Reversed-Phase High Performance Liquid Chromatography of Carbon Dioxide

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REVERSED-PHASE HIGH PERFORMANCE LIQUID CHROMATOGRAPHY OF CARBON DIOXIDE

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ABSTRACT

An investigation of the qualitative aspects of the liquid chromatography of carbon dioxide is described. It is demonstrated that although injected as a high pressure liquid, carbon dioxide dissolves in aqueous methanol mobile phases and elutes as a solution. The detector response is discussed in terms of the possible chemical interactions between the carbon dioxide molecule and the mobile phase; the effect of eluent pH upon the response is described. The variation of relative retention with mobile phase composition is detailed and the results discussed in terms of Horvath's solvophobic theory.

INTRODUCTION

For some time, this laboratory has been engaged in the development of a technique for the determination of the limits of solubility of organic compounds in liquid carbon dioxide. Although the basic technique for the sampling and analysis of high pressure liquefied gas solutions has been established (1),

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solubility data cannot be obtained from substrate quantitation
alone; it is necessary to determine the quantity of liquid
carbon dioxide present in solution. Reversed-phase high per-
formance liquid chromatography facilitates quantitation of the
organic species and it would be experimentally expedient if the
solvent could be simultaneously quantitated.

However, before any progress toward this objective can be
made, it is first necessary to investigate the qualitative
aspects of the liquid chromatography of carbon dioxide to ensure
that this technique is in fact appropriate. This contribution
describes such an investigation within the following framework:

* Is carbon dioxide detectable?
* Is carbon dioxide eluted as a gas or as a solution
  in the mobile phase?
* Does carbon dioxide interact chemically with the
  eluent?
* Is retention of carbon dioxide observed in the
  reversed-phase chromatographic system?

EXPERIMENTAL

The instrument employed in this investigation was of
modular design comprising components obtained from Waters
Associates Australia Pty. Ltd: a model 6000A solvent delivery
system and a model R401 differential refractometer. Samples
were introduced via a 4 port internal-volume sample injection
valve (Valco, Texas), of nominal volume of 2 mm^3, into a Waters
Associates 30 cm x 3.9 mm ID μ-Bondapak C_{18} analytical column.
The detector signal was relayed to a Hewlett-Packard reporting
integrator, model 3390A. Binary mobile phases of various
compositions were prepared from methanol and water, each of
which had been freshly distilled in glass apparatus. Eluents

were filtered and degassed prior to use. All chemicals were of analytical reagent grade and were obtained from local suppliers. Liquid carbon dioxide of "FOOD GRADE" (99.8% minimum purity) was obtained from The Commonwealth Industrial Gases Limited (Sydney, Australia). The liquefied gas is supplied in aluminium cylinders equipped with an internal tube attached to the valve to permit withdrawal of the liquid phase. Liquid carbon dioxide was introduced to the chromatograph by the procedure described elsewhere (1) after filtration to 2 μm by a Nupro in-line filter (Sydney Valve and Fitting Co., Sydney, Australia). All analyses were performed at approximately 22°C.

RESULTS AND DISCUSSION

Detection of Carbon Dioxide

Although the carbon dioxide molecule does not absorb radiation in the ultraviolet and visible regions of the electromagnetic spectrum, it does possess a small refractive index (2). This value varies with the temperature and pressure of the gas and increases upon liquefaction. As indicated in Table 1, the refractive index of carbon dioxide is significantly different

TABLE 1

Indices of Refraction

Substance	Refractive Index	Reference
Methanol	1.329	(3)
Ethanol	1.361	(3)
Isopropanol	1.38	(3)
Acetonitrile	1.344	(3)
Water	1.333	(3)
CO_2, 0°C, 101.3 kPa	1.0004	(2)
CO_2, liquid, 25°C	1.173	(2)

from that of compounds frequently employed as mobile phases in reversed phase liquid chromatography.
It is to be expected, therefore, that the differential refract-ometer should be capable of detecting the presence of eluted carbon dioxide. Figure 1 represents the response of the differential refractometer when pure liquid carbon dioxide was introduced into the chromatograph. As implied by the data in Table 1, the peak obtained was of negative polarity.

