A New Method for the Determination of Mobile Phase Volume in Normal and Reversed-Phase Liquid Chromatography

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Key Words
Column liquid chromatography
Mobile phase volume
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Eluent electrolyte

Summary
A new method for the determination of the mobile phase volume (V_m) in liquid chromatography is presented based on the model regarding the retention of ionic solutes in the presence of eluent electrolytes. The V_m value can be determined by measuring the retention volumes of two ions that have the same charge in two eluent electrolyte systems. Compared with the methods using isotopically labelled eluent components or inorganic salts as V_m markers, the method presented is proved to give more reasonable V_m values for both normal and reversed-phase liquid chromatography. As well as in binary mixed solvent systems, the V_m values in single solvent systems can be determined by this method.

Ideally, the mobile phase volume is obtained as the retention volume of an inert compound that explores the mobile phase, but does not interact with the stationary phase. Many different solutes have been proposed as unretained compounds, such as the isotopically labelled eluent components [1, 2, 4–9], inorganic salts [10–14] and organic compounds [9, 15–18]. In general, however, no completely unretained tracer compound is available, except in the cases where the polarity of the mobile phase is extremely different from that of the stationary phase. Further complications arise, in some cases, as a result of size or electrostatic exclusion of the tracer compounds from the pores of the column packing.

Some investigators believe that only the total column porosity has a true physical meaning [19, 20]. Knox and Kaliszanz [20] have proposed that the mobile phase volume be defined as the total volume of all the components of eluent present within the column bed. However, this concept seems to be unsatisfactory because the solvent molecules that penetrate into the pores of the packing may form the solvation layer as a result of interaction with the packing materials; they are then chemically different from those in the mobile phase.

Melander et al. [2] have proposed the convention that the most weakly bound eluent component is not present in the solvation layer and, consequently, that its retention volume corresponds to the mobile phase volume. The main drawback of this model is that it is not applicable to a system where a single solvent is used as eluent.

It is well known that a linear dependence of the logarithmic capacity factor on the carbon numbers of successive members of the homologous series is observed in liquid chromatography as well as in gas chromatography [34]. Therefore, it is possible to calculate the mobile phase volume from the retention volumes of members of the homologous series if such a relationship exists [21–26]. However, several investigators have pointed out that in some cases, the value of mobile phase volume obtained by this method is significantly dependent on both the number and choice of homologues used [2, 3, 12, 24, 25].

In this paper we propose a new method for determining the mobile phase volume based on the model regarding

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the retention mechanism of ionic solutes in partition chromatography in the presence of eluent electrolytes [35]. It will be shown that this method produces reasonable values of mobile phase volumes, not only for binary solvent systems (both normal and reversed-phase systems), but also single solvent systems.

Principle of the Method for the Determination of Mobile Phase Volume

We have previously revealed that the ratio of the capacity factors of the analyte ions that have the same charge is constant, regardless of the type of the eluent electrolyte in the system where the following conditions are satisfied [35]:

(a) The amount of ionic groups in the stationary phase is so small that their electrostatic effect on the retention of analyte ions can be suppressed by adding an electrolyte to the eluent at an ionic strength of 0.1 or below; the ionic activity coefficient in dilute solution, of which the ionic strength is not more than 0.1, is independent of the type and concentration of co-existing ions [36].

(b) The association of analyte ions with counter-ions can be neglected in both the mobile and the stationary phases.

(c) The size of the eluent ions is so small that size-exclusion effect on the retention of the ions is negligible.

(d) The concentration of analyte ions is negligibly smaller than that of eluent electrolyte.

(e) When the adsorption of the analyte ion and/or eluent ions onto the surface of the column packing occurs, the area of the surface that is not occupied by the ions is so large that it can be regarded as independent of the type of eluent electrolyte.

Hence, we can write

\[ k'_{YX}^{A}/k'_{YX}^{B} = k'_{WZ}^{A}/k'_{WZ}^{B} \]  

(1)

where the subscripts \( A \) and \( B \) stand for the analyte ions that have the same charge, and the superscripts \( YX \) and \( WZ \) stand for the eluent electrolytes arbitrarily chosen.

