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SOLVENT DEGASSING AND OTHER FACTORS AFFECTING LIQUID CHROMATOGRAPHIC DETECTOR STABILITY

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SUMMARY

We have measured the absorbance of methanol in the region 200–300 nm as a function of dissolved helium, nitrogen and air, and shown that a 1 % change in oxygen level can cause a $4 \cdot 10^{-3}$ a.u. change at 210 nm. We have also measured the amount of oxygen that can be removed from different solvents by the degassing techniques of refluxing, helium sparging, vacuum degassing and ultrasonic agitation. Using these results we have determined the operating conditions for minimum baseline drift in high-performance liquid chromatographic detection at 0.005 a.u.f.s. sensitivity in the range 190–220 nm. We observed a residual change in the absorbance of methanol, related to temperature, even after removal of oxygen.

INTRODUCTION

The increasing importance of high sensitivity in high-performance liquid chromatography (HPLC) systems, particularly at low UV wavelengths, makes it necessary to look at all factors affecting total system stability. A major group of such factors is that concerned with gases dissolved in solvents which affects not only flow stability but also the UV absorption, causing baseline drifts and random noise in UV detector outputs. These arise not only from actual absorbance changes but also from the low energy through-put of detectors operating with highly absorbing solvents.

Dissolved gases are usually present in mobile phases, and Bakalyar *et al.*[1] and others[2] have written about the effects of dissolved oxygen on UV detectors. Most published results have been in the region of 254 nm, however, despite the fact that many workers routinely use methanol-based mobile phases at 220 nm and below. We have measured the absorbance of methanol over the range 200–300 nm when saturated with helium, nitrogen air and carbon dioxide, and the efficiency of various techniques for removing dissolve ' oxygen. Using these results we have determined the operating conditions for minimum baseline drift at 0.005 a.u.f.s. sensitivity in the wavelength range 190–220 nm.

Although most   ͬ the results relate to methanol the efficiency of oxygen removal has been measured for other solvents.

EXPERIMENTAL

Equipment

A Pye Unicam LC-UV detector, XPD pump and Partisil 5 ODS column (25 cm × 4.6 mm I.D.) were used for all HPLC experiments. A Pye Unicam SP8-250 UV–visible spectrophotometer equipped with a thermostated (0.05°C) 1-cm cuvette was used for absorbance against temperature measurements. Oxygen levels were measured on a Pye Unicam GCD gas chromatograph fitted with an electron-capture detector and a back-flush system.

Solvents

All solvents were "HPLC Grade" from Rathburn Chemicals, Walkerburn, Great Britain.

Gases

Helium, air and nitrogen (white spot quality) were from British Oxygen Co. (London, Great Britain).

High-performance liquid chromatography

Solvents were contained in 2.5-l glass containers having a single opening of 20 mm diameter through which all connecting tubing passed. In some experiments this opening was sealed and vented through a separate Dreschel gas wash bottle of solvent to eliminate any back diffusion of air. Solvents were saturated with the appropriate gas by bubbling it through a 15-μm sinter into the solvent for 10–15 min at a flow-rate of 300–600 ml min^{-1}. Subsequently a continuous purge of gas was maintained at the flow-rate appropriate to the experiment.

Flow-rates were between 1 and 2 ml min^{-1} and column pressures 30–130 bar. Temperature measurements were made using a platinum resistance thermometer in a Wheatstone bridge configuration.

Oxygen removal

Oxygen was displaced by another gas as described above or removed by one of the following methods:

    (1) Boiling the solvent in a glass vessel equipped with a reflux condenser;
    (2) Evacuation of the air above the liquid in the solvent contained by a vacuum pump;
    (3) Immersing the solvent container in an ultrasonic bath (10 W).

Oxygen was measured by injecting 10-μl of liquid directly into a Porapak T column (17 cm × 0.4 cm I.D.) at 110°C which was connected via a magnesium perchlorate guard column (10 cm × 0.4 cm I.D.) to a 5A molecular sieve column (90 cm × 0.2 cm I.D.). The latter two columns were operated at 20–25°C. The sample was allowed to flush through all the columns to an electron capture detector operated in the pulsed mode and subsequently the Porapak was back flushed for 4 min.