The solubility of carbon dioxide in methanol and water is appreciable at ambient temperature and atmospheric pressure. Typical values are, at 25°C and 101.3 kPa, 38 1 and 0.83 1 of carbon dioxide per litre of the respective solvents (4). Con-sidering that the operating pressures of modern liquid

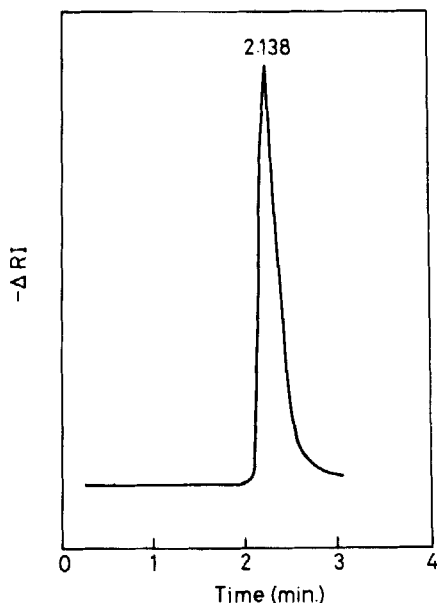Figure 1.   Detector response for carbon dioxide injection.
             HPLC conditions: mobile phase - 70:30 methanol/
             water; flowrate - 1.8 $cm^3 min^{-1}$; detector
   attenuation - 4X; chart speed - 1.0 cm min^{-1}.

chromatographs may reach 34 MPa (5), there can be no doubt that liquid carbon dioxide dissolves in the mobile phase at the point of injection. There is, however, a pressure gradient along the length of the column, the system pressure reducing to atmospheric in the regime of the detector(s). The possibility of the dissolution of carbon dioxide under these conditions could not be discounted. Were the response illustrated in Figure 1 due to the presence of gaseous carbon dioxide, then quantitation would be impossible since such response would depend on the equilibrium

$$CO_2 \text{ (soln)} \rightleftharpoons CO_2 \text{ (g)} \tag{1}$$
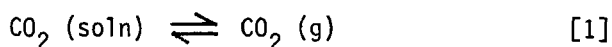
In order to demonstrate that the detector was responding to CO_2 (soln) and not gaseous carbon dioxide, the pump was directly connected, via the sample injection valve, to the differential refractometer. Introduction of a sample of low pressure carbon dioxide vapour produced the response illustrated in Figure 2. We concluded that the sharp peaks were due to a gas bubble passing through the detector cell; the broad peak was attributed to dissolved carbon dioxide.

Chemical Interactions

It is well known that carbon dioxide and water react to form carbonic acid. This compound then dissociates to form a proton and bicarbonate ion (6):

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \tag{2}$$

$$H_2CO_3 \rightleftharpoons H^+ + HCO_3^- \tag{3}$$
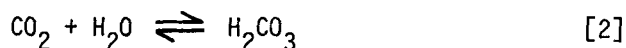
The total equilibrium can be represented by

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^- \tag{4}$$
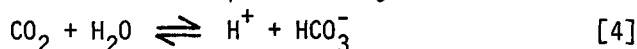
An equilibrium such as [4] would be readily affected by a change
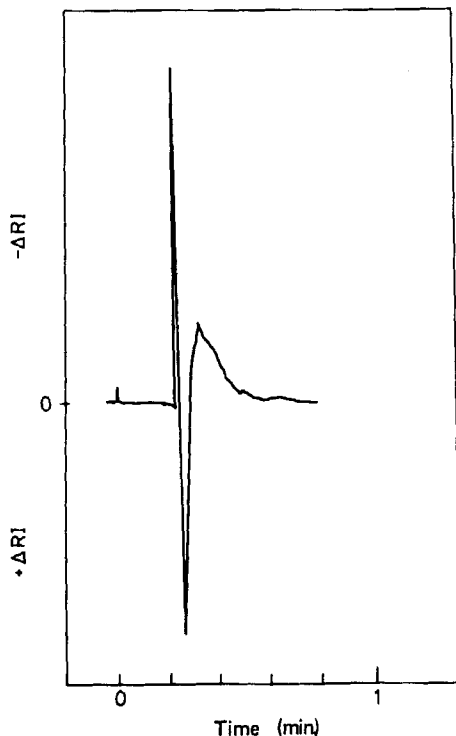
Figure 2.   Detector response for vapour - phase carbon dioxide.
             HPLC conditions: mobile phase - 80:20 methanol/
             water; flowrate - 1.5 $cm^3 min^{-1}$; detector attenuation
             - 4X; chart speed - 5 cm min^{-1}.

in pressure or pH: at high pressures and/or high pH, bicarbon-
ate ion formation would be favoured; acidic conditions would
preserve the non-reactivity of carbon dioxide.

       In order to test whether or not the response illustrated in
Figure 1 was due to a reaction product, liquid carbon dioxide
was injected into an eluent of pure (anhydrous) methanol: there
was no difference in the detector response, indicating that
reaction products do not account for the observed response for
carbon dioxide. This is to be expected since the equilibrium

constants for reactions [2]-[4] have values of 3.6×10^{-5} (7), 2×10^{-4} (8) and 7.2×10^{-9} (9), respectively.

