Solute Retention in Column Liquid Chromatography. XI. Exposition of the Minor-Disturbance Method of Measurement of the Void Volume

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SOLUTE RETENTION IN COLUMN LIQUID CHROMATOGRAPHY. XI. EXPOSITION OF THE MINOR-DISTURBANCE METHOD OF MEASUREMENT OF THE VOID VOLUME

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ABSTRACT

The retention volumes corresponding to the disturbance-peaks produced by injection of the components of aqueous blends of organic liquid carriers (reverse-phase stationary packings) have been claimed by some groups to agree to within an experimental error of ca. 1% while, in other instances, discrepancies of slightly greater than this figure have been reported. These results are accounted for in terms of the concentration-based solute partition coefficients K_R. Thus, taking water–methanol mobile phases as an example, the injection of a small amount of either compound is said to alter its partition coefficient due, if nothing else, to the resultant change of the composition of the bulk mobile phase. Further, the respective partition coefficients of the mobile-phase components (hence their retentions) will in all likelihood not be identical. Moreover, for liquid-liquid and reverse-phase LC systems at least, while the magnitude of the change of each of the component K_R is difficult to forecast, the direction is said to be predicated on the finite-concentration activity coefficients of the solutes in the blended solvent. For example, because water gives substantially larger negative deviations from Raoult's law with methanol-rich solvents than does methanol, the former is expected to be retained relative to the latter when such blends are employed as LC mobile phases; the converse should then obtain for water-rich carriers. Both situations have been verified experimentally.

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INTRODUCTION

The optimization of column liquid chromatographic (LC) separations in terms e.g. of mobile- and/or stationary-phase composition (including gradients of whatever form), temperature, and so forth, is often said to be carried out most conveniently in terms of solute capacity factors k' (1-3). However, doing so requires that the column void ("dead") time t_M or volume V_M be known or determined; where $k' = (t_R - t_M)/t_M = (V_R - V_M)/V_M$, and where t_R is the solute raw retention time and V_R the corresponding elution volume. Furthermore, determination of the column void volume in LC is not a trivial matter, as discussed most recently by Djerki and Laub (4). Nevertheless, a variety of techniques have been developed to this end, one of the simplest being the "minor disturbance" method: a small amount of one of the mobile-phase components is injected, which produces a "system" peak (i.e., a baseline disturbance, that may be either positive or negative). The void volume is then taken as the volume of mobile phase that passed through the column from the moment of injection to the maximum of the disturbance.

McCormick and Karger (5) carried out measurements of the disturbance-peak retention volumes given by three separate binary systems comprised of aqueous methanol, tetrahydrofuran, and acetonitrile over the full volume-fraction (v/v) compositional range, 0-100%. They found that what differences there were between the two disturbance peaks with a given mobile phase lay within experimental error, and amounted to less than 1% for all but the data obtained with neat solvents and with 80% methanol. (For the latter system the methanol peak gave a smaller retention volume than did water.)

It has been said in a number of other studies (6-8) that such minor disturbance peaks are in fact a measure of the derivative of the composite sorption isotherm of the mobile-phase components with the stationary phase; and that the system peak due to any one of the solvents should therefore be identical to that observed for any other. However, if this were proved to be so, one would need to know the relevant sorption isotherm(s) in order to calculate the void volume from such data, or at least to have made the void-volume measurement in the linear region of

the composite isotherm. Since linearity is extant only over very limited ranges with typical LC systems (e.g., aqueous methanol mobile phases with reverse-phase packings), Slaats, Markovski, Fekete, and Poppe (6) claimed that the full sorption isotherm must usually be determined in order to assess V_M in this way.

The results obtained for minor-disturbance peaks in our previous work (4) with liquid-liquid chromatographic systems (LLC: aqueous methanol mobile phases, polydimethylsiloxane stationary phase) were similar to those reported by McCormick and Karger. Thus, void volumes calculated from the methanol and water disturbance peaks were in good agreement for water-rich mobile phases whereas, for 70% and 90% v/v methanol, there was a difference between the two of slightly more than 1%. Moreover, water was retained relative to methanol with methanol-rich mobile phases; whereas the converse was true for water-rich carriers. Engelhardt, Muller, and Dreyer (9) also reported that deuterated water was retained relative to deuterated methanol with methanol-rich mobile phases.

