NOBLE, DIATOMIC AND ALIPHATIC GAS ANALYSIS BY AQUEOUS HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Dissolved non-polar gases (five noble, three diatomic, and four unbranched aliphatic hydrocarbon gases) were resolved by aqueous high-performance liquid chromatography using an ion-moderated partition chromatography column (Bio-Rad HPX-87P) originally designed for monosaccharide analysis. The dissolved-gas peaks, which were detected and quantitated by high-sensitivity refractive-index detection, are eluted at 25°C in the order Ne, He, N_2, H_2, O_2, Ar, CH_4, Kr, Xe, C_2H_6, C_3H_8, n-C_4H_{10}. Based on the general elution order, and on the temperature dependences (4–72°C) of elution times for the individual gases, a mixed chromatographic mechanism is proposed, in which separation of the gases results from a mixture of size-exclusion, ion–ion-induced dipole, and classical hydrophobic effects, plus a second type of "hydrophobic" effect involving binding of the clathrate hydrate cages surrounding non-polar solutes to similar cages formed around hydrocarbon portions of the column packing material.

INTRODUCTION

Our laboratory routinely monitors enzymatic digestion of cellulosic biomass through analysis by high-performance liquid chromatography (HPLC) of the soluble sugars produced, using a Bio-Rad HPX-87P carbohydrate analysis column with deionized water as eluent and with refractive index (RI) detection. During these analyses we observed baseline perturbations in the form of negative RI peaks in the glucose–xylose–galactose region of the chromatograms. A few simple experiments revealed that the occurrence of the negative peaks was related to the entrainment of air in the injected sample. Sample injections known to have contained actual air bubbles resulted in unusually large negative peaks, whereas injection of water degassed by boiling resulted in positive RI peaks at the same positions on the chromatogram. In this latter case, the sample injected apparently contained less dissolved air than did the mobile phase, since although the filtered, deionized water used for the mobile phase had been degassed prior to being placed in the solvent reservoir, it had not been maintained rigorously gas free after that time.

It was not especially surprising that differences in the dissolved-gas levels of aqueous mobile phase and injected aqueous sample should result in peaks detectable by RI monitoring, since the solution of gas in water is known to result in a decrease in the density of the water[1]. What was remarkable, however, was that entrapment of air in the injected sample resulted in not one, but two, well-defined, reproducible, and reasonably well-resolved peaks. Since the ratio of the areas of the two peaks was approximately 4:1, it occurred to us that the column might be separating nitrogen and oxygen, which are present in the atmosphere at approximately the same ratio. Preliminary experiments involving purging of samples with helium and nitrogen confirmed that this was indeed the case.

A number of studies have dealt with problems such as bubble related noise, baseline drift, and reduction in detector response caused in liquid chromatography (LC) and HPLC detection by the presence in the mobile phase of dissolved gases in general[2] and oxygen in particular[2–5]. To the best of our knowledge, however, there have been no reports of the separations of dissolved permanent gases by aqueous chromatography.

The observation that individual gases were separated by the column meant that the phenomenon of the negative peaks was of considerable interest in itself, in addition to being of practical importance in connection with analysis of monosaccharide and other solutes conveniently followed using RI detection. The practical importance of the gas peaks is of course that, with the advent of high-sensitivity RI detectors capable of being operated usefully at full-scale expansions of $0.25 \cdot 10^{-5}$ RI units (RIU) or even $0.125 \cdot 10^{-5}$ RIU, baseline perturbations that were mere annoyances in chromatography using less sensitive detection now become a major factor in determining the lower limit of quantitation for certain solutes. In the system we use for monosaccharide analysis, the solutes the quantitations of which are most interfered with are xylose and galactose, important constituents of fibrous biomass. The separation of three permanent gases (including helium) that do not react with water and are relatively inert with respect to the column packing material is also of considerable interest in terms of chromatographic theory. In order to determine whether the separation was general for dissolved permanent gases, we designed and had fabricated a solvent reservoir that maintains the mobile phase rigorously gas-free, thus giving a consistent baseline for gas-peak analysis, and then proceeded with determination of the chromatographic characteristics of three diatomic, five noble, and four hydrocarbon gases. The results demonstrate that aqueous HPLC can be used to separate and quantitate dissolved gases. A rationale is presented which correlates separation of the gases with known molecular properties of the solutes.

EXPERIMENTAL

Instrumentation

The chromatographic system used in these experiments consisted of a Beckman Model 100 A pump delivering eluent (deionized, degassed water) through a Beckman/Rheodyne Model 210 injection valve, to a Bio-Rad carbohydrate analysis precolumn and lead-loaded (Aminex HPX-87P) carbohydrate analysis column (300 × 7.8 mm). Column and precolumn were water-jacketed and controlled at various operating temperatures from 4°C to 72°C by a Forma Model 2006 refrigerated water

circulator. Detection of peaks was by means of a Hewlett-Packard high-sensitivity RI detector (Erma Instruments) used routinely at attenuations of 1.0 to 0.25 RIU per 10-mV full-scale output. Data were collected using a Shimadzu Model CR 1-A integrating recorder. A 0.250-ml sample loop was used for all injections. Gastight® (Hamilton) syringes and Mininert® syringe valves were purchased from Alltech.

Thoroughly degassed eluent (deionized water) was provided by a Pyrex reservoir (custom-fabricated from a 2000-ml suction flask) that permitted continuous gentle boiling of the eluent, with steam release through a constricted vent to prevent back-diffusion of air into the reservoir flask. Degassed water was pumped from the reservoir through stainless-steel tubing introduced through a custom side-arm; this design left the neck of the flask itself available for replenishing the water supply by adding filtered and degassed (boiled) water during extended chromatographic runs. The tubing that carried the eluent from reservoir to pump inlet was of stainless steel, Swage-Lok-connected, to avoid problems due to the oxygen- and nitrogen-permeability of tubing formed of PTFE or other flexible materials commonly used for HPLC eluent inlet lines.

