in each table.

*** Non-significant digits are shown only to indicate results of the computations.

Eluotropic scale

The solvent eluotropic value is a measure of solvent strength relative to a reference solvent, and can be shown to represent the adsorption energy of the solvent molecule per unit area (ref. 33, p. 189). Hence, solvents of large eluotropic value strongly bind to the surface of the adsorbent. Eon⁴⁰ has shown that the eluotropic value can be interpreted to represent the relative interfacial tension that exists between solvent and adsorbent. Thus the eluotropic value for a given solvent-adsorbent pair is invariant. In some instances it appears that one solvent may change in relative strength and become stronger than another, as in binary aqueous solutions where K_D versus solvent composition curves may cross one another. One explanation for such behavior is that secondary solvent effects such as specific solute-solvent or solvent 1-solvent 2 interactions have become significant, and must be considered to adequately account for the distribution behavior.

A semi-quantitative eluotropic scale for SVB adsorbents can be developed from the application of the LEAC theory. As a first approximation eluotropic values of solvents can be established by measuring the K_D value of a solute on a given adsorbent in two different solvents, where K_1 and K_2 are the K_D values in the weaker and stronger solvents, respectively (ref. 33, p. 190). Neglecting secondary effects, *e.g.*, assuming $\Delta_{\text{ess}} = 0$, it readily can be shown that

$$\varepsilon_2^0 - \varepsilon_1^0 = \left(\frac{\log(K_1/K_2)}{\alpha A_s} \right)$$

For clean XAD adsorbent batches we will assume $\alpha = 1$, as water does not deactivate the adsorbent surface; however, it is conceivable that strongly adsorbing species could deactivate the copolymer by adsorbing on the surface and blocking available adsorption sites. A batch of adsorbent that is *not* thoroughly cleaned before use would be analogous to a polar adsorbent in which the degree of deactivation was not controlled, as it is the usual practice to activate (clean) the adsorbent by heating prior to a controlled deactivation step.

The K_D value of benzo[α]pyrene in methanol on XAD-2 will be chosen as the reference point to establish an eluotropic scale for SVB adsorbents, and the ε^0 value of methanol will be assigned as 0. A similar approach was used in an investigation of carbon black where an eluotropic scale was developed for fourteen pure solvents after assigning methanol an ε^0 value of 0⁴¹. Benzo[α]pyrene was chosen as a reference solute in the present study because its degree of adsorption (range of K_D values) varies sufficiently to show differences in the various solvents. A solute such as *n*-hexadecane would not be acceptable as it does not distinguish any differences among the aliphatic and aromatic hydrocarbon solvents. In addition, benzo[α]pyrene as a reference solute is not likely to be involved in as many specific interactions (such as hydrogen bonding) as reference solutes having polar functional groups; however, it is recognized that benzo[α]pyrene could possibly associate with some solvents by π - π interactions or be involved in molecular complex formation and thus give erroneous ε^0 values. Such cases may arise in the future as SVB adsorbents are characterized in greater detail. While calculating the ε^0 values given in Table X, it was observed that the values obtained for the benzo[α]pyrene/XAD-4 system were 0.01 to 0.02 units lower than for the other three systems. Two systems, benz[α]anthracene/XAD-2 and benzo[α]-

pyrene/XAD-2 which utilizes the same adsorbent were chosen for the average ϵ^0 scale shown in Table X, where the solvents are ranked in order of increasing ϵ^0 value. The difference between the weakest and strongest pure solvent that can be assigned an eluotropic value is about 0.25, only about one-fifth the range Snyder obtained for

