Journal of Chromatography A, 1220 (2012) 75–81

Contents lists available at SciVerse ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Comparison of concentration pulse and tracer pulse chromatography: Experimental determination of eluent uptake by bridged-ethylene hybrid ultra-high performance liquid chromatography packings

Mei Wang[a], Bharathi Avula[a], Yan-Hong Wang[a], Jon F. Parcher[a], Ikhlas A. Khan[a,b,c,*]

[a] National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA
[b] Department of Pharmacognosy, School of Pharmacy, University of Mississippi, University, MS 38677, USA
[c] Department of Pharmacognosy, College of Pharmacy, King Saud University, Riyadh, Saudi Arabia

ARTICLE INFO

Article history:
Received 29 September 2011
Received in revised form 8 November 2011
Accepted 24 November 2011
Available online 2 December 2011

Keywords:
Tracer pulse chromatography
Concentration pulse chromatography
Excess volume isotherm
Liquid chromatography retention mechanisms

ABSTRACT

Excess volume isotherms of acetonitrile and methanol sorbed on a C_{18} BEH UHPLC packing were determined over a range of pressure, temperature, flow rate and eluent composition. The isotherm measurements were carried out by two independent experimental methods, *viz.*, concentration pulse and tracer pulse chromatographies. Isotherms were measured with both experimental techniques at 30, 45 and 60 °C. The excess isotherms increased with decreasing temperature although the variations were relatively small. Direct comparison of the two experimental techniques showed that the measured void volumes were identical within experimental error. The measured excess volumes by both techniques were comparable with the concentration pulse experiments producing slightly higher excess volume data with highly aqueous eluents. Both experimental techniques show some variations of the retention volumes with sample volume, sample composition, flow rate and column inlet pressure. The results confirmed the validity of both concentration and tracer pulse chromatographies for the determination of column void volumes and the excess volume of eluent taken up by UHPLC packings.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

The separation and analysis of the chemical components of natural products is critically dependent upon modern liquid chromatographic techniques and instrumentation. Probably the most obvious and timely applications involve the separation of peptide mixtures by ultra-high performance liquid chromatography (UHPLC) in the field of proteomics. The use of high efficiency LC with modern bridged-ethylene hybrid, small particle packings combined with fragmentation patterns and accurate mass determination by Q-ToF mass spectrometers has produced truly impressive results in the various fields of "omics." However, despite the obvious successes of reversed-phase liquid chromatography, the exact retention mechanisms controlling resolution and retention are unclear principally because of the very complex nature of the interfacial region between the mobile and stationary phases in a RPLC column. One of the primary problems encountered in the study of LC retention mechanisms is the inability to clearly define and differentiate the mobile and stationary phases in typical RPLC columns. For example, it has been shown repeatedly that aqueous-organic eluents interact with the bonded-phase packings and even the silica surface of most packings. If the eluent is retained by the packing, then the retained portion of the eluent will usually have a different composition than the bulk eluent due to the influence of the solid adsorbent. In this case, the immobilized eluent can act as a component of the stationary phase. The full extent of such interactions depends upon the packing, eluent composition, temperature and pressure. The exact role or influence of the uptake of eluent on the efficiency and resolution of modern LC separations is uncertain. It is clear, however, that understanding the distribution of eluent components between the stationary and mobile phases is crucially important for a clear comprehension of the distribution of infinite dilution analytes.

Because of the dynamic nature of the physical and chemical structure of the interfacial region in a liquid chromatographic column, any experimental measurement involving this interface must be carried out in a dynamic system rather than a static equilibrium system. There are currently three principle chromatographic methods for measuring the uptake of multicomponent eluents by a given packing. These are (i) concentration pulse (CP) chromatography, (ii) frontal analysis (FA) chromatography and (iii) tracer pulse (TP) chromatography. These techniques are complementary and the

* Corresponding author at: National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, MS 38677, USA.
Tel.: +1 6629157821, fax: +1 6629157989.
E-mail address: ikhan@olemiss.edu (Ikhlas A.khan).