The capacity factor is calculated from the equation

\[ k'_{YX}^{A} = (V_{YX}^{A} - V_{m})/V_{m} \]  

(2)

where \( V_{YX}^{A} \) and \( V_{m} \) are the retention volume of the analyte ion, \( A \), when eluted with the solution of \( YX \) and the volume of mobile phase, respectively. Substituting eq. (2) and the corresponding equations for \( k'_{YX}^{B} \), \( k'_{WZ}^{A} \) and \( k'_{WZ}^{B} \) into eq. (1), we obtain

\[ V_{m} = \frac{V_{YX}^{A}V_{WZ}^{B} - V_{WZ}^{A}V_{YX}^{B}}{V_{YX}^{A} + V_{WZ}^{A} - V_{WZ}^{B} - V_{YX}^{B}} \]  

(3)

This equation reveals that the mobile phase volume can be calculated from the retention volumes of two equally charged analyte ions determined in two eluent electrolyte systems.

The error of \( V_{m} \) value calculated by eq. (3) is determined by the precision of the experimental values of retention volumes. In order to minimize this error, the differences between \( V_{YX}^{A} \) and \( V_{YX}^{B} \) and between \( V_{WZ}^{A} \) and \( V_{WZ}^{B} \) should be as large as possible. It is also preferable to select the eluent electrolyte as one of the two eluent electrolytes that gives the smallest retention volumes of an analyte ion in a given eluent solvent system.

Experimental

Materials

All chemicals used in this study were of reagent grade quality and they were used without further purification unless otherwise stated. HPLC grade acetone and acetonitrile were purchased from Wako (Osaka, Japan). Deionized and distilled water was used for the preparation of sample solutions and eluents.

A cross-linked dextran gel, Sephadex G-25 (particle size = 10–40µm, Pharmacia Fine Chemicals, Uppsala, Sweden) and a cross-linked polyethylene glycol gel, TSK gel Ether-250 (5µm average particle size, Toyo Soda, Tokyo, Japan) were slurry-packed into a Pyrex column (300 x 5mm i.d.) and a stainless steel column (500 x 5mm i.d.), respectively. A 15cm by 4.6mm i.d. prepacked stainless steel column, Shodex RS pak DS-613 (poly(styrene-divinylbenzene) copolymer, Showa Denko, Tokyo, Japan), was also used in this study for reversed-phase systems.

Blue Dextran 2000 (Pharmacia) was used as a reference material for evaluating the interstitial volume (interparticle volume) in the column.

Chromatographic Conditions

The pumping system was a Kyowa Seimitsu (Tokyo, Japan) Model KHP-010. The solvent reservoir was a commercially available glass syringe with the 200ml capacity [37]. The columns were thermostated at 25.0 ± 0.1°C through a column jacket, using a Yamato (Tokyo, Japan) Model BH-71 constant temperature circulator.

The eluents used were water or mixtures of water and acetone or acetonitrile containing alkali metal salts. Test solutions (0.1–1.0mM) were prepared by dissolving the salts of analyte ion in the eluent solvents. The solution of Blue Dextran 2000 was prepared at a concentration of 0.05% (w/v); at higher concentrations, depending on the type of eluent solvent, a skewed peak (leading) was observed. A 10μl portion of a solution was introduced into the column.

Eluents were carried out at a constant flow rate of ca. 0.8ml/min. Exact values of the volumetric flow rate were measured using the buret designed so as to prevent the vaporization of solvent.

Inorganic anions were detected with a Kyowa Seimitsu Model KLC-800 UV-visible variable wavelength spectrophotometer, a Denkako (Sakado, Japan) Model ICP-70 potentiometric detector or a Toyo Soda (Tokyo, Japan) Model CM-8 conductivity detector. A Shimadzu (Kyoto, Japan) Model MAF-1 atomic absorption spectrometer was used for the detection of alkali metal ions in a similar manner as described in the literature [38]. D₂O and CD₃OH.