TABLE X

ELUOTROPIC SCALE FOR VARIOUS SOLVENTS ON AMBERLITE XAD-2 AND XAD-4, AND BIO-BEADS SM-2

| <i>Solvent</i> * | ϵ^{0**} , XAD-2 | ϵ^{0***} , SVB |
|---------------------------------------|-----------------------------|----------------------------|
| <i>tert.</i> -Butanol | -0.072 | -0.072 |
| 2,2,4-Trimethylpentane | -0.018 | -0.022 |
| Isopropanol | -0.016 | -0.017 |
| Isobutanol | -0.016 | -0.018 |
| 1,1,2-Trichloro-1,2,2-trifluoroethane | -0.003 | -0.023 |
| Methanol | 0 | 0 |
| <i>sec.</i> -Butanol | 0.010 | 0.010 |
| Ethanol | 0.014 | 0.017 |
| <i>n</i> -Propanol | 0.015 | 0.014 |
| <i>n</i> -Butanol | 0.030 | 0.030 |
| Propylene carbonate | 0.052 | 0.049 |
| Isohexanes | 0.062 | 0.055 |
| Cyclohexane | 0.063 | 0.053 |
| <i>n</i> -Nonane | 0.067 | 0.068 |
| <i>n</i> -Pentane | 0.072 | 0.067 |
| Acetonitrile | 0.072 | 0.065 |
| Light petroleum | 0.075 | 0.066 |
| <i>n</i> -Heptane | 0.078 | 0.071 |
| Hexanes | 0.079 | 0.074 |
| Isopropyl ether | 0.089 | 0.083 |
| Dimethyl sulfoxide | 0.097 | 0.089 |
| Carbon tetrachloride | 0.104 | 0.079 |
| Cyclohexane | 0.104 | 0.088 |
| Acetone | 0.105 | 0.089 |
| Methyl isobutyl ketone | 0.122 | 0.144 |
| 1,1,1-Trichloroethane | 0.132 | 0.105 |
| Methyl ethyl ketone | 0.150 | 0.127 |
| 2-Pentanone | 0.150 | 0.150 |
| Diethyl ether | 0.157 | 0.151 |
| N,N-Dimethylformamide | 0.167 | 0.160 |
| 1,2-Dichloroethane | Large | 0.155 |
| Chloroform | Large | 0.185 |
| Methylene chloride | Large | Large |
| Ethyl acetate | Large | Large |
| Tetrahydrofuran | Large | Large |
| 1,2-Dimethoxyethane | Large | Large |
| Benzene | Large | Large |
| Toluene | Large | Large |
| <i>o</i> -Xylene | Large | Large |

* Solvents are ranked in order of increasing strength using the ϵ^0 values in the second column.

** An average calculated from the individual values obtained on the benz[*a*]anthracene/XAD-2 and benzo[*a*]pyrene/XAD-2 systems.

*** An average obtained from the four values calculated for the three adsorbents SM-2, XAD-2, and XAD-4.

alumina (ref. 33, see p. 194); however, the range observed here for SVB adsorbents is about the same as that obtained by Colin *et al.*⁴¹ for graphitized carbon black. The range for SVB adsorbents (as well as reversed-phase C_{18} sorbents, and supposedly carbon black) can be increased significantly by using binary solvent mixtures of water (the weakest eluent for SVB adsorbents) and water-miscible organic solvents such as alcohols, tetrahydrofuran, acetone, *N,N*-dimethylformamide, dimethyl sulfoxide, and acetonitrile. The addition of water decreases the solvent strength to -0.2 or more negative values.