RESULTS AND DISCUSSION

Absorbance of methanol saturated with various gases

Table I shows the absorbance of methanol measured over the range 200–300 nm when saturated with helium, nitrogen, air and carbon dioxide. It shows not just that oxygen is the main cause of higher absorbance but that the effect is very large indeed at wavelengths below 230 nm. Either helium or nitrogen replacement of the oxygen cuts the absorbance by half and extends the useable wavelength range 5 or 10 nm by significantly increasing the energy through-put of the detector, effectively extending the UV window of the methanol. It is interesting that CO_2 gives similar results.

The measured difference in absorbance at 210 nm between air-saturated and nitrogen-saturated methanol corresponds to a drift of $4 \cdot 10^{-3}$ a.u. for every 1 % change in dissolved oxygen. The significance of this in chromatographic terms is shown in Fig. 1, where displacement of oxygen by nitrogen causes a reversible 0.41 a.u. fall in absorbance. Significantly the trace shows helium is less efficient than nitrogen at replacing oxygen in this experiment and this was traced to air back-diffusing against the lighter helium.
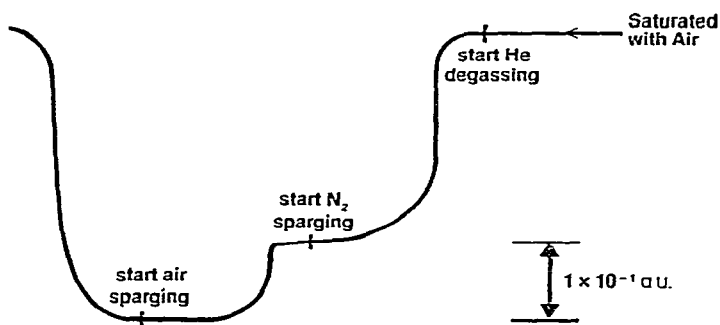

Fig. 1. Baseline changes in methanol at 210 nm and 2 ml min^{-1} on removing oxygen. Replacement of oxygen by nitrogen decreases the absorbance by 0.407 a.u. The effect is reversible.

Baseline stability under helium sparging

It is clear that temperature changes of the mobile phase will cause changes in dissolved gas content. If oxygen has not been removed from a methanol mobile phase, for instance, a 1.0°C rise will cause a 0.3 % decrease in oxygen level at 300°K and a drift of $1.2 \cdot 10^{-3}$ a.u. will result. Fig. 1 suggests that complete oxygen replacement by helium is not always achieved and this probably accounts for the random drifts reported by workers using helium sparging. Incomplete replacement of the oxygen by helium appears to be a serious problem that has not been reported before, and we found replacement was variable and incomplete even when large amounts of methanol in narrow-necked containers were sparged. The problem was traced to back-diffusion of air against the lighter helium gas.

Chromatographic results with methanol at 210 nm show random variations of $2 \cdot 10^{-3}$ a.u. for such a system, but closing the solvent container through a Dreschel bottle to prevent back-diffusion improved the baseline stability ten-fold (Fig. 2). This reduction was achieved at helium sparging rates of only 4 ml min^{-1}, a considerable

TABLE I

ABSORBANCE OF METHANOL SATURATED WITH DISSOLVED GASES AT 20°C

Methanol was saturated with helium, nitrogen, air or carbon dioxide at 20°C and pumped through the flow cell of a Pye Unicam LC-UV detector at 2 ml min^{-1}. The absorbance was recorded, in a.u., at various wavelengths in the range 200–300 nm.

Gas	Wavelength (nm)											
	300	280	270	260	250	240	230	220	215	210	205	200
Helium	0.058	0.074	0.091	0.100	0.141	0.154	0.179	0.202	0.243	0.416	0.896	>1.28
Nitrogen	0.054	0.074	0.091	0.111	0.141	0.154	0.178	0.195	0.230	0.387	0.909	>1.28
Air	0.055	0.076	0.096	0.116	0.157	0.202	0.288	0.429	0.547	0.794	>1.28	>1.28
CO$_2$	0.051	0.073	0.091	0.111	0.141	0.166	0.229	0.278	0.352	0.448	0.896	>1.28