Materials

Nitrogen, oxygen, helium, argon and hydrogen (ultra-high-purity, >99.999%), neon (research grade, >99.998%), ethane (C.P., 99.2%), propane and butane (instrument grade, 99.5%) were all purchased in high-pressure steel cylinders from Scientific Gas Products. Methane (>99.99%) was obtained from Liquid Carbonic. Krypton and xenon (both 99.995%) were purchased in 1-l glass flasks from Scott Specialty Gases. The deuterium oxide used as a marker for total column volume was from Sigma.

Sample preparation for retention time studies

Solutions of all gases, except krypton, xenon and air, were prepared simply by bubbling the gas through a small quantity of Millipore-filtered deionized water. Samples of krypton and xenon were prepared by withdrawing 4 ml of the gas from the septum-capped glass vial into a 10-ml Gastight syringe fitted with a Mininert syringe valve and a 6-in. 20-gauge septum-taper needle, and containing 2 ml of deionized, degassed water. [Degassed water (4 ml) had been injected immediately before from the syringe into the flask to give the flask a slight overpressure.] The valve-sealed syringe was then attached to a laboratory rotator for 20 min to provide thorough equilibration of gas and water, after which the injection was made directly from the syringe. Samples of deionized, filtered water saturated with air were prepared simply by vortexing the water sample in contact with the atmosphere.

Standards for quantitation of dissolved gases

The apparatus shown schematically in Fig. 1 was used to prepare, simultaneously and separately, supplies of deionized water saturated at 25°C and ambient pressure with either helium (G_1) or argon (G_2). Each gas was first saturated with water vapor by bubbling through vigorously stirred water in a 250-ml flask containing a filter-paper wick, and then bubbled into the sample preparation flask, which was also vigorously stirred with a PTFE-coated magnetic bar. Both of the water-saturating chambers and both of the sample preparation chambers were maintained at
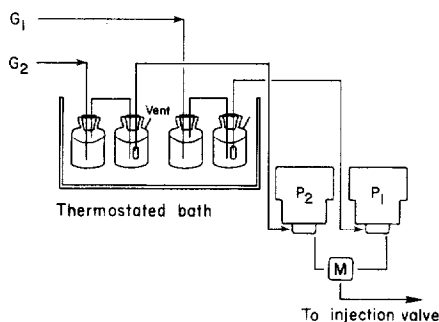
Fig. 1. Apparatus used to generate standard solutions for quantitation of dissolved gases. Aqueous solutions of two different gases in varying ratios are prepared by adjusting the relative rates at which two HPLC pumps (P_1 and P_2) deliver saturated (25°C, 1 atm) solutions of each of two gases (G_1 and G_2) to the mixing chamber M. (See text for further details.)

25°C in a water bath; both sample preparation chambers were vented to the atmosphere through water traps (not shown) with 2-cm heads of water. Gas-saturated water was withdrawn from each sample vessel via stainless-steel tubing (1/8-in. O.D.) by one of two HPLC pumps, P_1 and P_2 (Waters Assoc., Model 6000A solvent delivery systems). The output from pump P_1 was connected to the mixing chamber at the outlet of pump P_2. The combined output of the two pumps was connected directly to the injection valve of the HPLC column through 4 ft. of 0.010-in. I.D. stainless-steel tubing. Samples of water at a variety of precisely-known degrees of saturation (with respect to a solution saturated with each gas at 25°C, ambient pressure) were prepared by varying the pumping speed of the two pumps (total flow-rate 1.0 ml/min). The mixing chamber and sample loop were flushed for a minimum of 6 min at each pump-speed ratio before the sample was injected onto the column. Gas saturation of the water in each sample preparation chamber was confirmed by observation of peaks of constant size for samples prepared with only one sample-mixing pump running, and injected at 15-min intervals over a period of an hour. For the gas-quantitation studies, the column operating temperature was 60°C.

RESULTS

Figs. 2–4 illustrate the separation of three series of permanent gases by an HPX-87P (Bio-Rad) carbohydrate analysis column, operated at 60°C with degassed, deionized water as eluent. Fig. 2 shows the separation of the principal constituents of air, which was the initial observation leading to this study. The third diatomic gas used in this study, hydrogen, has a retention time of 31.6 min at 60°C with a 0.3 ml/min flow-rate. Hydrogen (not shown) therefore is eluted so shortly after nitrogen that it is not resolved from nitrogen at 60°C and is poorly resolved from oxygen.

Of the five noble gases, four (argon, krypton, xenon, and helium or neon) can be resolved with baseline separation (Fig. 3). Helium and neon however, are eluted so close together that no separate peaks, or even shoulders are obtainable. In the composite chromatographic pattern shown in Fig. 3, the peaks for helium, neon, argon and krypton are negative peaks; that is, they were recorded with the polarity
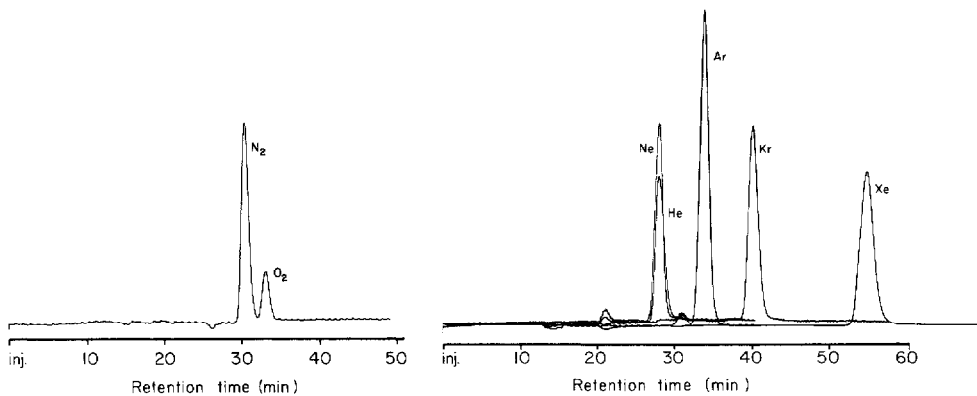
Fig. 2. Chromatography on the HPX-87P of a 0.25-ml sample of deionized water saturated with air at ambient conditions. Flow-rate, 0.3 ml/min; column temperature, 60°C; detector sensitivity, $0.25 \cdot 10^{-5}$ RIU full scale, detector polarity reversed.