0021-9673/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.chroma.2011.11.051

selection of methods is usually dictated by the available detection system. Eluents are usually designed to be invisible (UV) or ignored (MS) by the detection system in order to minimize background interference. However, often a slight response is observed for some eluent component and this allows the use of UV detection for CP and FA applications. In other cases, bulk property detectors, such as refractive index detectors, can be used to detect concentration changes in the eluent. UV detectors operated at low wavelengths can also be used to detect concentration changes or isotopically labeled analytes.

These three chromatographic techniques produce similar experimental data but differ in several critical aspects. In the case of concentration and tracer pulse chromatographies, the eluent composition is maintained uniform throughout the column and the interfacial region is at equilibrium when the system is perturbed by an injected sample. An isotopically labeled eluent component is injected in a tracer pulse experiment, whereas a sample of eluent with a composition slightly different from the column eluent is injected for concentration pulse experiments. In FA, the eluent composition is changed in a step function and no sample injection is performed. The experiment data differ for the techniques because the FA and TP methods produce excess and void volume data directly. The CP experiments result in data that can be integrated to obtain excess or void volume data. An alternative approach is to assume an isotherm model and determine the isotherm parameters that produce the best fit of the model to the data [1–4].

Direct comparison studies have been published for frontal analysis and concentration pulse chromatography [5–9]. Validation of the mass spectrometric tracer pulse chromatography compared to concentration pulse chromatography was carried out by Samuelsson et al. [10] for absolute sorption isotherms of methyl- and ethyl-mandelate at a single, fixed eluent composition of 30% acetonitrile. The various experimental methods for the determination of sorption isotherms for RPLC systems have also been discussed in Guiochon's comprehensive treatise [11].

Recently, Boswell et al. [12,13] used mass spectrometric tracer pulse chromatography to determine the effect of eluent uptake on the kinetic void volume (mobile phase volume) in a series of studies of the prediction of gradient and isocratic retention data from a C_{18} BEH UHPLC column. The mobile phase volume was determined from a combination of the retention volume of D_2O and a linear regression technique proposed by Schay [14]. This approach requires the use of excess volume data that is necessarily based on an assumed convention. The convention most commonly used is the/vNA convention proposed by Riedo and Kovats [15].

The objectives of the present investigation were (i) to compare and validate concentration and tracer pulse experimental methods for the determination of the excess volumes of eluent components taken up by RPLC packings over a range of temperature, pressure, flow rate and eluent composition, (ii) to determine the effect of various experimental parameters on the accuracy of the measured excess volumes, and (iii) to measure the excess volumes of acetonitrile and methanol on modern BEH UHPLC packings.

2. Theory

The mass balance equation for concentration pulse experiments with binary eluents is:

$$V_R(\theta_i^M) = V^M + \frac{\partial V_i^S}{\partial \theta_i^M} \quad (1)$$

where $V_R(\theta_i^M)$ is the retention volume of a concentration pulse at an eluent volume fraction of θ_i^M, V^M is the volume of the mobile phase in the column, and V_i^S represents the volume of eluent component i in the stationary phase at the same eluent composition. The equivalent model for frontal analysis and tracer pulse chromatography is:

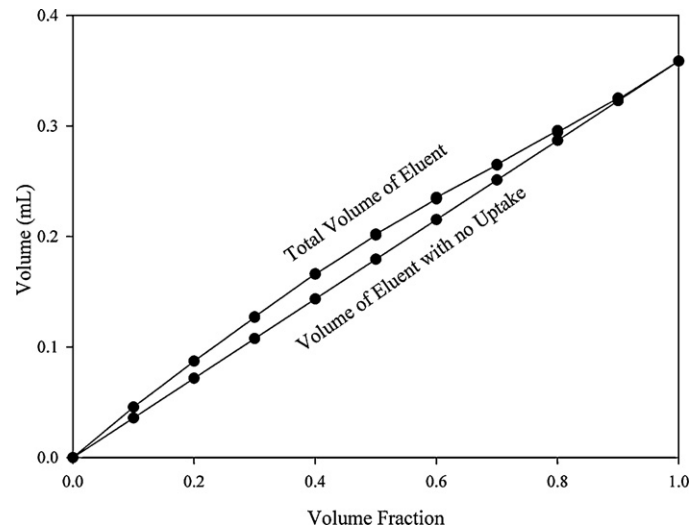

Fig. 1. Pictorial representation of the concept of excess volume.

$$V_{R,i}^*(\theta_i^M) = V^M + \frac{V_i^S}{\theta_i^M} \quad (2)$$

where $V_{R,i}^*(\theta_i^M)$ represents the retention volume of a detectable isotope (TP) or a step change in eluent composition (FA). The linear, as opposed to differential, form of this equation is one of the major advantages of the latter experimental methods.