The eluotropic ranking of solvents obtained in this work can be compared with solvent strength rankings derived from the results of other investigations on SVB adsorbents. For example, the k' values reported by Mori (ref. 16, see Table I) for three PAHs and four alkyl benzenes on Shodex Hp-225 were used to calculate average ϵ^0 values for ethanol ($\epsilon^0 = 0.013$), cyclohexane ($\epsilon^0 = 0.067$), hexane ($\epsilon^0 = 0.068$), and chloroform ($\epsilon^0 = \text{large}$). These values agree reasonably well with the semi-quantitative values reported in Table X for XAD-2, and are used to rank the solvents as shown in Table XI. Distribution data of 4-chloroaniline on XAD-2 reported by Chu and Pietrzyk¹² for 10% solutions of organic solvent in 0.1 *F* NaOH permit a relative solvent ranking of methanol < ethanol < acetonitrile < 1-propanol. Similar data for 10% solutions in 0.1 *F* HCl show a reversal between acetonitrile and 1-propanol. Another study by Pietrzyk and Chu¹¹ of K_D values of *p*-chlorophenol on XAD-2 in binary solvent mixtures gives a relative ranking of methanol < methyl cellosolve < ethanol < 1-propanol < acetonitrile < dioxane < acetone. Moreover, Fig. 2 in ref. 11 shows a cross-over effect in aqueous solutions where one organic modifier may change in apparent solvent strength ranking with another organic solvent. This was observed for the pairs methyl cellosolve-ethanol and dioxane-acetonitrile. Other solvent rankings established in the above study for non-polar type solutes were: ethanol < heptane < ethyl acetate ($\epsilon^0 = \text{large}$) and methylene chloride ($\epsilon^0 = \text{large}$). Earlier work⁴² that established the regeneration efficiency of SVB adsorbents gives a relative solvent ranking of methanol < ethanol < propanol < acetone, which is consistent with our results in Table X.

Table XI provides a comparison of eluotropic scales of three non-polar adsorbents, SVB, octyl and octadecyl chemically bonded adsorbents, and graphitized carbon black. A qualitative type of solvent eluotropic trend generally shown by the various adsorbents is: water < alcohols \approx acetonitrile < aliphatic hydrocarbons < ethers < aromatic solvents, which tends to verify the adage that solvent strength on non-polar adsorbents increases from small polar to large non-polar solvents³³. The similarities shown by the various adsorbents is remarkable when it is considered that some of the scales (Permaphase ODS, and LiChrosorb RP-8) were developed from retention data obtained with solvent mixtures.

A potential application of the eluotropic scale presented in Table X would be its use as a guide in selecting solvents for separation on SVB adsorbents. The eluotropic table not only indicates overall differences in solvent strength but also shows the effect of branching in the hydrocarbon chain, and more qualitatively the effect that the functional group has on solvent strength (alcohol < ether \approx ketone < halogen < aromatic). A consideration of these factors could lead to the selection of eluents that would give unusual and/or selective separations. As one example, three of the solvents which contain isopropyl groups (2,2,4-trimethylpentane, isopropanol,

and isobutanol) are very close in solvent strength, yet another solvent (isopropyl ether) containing the isopropyl group is much stronger, suggesting that the ether group behaves quite differently than the alcohol group. The separation of PAHs from ALHs with 2,2,4-trimethylpentane has been cited previously as one application of fundamental K_D studies and the eluotropic scale. Since many of the separations on SVB adsorbents utilize the reversed-phase mode of operation with aqueous binary mixtures, the solvent ranking in Table X also establishes the relative strength of several water-miscible solvents that can be used as the organic component in binary mixtures. The relative ranking of some of the solvents is: 2-propanol < methanol < ethanol \approx 1-propanol < 1-butanol < acetonitrile < dimethyl sulfoxide < acetone < methyl ethyl ketone < N,N-dimethyl-formamide < tetrahydrofuran. Unpublished work in our laboratory with binary aqueous mixtures has verified this general ranking.

CONCLUSION

XAD-2 as an adsorbent displays reversibility for the adsorption of PAHs, and has a linear sample capacity that is equal to that of other adsorbents. Distribution behavior on XAD-2 can be described using the theory of linear elution chromatography. Consequently a solvent eluotropic scale has been developed as a first step in applying this theory to the characterization of SVB adsorbents in greater detail. Presently we are investigating the adsorption of PAHs by XAD-2 from aqueous binary solutions in order to establish the eluotropic strength of water. A second step in developing LEAC theory for SVB adsorbents will be to assign adsorption energies to a variety of functional groups so that S^0 can be calculated for various solutes. Work in this direction with homologous series is in progress.

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