High Pressure Gas Chromatography of Nonvolatile Species

Compressed gas is used to cause migration of intractable solutes.

J. Calvin Giddings,
Marcus N. Myers, Lilian McLaren, Roy A. Keller

As gases are subjected to increasing pressures of the order of 1000 atmospheres, their density approaches that of liquids and they acquire solvent properties much like liquids. Hence these gases can be incorporated into a chromatographic system which, unlike gas chromatography, can handle macromolecules and other species of low or negligible volatility.

This system promises three major advantages over liquid chromatography, which presently offers the only means for chromatographing complex molecules. First, solvent power is a sensitive function of pressure and can be quickly and precisely varied throughout the system by simple mechanical means. Such control is a particularly important element in macromolecular separations and is ordinarily achieved by changes in solvent composition, which only slowly reach remote parts of the system. Second, the relatively low viscosity and high diffusivity of dense gases, on theoretical grounds, speeds the separation by as much as tenfold, a not inconsiderable advantage in view of the natural sluggishness of macromolecular liquid chromatography. Third, we can expect to realize to a degree the sensitive detection and precise quantitation of gas chromatography.

The limitations are also important. High pressure instrumentation is complex, expensive, and difficult to handle compared to the simplistic systems typical of liquid chromatography. Leaks occur frequently and explosions must be carefully guarded against. Other problems relate to unsteady flow, injection, and detection. Few of these difficulties are basic; they reflect mainly, as we shall explain, the primitive state of the art in this new area.

Solvent effects in dense gases are relatively neglected but potentially important in several fields. In this paper we emphasize these effects and their utilization in chromatography. We show the results of migration experiments on some 80 polymers and biochemicals at pressures to 2000 atm. We discuss the unique instrumental problems and characteristics of such chromatographic systems with suggestions for new approaches. We also attempt to explain some present observations and anticipate future experimental requirements, particularly in regard to pressure, by applying to these compressed gases the concept of the Hildebrand solubility parameter.

Solubility in "supercritical" gases was frequently observed in the latter half of the last century in the midst of intense activity revolving around the critical state of gases. The observation was first made by Hannay and Hogarth (*1*), within a decade of the discovery of the critical phenomenon by Andrews and the development by van der Waals of his equation of state.

Engineers later became aware of the situation because of the deposition of quartz, amorphous silica, and inorganic salts on the convex sides of steam turbine blades. Geologists have also recognized the importance of compressed gases as vehicles of material transport.

The solubility of solid substances in such gases was reviewed by Rowlinson and Richardson (*2*) and by Booth and Bidwell (*3*). The collected evidence indicates that the solubility of the solid is not discontinuous at the critical point of the solution. The compressed gas seems capable of dissolving solids to about the same extent as a liquid of the same density. The solution may be ionic; for example, Hannay and Hogarth found that an ethanol solution of cobalt chloride above the critical point had essentially the same absorption spectrum as the liquid solution, which indicated the existence of ionic solute in the vapor. For iodine in carbon dioxide, the absorption spectrum more closely resembles that of iodine in solution than that of iodine vapor. In all reported instances of enhanced solubility in a compressed gas the mixture is believed to be a true molecular solution and not a colloidal dispersion. It is more sensible to conceive for this solubility a critical region where there is no clear distinction between liquid and vapor than a critical point which suggests a sharp discontinuity. One may employ the continuous range between dilute gases and liquids and view the phenomenon as one of enhanced volatility when approached from the former and as one of solubility when approached from the latter. Both ultimately depend upon the increase in intermolecular interactions with density.

The feasibility of utilizing this phenomenon in high pressure gas chromatography was first demonstrated by Klesper, Corwin, and Turner (*4*), who verified the enhanced solubility of some porphyrins in dichlorodifluoromethane and monochlorodifluoromethane at pressures up to 136 atm. Increased solubility was not observed with monochlorotrifluoromethane, trifluoromethane, and nitrogen. A column 0.77 meter long containing 33 percent polyethylene on 60–80 Chromosorb W at 110 to 125 atm of dichlorodifluoromethane separated the two porphyrins.

In 1964, in a critique of gas chromatography, Giddings (*5*) suggested the great separating potential to be derived from pressures at about 1000 atm, which would cause the convergence of gas chromatography with classical liquid chromatography. The magnitude of possible equilibrium shifts was later discussed (*6*).

In 1966 Sie, van Beersum, and Rijnders (*7*) reported the separation of C_7 to C_{13} *n*-paraffins in 90 minutes on a column (1 meter long and 3 millimeters in inside diameter) filled with 25 percent by weight squalane on 100/120 mesh Sil-O-Cel with carbon dioxide at 40°C and 78 atm; benzene, toluene, *p*-xylene, and *cis*-decalin were

---

Dr. Giddings is professor, Dr. Myers is assistant research professor, and Dr. McLaren is research associate in the Chemistry Department at the University of Utah. Dr. Keller is head of the Chemistry Department at the State University College, Fredonia, New York.

separated in 130 minutes and toluene, *n*-octane, cumene, and *sec*-butylbenzene in 60 minutes on the same column with carbon dioxide at 49 atm. They also discussed the calculation of the second cross virial coefficients of binary mixtures from their data. They noted that at the lower temperatures solute volatility is decreased, but compensating this are the increased nonideal gas phase interactions at high pressure which enhance volatility. The versatility for the separation of solutes of high molecular weight was demonstrated by the development of "fluid-liquid" (*8*) and "fluid-solid" techniques for polynuclear aromatics, such as the tars. Sie and Rijnders used *n*-pentane at 44 atm and 210°C and isopropanol at 48 atm and 245°C. A noteworthy accomplishment was the separation of 1,2-benzopyrene from 3,4-benzopyrene (*9*), compounds which have widely different carcinogenic properties and occur in particulate air pollutants.