4×10^{-3} a.u.   He 30 ml min^{-1}

4 min

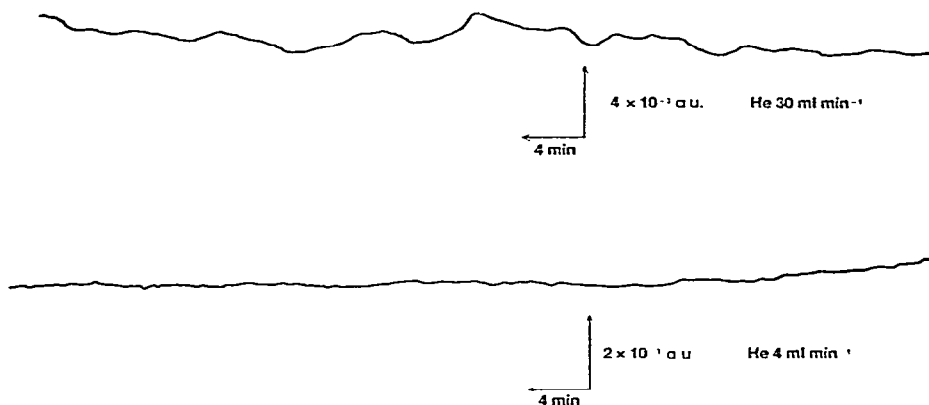2×10^{-3} a.u.   He 4 ml min^{-1}

4 min

Fig. 2. Baseline drifts with helium sparging (210 nm). Above: random drifts due to air back-diffusing through 20-mm hole. Below: ten-fold improvement in random drifts when container closed with bubble trap.
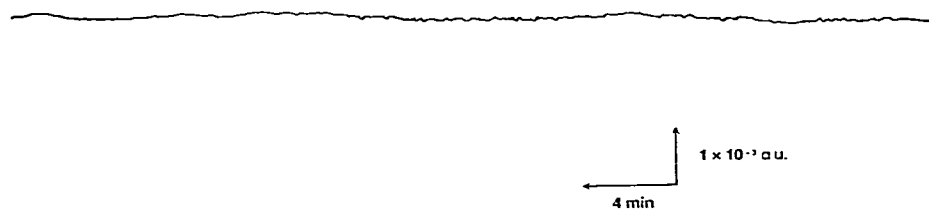
1×10^{-3} a.u.

4 min

Fig. 3. Marginal improvement of fast sparging. Increasing the sparging rate to 120 ml min^{-1} in a closed container gives a two-fold improvement.

saving in helium gas. In fact increasing the sparging rate from 4 to 120 ml min^{-1} with a closed container only improved the stability to $1 \cdot 10^{-4}$ a.u. (Fig. 3).

It is clear therefore that if helium sparging is to give a stable baseline at high sensitivity and low wavelength the solvent container must be closed to a narrow opening of perhaps 1 mm or less cross-section to eliminate back-diffusion of air.

Temperature dependence of methanol absorbance

Further studies of absorbance changes showed that even when oxygen was apparently completely removed from the mobile phase the baseline still drifted in phase with temperature change (Fig. 4) to the order of $+3 \cdot 10^{-3}$ a.u. deg^{-1} for methanol at 10 nm. We have not yet established the cause of this drift but it is not due to residual oxygen since increasing temperature would decrease the oxygen level and

210nm 2×10^{-3} a.u.

