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THE ROLE OF DISSOLVED GASES IN HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY

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SUMMARY

Dissolved gases are usually present as components of the mobile phase in high-performance liquid chromatography. Each gas has its unique properties and affects the chromatographic system in different ways.

The solubility in pure and mixed solvents is explored experimentally and compared with data already in the literature. It is found that the non-linear solubility characteristics in binary solvent systems account for the observed evolution of large quantities of gas when air-saturated solvents are mixed in chromatographs. The degassing requirements in one-pump, low-pressure-mixing gradient architectures are compared with those of conventional two-pump, high-pressure-mixing systems.

Dissolved oxygen affects detector performance in several ways. It forms a UV light-absorbing complex with many solvents. Changes in oxygen concentration therefore cause UV detector drift. The magnitude of this effect varies markedly with different solvents, and is particularly pronounced at wavelengths below 260 nm. Dissolved oxygen quenches fluorescence of both solvents and solutes. As a consequence, fluorescence detector drift and responsivity depend on oxygen concentrations. Maximum fluorescence sensitivity can only be achieved with deoxygenated mobile phases.

Because of these facts, analytical precision requires that gas concentrations be carefully controlled. The various control techniques are discussed: heating, boiling, vacuum, ultrasonics and gas sparging. A new method of helium degassing is described which eliminates bubble formation and maintains the level of all other gases at zero concentration.

INTRODUCTION

Because air is ubiquitous, the gases which comprise it tend to be found in high-performance liquid chromatography (HPLC) mobile phases. Their presence accounts for a variety of effects, many of which interfere with sensitive, precise, trouble-free chromatographic analysis. Although some of these problems have been understood for some time, others have received scant attention. There is little overall awareness of the important role of gases, and that which does exist is often based on misunderstanding.

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The importance of excluding dissolved oxygen from the mobile phase in order to protect labile stationary phases has been mentioned in most standard texts on HPLC[1–3]. Leitch documented the improved column lifetime and analytical precision resulting from deoxygenated solvents[4]. Exposure of the liquid–liquid partition column (3,3′-oxydipropionitrile) to dissolved oxygen during constant or heavy use severely reduced column life.

The potential for harm to the mobile phase has also been pointed out[2]. Butyl ether mobile phase was oxidized during handling and storage in the reservoir, forming peroxides which reacted with the stationary phase and changed its polarity. The susceptibility of other ethers, such as tetrahydrofuran, may also be a problem[5].

Snyder[6] has counciled about the benefits of excluding oxygen in liquid–solid (adsorption) chromatography, since sample oxidation is often increased by the presence of the adsorbent. A recent example[7] in reversed-phase chromatography described the ease with which aniline and its metabolites were oxidized during chromatography, and the improved results with deoxygenated mobile phases compared to mobile phases to which antioxidants had been added.

The improvement of detector performance resulting from deoxygenating the mobile phase has also been reported. Fox and Staley[8] showed that deoxygenated mobile phases in polycyclic aromatic hydrocarbon analysis produced a limit of detection for benzo[*a*]pyrene which was nearly four times as sensitive than when air-saturated mobile phase was used. Different but linear calibration curves were obtained in the presence and absence of dissolved oxygen. Compared to pyrene, oxygen quenching of fluorescence is less important in chrysene but more important in benzo- and dibenzopyrenes. They did not determine how effective or reproducible their deoxygenation process was. Chamberlain and Marlow[9] demonstrated that oxygen dissolved in the mobile phase caused increased noise levels and decreased standing current in the LC electron capture detector they used.

Perhaps a more generally encountered problem than any of the above is the occurrence of gas bubbles in the detector[1–3]. Air dissolved in the carrier at high pressure can subsequently form bubbles in the mobile phase as it passes through the detector, causing noise and drift. Gas bubbles also affect pump performance, but this has not been a serious concern prior to the advent of one-pump, low-pressure-mixing chromatograph architectures[10–13].

Some of the above reports contain misunderstandings about the solubility behavior of air gases. For example, it has been said that the more polar the mobile phase, the greater the tendency to dissolve air[1,9]. Actually the opposite is true. Water is the least hospitable solvent for gases. And the bubble problems are attributed to oxygen[1,4,14], whereas nitrogen is as much a source of difficulty.

The purpose of this paper is to report new findings on gas behavior of chromatographic significance, and to describe ways of improving the reliability, sensitivity and analytical precision of HPLC by careful control of dissolved gases. We will proceed by (1) reviewing gas solubility behavior in pure solvents and binary mixtures, (2) comparing the one-pump, low-pressure-mixing gradient architecture with the two-pump, high-pressure mixing gradient architecture, as to gas solubility problems, (3) describing methods of controlling dissolved gas concentrations, (4) discussing the "optical properties" of dissolved gases so as to explain observed UV and fluorescence detector artifacts, including UV detector behavior which has not been reported before

in the chromatographic literature and (5) commenting on other aspects of dissolved gases, including the effect of carbon dioxide on mobile phase pH and therefore on the reproducibility of retention times and peak areas.

EXPERIMENTAL

Column, solvents and samples

Stainless-steel columns (250 × 3.1 mm I.D.) packed with 10-μm Spherisorb ODS and 250 × 4.6 mm I.D. stainless-steel columns packed with 10-μm LiChrosorb RP-8 were used. Both are totally porous, bonded reversed-phase packings which are respectively octadecyl (C_{18}) functionality on spherical silica and octyl (C_8) functionality on irregular silica. (Spectra-Physics, Santa Clara, Calif., U.S.A.)

Mobile phases were prepared from distilled-in-glass solvents (Burdick & Jackson, Muskegon, Mich., U.S.A.). Water was prepared by a Milli-Q system, fed by a Milli-RO system, in turn fed by Santa Clara (Calif., U.S.A.) tap water; the Milli-Q had four cartridges, two mixed-bed ion exchangers followed by two activated carbon units (Millipore, Bedford, Mass., U.S.A.). Solvents were degassed as indicated in the text.