Recently McLaren, Myers, and Giddings (*10*) reported the enhanced solubility and differential migration of various polymers and biomolecules in compressed carbon dioxide at 40°C and 70 to 2000 atm and in ammonia at 140°C and 100 to 340 atm. This work included the migration of species with molecular weights up to 400,000.

In order to reduce the experimental conditions of the different laboratories to a common basis, we have constructed a reduced states curve in which pressure and density are plotted as coordinates (Fig. 1). We have superimposed on this curve the experimental range explored with success by the different groups. We assume that the law of corresponding states will have relevance and that close-lying points on this plot will be related in solvent characterization, even though the actual conditions of temperature and pressure may be far removed from one another. We will return to this approach later because it seems to offer the most immediate hope for making sense out of an immense variety of possible experimental conditions and solute-solvent pairs. Most striking about Fig. 1 is the wide range of conditions already known to yield positive results.

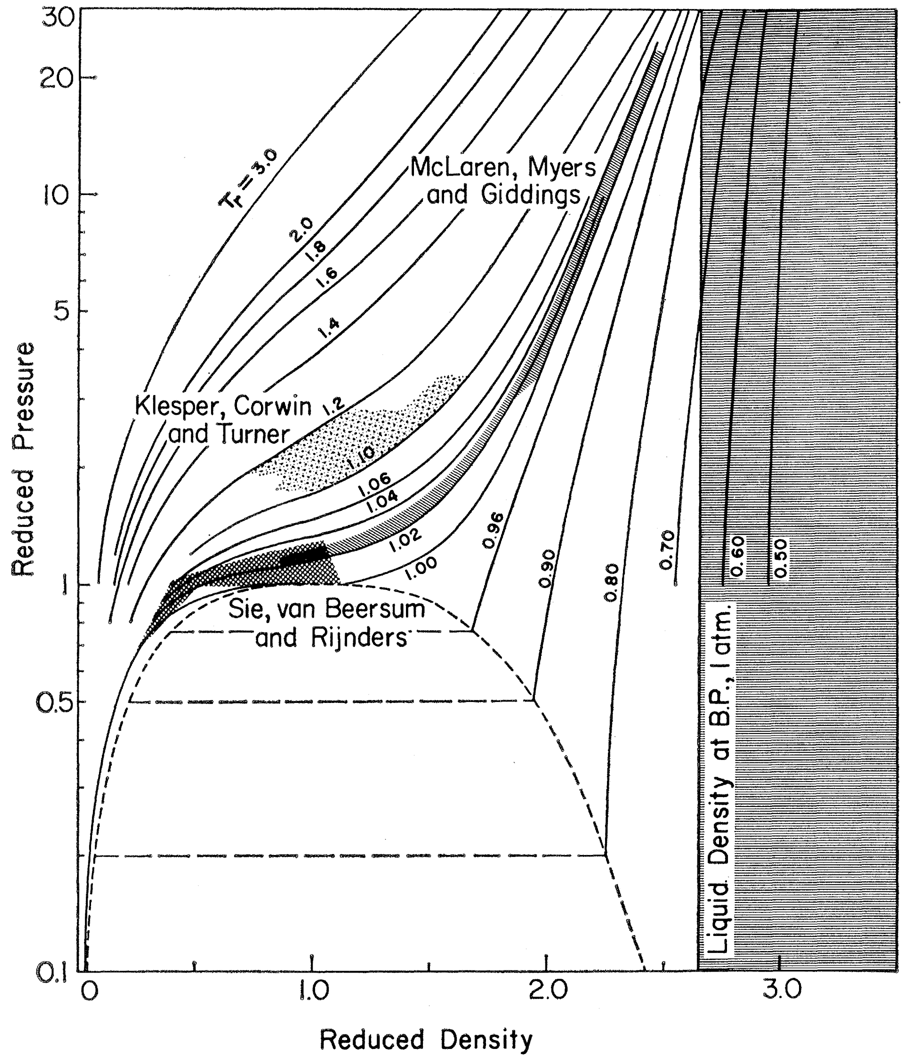

Fig. 1. Known regions of enhanced chromatographic migration on a curve of reduced state.

Instrumental Approaches

Chromatography at high pressures sequentially resembles that at ordinary pressures. We must develop a driving force for flow, however large or small; we must insert a narrow, multicomponent pulse; we must detect (and sometimes collect) the eluted fractions. The high pressure requirement further complicates each of these steps. Commercial apparatus will generate an adequate flow stream to about 2000 atm, although with less than satisfactory reliability; commercial tubing, which is readily available for column construction, is safe to about 3000 atm. Injection and especially detection present unique problems to be discussed later.