Unfortunately, in RPLC systems it has proven to be impossible to unambiguously delineate the exact volumes of the mobile and stationary phases because of the complex nature of the interfacial region [1]. So Eqs. (1) and (2) are not practical. However, the concept of excess volumes presents a useful, but empirical, solution to this problem. The excess volume, $V_i^{XS}(\theta_i^M)$, of any component i in the stationary phase is defined as [16]

$$V_i^{XS}(\theta_i^M) = V_i(\theta_i^M) - V^0\theta_i^M \quad (3)$$

where $V_i(\theta_i^M)$ and V^0 represent the volume of component i and the total volume of all eluent components in the column, respectively. The quantity V^0 is the thermodynamic void volume as defined by Knox and Kaliszan [17]. The term $V^0\theta_i^M$ represents the theoretical volume of component i that would be in a system if there were no interaction between the eluent and the stationary phase. In order to derive practical chromatographic models involving excess quantities, an empirical convention must be adopted. The most common convention used with liquid systems is the/vNA convention described by Riedo and Kovats [15]. The convention is based on the assumption $\sum_i V_i^{XS} = 0$. This convention requirement is one of the problematic issues involved in the concept of excess volumes.

A simple explanation of the concept of excess volume is illustrated in Fig. 1. The upper line represents a plot of the total volume of eluent, $V_i(\theta_i^M)$, in a real RPLC column. The lower line is a plot of the volume of eluent that would be in V^0 under ideal conditions. This ideal volume is determined from the product of the thermodynamic void volume and the eluent composition, i.e., $V^0\theta_i^M$. The excess volume, $V_i^{XS}(\theta_i^M)$, is the difference between these two lines.

Using these excess concepts and the convention that $\sum_i V_i^{XS} = 0$, Eqs. (1) and (2) can be recast in the form

$$V_R(\theta_i^M) = V^0 + \frac{\partial V_i^{XS}(\theta_i^M)}{\partial \theta_i^M} \quad (4)$$

$$V_{R,i}^*(\theta_i^M) = V^0 + \frac{V_i^{XS}(\theta_i^M)}{\theta_i^M} \quad (5)$$

for concentration pulse and tracer pulse chromatographies, respectively.

The excess volume of an eluent component taken up by the stationary phase at any eluent composition θ_i^M can then be calculated from the relations:

$$V_i^{XS}(\theta_i^M) = \int_0^{\theta_i^M} V_R(\theta_i^M) d\theta_i^M - V^0 \theta_i^M \quad (6)$$

$$V_i^{XS} = (V_{R,i}^*(\theta_i^M) - V^0)\theta_i^M \quad (7)$$

for concentration pulse and tracer pulse or frontal analysis chromatography, respectively.

Obviously, accurate determination of the thermodynamic void volume V^0 is critically important for the experimental determination of excess volumes. The thermodynamic void volume can be calculated from the relations [17,18]:

$$V^0 = \int_0^1 V_R(\theta_i^M) d\theta_i^M \quad (8)$$

$$V^0 = \sum_i V_{R,i}^*(\theta_i^M)\theta_i^M \quad (9)$$

In the case of concentration pulse chromatography, retention volume data must be obtained over the full eluent composition range in order to obtain an integrated void volume. With tracer pulse methods, the void volume can be determined at any eluent composition. In particular, the void volume can be obtained from the retention volume of an isotopically labeled solute with a single, pure eluent. In the case of frontal chromatography, theoretically the void volume could be determined from a single step experiment in which the composition of one eluent component was changed from $\theta_i^M = 0$ to $\theta_i^M = 1$. In practice, this type of measurement is impractical because of the problems encountered with pure eluents. In most cases, an "unretained" solute is commonly used to determine the void volume.