Sparging gases were high purity grades, exceeding 99.99 mole% purity. Air was "breathing quality". Samples were from Chem Service (West Chester, Pa., U.S.A.) and Aldrich (Milwaukee, Wisc., U.S.A.). They were dissolved in water–methanol and water–acetonitrile mixtures.

Control of flow, composition and temperature

A Spectra-Physics Model SP 8000 research liquid chromatograph and a Spectra-Physics Model 3500B gradient liquid chromatograph were used. The former employs a single pump, attached to a low-pressure composition forming module (ternary proportioning valve). It has $\pm$0.1 °C column temperature control via a forced air oven. The 3500B system employes a dual reciprocating piston pump for solvent A and an identical but independent one for solvent B. The composition is formed at high pressure in a dynamically stirred chamber. Temperature control was via a water bath.

The detectors were Spectra-Physics Model SP 8310 operated at 254 nm, Model SP 770 variable-wavelength detector and Model SP 970 fluorescence detector.

RESULTS AND DISCUSSION

Gas solubility in pure solvents

The solubilities of gases in liquids have long been an area of active interest to chemists. Practical concern has been related to such diverse fields as industrial processes and the composition of artificial atmospheres. Theoretical concern has been related to the small solubility and the variety of gases available to use as probes for the investigation of liquid and solution structure and properties. A number of excellent review articles exists[15–18]. There are several major sources[19–21] for gas solubility data in pure solvents in addition to these reviews. Solubility data in mixed solvents is of course of the greatest interest to the chromatographer since the use of a pure solvent mobile phase is rare except in exclusion chromatography. Unfortunately, the

data for such realistic mobile phases is scanty, although a few papers exist[22–25] which describe the solubility of some air gases in aqueous alcohol solutions.

Before proceeding further it may be instructive to review briefly a few of the salient facts about gases. Air comprises 78.08% N_2, 20.95% O_2, 0.93% Ar, 0.03% CO_2 and less than 0.01% other gases. Gases in a mixture behave essentially independently, the solubility of the individual gases in a mixture of gases being directly proportional to their partial pressures (Dalton's law). Different gases have different solubilities in a given solvent. Different solvents have different solubility properties towards a given gas. The solubility of gases in liquids usually decrease with increasing temperature, but there are numerous exceptions and the correlation of solubility data as a function of temperature is not simple. Gas solubilities in most solvent mixtures, like so many physical phenomena in non-ideal solutions, are not a linear function of the composition expressed in mole fraction.

Fig. 1 shows the solubility of several gases in many solvents using data taken from refs. 17 and 18. The solubility is expressed as the mole fraction. A feel for the magnitude of these values can be gained by realizing that the carbon dioxide solubility

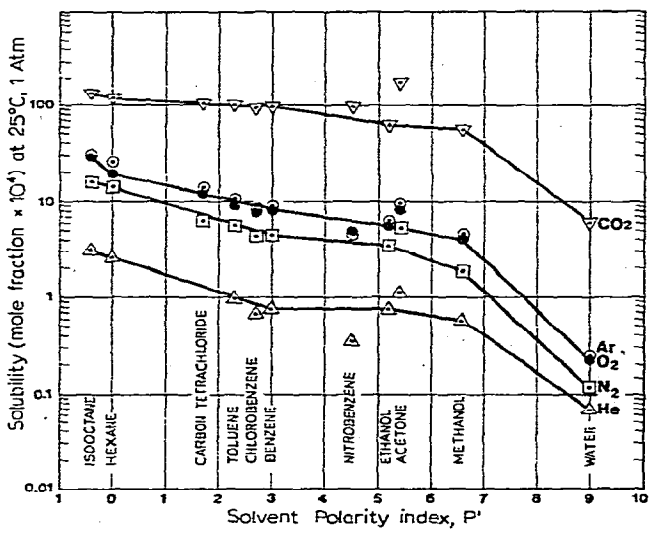

Fig. 1. Gas solubility *vs.* solvent polarity index. The solubility is expressed as mole fraction $\times 10^4$ for pure gases in equilibrium with the solvent at 1 atm and 25 °C. The polarity index is the Snyder parameter. ▽, CO_2; ⊙, Ar; ●, O_2; ⊡, N_2; △, He.

of mole fraction 0.01 in benzene is equivalent to about 2.4 ml of carbon dioxide at 1 atm and 25°C per ml of benzene; and the nitrogen solubility of mole fraction 0.000014 in water is equivalent to about 16 μl of nitrogen at 1 atm and 25°C per ml of water. The solubility of the various gases in the various solvents thus ranges over a factor of 1000. The solubility is plotted against solvent polarity, expressed as Snyder's polarity index, P'[26]. This solvent characterization parameter is but one of several which have been proposed recently. It can be compared with the Hildebrand solubility parameter δ[27,28] in that values of P' roughly parallel values of δ, and have similar significance. However, whereas δ is measured for the pure solvent, and only reflects interactions that exist in the pure solvent, P' is measured against a variety

of solutes that encompass all possible types of interaction. The Snyder parameter therefore may not be the most reasonable one to use if a smooth curve is desired, but it is employed here since it is currently popular in chromatographic literature[12]. The important point to note is that gases, being non-polar, behave as one would expect, having increasing solubility as the solvent polarity decreases.

It should be pointed out that these solubility values represent the amount of gas which will be found dissolved in the solvent at equilibrium with one atmosphere of the pure gas over the solvent. So the previously mentioned 2.4 ml of carbon dioxide in benzene only exists in a pure carbon dioxide atmosphere. Much less is in the solvent exposed to air. Hydrogen is not plotted. It falls midway between the helium and nitrogen curves, except in water, where it is slightly more soluble than nitrogen. The solubility of the rare gases increases with increasing atomic weight. In water the mole fractions for He, Ne, Ar, Kr, Xe and Rn are 0.07, 0.08, 0.25, 0.45, 0.78 and 1.68, respectively.

It is clear that water dissolves the least amount of air gases. The common misconception that it is the best solvent may derive from the fact that gas is often seen to come out of solution in water. But this may be because, once degassed, it does not take much gas to redissolve before saturation is reached. The real problem with regard to gas solubility in HPLC, however, comes with mixed solvents, either in isocratic mode where two solvents are mixed by the instrument, or in gradient mode.