More precautions are necessary in the high pressure form of gas chromatography. There is some danger of a violent bursting in some part of the flow chain. Many of the potentially interesting polar gases are highly toxic. The combination of toxicity and volatility necessitates careful shielding and ventilation of the apparatus for such work.

We begin our discussion of the high pressure sequence with a consideration of flow sources. Reliable, adequate, and steady sources for gas flow above normal tank pressure, ~ 170 atm, are uncommon. There are several models of diaphragm compressors and intensifiers and schemes which rely on heating of liquid-filled vessels, but the above three criteria are not fully satisfied with any present apparatus. Our program has relied mainly on an air-driven diaphragm compressor (AMINCO 46-14021) which delivers about 0.1 cubic centimeter per second at pressures as high as 2000 atm. This compressor, coupled with a 1-liter surge tank, is satisfactory except for a reliability problem associated with the air drive transfer system, a defect which the manufacturer intends to correct soon. We have also used two high pressure liquid pumps (Whitney LP-10 and AMINCO 46-13715) whose streams can be heated above the critical point after compression.

The column for high pressure gas chromatography should be generally designed and operated by using the same criteria which optimize other forms of chromatography (*11*). However, there are several special considerations. The column and its fittings must be structurally designed to withstand the pressures involved. Furthermore, dense gases can be excellent solvents and may strip away the kinds of liquid phases of high molecular weight which serve so well for ordinary gas chromatography. Also, the internal column diameter should be small for multiple reasons: (i) small diameter columns possess reduced nonequilibrium effects and thus higher resolution; (ii) less flow is demanded from the high pressure source for a given velocity, and the reduced volume ameliorates the hazards of explosion and toxicity.

Small bore, high pressure tubing is readily available (High Pressure Equipment Company) for column construction. We prefer columns with internal diameter about 0.5 millimeter. With the AMINCO 46-14021 compressor we can achieve linear velocities of 10 centimeters per second and higher in such columns.

High pressure creates unique injection problems. Injection, basically, is the transfer of a small amount of material from the laboratory environment to the column inlet; thus it is a transfer against increasing pressure. Ordinarily such transfer, against pressure increments of only 1 atm or so, can easily be accomplished as by syringe. At a thousand times this pressure one must be concerned with the greater mechanical force needed to drive the sample in and with the increased tendency for the mobile fluid to leak out; the latter problem is greatly aggravated by the necessary presence of moving parts.

We have approached the first problem by letting the high pressure flow source do the work of compression and transfer, and the second by using commercial valves for the dynamic components. This approach, one of many conceivable, is implemented by a simple bypass system whose basic structure has been described (*12*).

Detection Problem

Component detection is the most significant and challenging instrumental problem. We envision three broad approaches to detection, in which the exiting stream of compressed gas can be converted to a dilute gas, liquid, or kept as a dense gas prior to detection.

If the dense fluid is decompressed immediately after exiting from the column and thereby becomes a dilute gas, it can be led into any one of the powerful detector arsenal of gas chromatography. Here, theoretically, one could detect part-per-billion quantities with various degrees of selectivity. The difficulty with this concept stems from the extreme thermodynamic instability, reflected in the observed nonvolatility, of systems of isolated macromolecules in dilute gases. One can expect the immediate onset of molecular association and wall condensation.

The problem is diametrically opposite to that sometimes encountered in gas chromatography sample collection. Here the effluent gas is cooled and the condensed sample recovered. Recovery is less than 100 percent, which reflects the escape of fog particles and molecules with the discarded carrier gas. In theory we can rely on the escaped portion, which is highly vexing to preparative scale workers, and direct it into an appropriate detector. Since considerable research has been done on the improvement of collection efficiency, we should eventually be able to apply the information learned to improve the escape efficiency.

We have detected successfully in the dilute gas mode by using a flame ionization unit. With dilute samples little apparent difficulty stems from the association-condensation phenomenon. However, large samples lead to line clogging and spiked peaks. One such peak, that for carotene in CO_2 at 500 atm, is shown in Fig. 2. We believe that the spikes are a consequence of single fog particles which enter the detector and create short ion bursts. A visible fog often exists under such circumstances.

A promising approach under evaluation, which may circumvent condensation, employs pyrolysis conditions at the column exit. The goal is to achieve small, volatile decomposition species which, if necessary, can be additionally characterized by mass spectroscopy and low pressure gas chromatography to permit identification of the parent species. Pyrolysis is conventionally used at the column entrance to characterize unseparated mixtures.

An alternative detection technique allows the exiting stream to be cooled to and decompressed as a liquid. This method of liquid detection, which is based on sample collection and ultraviolet quantitation, has been used by Sie and Rijnders. This approach, compared to dilute gas detection, generally averts thermodynamic instability but must rely on the less satisfactory methodology of liquid detection. Furthermore, the small specific volume and diffusivity of liquids put severe requirements on detector dead volume and geometry, a problem magnified if the column diameter is small.

Finally, we can imagine detection at column pressure and temperature. The detector must be able to withstand considerable pressure and be relatively insensitive to existing pressure fluctuations. Dead volume requirements are demanding but less so than in liquid detection. The change of state needed for detection in the other two modes is avoided altogether.