Eq. (7) can be recast in a simpler form where the eluent consists of components i and j by using Eq. (9):

$$V_i^{XS} = (V_{R,i}^* - V_{R,j}^*)\theta_i^M \theta_j^M \quad (10)$$

Eq. (10) is particularly useful because it does not require knowledge of either the column void volume or any extra-column volume present in the chromatographic system. Accurate determination of the extra-column volume is a particular concern with frontal analysis experiments [5,6].

The void volume of liquid chromatographic columns can be measured in several other ways in addition to Eqs. (8) and (9). Numerous reviews have been published on the methods for the determination of this particular chromatographic parameter [19–21]. One of the most comprehensive investigations compared the void volumes determined by tracer pulse (with pure acetonitrile), concentration pulse, and pycnometry with the void volume calculated from geometric considerations alone [21]. The general conclusions were that (i) the techniques produced void volume data that agreed within about 6%, (ii) the pycnometric values were consistently lower than the chromatographic data, (iii) the void volumes determined from concentration pulse experiments varied significantly with eluent type, and (iv) the tracer pulse data agreed well with the concentration pulse data for weak eluents.

3. Experiment

3.1. Chemicals

The eluents were HPLC grade obtained from Fisher Scientific. The isotopic solutes were methanol-d_3 (99.8 atom % D), acetonitrile-d_3 (99.8 atom% D) and water-d_2 (99.8 atom% D) obtained from Sigma–Aldrich.

3.2. Column

The column was a Waters Acquity UHPLC BEH C_{18} column. The particle size was 1.7 μm. The column size was 2.1 mm × 150 mm.

3.3. Instrumentation

The UHPLC/MS system consisted of an Agilent 1290 Infinity series chromatograph with a dual pump, autosampler, thermostated column compartment, and a diode array detector. The temperature stability specification for the thermostated column compartment was ±0.05 °C and the accuracy specification was ±0.5 °C. The chromatograph was coupled with an Agilent 1260 single quadrupole mass spectrometer with a dual APCI/APESI source operated in the positive mode. The system was controlled by ChemStation software. The eluent components were mixed by the binary pump. The MS was manually tuned for low masses (m/z <50).

3.4. Procedures

Tracer pulse and concentration pulse experiments were carried out sequentially at each eluent composition. A sample of eluent with a slightly different composition was injected for the concentration pulse experiments and the concentration change was recorded from the DAD. In the same run, a sample of deuterated eluent components was injected and detected by the mass analyzer.

3.4.1. Concentration pulse experiments

The concentration pulse experiments were carried out over the full range of eluent composition. Two microliter samples of the same eluent with a composition of ±10% of the column eluent were injected in triplicate. That is, a different sample was required for each eluent composition. The DAD was operated at a wavelength of 191 nm. The extra-column volume to this detector was determined to be 30 μL by injection of a sample with the column removed. The concentration pulse data were integrated both numerically and by fitting a third-order polynomial to the retention volume data.

3.4.2. Tracer pulse experiments

One microliter samples of the deuterated eluent components were injected in triplicate. The same injection sample was used for all of the eluent compositions. The tracer pulse samples were detected as $[M+H]^+$ ions by the quadrupole mass analyzer operated in the selected ion monitor (SIM) mode to eliminate background from the column eluent. In the case of D_2O, the base peak was m/z = 19 and this m/z value was used for the SIM detection. The extra-column volume for this detector was 60 μL.