The effect of low pH upon the detector response was determined by injecting liquid carbon dioxide into an eluent comprising 40 v % methanol/60 v % water which had been acidified with glacial acetic acid; the pH of this solution was approximately 3.5. That no change in the response was observed confirmed the non-reactivity of carbon dioxide under acidic conditions.

In order to produce an eluent of high pH, a solution of 60 v % methanol/40 v % 2.6×10^{-4} M Na_2CO_3 was prepared. Such a mobile phase would be alkaline due to the equilibrium

$$CO_3^{2-} + H_2O \rightleftharpoons HCO_3^- + OH^- \qquad [5]$$
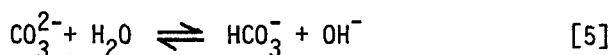
Injection of liquid carbon dioxide into this medium resulted in the chromatogram illustrated as Figure 3. The appearance of the broad peak of positive polarity demonstrated that carbon dioxide undergoes chemical reaction in alkaline solution. Such reaction proceeds according to the following equilibrium (9):

$$CO_2 + OH^- \rightleftharpoons HCO_3^- \qquad [6]$$
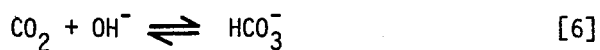
However, the injection of a solution of eluent saturated with $NaHCO_3$ failed to effect a detector response, indicating that the first peak in Figure 3 could not be attributed to the presence of bicarbonate ions.

Consideration of the total reaction

$$CO_2 + CO_3^{2-} + H_2O \rightleftharpoons 2HCO_3^- \qquad [7]$$
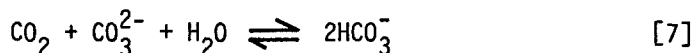
suggests that the observed response may arise from a local change in eluent composition due to the consumption of water. That is,
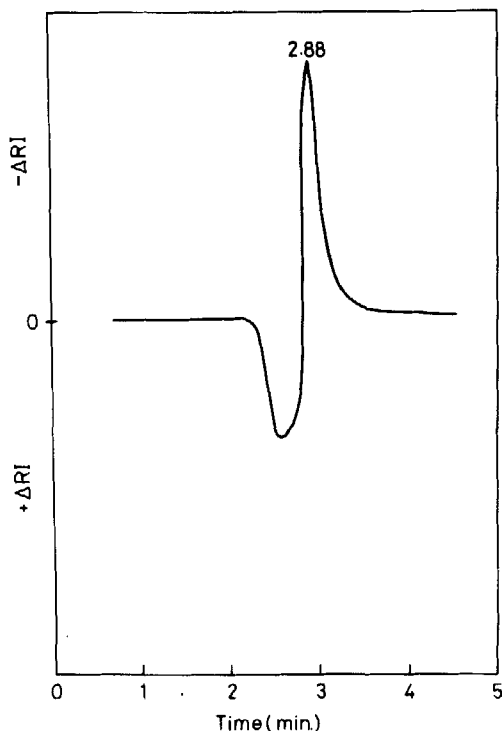
Figure 3.   Chromatogram of carbon dioxide in alkaline eluent.
             HPLC conditions: mobile phase - 60:40 methanol/
             2.6×10^{-4} M Na_2CO_3; detector attenuation - 4X; chart
             speed - 1.0 cm min^{-1}.

the first peak in Figure 3 reflects an increase in the methanol
concentration with respect to the composition of the eluent
trapped in the reference cell. In any event, it is apparent
that quantitation of carbon dioxide is impossible if alkaline
eluents are employed.

Retention of Carbon Dioxide

     The accuracy of quantitation depends to a large extent on
the resolution of adjacent peaks (10). Resolution in turn
depends on, among other parameters, the capacity factor, k'.
It is therefore of interest to investigate the variation of the

capacity factor of carbon dioxide with mobile phase composition. This study was also restricted to the use of binary methanol-water eluents.

Aqueous methanol solutions of various concentrations were prepared and the absolute retention times of carbon dioxide and several other compounds measured by means of the reporting integrator. A nominal flowrate of 2 cm^3min^{-1} was used except where this would have resulted in exceeding the column's recommended pressure limit of 21 MPa. In those instances, the maximum attainable flowrate was selected. The exact flowrate was obtained from the time taken to fill a 25.0 cm^3 volumetric flask. Absolute retention times were converted to retention volumes in the usual fashion (10). The column hold-up volume was estimated by the injection of a volume of mobile phase diluted slightly with water (11).

The data obtained in this study are presented in Figure 4 as plots of ln k' against the percentage methanol in the mobile phase. Several authors (12-15) have remarked that plots of this type are frequently quasi-linear.