High Pressure Gas Chromatography at Utah

Our first apparatus, operational to 170 atm, was built in 1964 to exploit the deductions by Knox (*13*) and this group (*14*) on the potential increase in separation speed and resolution under high pressure conditions. This objective was pursued with diverse instrumentation, the last resembling our present

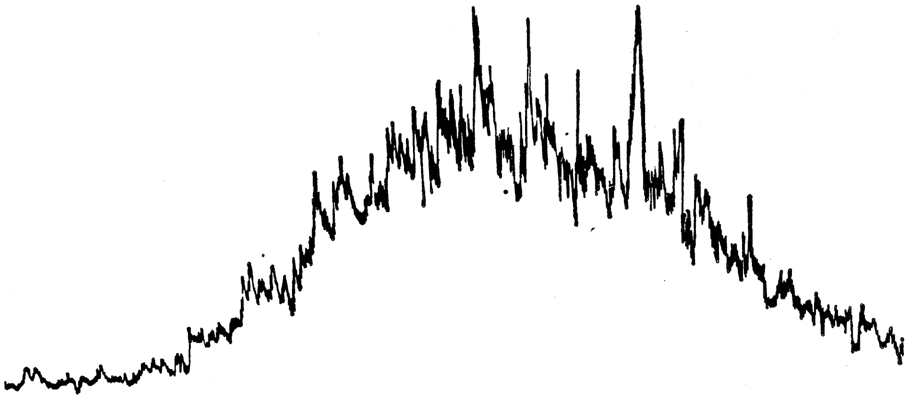

Fig. 2. Carotene peak obtained in CO_2 at 500 atm. Spikes may reflect ion bursts from the fog particles that form.

2000-atm equipment. In this work we observed theoretical plate counts which were in excess of 10^6 total (*15*), 10^4 per meter (*12*), and which approached 10^4 per second, the latter while demonstrating the feasibility of turbulent gas chromatography (*16*). Quite often our carrier gas was compressed to about 90 percent of the normal liquid density, so that we naturally inquired whether the gas also possessed liquid-like "solvent power."

In 1965 we began a serious attempt to exploit the solvent properties of dense, supercritical gases for chromatographic ends. Our goal was a rather complete exploration of the density hiatus between ideal gases and liquids and required pressures higher by an order of magnitude than had previously been used in chromatographic systems.

We first confirmed that helium, even when compressed to 2000 atm, is an extremely poor solvent for complex molecules. Nitrogen also failed to cause chromatographic migration. Then the complementary gases, carbon dioxide and ammonia, were run in the neighborhood of 40°C and 140°C, about 8°C above their respective critical temperatures, 31.1°C and 132.3°C.

Table 1 reports the ability of these gases to migrate various substances to a detector. In total, more than 80 compounds of limited or negligible volatility have been tested. The ease of migration, which is related to the ability of dense gas to dissolve the solute, was ascertained from the magnitude of the detector signal. In many cases migration was confirmed by visible solute loss from the sample chamber, and in a few cases it was verified by the infrared spectra of the effluent. We report a "threshold pressure" for some solutes in CO_2, which indicates the pressure at which detectable migration commences. All NH_3 experiments were confined to a lower pressure, 200 atm, a condition dictated mainly by the liquid pump.

Table 1 confirms the analogy between dense gases and liquids as solvents. Dense ammonia will most suitably migrate highly polar compounds such as amino acids and sugars, whereas carbon dioxide is best for nonpolar, unsaturated systems like the terpenes. This subject was discussed in an earlier paper (*10*).

Table 1. Relative migration of various species in ammonia and carbon dioxide. Other solutes that migrate well in NH_3 include: xylose, fructose, mannose, sorbose, alanine, leucine, tryptophane, tyrosine, arginine, lysine, glycylglycine, glycyl-L-leucyl-L-tyrosine, caffeine, adenilic acid, quercitin, silicon gum rubber (OV-17), α-glucose pentaacetate, and Carbowax 20 M. Those with no apparent migration in NH_3 include amygdaline, hippuryl-L-arginine, xantheine, adenosine monophosphate dihydrate, trypsin, lysozyme, apoferritin, γ-globulin, bovine albumin, bovine hemoglobin, gelatin, β-carotene, silicone gum rubber (OV-1 and SE-30), polysulfone, Polyox, polystyrene 900, Versamid 900, and cellulose acetate. Other solutes which migrate well in CO_2 include: officinilic acid, *n*-octadecane, *n*-octacosane, *n*-docosane, silicone oil DC 200, Carbowax 20 M, and polyvinyl chloride. Those with no apparent migration in CO_2 include polyethylene and amino acids.