4. Results and discussion

Experimental variables that could influence the chromatographic determination of excess isotherms include flow rate, temperature, pressure, sample size and sample composition. Any

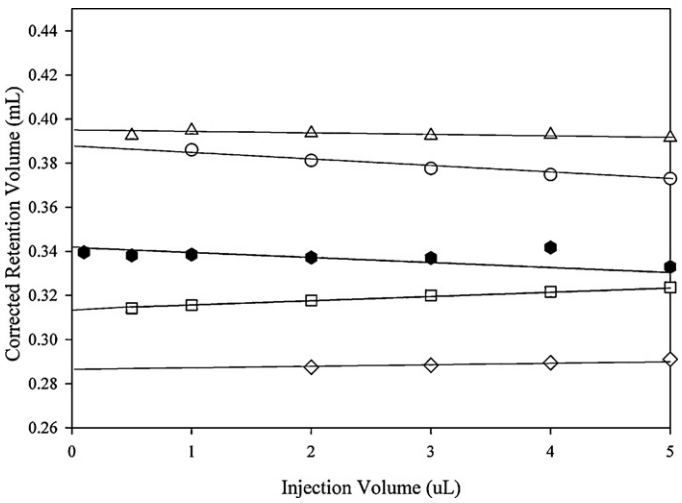

Fig. 2. The effect of injected sample volume and composition on the retention volume of analytes. Open symbols represent CP experiments and closed symbol represents TP experiments. (△) eluent: 20% ACN, sample 30% ACN; (○) eluent: 20% ACN, sample 80% ACN; (□) eluent: 90% ACN, sample 100% ACN; (◇) eluent: 70% ACN, sample 80% ACN; (⬢) eluent: 80% ACN, sample: D_2O and ACN-d_3.

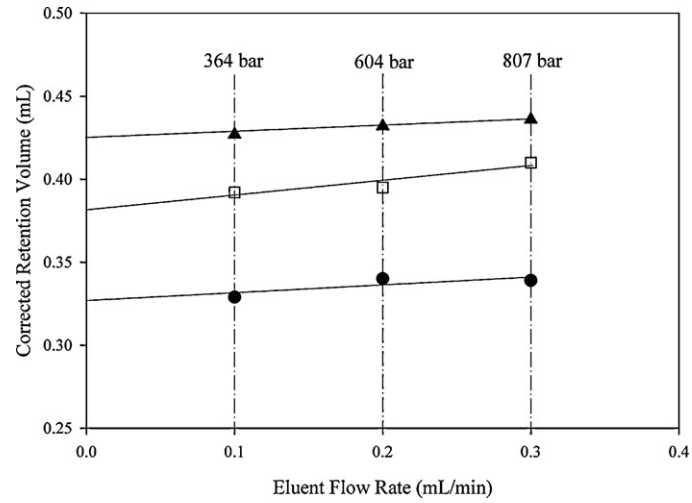

Fig. 3. Effects of eluent flow rate and column pressure at 30 °C. Open symbol is for CP experiments, close symbols are for TP experiments. (●) D_2O; (▲) ACN-d_3; (□) CPC.

comparison of experimental techniques must take into account the effect of these variables.

4.1. Sample volume and composition effects

The volume of sample injected may influence the peak shape and hence the retention volume. Fig. 2 illustrates the variation of the retention volume of CP and TP samples with sample volume. In general, the retention volumes of the concentration pulses increased slightly with sample size if the injected sample composition was close to that of the eluent. The sample size dependency was exaggerated when the sample composition was not within 10% of the eluent composition. The TP experiments showed no discernible variation with sample volume.

4.2. Flow rate and pressure effects

Fig. 3 illustrates the effect of pressure and flow rate on the retention volumes from TP and CP experiments. The numbers shown in the figure represent the inlet pressures. In each case, the retention volume increased slightly with flow rate and pressure. For this reason, all experiments were carried out at either 0.1 or 0.2 mL/min.