Gas solubility in mixed solvents

As previously mentioned, there is not much data in the literature on gas solubility in mixed solvents. Most data which exists is for water–alcohol mixtures, and we can infer from it what may happen in other binary mixtures of solvents, especially those binary mixtures where the two solvents interact strongly.

Ben-Naim[22] studied the solubility of argon in water–methanol systems. A plot of solubility *vs.* mole fraction of methanol shows the solubility generally increasing with increasing methanol content as one would expect. However, it goes through a maximum and then a minimum, especially at low temperature. Cargill and Morrison[23] extended the argon study over a wider temperature range, and included water–*tert.*-butanol systems. The latter exaggerated the peculiar behavior of alcohol binary mixtures. Cargill[24] also studied oxygen and found it to behave like argon. Fig. 2 shows a few of the solubility curves from the Cargill paper[24]. In the paper the solubility was expressed by S_0, defined as the volume of gas in ml, corrected to 273 °K and 1 atm, dissolved by 1 kg of solvent, under a gas pressure of 1 atm. In Fig. 2 it is expressed as mole fraction.

Fig. 2 can be contrasted with Fig. 3, which shows the amount of gas actually present in the solution at various mole fractions, starting with the A and B solvents air saturated. The significant observation is that: when two solvents, such as water and ethanol, are at equilibrium with the atmosphere (*i.e.*, air saturated) and when they are blended to form a mixture (such as in gradient elution), supersaturated conditions exist during much of the run, which cause gas to come out of solution until the concentration is at the allowed saturation level.

Fig. 4 illustrates what happens in a real chromatographic situation. It is a record of the column inlet pressure during a gradient run of 0 to 100% methanol in water in 5 min. The pumping system is the Spectra-Physics SP 8000 liquid chro-

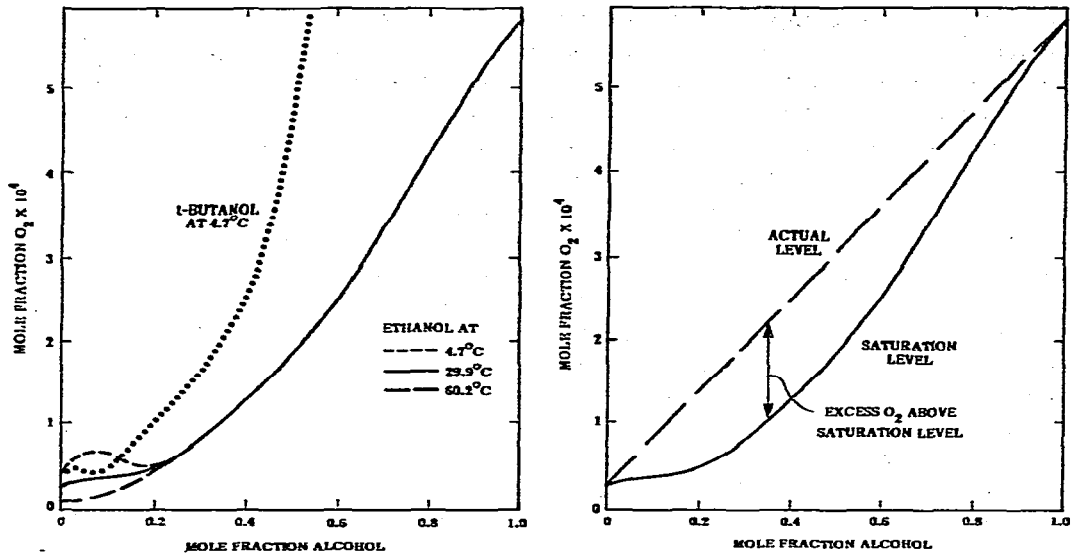

Fig. 2. Oxygen solubility in aqueous alcohol mixtures: mole fraction oxygen *vs.* mole fraction alcohol. The solubility is expressed as in Fig. 1. The original data of Cargill[24] was plotted in terms of S_0, defined as the volume of gas in ml, corrected to 0 °C and 1 atm, dissolved by 1 kg of solvent, under a gas pressure of 1 atm, on a logarithmic scale. Data reproduced by permission of the publisher.

Fig. 3. Oxygen solubility *vs.* oxygen concentration. The actual level represents the amount of oxygen in the admixtures, starting with air saturated pure water and air saturated pure ethanol. The saturation level is the allowed solubility level, the 29.9 °C curve from Fig. 2. Note the non-linear behavior, particularly at the low mole fractions of ethanol.

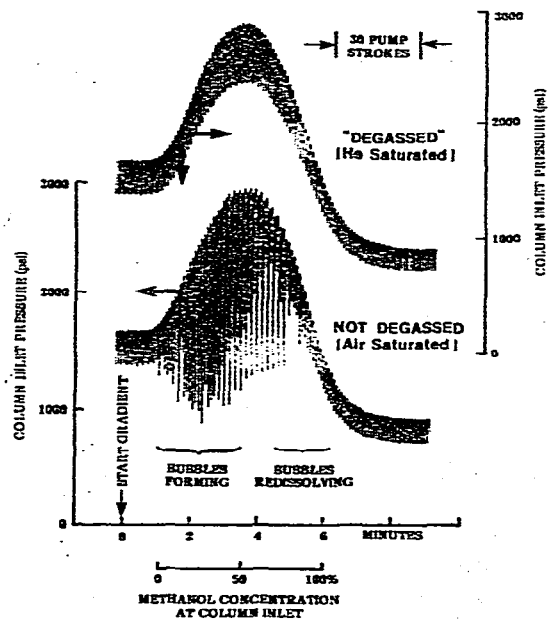

Fig. 4. Bubble formation in pump chambers with undegassed solvents. Column, 250 × 3.1 mm I.D.; packing, 10-μm Spherisorb ODS; solvent, 0–100% methanol in 5 min; flow-rate, 5.0 ml/min; pressure, as indicated; temperature, 25 °C. Recorded on the SP 8000 printer/plotter using the signal from the pre-column pressure transducer.