4 min
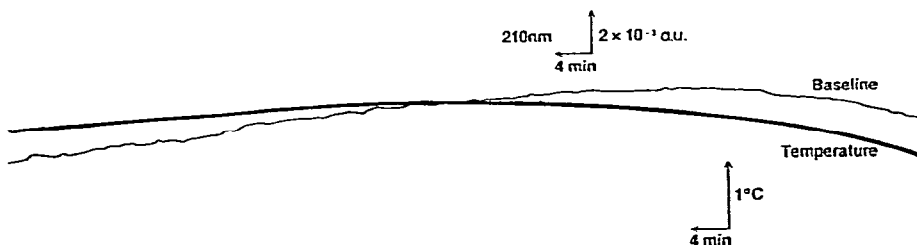
Baseline

Temperature

1°C

4 min

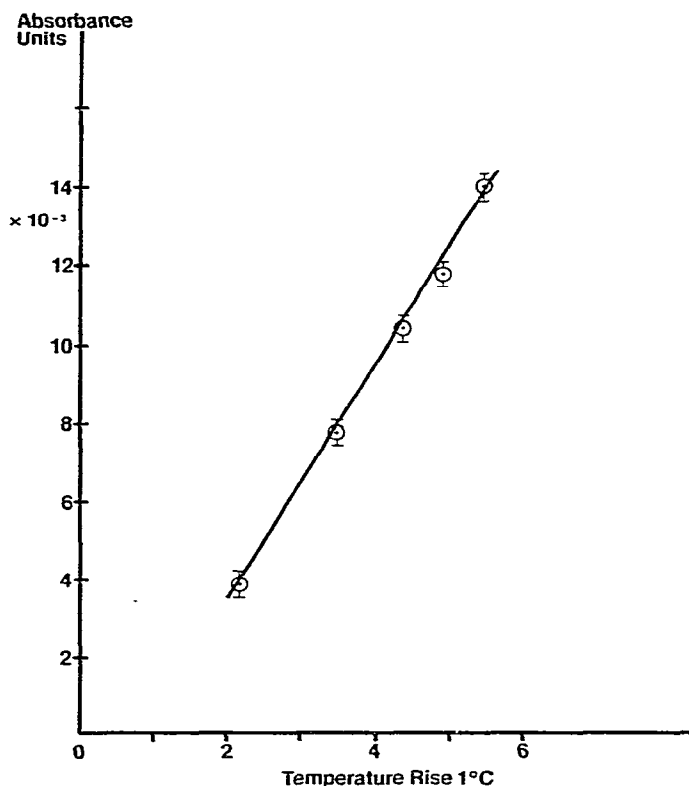Fig. 4. Detector drift following ambient temperature.

Fig. 5. Absorbance as function of temperature. A plot showing the residual change in absorbance with temperature of methanol, after removing oxygen.

hence decrease the absorbance. Spectrophotometric measurement of this apparent temperature effect confirmed its presence (Fig. 5) and work continues.

Various methods of oxygen removal

In view of the importance of removing dissolved oxygen from mobile phases we compared the efficiency of solvent refluxing, helium replacement of oxygen, ultrasonic degassing and vacuum degassing. Solvent refluxing completely removed all detectable dissolved oxygen in any solvent. The efficiency of the other methods were dependent on the solvent used, water and methanol being the most difficult to degas, hexane and acetonitrile less so. Ultrasonic degassing was particularly disappointing and on occasions actually increased oxygen levels. Fig. 6 and 7 show the comparative efficiencies of the various methods for water and methanol.

Nitrogen was not included in these early studies but its effect is identical to helium without the disadvantage of back-diffusion problems.