In this work we account for these findings in terms of mobile-phase component partition coefficients. The considerations apply to LLC and reverse-phase LC systems at least, and, by straightforward extension, to LSC as well.

THEORY

Let $K_{R(W)}$ be the concentration-based partition coefficient of water at some mobile-phase composition of water + methanol with a reverse-phase packing, and let $K_{R(Me)}$ be the partition coefficient of methanol with the same solvent. Each is defined by the general expression:

$$K_{R(i)} = C_i^S / C_i^M \qquad (1)$$

where i = water (W) or methanol (Me), and where C_i^S and C_i^M are the concentrations of water or methanol in the stationary (S) and mobile (M) phases, respectively. Clearly, each of these equilibrium constants will depend upon the compositions of S and M.

It is often assumed, next, that if some amount of methanol-rich sample is injected into such a system, the compositional change of the mobile

phase can be reckoned on the basis of volume additivity. However, blending methanol with water results in volume contraction. The solution viscosity is also composition-dependent. Moreover, the addition of methanol to a water-methanol mixture clearly will not result in the same changes to the solution properties as will the addition of water. Thus, contrary to the assumption implicit in refs. 5-8, even were volume additivity to be assumed, the partition coefficients of the mobile-phase components obviously need not be identical. Therefore, the respective baseline disturbance peaks need not necessarily correspond.

Injection of a small amount of one or the other of the mobile-phase components will also affect the solubility of that component in the mobile phase and, potentially at least, in any sorbed layer that might comprise part of the stationary phase as well. We therefore write the partition-coefficient expression at this point in the form:

$$K_{R(i)} = (C_i^S + dC_i^S)/(C_i^M + dC_i^M) \qquad (2)$$

where eqn. 2 emphasizes that, while K_R depends upon the compositions of the stationary and mobile phases, it is independent of the total concentration of solute (10). Thus, assuming that C_i^S hold roughly constant (i.e., that any surface-sorbed layer remains undisturbed), an injection of, say, 10 mm^3 either of pure methanol or pure water will certainly affect the denominator of eqn. 2, and will therefore result in a change in the associated solute partition coefficient.

The sign of the change in K_R will depend upon how the solubility of the solute varies with the composition of the solvent. For example, the (few) available data for aqueous methanol systems indicate that the solubility of methanol in itself is in fact less favored than that in aqueous solutions, since the activity coefficient of methanol in water/methanol mixtures is less than unity (methanol is attracted into solution). Thus, $K_{R(Me)}$ would be expected overall to decrease upon the injection of pure methanol, since this would result in an increase in its concentration in the bulk mobile phase (11). The same considerations hold as well for water solute, and $K_{R(W)}$ should therefore also decrease upon the injection of pure water. However, the magnitudes of the decreases in $K_{R(W)}$ and $K_{R(Me)}$ will in all probability not be identical: Raoult's-law data are invariably distributed

asymmetrically about the concentration axis, such that the activity coefficient of methanol in slightly-wet methanol is close to unity, while that of water in the same solution is considerably less than unity. Thus, the change in $K_{R(W)}$ due to the injection of a small increment of water into methanol-rich mobile phases will be substantially greater than that in $K_{R(Me)}$ upon the injection of a small amount of methanol into the same solution.

In any event, solely on the basis of the solute activity coefficients, the partition coefficient of methanol (hence, its retention volume) would be expected to be smaller than that of water in methanol-rich mobile phases. Further, just the converse should hold for water-rich phases, that is, methanol should then be retained relative to water. These were indeed the trends observed for the liquid–liquid systems comprised of polydimethylsiloxane stationary phase with water/methanol carriers studied by us previously (cf. Table 4 of ref. 4). However, it may well turn out that the order of elution of minor–disturbance peaks cannot be rationalized in all instances solely in terms of solute solubility in the mobile phase, since such an approach fails to take into account e.g. the interactions of LC stationary phases with specific structures extant within aqueous mixtures of organic carrier liquids (12,13). The matter thus invites further and comprehensive study; systems comprised of cyano phases with pyridine-water carriers would seem for example to be of particular interest.

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