matograph, which has a single pump with two pump chambers each of 400-μl displacement. The flow feedback control has been turned off so that the pulsation (pressure dip) at the be ginning of each pump stroke is evident. This pulsation is a measure of the compliance in the chamber, which in turn is the sum of mechanical elasticity, fluid compressibility and the compressibility of any undissolved gas present. The upper curve exhibits the expected rise and fall of pressure during the gradient, which reflects the viscosity profile of the gradient mobile phase. The pressure pulses are uniform, demonstrating that there are no undissolved gas bubbles in the chamber. This curve was made using solvents which had been helium "degassed" (see below). The lower curve is similar, but the pressure pulses are irregular. The solvents were not degassed, but were simply equilibrated with air. These irregular pulses result from bubbles of gas entering the pump during the first part of the gradient. Later in the gradient, the pressure and solvent composition are such as to redissolve them. In some cases, especially at low operating pressures, the pulses persist because the gas never redissolves. Such behavior not only causes flow-rate errors, but is deleterious to composition precision.

The bubbles in the above example are formed in the low-pressure ternary proportioning valve, which mixes the two pure solvents in the proper proportion during the run. Fig. 5 diagrams such a system, and compares it with the conventional two-pump, high-pressure-mixing architecture. It is clear that the environment where the pure solvents are mixed is radically different in the two types. In the conventional architecture, the solvents are mixed at high pressure, where the solubility is much higher. Thus gases do not come out of solution at that point. However, if there is air in the solvents, it will come out of solution when the pressure again reaches one atmosphere, and sometimes sooner if some gas has been picked up at high pressure via a small leak in the system. For this reason, a flow restrictor is often put at the detector outlet, so that only after the detector does the pressure reach a level where gas bubbles form (see the pressure profile in Fig. 5).

Gas control methods

There are two approaches to gas control. One strives to eliminate all dissolved gases, the other to eliminate or control the concentrations of only certain gases. The former has been the most common approach directed at remedying the various problems described above, *i.e.*, gas bubbles, oxidative degradation of samples and phases, and detector artifacts. The approach used most often is vacuum degassing[1,2], the application of a vacuum to the mobile phase just prior to chromatography. Heating[2,3] and ultrasonic treatment[14,29] have also been employed. One of the gas solubility reviews[16] has a good discussion of degassing methods. It reports that the most common method to degas a solvent in non-chromatographic work is to boil away a portion of it under vacuum, a batch binary distillation. The Ramsey–Rayleigh equation for this type of distillation predicts that the evaporation of as little as 0.1% of the solvent should reduce the gas content by several 1000-fold. But the assumption of equilibrium is incorrect. In practice 10–20% of the solvent is evaporated. Other methods include pumping on the frozen solvent or boiling followed by spraying into an evacuated flask.

Sparging with a pure gas (bubbling it through the solvent) has most frequently been employed for the elimination of only certain gases[1,2]. This technique, also referred to as purging or stripping, has been used in gas chromatographic studies[16,30].

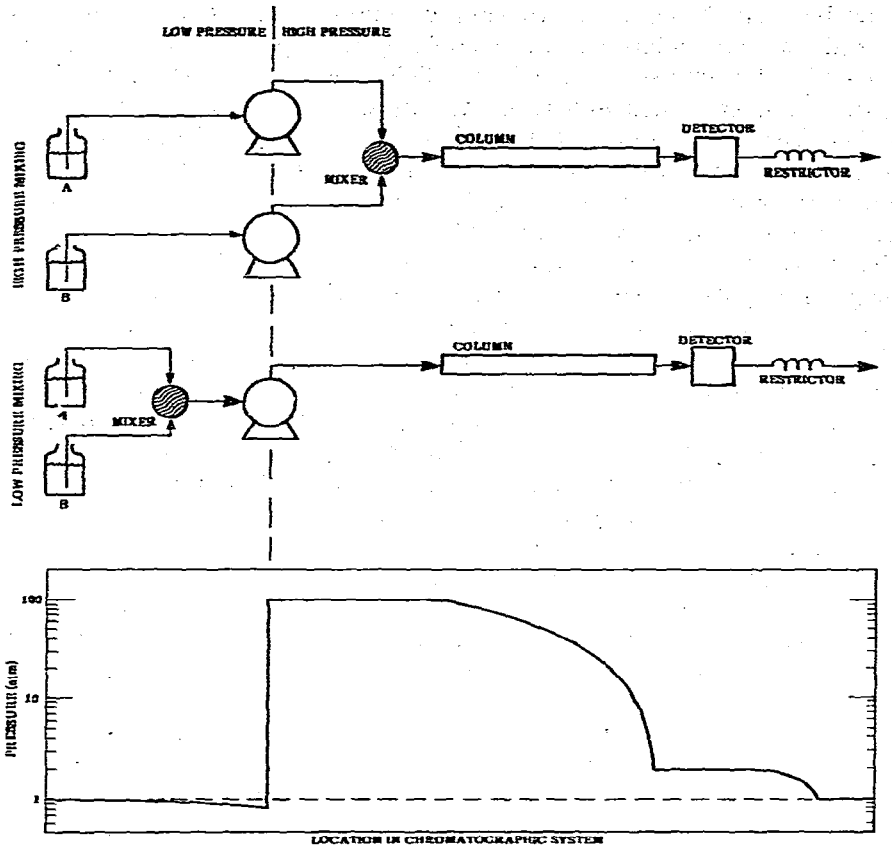

Fig. 5. Comparison of one-pump and two-pump architectures. The upper system represents a two-pump, high-pressure-mixing system such as the SP 3500B. The lower system represents a one-pump, low-pressure-mixing system such as the SP 8000. The pressure drops appear non-linear along the column length only because it is a semi-logarithmic plot.