According to the solvophobic treatment of the phenomenon of retention in reversed-phase liquid chromatography (13,15) the magnitude of solute retention depends on the balance of the solute-stationary phase and solute-mobile phase interactions. Approximate linearity of the theoretical ln k'- composition plots has been obtained from consideration of the effects of the surface tension of the eluent and the surface area change which occurs upon binding the solute to the hydrocarbonaceous station-ary phase (15). Were the surface area change to decrease with the reduction in concentration of organic modifier in the eluent, then departure from linearity towards lower ln k' values could reasonably be expected.
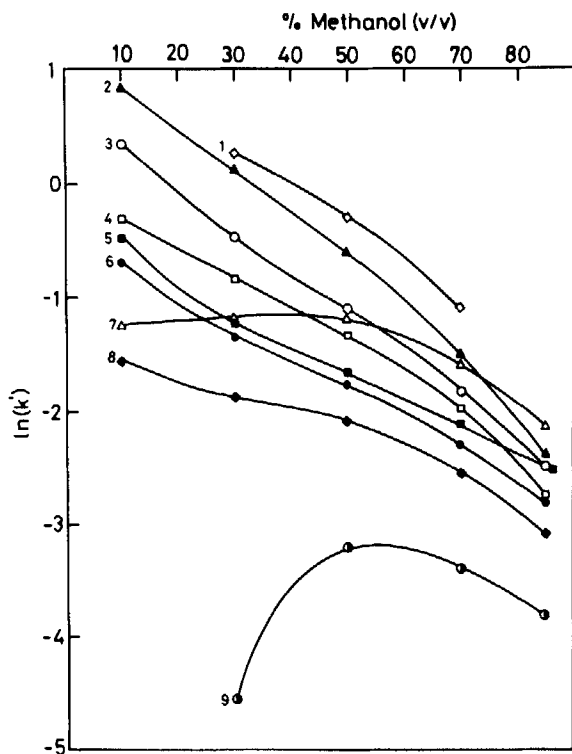
Figure 4.   Variation in capacity factor with eluent composition.

(1) methylene chloride; (2) n-butanol;
(3) methyl ethyl ketone; (4) n-propanol;
(5) 1,4 dioxane; (6) acetone; (7) carbon
dioxide; (8) acetonitrile; (9) formamide.

        Such a departure from linearity could result also from the
strengthening of the solute-solvent interaction relative to
solute-stationary phase interaction. In the case of formamide,
this is a plausible explanation of the retention behaviour since
the solvent properties (16,17) of this compound are almost
identical to those of water. Consequently, solute-solvent
interactions are enhanced when the eluent assumes properties
similar to those of pure water and decreased retention results.

An explanation of the retention behaviour of carbon dioxide is somewhat more complex, since this compound is considered to be non-polar, and significant interaction between the solute and stationary phase is to be expected. However, the carbon-oxygen bonds of the linear carbon dioxide molecule are undoubtedly polarized (18), thus offering opportunities for hydrogen bonding with the polar eluent. Association of the carbon dioxide molecule with the mobile phase would therefore result in the formation of a complex, the polarity of which would be greater than that of carbon dioxide alone, leading to a reduction in the retentive forces (15). If, in fact, interaction between the aqueous eluent and carbon dioxide resulted in hydration of the latter (as distinct from reaction to form carbonic acid, then the decreased retention of carbon dioxide at high concentrations of water could be explained, despite the fact that pure water is a poorer solvent for carbon dioxide than is pure methanol. That is, since carbon dioxide is more soluble in methanol than in water, we would expect enhanced retention as the concentration of methanol in the eluent decreases due to the weakening of the solute-solvent interaction because of dilution effects. While this phenomenon is observed for a range in methanol-water composition, it does not persist; therefore carbon dioxide must interact with the added water. Such interaction may be due to hydrogen bond formation or hydration of the dissolved carbon dioxide.

While carbon dioxide may physically associate with the mobile phase, there is no change in the detector response with mobile phase composition, indicating that chemical reaction products do not account for the observed response for carbon dioxide injections into neutral or acidic media. Therefore we consider that the phenomenon of physical association is not detrimental to the viability of quantitating dissolved carbon dioxide.

CONCLUSION

Carbon dioxide has been found to be soluble in binary
methanol water mobile phases and can be detected by monitoring
the refractive index of the eluent. Although alkaline solvents
are unsuitable for carbon dioxide analysis, no evidence for
chemical reaction in neutral or acidic media has been found. A
semi-logarithmic plot of capacity factor versus methanol content
has been found to be non linear for both carbon dioxide and
formamide. Such a phenomenon may be explained by proposing that
these compounds interact preferentially with the eluent. Carbon
dioxide may interact via hydrogen bond formation or hydration
with the aqueous component of the mobile phase.

ACKNOWLEDGEMENTS

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