Fig. 3. Superimposed chromatograms (HPX-87P) of five noble gases. Operating conditions same as Fig. 2, except that for xenon, polarity was normal and detector sensitivity was $1.0 \cdot 10^{-5}$ RIU full scale.
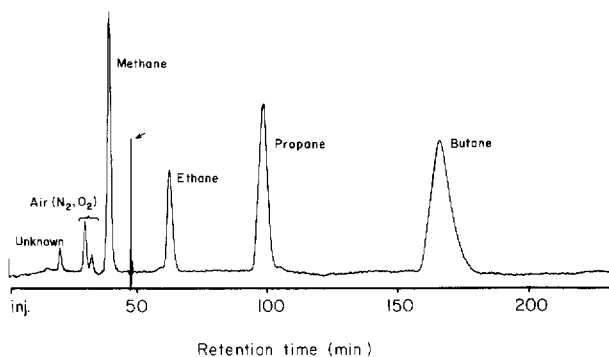

Fig. 4. Co-chromatography of four hydrocarbon gases. Detector polarity was reversed for methane, switched to "normal" for the remaining three gases. The spike at approximately 47 min (arrow) resulted from polarity change and baseline readjustment.

of the RI detector reversed. The xenon chromatogram, however, was monitored using normal detector polarity, and a detector sensitivity setting one-fourth of that used for the other noble gases. Whereas dissolving any of the four lighter noble gases in water results in a solution with RI less than that of pure water, a solution of xenon in water has an RI higher than that of water, as is the case for sugars, fatty acids and others of the more usual RI-detectable solutes. A similar division in terms of effect on RI is seen in Fig. 4, which shows a chromatogram at 60°C of the four n-alkanes that are gases under normal conditions. Here, methane is detected as a negative peak, but the three larger members of the series produce positive RI peaks. Like the noble gases (Fig. 3) the n-alkanes are eluted from the column in order of increasing molecular size. Deuterium oxide, used as an RI-detectable marker for a solute neither adsorbed nor sterically excluded by the column, had a retention time of 34.5 min at 60°C, 0.3 ml/min.

The highly stable, reproducible RI baseline provided by use of thoroughly degassed water as eluent allows the quantitation of dissolved permanent gases by measurement of peak areas. Fig. 5 shows standard curves constructed for helium and argon using as samples mixtures in varying ratios of saturated (25°C, 1 atm) water solutions of the two gases. Samples were mixed using the system shown schematically in Fig. 1 and described in the Experimental section. From the regression lines in Fig. 5 we see that for a given degree of saturation of the sample at 25°C, 1 atm partial pressure, the ratio of peak areas for argon and helium is 2.88. The ratio of the water solubility of argon to that of helium under these conditions is, however, 3.57 (ref. 6). On a molar basis, therefore, the (negative) RI response of argon is only 80.6% of that of helium.
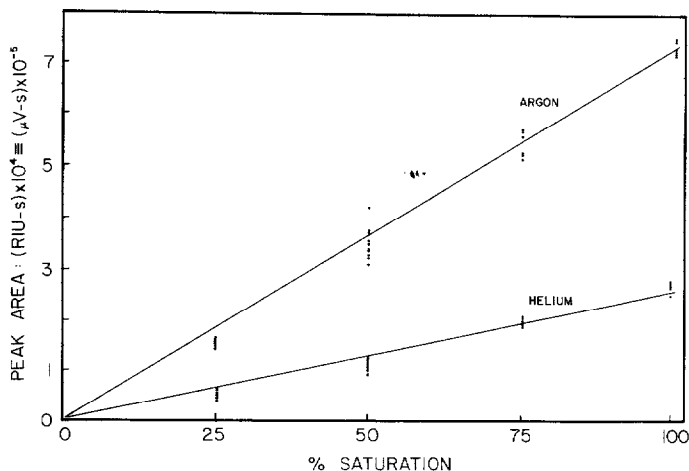

Fig. 5. Standard curves for quantitation of dissolved helium and argon. Samples prepared as described in Experimental section, using the apparatus shown schematically in Fig. 1.

The dependence of elution times on column operating temperature is shown in Figs. 6–8. Both the diatomic gases (Fig. 6) and the "noble", or helium-group gases (Fig. 7) have relatively simple temperature dependences, with elution times uniformly increasing as temperature is increased. The differences in slope between the diatomic and noble gases are more apparent than real, arising from the fact that, for the sake of clarity and to facilitate comparisons within the groups, the diatomic and noble gas elution time–temperature profiles have been plotted separately with different abscissa scales. In fact, the temperature dependences of elution for the two groups are quite similar; oxygen essentially co-elutes with argon over the temperature range studied, for instance.