Williams and Miller[31] compared several techniques for purging water: dynamic and static vacuum, with and without manual and ultrasonic agitation; ultrasonic treatment alone; and purging with an inert gas. The most effective system tested was inert gas purging at flow-rates of about 1000 ml/min of 100-ml water samples. This technique removed 95–98% of the dissolved oxygen in 15–30 sec, where the next best technique of dynamic vacuum with agitation took 1–2 min to remove the same quantity of gas.

The degassing technique we used was that employed by the SP 8000 chromatograph. This has been briefly described only once[10] and is the subject of a patent application. The method uses helium to sparge all pure solvents (up to three in the ternary-type mobile phase control system of the chromatograph). It was found that this is the only gas, with the possible exception of neon, which is capable of eliminating all the previously mentioned problems. That is, it not only prevents bubble formation but eliminates all gases except helium from the mobile phase. (There is no literature on the helium solubility in binary mixtures.) Our experimental evidence

suggests two possible explanations for this. The solubility curve for helium may be non-linear, like the oxygen shown in Fig. 3. But the amount of gas involved may be so low, *i.e.*, the absolute value of the gas volume which is supersaturated may be so small, that the microbubbles formed do not manifest themselves. Alternatively, the solubility curve may be nearly linear, leading to only small amounts of gas which are above the saturation level. Both of these may be operating.

The effectiveness of this technique for eliminating bubble generation during low-pressure mixing is illustrated in Fig. 6. This plots the volume of air evolved per ml of mobile phase formed by mixing two pure, air-saturated solvents. A Model 740B pump (dual reciprocating piston, feedback-controlled type) was used for each solvent. The outputs of the pumps were teed together and the tee exit line directed to a 1.0-ml mixing chamber. The position of the inlet and outlet lines, and the shape of the "roof" of the chamber were designed to trap any bubbles formed. After passage of a measured amount of total mobile phase through the chamber at a pre-determined solvent composition, the gas bubbles formed were sucked into a precision syringe which was connected to the top of the chamber. Replicate runs were not made, so the detailed shape of the curves is not to be taken as significant. Considerable scatter was suspected. The general shape, however, is no doubt accurate. These curves correspond well to the area of supersaturation in Fig. 3. Note that the more similar the two solvents, the less gas was evolved. The hexane–isooctane run produced no bubbles. In all cases, no bubbles were observed when the two solvents were helium degassed.

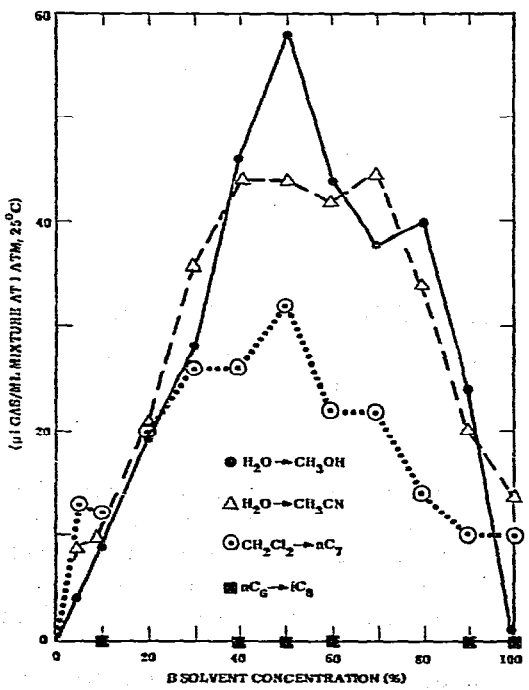

Fig. 6. Gas evolved *vs.* solvent composition. Apparatus and procedure described in text.

UV absorbance of dissolved gases

During the above studies of gas solubility and degassing techniques, we noticed that UV detector baselines sometimes drifted considerably when degassing was initiated. We have experimentally confirmed that this is due to the presence of oxygen, as illustrated in Fig. 7. It is a record of UV detector signal at 254 nm and 0.08 AUFS for a 1.0-cm cell with methanol flowing. The trace starts with the baseline after the methanol has been sparged with pure oxygen for some time. A stable signal was obtained. Then the sparging gas is changed to air, and the signal drifts down to a new equilibrated value. Then the gas is switched to helium. Yet another level is found. The ratios of these three signals are: (helium–air–oxygen) 0:0.223:1.00. The ratio of partial pressures, *i.e.*, the theoretical signal levels if due to oxygen concentration, are 0:0.209:1.00. This represents a 6.7% error from theory.