Solute	Migration in NH_3 at 200 atm and 140°C	Migration in CO_2	
		At 40°C	Pressure (atm)
	Waxes and oils		
Silicon gum rubber, SE-30	No	Yes	770*
Apiezon L	No	Yes	820*†
Carbowax 400		Yes	68*
Carbowax 1000		Yes	115*
Carbowax 4000	Yes	Yes	190*
Dinonyl phthalate		Yes	165*
Squalane	Yes	Yes	90*
Squalene	Yes	Yes	510
	Terpenes		
β-Carotene, α-carotene, lycopene	(Decompose)	Yes	170
	Purines		
Adenine	No	Very slight	1300
Guanine	No	No	1300
	Nucleosides		
Adenosine, guanosine, uridine	Yes	Slight	1350
	Corticol steroids		
Cortisone, hydrocortisone	Yes (react.?)	Moderate	1300
	Sterols		
Cholesterol, ergosterol, lanosterol	Yes	Yes	510
	Sugars		
Ribose, arabinose, glucose	Yes	Yes	1350
Galactose	Slight	No	1350
Maltose	Yes	Yes	1350
Lactose	Yes	No	1350
	Other solutes		
Glycine	Yes	Very slight	1350
Cytochrome c	Slight	No	1560
s-RNA from yeast, type III	No (react.?)	Slight	1560

* Threshold pressure. † A second threshold pressure is apparent at 1070 atm.

Migration of the less volatile compounds is the first step in an efficacious chromatographic system, but separation is the final criterion of chromatography. Earlier we reported several separations in CO_2, these being sterols, carotenes, squalane and squalene, and a stepwise pressure-programmed separation of squalane, dinonyl phthalate, and silicone gum rubber (*10*). In Fig. 3 we show a chromatogram of glycine and two simple peptides separated in a dense NH_3 system at 200 atm. For this we used a 1.5-meter column packed with Porasil B, a porous silica, at 140°C.

Our move to high pressure chromatography was not made without travail. Leaks were common, as would be expected with high pressure equipment having many valves and connections. Leakage was magnified by the very solvent power we desired to harness; all "O" ring gaskets made of neoprene or Viton quickly decomposed in the NH_3 system, which led to valve leakage. One Viton test ring disappeared entirely except for a small residue. Ethylene propylene "O" rings resisted ammonia but were affected by temperature and required frequent replacement.

Unsteady flow complicated detection and system characterization. The flow of both CO_2 and NH_3 commonly decreased with time, apparently owing to Joule-Thomson cooling with attendant dimension changes and condensation at the reduction valve. With all but the smallest samples, the solute would itself condense and plug the lines following expansion. Our initial efforts to avoid this condensation by using pyrolysis resulted instead in a carbonaceous residue.

Contamination has been responsible for a number of spurious signals and a few uncertain results. Peaks have been traced back to fingerprints, thread lubricants, cleaning solvents, and solutes incompletely removed in prior experiments. Solutes such as commercial polystyrene contain small quantities of solvent of low molecular weight which is difficult to remove. The latter yields a spurious signal at pressures of 150 atm, whereas a true threshold pressure for polystyrene has not yet been observed. In this case the contaminant and its signal were removed by dissolving the polymer in carbon disulfide, a solvent which does not give a flame ionization signal. The polymer was then reformed by boiling away the new solvent, and presumably the traces of the old, under reduced pressure.

Such problems are perhaps to be expected in the beginning. None of them seems in any sense insurmountable as we view the picture presently. Perhaps a few more years will be needed to develop truly useful analytical gas chromatographs operating at approximately 1000 atm. It would be simpler if such high pressures could be avoided, and perhaps they can for some applications, as the work of Sie, van Beersum, and Rijnders suggests. However, the less volatile species may require pressures of at least this magnitude, for reasons suggested below.

Compressed Gases as Solvents

The theoretical description of gases at high densities entails considerable complexity (*17*). At present, therefore, we cannot rely on clear, rigorous guidelines for the choice of gas solvents, temperatures, and pressures. We seek, instead, to uncover the gross elements of gaseous solubility and to find a common mathematical framework in order to correlate gases with one another and with the liquid solvents against which they must compete.

We suggested above that the "solvent power" of a gas depends in part on its state relative to its critical condition. The influence of state, that is, relative compression and temperature, will be termed a "state effect" or "physical effect." Separate from this there is a "chemical effect," unique to each chemical species and dependent on its polarity, acid-base properties, and hydrogen bonding tendencies. Classical solubility differences among liquids depend mainly on the "chemical effect," since most liquids are in similar states or can be simply reduced to such without much alteration of their solvent characteristics. The response of "state effects" to compression gives the additional degree of freedom so advantageous to dense gases when these are employed as solvents.

One feels intuitively that reduced density must be the principal variable of the "state effect," which reflects mean distances and interactions between molecules. If a gas is compressed to a liquid-like density, we expect a liquid-like solvent effect. This is supported by the ability of the most highly compressed gases to migrate large polymers. The assertion made by Hannay in 1880 appears still to have considerable insight (*18*):

> The liquid condition of fluids has very little to do with their solvent power, but only indicates molecular closeness. Should this closeness be attained by external pressure instead of internal attraction, the result is that the same or even greater solvent power is obtained . . .

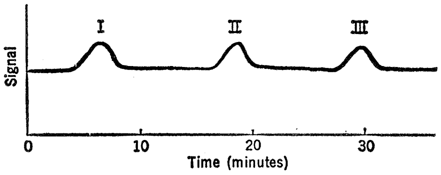

Fig. 3. Separation of glycine (I), glycylglycine (II), and glycylleucyl tyrosine (III) in NH_3 at 200 atm and 140°C on a Porasil B column 1.5 meters long.