The temperature dependence of elution time for the smallest of the aliphatic series, methane, also does not vary strikingly from that of a noble gas with a similar average elution time; over most of the temperature range, the elution behavior of methane is quite similar to that of krypton. As the number of carbon atoms is increased, the remaining members of the n-alkane series begin to show temperature
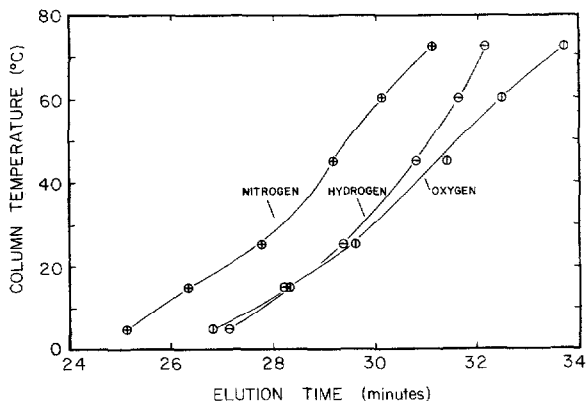
Fig. 6. Dependence of elution time on column operating temperatures for three diatomic gases. Flow-rate, 0.3 ml/min.
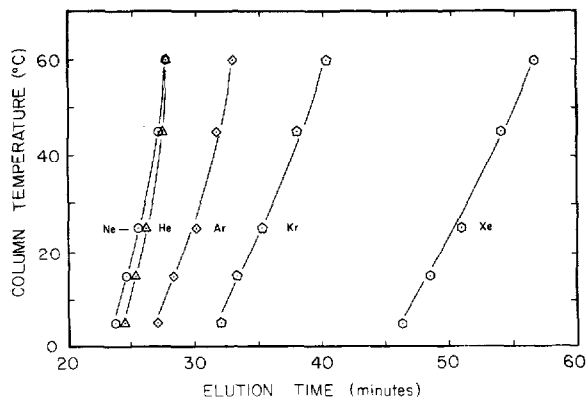

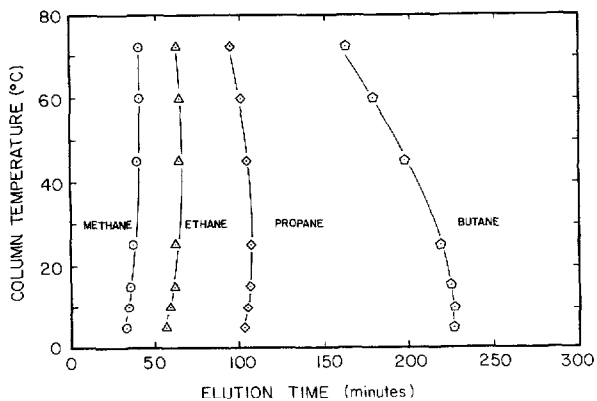Fig. 7. Temperature dependence of elution time for five noble gases. Flow-rate, 0.3 ml/min.

Fig. 8. Temperature dependence of elution time for four paraffin hydrocarbon gases. Flow-rate, 0.3 ml/min.

dependences significantly different from that of the noble and diatomic gases. Although the elution time–temperature profile for ethane (Fig. 8) differs very little from that for methane, it is quite different from that for xenon, a noble gas eluting in the same general region of the chromatogram. The shapes of the curves for propane and butane are even more strikingly different from those for the noble and diatomic gases. As the column temperature is increased from 4°C, the elution time for ethane first increases, up to about 25°C, and then remains relatively constant up to 72°C. The elution time for propane increases with temperature up to approximately 25°C, but then decreases significantly as the temperature is raised further. The elution time for butane is relatively independent of temperature from 4 to 10°C, but above 10°C the elution time decreases markedly as temperature is increased. Although the temperature dependence of butane elution differs noticeably from that of methane, and from that of the noble and diatomic gases, there is no evidence (Figs. 7 and 8) of a sharp break in behavior, but rather a smooth transition from one shape of temperature dependence to another.

Not only are the noble and aliphatic gases eluted in order of increasing molecular size when considered as separate groups, but the molecular size and elution time data pairs for the two groups mesh comfortably to form a smooth progression of increasing elution time with increasing molecular size. (At 60°C helium and neon are co-eluted, but at lower temperatures, at which a difference in elution times can be shown by injections of the two gases separately, the gas that is eluted first is neon, which, though a heavier molecule, has the smaller Van der Waals radius[7–9]).

The diatomic gases considered as a group do not follow the "smallest-first-off" rule obeyed by the other two groups. Nitrogen, the diatomic molecule with the largest molecular volume as indicated by the emperical term b from the Van der Waals equation of state[9] is eluted earliest, while hydrogen, with the smallest volume of the three by a considerable margin, is eluted between nitrogen and oxygen over most of the temperature range. When the elution behavior of the diatomic gases is considered along with that of the noble and aliphatic gases, however, the diatomic molecules, in spite of the contrary ordering among themselves, do fall as a group in the general area of the elution time–temperature diagram they would be expected to occupy on the basis of the behavior of the other two groups.

The chromatographic behavior at 25°C of the solutes studied can be correlated with several of their molecular properties (Table I). In Fig. 9, the mean molecular polarizabilities[10–12] of the noble, diatomic, and aliphatic hydrocarbon gases, when plotted against retention time, fall along a smooth curve with an apparent intercept on the retention time axis at 24.8 min. When the value of this intercept, which corresponds to the elution time of a completely non-polarizable solute, is taken as t_{min} in eqn. 1

$$K_d = \frac{t_R - t_{min}}{t_{2H_2O} - t_{min}} \qquad (1)$$

the cube root[13,14] of the resulting chromatographic partition coefficient, (K_d) is a linear function of the molecular polarizability (Fig. 10). Here, t_R is the retention time for a given solute and t_{2H_2O} is the elution time for a solute neither adsorbed on the column material nor excluded from the pore volume.