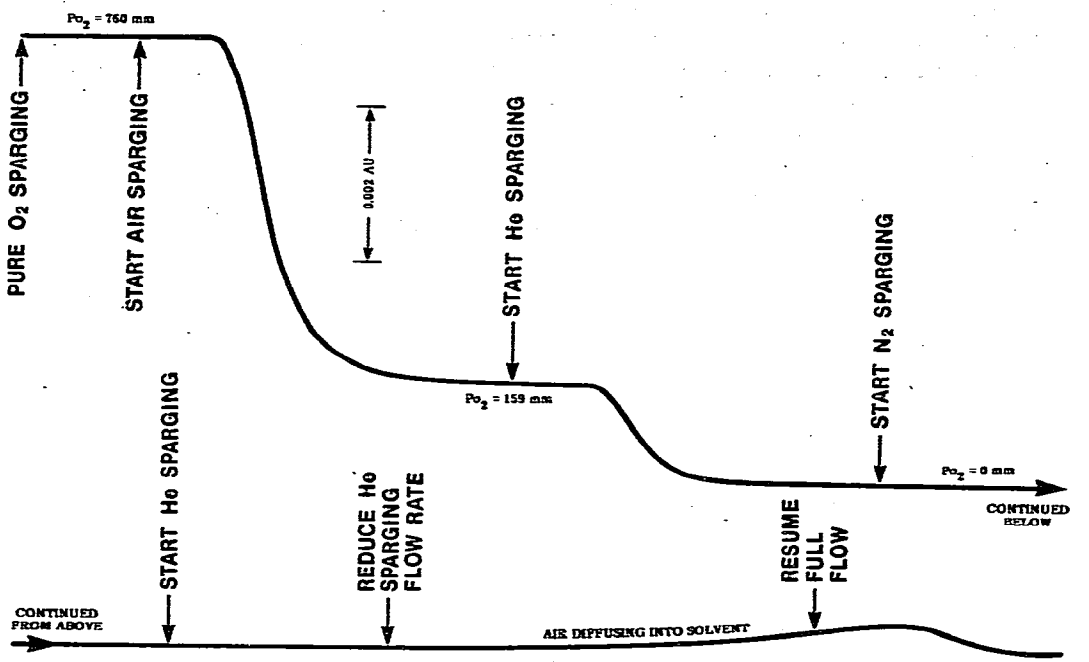

Fig. 7. UV detector standing signal *vs.* oxygen concentration in mobile phase. Solvent, methanol; flow-rate, 2.0 ml/min; pressure, nominally 1 atm; temperature ambient (approximately 25 °C); detector, Model SP 8200 at 254 nm with a 1-cm path cell.

The gas is further changed from helium to nitrogen and back to helium. The stable baseline is consistent with the hypothesis that it is the oxygen only which is responsible for the signal. Finally, the sparge rate was reduced, allowing air to back diffuse through the vent tube into the solvent bottle. The upscale drift due to oxygen absorbance is evident. Re-establishment of an adequate sparge rate rapidly brings back baseline stability.

The amount of absorbance varies considerably among the common chromatographic solvents, as shown in Fig. 8. The UV absorbance at 254 nm was monitored

at a sensitivity of 0.02 AUFS. Note that water exhibits only a very small effect at this wavelength, whereas the tetrahydrofuran baseline changed more than 0.16 AU. The baseline shifts are completely reversible; resumption of air sparging elevates the signal back to the original level. Evans[32,33] and Munck and Scott[34] demonstrated that dissolved oxygen gives rise to such absorption in the far UV in a number of organic solvents. This was observed for *n*-hexane, *n*-heptane, methanol, ethanol, diethyl ether and cyclohexane. The absorbance was found to be directly proportional to the partial pressure of oxygen above the solution. The absorbance increases at shorter wavelengths. Comparing the absorbance of the amount of oxygen dissolved in the solvent with the absorbance of an equivalent concentration of gaseous oxygen (negligible at the wavelengths examined) leads to a conclusion that the absorption of the solution must be due to interaction between oxygen and the solvent.

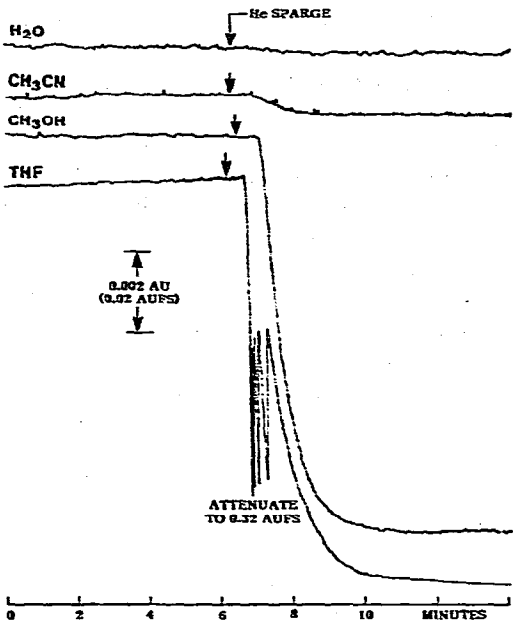

Fig. 8. UV detector standing signal with and without degassing for various solvents. Solvent, as indicated; flow-rate, 15 ml/min (splitter in front of detector providing 5 ml/min detector flow); pressure, 1 atm in solvent reservoir, 2 atm at pump outlet; temperature 23 ± 0.5 °C; detector, Model SP 8310 at 254 nm with a 0.6-cm path cell.

Heidt and Ekstrom[35,36] have examined this phenomenon in water. The absorption coefficient, ε, was independent of oxygen concentration, increased with decreasing wavelength, and increased with increases in temperature. They explained the results in terms of the existence of two different hydrates of molecular oxygen in water. Pure (deoxygenated) water also was found to absorb UV light and behave in a similar manner, but the magnitude of the effect was very much less than the light absorption due to molecular oxygen in water.

Thus dissolved oxygen affects HPLC mobile phases in two ways, one of which is reversible, the other non-reversible. The reversible effects have been described above

and are due to the absorbance of molecular oxygen itself (small) and to the absorbance of the molecular complex between oxygen and the solvent (large). Removal of oxygen gives rise to a decrease in UV detector baseline signal with this effect. The non-reversible effects are caused by the interaction of oxygen with the solvent to form relatively stable chemical species. The cyclic ether tetrahydrofuran (THF) appears to be particularly bad in this respect, as it is thought to form a hydroperoxide and a series of unstable peroxides. Fig. 8 has shown that the reversible effect with THF is also large.

Fluorescence effects of dissolved gases

The role of oxygen in fluorescence systems is quite complex and has been the subject of debate and experiment for many years. An early publication on the role of oxygen in fluorescence quenching was published by Bowen and Williams[37] who discussed in particular the quenching of aromatic hydrocarbon fluorescence. Later workers[38,39] found that the quenching effect of oxygen usually followed the Stern Volmer relationship $F_0/F = 1 + K[O_2]$. Results reported by Parker and Barnes[39] for the quenching of the borate–benzoin complex show that at 0.1% (v/v) oxygen in nitrogen in equilibrium with the solvent, ethanol, an 8% error occurs. At 0.8% oxygen the fluorescence is reduced 43%, and with air the fluorescence intensity is reduced 94%. The same authors showed that the majority of the effect was reversible, but that there was a second, slower reaction which was irreversible.

Bar and Weinreb[38] showed that in considering the mechanism of oxygen quenching, the mechanism of excitation is important. If the system is such that the solvent is absorbing the exciting radiation, and the energy is transferred to the solute, quenching occurs by competition between the oxygen and the solute. They report that as the concentration of solute decreases, the quenching effect of oxygen increases. With systems where the solute absorbs the exciting wavelength directly and the solvent does not absorb, oxygen quenching is again more efficient at lower concentrations but the magnitude of the effect with concentration is not so great. This is postulated to be owing to competition between oxygen quenching and self quenching, which increases considerably with increasing solute concentration.