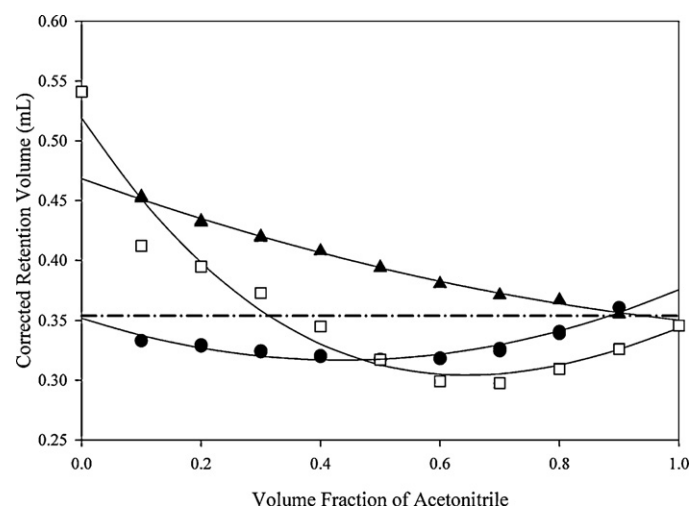

Fig. 4. Corrected retention volumes from TP and CP experiments at an eluent flow rate of 0.2 mL/min and temperature of 30 °C. (●) D_2O; (▲) ACN-d_3; (□) CPC; (–·–) V^0.

4.3. Thermodynamic void volumes

The corrected retention volumes of the TP and CP experiments with acetonitrile at 0.2 mL/min and 30 °C are illustrated in Fig. 4. The thermodynamic void volumes were calculated from this data using Eqs. (8) and (9) for CP and TP experiments, respectively. The results

Table 1
Thermodynamic void volume data for CP and TP experiments at various flow rates, pressures and temperatures.

Type of experiment	Temperature (°C)	Inlet pressure (Bar)	Flow rate (mL/min)	Void volume (μL)	
				Acetonitrile	Methanol
Concentration pulse	30	364	0.10	345	
	30	580	0.20	352	350
	30	807	0.30	359	
	45	511	0.20	346	
	60	438	0.20	357	
Tracer pulse	30	364	0.10	346[a]	
	30	580	0.20	354[a]	358[a]
	30	807	0.30	359[a]	
	45	511	0.20	349[a]	
	60	438	0.20	361[a]	

[a] The average of individual V^0 values at each eluent composition.

Table 2
Excess isotherm measured from tracer pulse and concentration pulse experiments.

Volume fraction	Acetonitrile (mL)						Methanol (mL)	
	Tracer pulse			Concentration pulse			Tracer pulse	Concentration pulse
	30 °C	45 °C	60 °C	30 °C	45 °C	60 °C	30 °C	30 °C
0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
0.1	0.011	0.010	0.012	0.013	0.013	0.011	0.004	0.004
0.2	0.017	0.016	0.016	0.020	0.020	0.018	0.006	0.005
0.3	0.020	0.019	0.018	0.023	0.022	0.021	0.006	0.005
0.4	0.021	0.018	0.018	0.022	0.021	0.021	0.006	0.004
0.5	0.020	0.017	0.016	0.019	0.018	0.018	0.005	0.003
0.6	0.015	0.013	0.012	0.014	0.014	0.014	0.004	0.002
0.7	0.010	0.007	0.006	0.010	0.010	0.009	0.003	0.001
0.8	0.005	0.002	0.001	0.005	0.006	0.005	0.002	0.001
0.9	0.000	−0.002	−0.002	0.002	0.002	0.001	0.001	0.001
1.0	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000

Table 3
Calculated V_i and V_i^S.

	Tracer pulse			Concentration pulse		
	30 °C	45 °C	60 °C	30 °C	45 °C	60 °C
Acetonitrile						
V_i (μL)	49.9	48.5	47.0	44.5	41.4	45.2
V_i^S (μL)	44.6	41.2	39.0	41.5	38.8	40.8
θ_i^S	0.89	0.85	0.83	0.93	0.93	0.98
Methanol						
V_i (μL)	11.2			8.4		
V_i^S (μL)	10.8			7.4		
θ_i^S	0.96			0.88		

for acetonitrile/water and methanol/water are given in Table 1. The average value obtained from the CP experiments over a range of temperatures, flow rates and pressures was 350 μL. The average for the TP experiments was 354 μL. The relative standard deviation for all of the void volume measurements was less than 2%. No systematic variation of V^0 with eluent type was observed in contrast with the results of Gritti et al. [21]. The CP and TP experiments produced equivalent void volume data. The major difference between the experimental techniques was the requirement for CP retention volume data over the full eluent composition range including both pure eluents.