Solubility data available in the literature[6,16–19] permit calculation of partition
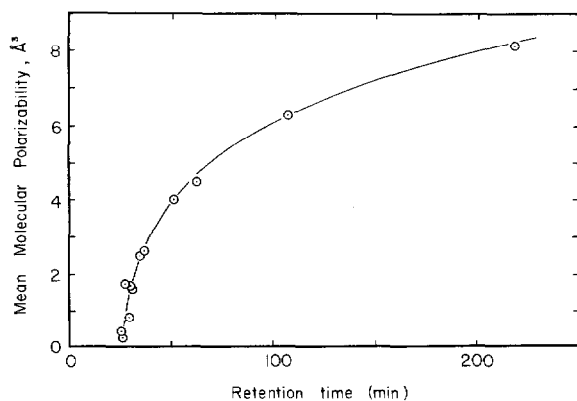
Fig. 9. Relationship between retention time (flow-rate, 0.3 ml/min, 25°C) and the mean molecular polar-izability (refs. 6–8) for all twelve gases studied. In order of increasing polarizability, the gases are: He, Ne, H_2, Ar, O_2, N_2, CH_4, Kr, Xe, C_2H_6, C_3H_8 and n-C_4H_{10}.

TABLE I

CORRELATION OF SEVERAL CHROMATOGRAPHIC RETENTION PARAMETERS FROM EQN. 1* WITH POLARIZABILITY (Å^3) AND MOLAR VOLUME (VAN DER WAALS b, l/mole)

Parameters	Correlation coefficient
Polarizability $vs.$ $K_d^{1/3}$	0.9900
b $vs.$ $\log t_R$	0.9879
b $vs.$ $K_d^{1/3}$	0.9843
Polarizability $vs.$ $\log t_R$	0.9788
b $vs.$ K_d	0.9572**
Polarizability $vs.$ $\log K_d$	0.9510**
b $vs.$ $\log K_d$	0.9207**
Polarizability $vs.$ t_R	0.9001**
Polarizability $vs.$ K_d	0.9001**

* t_{2H_2O} = 34.5 min; t_{min} = 24.8 min.
** Show distinct curvature.

coefficients[15] for the systems hexane–water and octanol–water, for most of the solutes in the present study. The partition coefficient $P_{organic}$ is calculated as the ratio, at 25°C, of solubility in the organic solvent to the solubility in water, with the partial pressure of the solute equal to 1 atm in both cases. P_{hexane}, the partition coefficient calculated with n-hexane as organic reference phase, is plotted against retention time (Fig. 11) for all of the solutes in this study except ethane, propane, and butane. Least-squares linear regression analysis of these data results in the best-fit straight line shown, with correlation coefficient r equal to 0.9631. A similar plot (not shown) with n-octanol as reference phase also shows a reasonably linear relationship (r = 0.9653, Table II). When the data in Fig. 11, for the n-hexane–water system, are replotted with the inclusion of data for ethane, the plot is significantly curved, with an r value of only 0.9315. Apparently the chromatographic retention of the noble and diatomic gases and methane is directly proportional to classical measures of "hydrophobicity", but the relationship breaks down for the higher hydrocarbons.
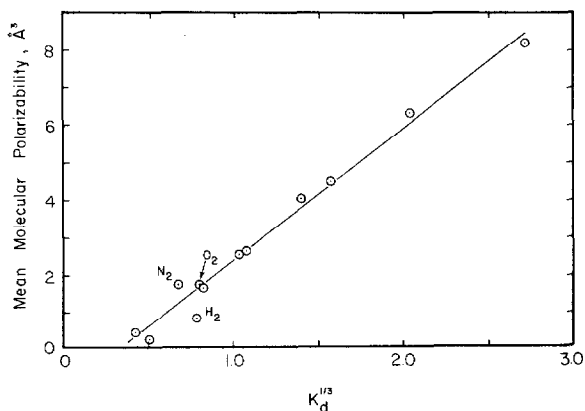
Fig. 10. Linear correlation between mean molecular polarizability and the cube root of the chromatographic partition coefficient K_d. The partition coefficient was calculated using eqn. 1, with the values 24.8 min and 34.8 min used for t_{min} and $t_{2_{H_2O}}$, respectively.

TABLE II

CORRELATIONS OF RETENTION TIME (t_R) WITH ORGANIC–WATER PARTITION COEF-FICIENTS ($P_{organic}$)

Partition coefficient	Correlation coefficient
P_{hexane}	0.9631*
$P_{octanol}$	0.9653**
P_{hexane}	0.9979***
$P_{octanol}$	0.9969§

     * Noble and diatomic gases plus methane.
   ** Noble and diatomic gases (except xenon and hydrogen) plus methane.
 *** Noble gases plus methane.
    § Noble gases (except xenon) plus methane. (Octanol solubility data not available for xenon.)
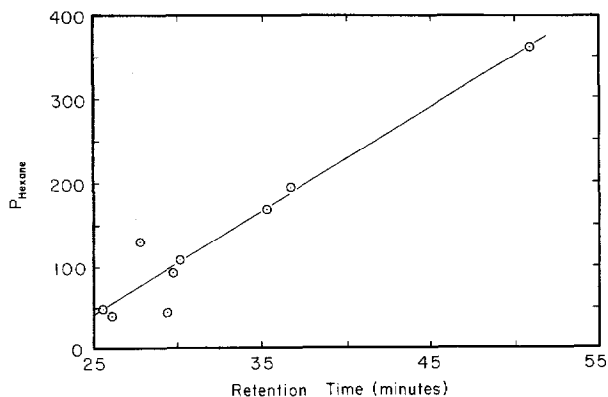

Fig. 11. Relationship between the hexane–water partition coefficient and retention time at 25°C for nine gases (those shown in Figs. 9 and 10, minus ethane, propane and butane). Partition coefficients calculated from solubility data found in refs. 6, 17 and 19.