In chromatography the "chemical effect" of the mobile phase is manifested in the eluotropic series which orders the elution strength of various liquids. Snyder (*19*) has developed an equation for liquid-solid adsorption chromatography which roughly separates the effects of the activity and extent of sorbent surface, the elution power of the solvent, and the solute structure. His scale is based on experimental behavior. Experiments with dense gases which would lead to a similar ordering are lacking. Snyder's series for alumina runs approximately: saturated hydrocarbons < unsaturated hydrocarbons < alkyl halides ~ ethers < carbonyls <amines <alcohols. Table 2 shows an eluotropic series which gives Snyder's ε^0 value (which measures elution strength) and the Hildebrand solubility parameter, δ, for those liquids (*20*). Despite the warning of Hildebrand and Scott (*21*) that it is dangerous to employ solubility parameters for esters, ketones, alcohols, and other polar liquids, we have included these (marked by an asterisk) as they were calculated from the molar energies of vaporization and the molar volume (*20*). The parallel between empirical elution power and the solubility parameter is striking. We suggest that the elution power of compressed gases may also relate to the solubility parameter, and that the latter may serve as a guide to the selection of a gas and its state.

Table 2. Elution power (ε^0 on alumina) and the solubility parameter (δ).

Liquid	ε^0	δ† (at temp. in °K)
Pentane	0.00	7.05(298)
Cyclohexane	.04	8.20(298)
CCl_4	.18	8.6(298)
Toluene	.29	8.90(298)
Benzene	.32	9.15(298)
Diethyl ether	.38	7.4(298)
Chloroform	.40	9.3(298)
Acetone	.56	9.5(293)
Dioxane	.56	10.0(298)
Ethyl acetate	.58	9.3(273)
Pyridine	.71	10.7(298)
Isopropanol	.82	10.8*(355)
Ethanol	.88	12.2*(351)
Methanol	.95	14.0*(273)

* Alcohols. † Taken from Hildebrand and Scott (*20*) unless marked by *.

We wish to see what can be deduced by assuming that (i) the solubility parameter is relevant to the "state effect" as well as the "chemical effect" of gaseous solvents and is thus applicable over a wide density range, and (ii) the gas obeys the simple van der Waals equation of state even at high densities. Hildebrand and Scott have noted that the van der Waals equation is reasonable for many liquids. They obtain

$$\delta = a^{1/2}/V \qquad (1)$$

where a is the intermolecular attraction term and V is the volume per mole. Some manipulations with the van der Waals equation and reduced variables lead to

$$\delta = (3\,P_c)^{1/2}\,\rho_r \qquad (2)$$

where P_c is the critical pressure and ρ_r the reduced density. Empirically, for liquids a slightly different coefficient prevails and, assuming the equivalence of gases and liquids at a common density, we are led to

$$\delta = 1.25\,P_c^{1/2}\,[\rho_r/\rho_r\quad(\text{liq})] \qquad (3)$$

where δ is in calories per cubic centimeter, P_c is in atmospheres, and ρ_r (liq), the reduced density of liquids, is normally about 2.66 (Fig. 1). Here the "state effect" may be identified with [ρ_r/ρ_r (liq)]. The other factor (1.25 $P_c^{1/2}$) may be associated with the "chemical effect" and has been used to correlate solubility phenomena in liquids (*20*).

The above equation suggests that reduced density is the principal variable of the "state effect" and that the relationship to δ is one of simple proportionality. Temperature does not enter into the expression, although if we had chosen the equation of Berthelot we would have found δ proportional to

ρ_r/T. However, the potential range of T is small compared with that of ρ_r.

We may now prepare a provisional eluotropic series for dense gases. This must include only the "chemical effect" since the "state effect" may be varied freely. Figure 4 shows such a series for various gases compared with a liquid series. These series occupy an overlapping range of δ values.

Assuming the above ideas to be valid, we can issue some guidelines for the use of high pressure gas chromatography. Small, volatile molecules will partition into a medium with $\delta = 0$ by virtue of entropy alone. This condition corresponds to classical gas chromatography where the ratio of gas to liquid density, $[\rho_r/\rho_r\ \text{(liq)}]$, is near zero. As solute molecules become larger and more complex, entropy alone becomes less able to establish a reasonable partition ratio and must be assisted by interactions in the gas, that is, δ must be finite. The magnitude of δ must therefore increase as "normal volatility" decreases. In the large polymer limit, δ of the solvent must be within about one unit of the polymer itself (*20*) and will therefore usually have a value greater than 6 to 10.

Klesper, Corwin, and Turner have worked in the range $\delta = 2.4$ to 4.5; Sie, van Beersum, and Rijnders, $\delta = 0.9$ to 2.8; and McLaren, Myers, and Giddings, $\delta = 3.8$ to 10.0. The latter range is higher because of the higher compression. If we must reach values from 6 to 10 for macromolecules, then, since the "chemical" or "liquid" δ itself is rarely much larger than 6 to 10, the "state" δ or $[\rho_r/\rho_r\ \text{(liq)}]$ must be kept near unity, that is, the density of the gas must be near that of the liquid. We see from Fig. 1 that this can only be realized with reduced pressures near 20, which for most gases corresponds to about 1000 atm.