4.4. Excess volumes of acetonitrile and methanol

The excess volumes of acetonitrile from aqueous solutions were measured at 30, 45 and 60 °C. The results are given in Table 2 and Fig. 5. The temperature dependence was slight; however, the volume of eluent taken up by the packing increased as the temperature decreased. Comparison between the CP and TP data indicated that the CP results were slightly higher than the TP data especially at composition of less than 50%. This difference is most likely caused by the difficulty of integration of the CP data due to the significant variation of the retention volumes with eluent composition in this range as shown in Fig. 4.

Table 4
Comparison of concentration pulse and tracer pulse techniques.

Type of chromatography	Advantages	Disadvantages
Concentration pulse	1. Simple, common detection systems such as RI or UV.	1. The concentration differences between the eluent and the injected sample must be small and thus the concentration pulses are often difficult to detect.
	2. Injected probe consists of only eluent with a composition slightly different from the column eluent.	2. The sample composition must be varied with the column eluent composition.
	3. The experimental procedure is simple.	3. Additional, so-called system peaks may interfere with the concentration pulse peak.
		4. The chromatographic data must be integrated or interpreted indirectly to give isotherm equation parameters.
		5. Determination of the void volume requires integration of retention volume data over the *full range* of eluent composition.
Tracer pulse	1. Produces isotherm data directly without the need for integration or isotherm models.	1. Requires a complex and expensive mass specific detection system.
	2. The same sample can be used for any eluent composition.	2. The isotopically labeled probes are often expensive or difficult to synthesize.
Both techniques	1. Allow the investigation of complex, dynamic interfacial regions such as those observed with RPLC systems.	1. Cannot obtain absolute isotherm data directly. Only excess data can be determined and this requires the adoption of a convention.

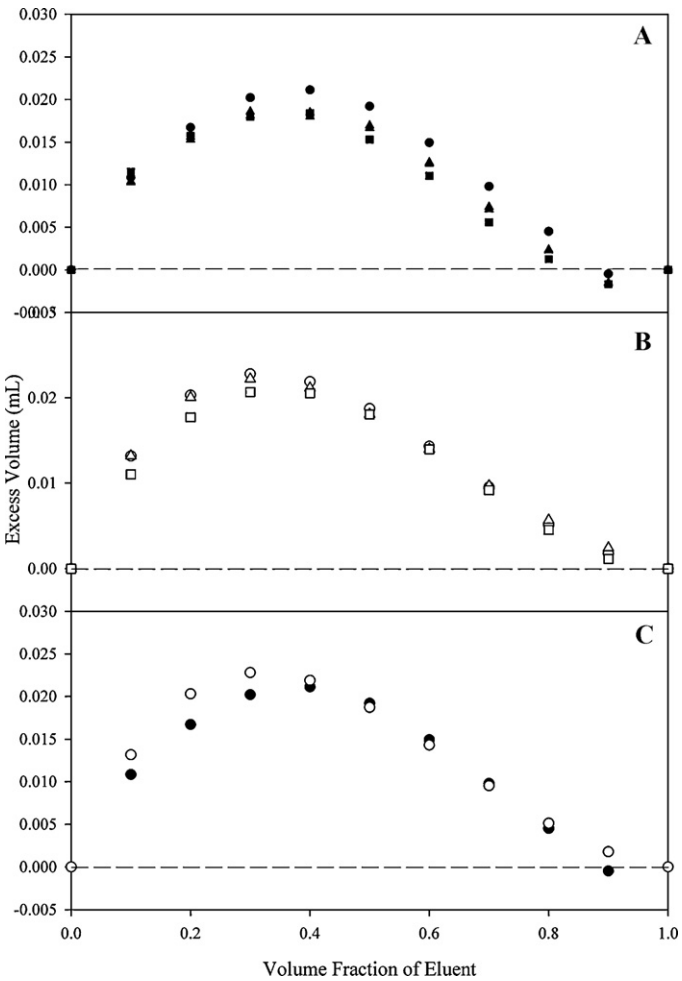

Fig. 5. Comparison of excess isotherms from TP and CP experiments. (A) Tracer pulse; (●) 30 °C; (▲) 45 °C; (■) 60 °C. (B) Concentration pulse; (○) 30 °C; (△) 45 °C; (□) 60 °C. (C) 30 °C data; (●) tracer pulse; (○) concentration pulse.