When only the spherical or roughly spherical gases (*i.e.*, the noble gases plus methane) are considered, plots of molecular polarizability *vs.* $K_d^{1/3}$, and plots of P_{hexane} and $P_{octanol}$ *vs.* retention time all show excellent linearity (Table II). The relationship shown in Fig. 10 between polarizability and $K_d^{1/3}$, is however the only relationship of those tested that exhibits good linearity for the entire collection of gases tested —the five noble gases, three diatomic gases, and four hydrocarbons.

For purposes of comparison with the results obtained with the HPX-87P column, brief studies were made of the chromatography of a small selection of gases on two other HPLC columns, both of which are designed purely for size-exclusion chromatography. With the Toyo Soda G2000PW (polyacrylamide bead) column and deionized, degassed water (0.3 ml/min) as eluent, neon, nitrogen, oxygen, ^2H_2O and methane eluted in the same order as on the HPX-87P. For columns of comparable dimensions (HPX-87P, 300 × 7.8 mm I.D.; G2000PW, 300 × 7.5 mm I.D.), however, resolution was considerably poorer on the G2000PW. Injection of an air-saturated sample produced two relatively broad and poorly-resolved peaks at 41.6 min (nitrogen) and 46 min (oxygen); use of two G2000PW columns in series was required for resolution comparable to that of the sharper peaks obained on the HPX-87P. The silica based-bead Toyo Soda G3000SW (300 × 7.5 mm I.D.) on the other hand, yielded an elution order distinctly different from that of the HPX-87P. Air (73.1 min) and methane (67–68 min) both were eluted after ^2H_2O (51.3 min). The two major components of air were not resolved at all, a single broad peak being obtained.

DISCUSSION

Quantitative applications

In the analysis of gases dissolved in water or aqueous solutions, the aqueous HPLC method described here offers at least one significant advantage with respect to other methods such as gas chromatography (GC). Whereas GC requires the establishment of a head space, equilibration of this space with the liquid, and back calculation from the composition of the head space to that presumed to have existed in the original liquid, the aqueous HPLC method allows direct injection of the liquid sample itself. The preparation of appropriate standards (Fig. 5) will then allow direct determination of each dissolved gas. This method should be especially useful in measurement of the more dilute gas solutions in water, for which direct injection of solution into a GC column would entail injection of unacceptably large amounts of water.

Mechanism of the separation

The separation of dissolved inert gases by the HPX-87P column is not readily explicable in terms of any single retention mechanism; instead, a combination of mechanisms must be invoked to explain the observed patterns. Of the variety of interactions made possible by the chemical nature of the column packing, four seem capable of making significant contributions to the retention of these neutral solutes:

(1) The bound heavy metal ion (Pb^{2+}), which contributes ion–dipole (ligand-exchange) interactions to the separation of monosaccharides, should be capable of ion–ion-induced dipole interactions in the chromatography of non-polar molecules.

(2) The polystyrene backbone contributes the potential for classical hydrophobic interactions.

(3) A second, indirect type of hydrophobic bonding, involving interactions between clathrate–hydrate shells around different non-polar groups or molecules, may be important, especially for the larger aliphatic hydrocarbon solutes tested.

(4) The overall elution pattern, primarily determined by a combination of the three effects listed above, may be overlaid by minor effects due to size exclusion processes resulting from bead porosity. We shall examine these potential retention mechanisms further.

The overall behavior of neutral molecules in this system is consistent with general adsorptive mechanisms. There is some element of size-exclusion, in that some of the solutes are eluted before ^2H_2O, which serves as a marker for V_t, the elution position of a solute that is neither adsorbed on the packing material nor sterically excluded from the pore volume of the beads. The void volume, V_0 cannot be determined with confidence, however, because of the several retention mechanisms that are almost certainly operating. Although size-exclusion effects may contribute to the chromatographic behavior of all of the neutral, non-polar solutes studied, the separation appears to be based for the most part on adsorptive, and not size-exclusion processes. For a series composed of the noble gases and the aliphatic hydrocarbons, the gases are eluted in order of increasing molecular size —the reverse of what would be expected for pure size-exclusion chromatography. In addition, from a theoretical point of view, eight of the twelve gases investigated (the four smaller noble gases, the three diatomic gases, and methane) are believed to exist in aqueous solution primarily as "guest" molecules in the same clathrate–hydrate structure— a "cage" formed by twenty water molecules hydrogen-bonded to each other and located approximately at the vertices of a regular pentagonal dodecahedron[20–23]. This twelve-sided structure will be some 11–12 Å in average diameter and may therefore contribute to the elution of some of the solutes at earlier times than would be predicted in terms of size exclusion, based on the relative Van der Waals dimensions of the solutes with respect to those of ^2H_2O. The enclosure of eight of the gas molecules in a package of relatively invariant size can also be expected to damp out any differences in chromatographic behavior that might have been expected from size-exclusion effects. Xenon, the largest of the noble gases studied, is more likely to be found in a larger, fourteen-sided cage in water[24–26]. The larger hydrocarbon gases are too large to fit into the dodecahedral cage. These have been shown, through X-ray crystallographic, NMR, and low-temperature dielectric relaxation studies[23,27,28] to occupy tetradecahedral (ethane)[23] and hexadecahedral (propane and butane)[23,27,28] cages in crystalline hydrates. Again, the gas molecules that occupy the larger cages (and therefore have larger effective sizes in solution) are found to elute from the column later, not earlier, than those molecules found in smaller cavities (Figs. 3 and 4). The separation of these gases must therefore be explained primarily in terms of effects other than size-exclusion.