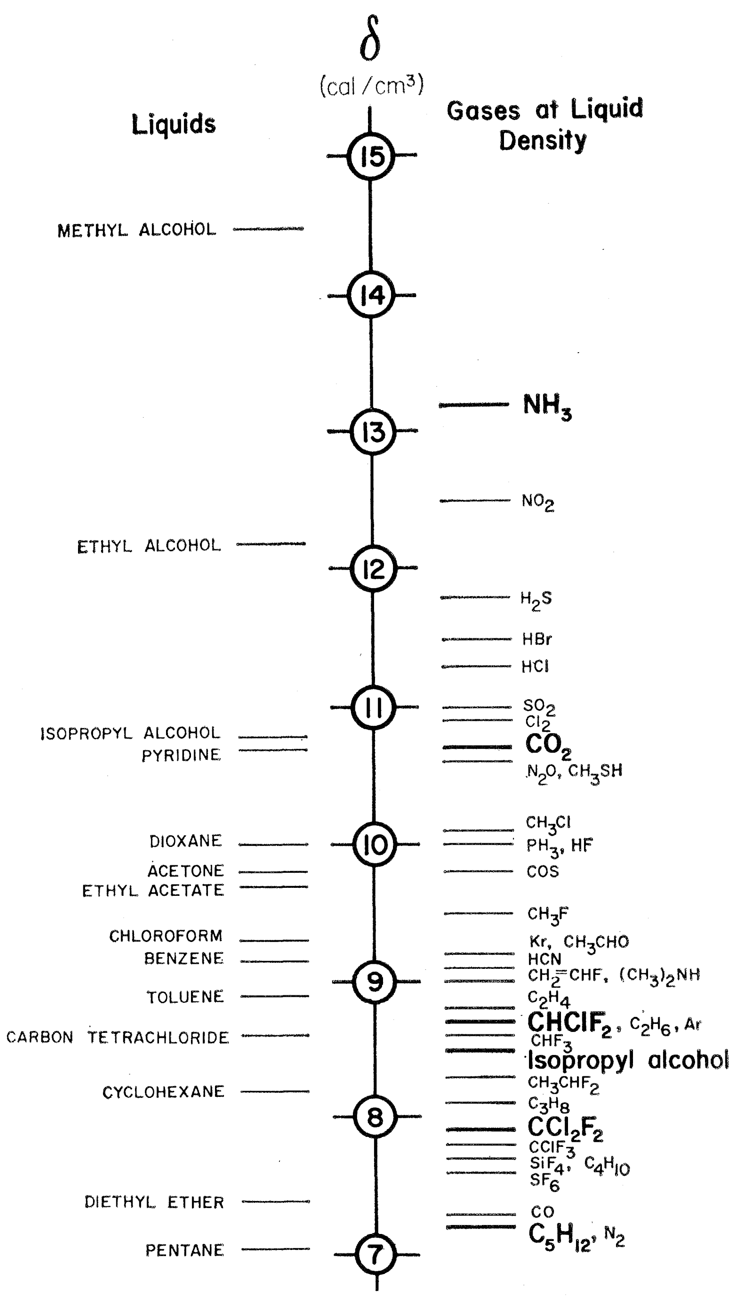

Fig. 4. Proposed eluotropic series of gases at liquid density compared with series for common liquids. Successful gases are shown in boldface.

Unfortunately, there are not sufficient data to test our conclusions quantitatively. Qualitative confirmation is provided by Table 1. Thus the CO_2 threshold pressure for the Carbowax series (400, 1000, and 4000) increases steadily in the sequence 68, 115, and 190 atm. Similarly squalane and silicone gum rubber have threshold pressures of 90 and 770 atm, respectively, which presumably reflect their wide difference in molecular weights. The threshold δ value increases from 4.2 to 9.1 in this particular case. Further qualitative confirmation comes from Hannay (*18*): "The gas must have a certain density before it will act as a solvent, and when its volume is increased more than twice its liquid volume its solvent action is almost destroyed."

The above approach is suggested as a simple means for correlating experimental results. It ignores certain quantitative factors such as the pressure-volume or "Poynting" type terms and "free volume" considerations. Also we have stretched the concept of the solubility parameter to the limit. The latter will be in doubt when clustering is significant and particularly when specific solvent effects exist. A species which is capable of hydrogen bonding with a solute which provides the conjugate function but which cannot hydrogen-bond with itself is likely to show a solvent power vastly different from that indicated by δ. Hence polar molecules are excluded from the considerations of Hildebrand. Likewise we warn here that hydrides of the halogens, phosphorus, and nitrogen are likely to show exceptional behavior.

We justify our tentative approach by noting that researchers who desire to utilize the technique face a vacuum when seeking rational rules for the design of their equipment. One does not need a capability of 2000 atm when 100 atm will suffice for the particular problem. If our conclusions on the "chemical effect" and "state effect" have even approximate validity, they will be useful to an increasing number of individuals who wish to chromatograph species too nonvolatile for conventional gas chromatography.