That the effect of quenching is specific to oxygen was confirmed by Furst *et al.*[40], who examined the fluorescence of a large number of compounds in solutions saturated with oxygen, nitrogen, argon, carbon dioxide, hydrogen and nitrous oxide. All gases except oxygen gave the same fluorescence intensity. Thus, the effect of the other gases is to remove oxygen from the solution without otherwise affecting the fluorescence.

The quenching effect varies with compound type, as was briefly mentioned in the introduction. Aromatic hydrocarbons, aliphatic aldehydes and ketones are particularly susceptible to oxygen quenching, whereas substituted aromatics and some heterocyclics are much less susceptible.

Clearly, the analyst using HPLC with fluorescence detection must be concerned with the variation of the magnitude of the quenching effect among different compounds, and in the non-linear character of this effect. This is particularly true when working at the trace level, where the limits of the detector are being stretched, and the oxygen quenching is at its most efficient.

UV and fluorescence effects in chromatography

Fig. 9 shows the effect of dissolved oxygen on both the detector baseline and the eluted peak response (height and area) for both UV and fluorescence detectors. The data, made on a dual channel recorder, was collected by repetitively injecting the one-peak sample every 1.8 min using the SP 8000 autoinjector. Initially, the mobile phase was helium degassed. At time 0, the helium was replaced by an air sparge. After 16 min, the helium degassing was resumed. The conditions are listed in the figure caption.

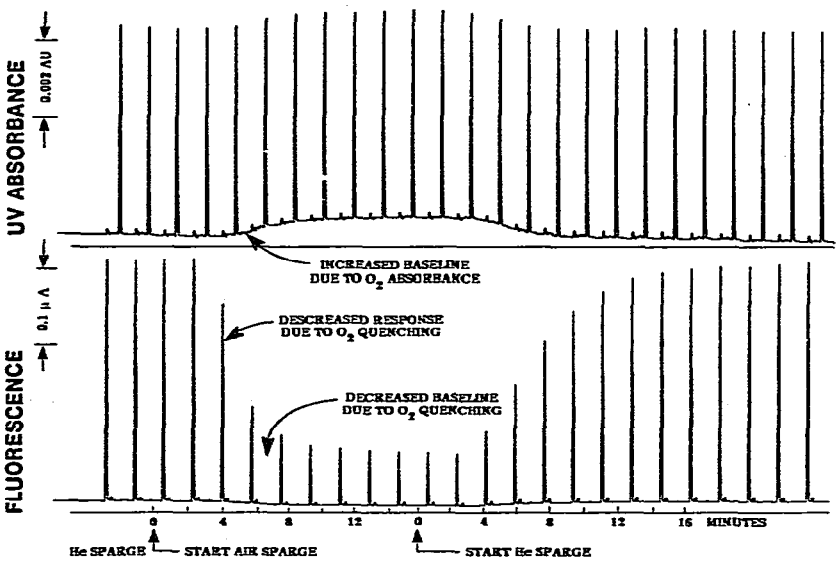

Fig. 9. UV absorbance detector and fluorescence detector response to mobile phase oxygen. Column 250 × 4.6 mm I.D.; packing, 10-μm LiChrosorb RP-8; solvent, water–acetonitrile (25:75); flow-rate, 5.0 ml/min; temperature, 25 °C; detector, Model SP 8310 UV detector at 254 nm with a 1.0-cm path cell, and Model SP 770 fluorescence detector at 250 nm exciting wavelength and with 340 nm emission wavelength filter, 1.0 μA full scale; sample size, 10 μl; sample, naphthalene.

The UV detector trace exhibits the baseline elevation previously described in association with Figs. 7 and 8. However, the response to the compounds does not change.

The fluorescence detector trace also exhibits a baseline shift. This has not yet been mentioned. The change is in the opposite direction to the UV drift. This is because the increased concentration of oxygen quenches the background fluorescence of the mobile phase, whereas in the UV detector the oxygen is responsible for increased absorbance. The magnitude of this drift is noteworthy. It is about 0.004 μA, or about 40% of the full-scale sensitivity of the detector (0.01 μA full scale). However, the most marked effect of the oxygen is in the sample response, which is only about 20% of that when the solvent is degassed.

Fig. 10 also shows these effects, as well as a number of general characteristics of the two detectors. Four separate chromatographic runs were made, using both a UV and a fluorescence detector on each run. Thus there are eight chromatograms.