Obviously refluxing is an inconvenient and potentially hazardous technique and helium or vacuum degassing is often the best compromise. Nitrogen sparging will replace oxygen and is more generally available than helium, but it may cause gas bubbles in pump inlets when used in conjunction with low pressure solvent mixing or similar flow restrictions.
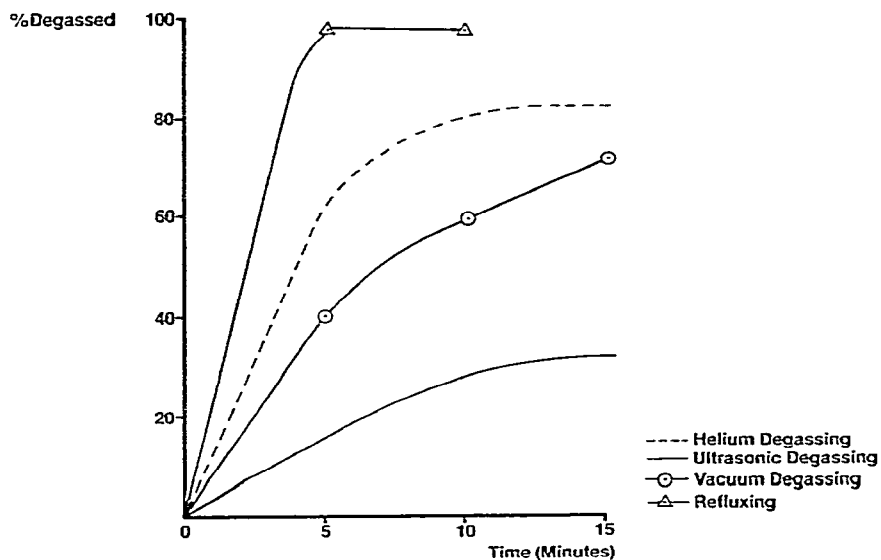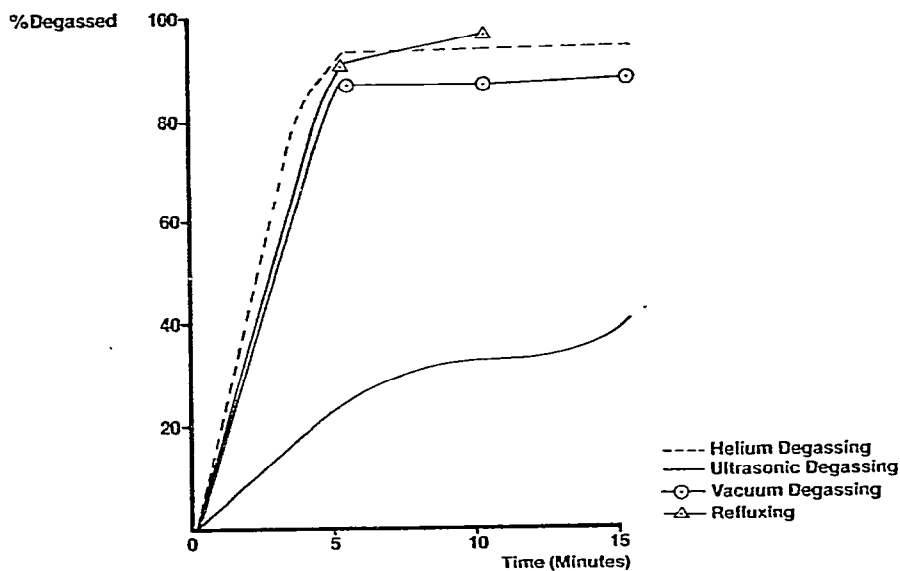
Fig. 6. Oxygen replacement in methanol.

Fig. 7. Oxygen replacement in hexane.

In all cases it is necessary to either keep the mobile phase continuously sparged, or continuously heated, to prevent re-absorption and variable oxygen levels.

CONCLUSIONS

(1) The absolute absorbance of a methanol mobile phase is critically dependent on its dissolved oxygen content. At low wavelengths the effect of oxygen is very much larger than might be inferred from previous papers[1].

(2) *Any* change in oxygen content will cause detector baseline drifts; for example, at 210 nm a 1 % change in dissolved oxygen level will cause a $4 \cdot 10^{-3}$ a.u. change in absorbance in methanol.

(3) Such changes can be brought about by changes in the ambient temperature of the mobile phase. A 1°C rise in temperature will cause a 0.3 % fall in dissolved oxygen in methanol, leading to a $1.2 \cdot 10^{-3}$ a.u. change. They can also be caused by variations in oxygen level resulting from air back-diffusing against a helium sparge.

(4) Nitrogen will effectively replace oxygen from a mobile phase and may be used to extend the UV window of methanol downwards providing gas bubble formation in the pump or detector is not a problem.

(5) Refluxing is completely effective in removing oxygen from all solvents studied but it is necessary to keep the solvent warm to prevent subsequent re-adsorption. Gas bubble formation will not be a problem since all other gases are also removed by refluxing.

(6) Vacuum degassing and helium replacement are moderately effective in removing oxygen from solvents such as water and very effective with hexane. Methanol and acetonitrile fall mid-way between the two. With such solvents the amount of oxygen (and nitrogen) removed is adequate to reduce bubble formation at the inlet to a pump, but without special care it is insufficient to achieve the lowest mobile phase UV absorption. In all cases continuous sparging, or warming, is necessary to prevent re-adsorption.

(7) Ultrasonic degassing is ineffective and can lead to increased oxygen levels under some conditions.

(8) After complete removal of oxygen from methanol we measured a reversible absorbance change of $3 \cdot 10^{-3}$ a.u. deg $^{-1}$ at 210 nm. Work is continuing to discover the cause of this effect.

REFERENCES

1 S. R. Bakalyar, M. P. T. Bradley and R. Honganen, *J. Chromatogr.*, 158 (9178) 277–293.
2 J. H. Williams and C. D. Miller, *Anal. Chem.*, 34 (1962) 657.