References and Notes

1. J. B. Hannay and J. Hogarth, *Proc. Roy. Soc. London* **29**, 324 (1879).
2. J. S. Rowlinson and M. J. Richardson, *Advan. Chem. Phys.* **2**, 85 (1959).
3. H. S. Booth and R. M. Bidwell, *Chem. Rev.* **44**, 447 (1949).
4. E. Klesper, A. H. Corwin, D. A. Turner, *J. Org. Chem.* **27**, 700 (1962).
5. J. C. Giddings, in *Gas Chromatography 1964*, A. Goldup, Ed. (Elsevier, Amsterdam, 1965).
6. ———, *Separation Sci.* **1**, 73 (1966).
7. S. T. Sie, W. van Beersum, G. W. A. Rijnders, *ibid.*, p. 459.
8. S. T. Sie and G. W. A. Rijnders, *ibid.* **2**, 729 (1967).
9. ———, *ibid.*, p. 755.
10. L. McLaren, M. N. Myers, J. C. Giddings, *Science* **159**, 197 (1968).
11. J. C. Giddings, *Dynamics of Chromatography*, Part I: *Principles and Theory* (Marcel Dekker, New York, 1965).
12. M. N. Myers and J. C. Giddings, *Separation Sci.* **1**, 761 (1966).
13. J. H. Knox, *J. Chem. Soc.* **433** (1961); *Gas Chromatography* (Methuen, London, 1962).
14. J. C. Giddings, *Anal. Chem.* **35**, 2215 (1963); **36**, 741 (1964).
15. M. N. Myers and J. C. Giddings, *ibid.* **37**, 1453 (1965).
16. J. C. Giddings, W. A. Manwaring, M. N. Myers, *Science* **154**, 146 (1966).
17. J. O. Hirschfelder, C. F. Curtis, R. B. Bird, *Molecular Theory of Gases and Liquids* (Wiley, New York, 1954); S. A. Rice and P. Gray, *The Statistical Mechanics of Simple Liquids* (Interscience, New York, 1965).
18. J. B. Hannay, *Proc. Roy. Soc. London* **30**, 484 (1880).
19. L. Snyder, in E. Heftmann, *Chromatography* (Reinhold, New York, ed. 2, 1967), p. 43.
20. J. H. Hildebrand and R. L. Scott, *The Solubility of Nonelectrolytes* (Reinhold, New York, ed. 3, 1950).
21. ———, *Regular Solutions* (Prentice-Hall, Englewood, N.J., 1962).
22. This work was supported by PHS research grant GM 01851-11 from the National Institutes of Health. We thank S. Hudson and R. Wagstaff for experimental work contributing to Table 1.

Computer-Assisted Instruction

R. C. Atkinson and H. A. Wilson

Ten years ago the use of computers as instructional devices was only an idea that was being considered by a handful of scientists and educators. Today that idea has become a reality. Computer-assisted instruction, like other aspects of electronic data processing, has undergone an amazingly rapid development. This rate of growth is partly attributable to the rich and intriguing potential of computer-assisted instruction for answering today's most pressing need in education—the individualization of instruction. Many useful ideas, however, have not achieved realization as quickly as computer-assisted instruction. The favored growth pattern of this method of instruction then must involve causes other than just a rich potential for meeting an educational need.

At least three other factors may be cited as contributing heavily to the growth of computer-assisted instruction. One of the most important was the development of programmed instruction. The surge of interest in programmed instruction during the 1950's, stemming primarily from the work of Skinner (*1*), focused the interest of educators on the problem of individualized instruction. Even though the actual results of programmed learning fell somewhat short of the glowing predictions of its early prophets, it left educators in a state of "rising expectations." The feeling remained that somehow through the use of science and technology the instructional process might eventually be tailored in a meaningful way to match the already known differences in motives and abilities among students.

The second factor contributing to the growth of computer-assisted instruction has been the mushrooming of electronic data processing in general. More specifically, however, the introduction of time-sharing systems and the design and production of third-generation computers has provided a major impetus to computer-assisted instruction. The early pioneering work at the University of Illinois on the Plato I system, which could handle only one student terminal at a time, furnished the foundation for further development. With the advent of time-sharing and the capability of the central processor to maintain more than one student terminal simultaneously, the wedding of programmed learning and electronic data processing got under way.

A third factor, and one of no less importance than those previously mentioned, has been the increasing aid to education by the federal government. In particular, the National Science Foundation and the various funding agencies which came into being under the Elementary and Secondary Education Act of 1965 have contributed substantially to the growth of computer-assisted instruction. Experimentation and development in the area of electronic data processing, particularly in the third-generation systems, has been an expensive process. Without supporting funds from the various government agencies and private philanthropic foundations (Carnegie, Ford, and others) the notion of applying electronic data processing capabilities to the problems of instruction might still be an idea discussed abstractly in a few technical journals.

Due to the interaction of the above factors, computer-assisted instruction has grown in less than 10 years to a point where during the school year of 1967–68 several thousand students ranging from elementary school to university level received a significant portion of their instruction in at least one subject area under computer control. In the Stanford projects alone approximately 3000 students were processed daily. Serious applications of computer-assisted instruction are now in progress in many universities throughout the United States: a list of those that have had major programs under way for two or more years includes Stanford University, University of California at Irvine, University of Texas, Florida State University, University of Illinois, Pennsylvania State University, University of Pittsburgh, State University of New

---

R. C. Atkinson is professor of psychology and member of the Institute for Mathematical Studies in the Social Sciences at Stanford University, Stanford, California. H. A. Wilson was a member of the faculty at Stanford University and is now vice president of the Computer Curriculum Corporation, Palo Alto, California.