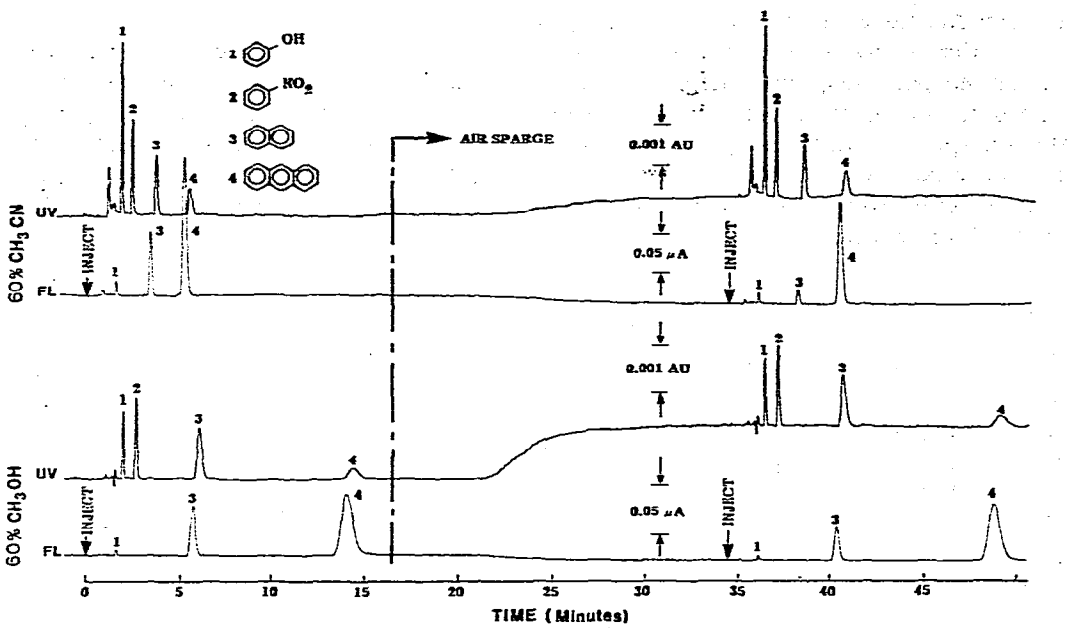

Fig. 10. Characteristics of UV and fluorescence detectors. Column, 250 × 4.6 mm I.D.; packing, 10-μm LiChrosorb RP-8; solvent, water–acetonitrile (40:60) and water–methanol (40:60); flow-rate, 3.0 ml/min; temperature, 25 °C; detector, same as Fig. 9, except UV at 0.01 AUFS and fluorescence at 0.5 μA full scale; sample size, 10 μl; sample, as shown.

The upper four chromatograms are run with a mobile phase of 60% acetonitrile in water. The bottom four chromatograms are run under identical conditions, except the mobile phase is 60% methanol in water. The four chromatograms on the left were made using helium-degassed mobile phases. The four chromatograms on the right were made using air sparged (air saturated) mobile phases. A vertical line indicates the point at which the gases were changed. The many aspects of the complex behavior of these two detectors are described below.

UV and fluorescent detector response differences

The upper left chromatograms illustrate the well known fact that the two detectors respond quite differently to different compounds. Solute 2, nitrobenzene, is not even detected by the fluorescence detector under these conditions. These two chromatograms will serve as a reference against which the other three sets will be compared.

Response differences caused by the solvent

The lower left chromatograms are quite different. First, quite obviously because the peaks are more retained. But a close inspection shows that, for some compounds, the detector response has changed. This is true for both detectors. The solvent composition of the mobile phase thus has a profound effect on response.

Baseline and response changes caused by dissolved oxygen

The upper right chromatograms, compared to the upper left ones, show the effects of oxygen. UV detector: baseline change but no peak height or area change. (For a description of the dependence of peak height and area on mobile phase composition, see ref. 41) Fluorescence: baseline change and peak height and area change. Note that peak 3 has been affected much more than peak 1. Thus an internal standard does not solve the problem. The sensitivity of the fluorescence detector has been reduced for all peaks, although only slightly in the case of peak 1.

The lower right chromatograms, compared to the lower left ones, show similar but not identical effects of oxygen on the fluorescence detector. Note for example that there is much less change in the relative sizes of peaks 1 and 3, compared to the upper (acetonitrile) chromatograms.

Other effects of dissolved gases

Two major effects of dissolved gases have not yet been mentioned: the changes in refractive index caused by changes in concentrations of various gases, and the changes in pH caused by changes in the concentration of carbon dioxide in unbuffered mobile phases. These effects will be discussed in subsequent papers, but a few comments will be made here.

The refractive index of the mobile phase is a function of the types and concentrations of dissolved substances, including gases. The effects of gases are small, but it is likely that, under some circumstances, careful attention to controlling dissolved gases will produce more stable refractive index detector baselines, in effect improving sensitivity.

The pH of the mobile phase is an important retention variable. Since dissolved carbon dioxide brings the pH 7.0 of pure water down to about 5.5, changing carbon dioxide levels are expected to effect retention times of basic compounds. UV and fluorescence detector response is also a function of pH, since the molar extinction coefficient is often pH dependent. Experiments of the type shown in Fig. 10 are expected to reveal such effects readily, when appropriate sample types are chosen.

CONCLUSIONS

We have demonstrated that gases dissolved in the mobile phase play a complex role in HPLC. Their non-linear solubility behavior in binary mixtures tends to cause the formation of gas bubbles when solvents are mixed, a process which degrades pump and detector performance.

Whereas the gas bubble problem has its solution in the reduction of the concentration level of all gases, most of the other problems can be dealt with by controlling just the oxygen concentration. Oxygen affects the standing signal levels of both UV and fluorescence detectors. It also affects the response characteristics of the latter. This is a complex phenomenon involving solute type, mobile phase solvent composition and oxygen concentration. Much work remains to be done before we will understand what is happening. In spite of limited understanding of the mechanisms, one can at least attempt to hold the oxygen level constant so as to provide reproducible chromatographic results. The most effective constancy is to take the oxygen concentration to zero.

The effect of dissolved gases on the performance of refractive index detectors is yet to be explored, as are the consequences of varying carbon dioxide concentrations on solute retention and on detector response characteristics. The techniques discussed here can be readily applied to such studies.

Much remains to be learned about gas solubility, and no doubt the main source of such knowledge will be the continued use of gases as probes to study the structure of liquids. The increasing use of ternary mixtures in both isocratic and gradient separations provides an even more difficult theoretical problem. In any event, there are a variety of techniques available for controlling gas concentrations and thereby eliminating problems which would otherwise exist. The new helium degassing method described appears to be a particularly simple and effective method.

NOTE ADDED IN PROOF

Subsequent to the submission of this paper, R. W. Cargill sent us a pertinent private communication. His data will be published in *J. Chem. Soc., Faraday Trans. I* (1978). It indicates that the solubility behavior of helium in aqueous alcohol mixtures is very similar (non-linear) to that shown in Fig. 2 for oxygen, although the magnitude of the solubility is lower. We had postulated two explanations of why helium degassing eliminates bubble problems (see the "gas control methods" section). The Cargill data suggests that the low-solubility postulate is correct, and not the linear-behavior postulate. The Cargill paper also makes a significant contribution to the understanding of water structure, and will be of interest to those who are working to elucidate retention mechanisms in reversed-phase liquid chromatography.

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