Dependence of the retention time on polarizability (Figs. 9 and 10) is consistent with general adsorptive phenomena, in particular with ion–ion-induced dipole interactions. Such interactions, which in this case can be ascribed to either the bound Pb^{2+} ions or to the sulfonic acid groups of the resin, are directly proportional to the polarizability of the neutral molecule, proportional to the square of the charge on the ion, and inversely proportional to the fourth power of the distance, r, between the nuclei[29]. The increase in retention time with increasing temperature that is shown

by the diatomic and noble gases (Figs. 6 and 7) may be due in part to an increase in the strength of such interactions. The decrease in the dielectric constant of water with increasing temperature[30] will increase the strength of electrostatic interactions in general. Probably more important in this case, though, is an effect due to the combination of the strong (r^{-4}) dependence of ion–ion-induced dipole interactions on internuclear distance[29], and to the structuring of water around non-polar solutes[20]. In order for the non-polar solute to approach sufficiently close to the stationary ion for induced-dipole effects to be significant, the clathrate "cage" about the solute must be disrupted. The rate of this disruption will be increased, and the position of the equilibrium[31] between "hydrophobically solvated" and "free" inert solute will be shifted toward "free" solute as the temperature is increased. A certain damping effect on the magnitude of the changes with temperature is to be expected, since the more polarizable solutes (those capable of stronger interactions with the ion) are also those that form the more stable clathrate cages, due to stronger Van der Waals interactions with the water molecules forming the cage[32].

A similar elution order (neon, nitrogen, oxygen, ^2H_2O, methane) was obtained with the Toyo Soda G2000PW column, which is a polyacrylamide-based size-exclusion column not advertized as containing heavy metal ions. These results might seem to indicate that separation of gases by the HPX-87P can be explained without reference to ion–ion-induced dipole interactions. A cautionary note should be interjected here, however. It has recently been pointed out that some commercial silica-gel size-exclusion packing materials contain significant [up to 0.3% (w/w)] contaminations of heavy metals, and that the presence of these metals can profoundly affect chromatographic performance[33,34]. The sand from which the silica-gel was manufactured is cited as the major source of the contaminant, but metal contamination from derivatizing reagents used in manufacture has also been listed as a possible source[34]. This last consideration could apply to resin-based gels as well as to silica-based gels. Since no effort was made to render the G2000PW and G3000SW rigorously metal-free prior to use, metal ion interactions with solutes remain as a possible contributor to separations by these columns.

Fig. 11 suggests another possible contributor to the overall adsorptive retention of non-polar solutes by the HPX-87P packing material. For methane and the noble and diatomic gases, retention time shows a reasonably good linear proportionality to P_{hexane}, the calculated (see figure legend) partition coefficient for the system hexane–water at 25°C and 1 atm partial pressure of the gaseous solute. As was the case in the plot of $K_d^{1/3}$ *versus* retention time, nitrogen and hydrogen are the poorly-fitting solutes. With the elimination of these two data points, the fit for oxygen, methane, and the noble gases becomes excellent, with $r = 0.9978$. (More will be said about the behavior of hydrogen and nitrogen later in the discussion.) For the remaining solutes in Fig. 11, the data are consistent with the existence of significant hydrophobic bonding between the solutes and the aromatic–aliphatic backbone of the resin beads. According to this idea, the solutes leave the aqueous phase and are "dissolved", with direct Van der Waals contact between solute and resin material, in the polystyrene–divinylbenzene copolymer. The possibility of reversed-phase partitioning by this type of column packing has been noted previously in connection with separations of fatty acids by Jupille *et al.*[35].

The linear relationship of retention time and P_{hexane} breaks down for the ali-

phatics larger than methane. The addition of data for ethane to that plotted in Fig. 11 would result in a distinct upward curve of the plot to the right of xenon; the retention times for the larger aliphatics do not increase with P_{hexane} in proportion to the increases seen for the smaller gases. This is understandable since the solubilities of the paraffin hydrocarbons increase very rapidly as the solute becomes more similar to hexane. Hexane is simply too similar to the aliphatic solutes to serve as a good model, with respect to these solutes, of a "hydrophobic milieu" in general, or of the styrene–divinylbenzene copolymer in particular.

The strengths of "hydrophobic" interactions in aqueous solution have been shown to increase with increasing temperature[20,36,37]; the increased retention times shown at higher temperatures by the diatomic and noble gases are therefore quite consistent with a substantial contribution of such binding to the retention mechanism. One aspect of the temperature dependence of inert-gas chromatography on the HPX-87P column that is not, however, explained by the interactions described so far is the temperature dependence of the elutions of n-butane and n-propane, and to a lesser extent, that of ethane. As the column operating temperature is raised from 4°C, methane, ethane, and propane first show increasing retention times, but above 25°C, an opposing (retention-time shortening) tendency effectively halts the lengthening of retention time for methane, and actually causes the retention times for ethane and propane to shorten as column temperature is increased further. This latter temperature effect is even more pronounced in the case of butane, to the extent that even at 4–10°C, there is no interval over which retention time clearly increases with temperature. Instead, as temperature is increased, the retention time for butane decreases steadily from its maximum at 4–10°C to a retention time at 72°C that is only 72% as long as that at the lowest temperatures studied. This type of temperature dependence is not predicted on the basis of either of the two adsorptive mechanisms proposed so far; both ion–ion-induced dipole interactions and classical hydrophobic bonding would be expected to increase in strength with increasing temperature[20,30,38].

The temperature dependence of butane elution, and the similar, though less pronounced "anomalous" behavior of the other aliphatics can be explained on the basis of a second type of "hydrophobic bonding". Stillinger[21] has proposed that at least part of "hydrophobic bonding", may be due to the tendency of clathrate "cages", whether occupied by a "guest" molecule or empty, to share edges or faces[21,22,39]. Whereas the type of hydrophobic bonding discussed earlier involves direct solute–solute (Van der Waals) contact and interaction, this second type of bonding is indirect, involving water–water interactions between molecules in the cages around the two solute molecules. Solute molecules "bonded" in this fashion inhabit adjacent, face-sharing and mutually-stabilizing clathrate structures.

Clathrate cages are likely to be formed, not only around isolated non-polar molecules, but also around the hydrocarbon portions of the packing material. Face-sharing interactions between the clathrate sheath around the packing material and the clathrate cages of dissolved gas molecules should result in retention of the gas molecules on the column, with the extent of retention of a given gas depending both on the strength of interactions between clathrate cages and upon the stability of the solute cage itself[31]. It is possible that the longer retention times of the larger hydrocarbons are due to more extensive interaction between the packing-material hydrate

sheath and the larger and less convex[40] sides of the clathrate cages surrounding the larger hydrocarbons. The shortening of the retention times for hydrocarbons at higher temperatures may result from greater disruption of the more extended clathrate structures. The fact that butane retention is far more sensitive to temperature than is retention of the smaller hydrocarbons may simply indicate that the overall retention mechanism for butane has a much larger contribution from clathrate–clathrate interactions, and thus has more to lose when these interactions are disrupted. It may also be that the butane clathrate cage itself is less stable than the cages enclosing the smaller molecules. This last possibility is difficult to evaluate on the basis of available information, but the results of NMR and dielectric relaxation studies[27,28] and phase diagram studies[41–43] of natural gas hydrates provide reason for believing that this may be the case.

The retention model proposed above could with some justification be considered a specific case of the more general liquid–liquid partition effect suggested by Samuelson[44] as the primary retention mechanism in cation-form ion exchange chromatography with aqueous–organic mobile phase. Although water is the only component of the mobile phase here, the formation of the clathrate sheath around hydrophobic portions of the packing results in two distinct water phases —bound, stationary (clathrate) water and mobile, bulk water.

Earlier in the discussion the statement was made that the separation of gases on the HPX-87P will have to be explained on the basis of principles other than those of size-exclusion. While generally true, this statement requires some qualification, particularly in the case of diatomic gases hydrogen, nitrogen and oxygen. In Fig. 10 it can be seen that nitrogen elutes earlier, and hydrogen later, than would be predicted for these two gases on the basis of their polarizabilities as compared with those of the other ten gases studied. One possible explanation for this observation is to be found in the partial molar volumes of the gases in water at 25°C. Oxygen has a partial molar volume in the same range as those of argon and krypton (32.6–33.2 cm^3/mole)[1] with the partial molar volume for helium being slightly smaller (29.7 cm^3/mole)[45]. The partial molar volume of hydrogen (26.7 cm^3/mole)[1] is significantly smaller than that for the oxygen, argon, krypton group, and that of nitrogen (35.7 cm^3/mole) is larger. This ordering of the partial molar volumes is consistent with the supposition that oxygen, argon, and krypton fit comfortably into dodecahedral clathrate cages, while nitrogen may spend at least part of the time in a larger (tetradecahedral) cage. Hydrogen, appears to spend a significant portion of time in a more compact solvation structure which can be thought of as resulting from the collapse of the dodecahedral structure. Both the "late" elution of hydrogen (compared with that predicted by polarizability correlations) and the "early" elution of nitrogen can be rationalized on the basis of size-exclusion effects due to the different effective sizes of these gas molecules and their attendant solvent cages. While the overall chromatographic behavior of the twelve gases is best correlated with molecular polarizabilities or organic–water partition coefficients, these correlations probably are overlaid by some size-exclusion effects. For pairs of gases such as oxygen and nitrogen, in which the molecular polarizabilities are essentially the same (1.70 and 1.73 Å^3, respectively), the size-exclusion effects may well dictate the order of elution.

The foregoing description of a possible mixed mechanism for the separation of dissolved gases by the HPX-87P is summarized in eqn. 2.

$$V_e = V_t - K_{EX}V_p + K_{PO}V_s + K_{HP}V_s + K_{CL}V_s \qquad (2)$$

In eqn. 2 V_e, the measured elution volume for a given solute, is depicted as the sum of five terms that reflect the various retention/exclusion mechanisms discussed above. The first two terms represent size-exclusion phenomena; V_t is the total volume available to the solvent (measured by injection of ^2H_2O in this instance) and is equal to the geometric volume of the column, *minus* the volume actually occupied on a molecular level by the packing material itself (V_s). K_{EX} represents the proportion of the total pore volume (V_p) from which a given solute (gas molecule plus hydrate shell) is sterically excluded. Since for a mechanism involving adsorptive as well as size-exclusion effects, exclusion of a solute from a portion of the pore volume results in denial of access to some of the adsorptive interaction sites, this term also represents losses in adsorptive retention. For this reason, K_{EX} may have values greater than 1.

K_{PO} is proportional to the strength of ion–ion-induced dipole interactions between the solute and packing material Pb^{2+} ions. K_{HP} is proportional to the strength of classical hydrophobic (direct contact) interactions between the solute and the resin hydrocarbon backbone. K_{CL} is a measure of the strength of water–water interactions between the clathrate hydrate shells around the solute molecules and the hydrate sheath around the hydrocarbon portions of the packing material.

The mixed retention mechanism described above is of course rather speculative, and is presented not as a finished analysis but as a starting point for further discussion of the observed separations.

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