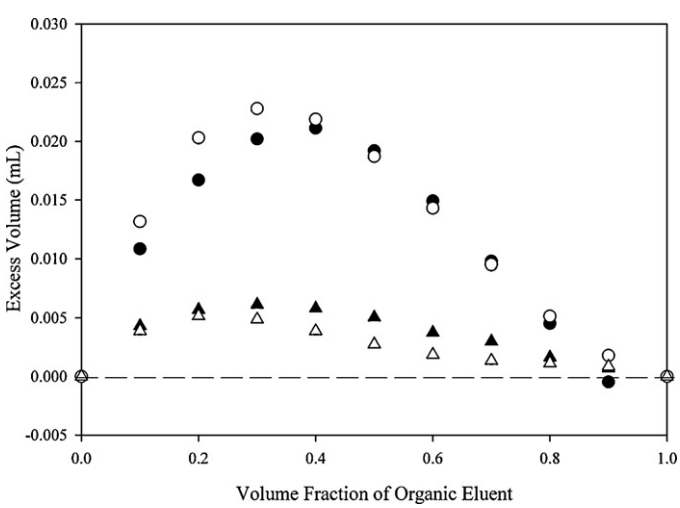

Fig. 6. Excess isotherms for methanol and acetonitrile experiments at 30 °C and 0.2 mL/min. (○) CPC: acetonitrile; (●) TP: acetonitrile; (△) CP: methanol; (▲) TP: methanol.

The results for the sorption of methanol were similar to those obtained for acetonitrile but lower in magnitude. The volume of methanol sorbed at 30 °C was approximately 1/4 of the volume of acetonitrile taken up under the same conditions. This ratio is in excellent agreement with previous results [22–24].

4.5. Absolute volumes of the stationary and mobile phases

An alternative way to define excess volume is given by the relation [25]

$$V_i^{XS}(\theta_i^M) = V_i^S(\theta_i^M) - V^S\theta_i^M \quad (11)$$

The excess isotherms illustrated in Figs. 5 and 6 follow this equation explicitly with constant values of $V_i^S(\theta_i^M)$ and V^s within the range $0.5 \le \theta_i^M \le 0.8$. The calculated values for the volume of acetonitrile in the stationary phase, $V_i^S(\theta_i^M)$, and the total volume of eluent in the stationary phase, V^s, are given in Table 3 along with the calculated values of the volume fraction of eluent taken up by the stationary phase, θ_i^S.

5. Conclusions

With careful experimentation, tracer pulse and concentration pulse experiments produce equivalent results. However, there are considerable differences in the experimental procedures as well as time and labor requirements. The primary advantages and disadvantages of each experimental technique are summarized in Table 4.

Both experimental techniques can and have been used to study the very complex, multicomponent, multiphasic, dynamic interfacial domain that exists between the stationary and mobile phase in RPLC systems. The two experimental techniques studied herein produce accurate void volume and excess volume data for systems that are very difficult to investigate by other methods.

The data presented here indicate that even the most modern BEH UHPLC packings either adsorb or absorb significant amounts of eluent from aqueous-organic mobile phases commonly used in LC applications. While this uptake of eluent is not the controlling factor in the retention and resolution of analytes, it is a phenomenon that should be taken into account in proposed theoretical models.

Acknowledgements

This research was supported by grant nos. 5U01FD00207-10 and 1U01FD003871-02 from the Food and Drug Administration. Additional support was provided by Specific Cooperative Agreement No. 58-6408-2-0009 from the United States Department of Agriculture, Agricultural Research Service.

This work was partially supported by Global Research Network for Medicinal Plants (GRNMP) and King Saud University.

Instrumentation for this research was donated to the University of Mississippi by Agilent Technologies.

References

[1] F. Gritti, G. Guiochon, J. Chromatogr. A 1099 (2005) 1.
[2] F. Gritti, G. Guiochon, J. Chromatogr. A 1155 (2007) 85.
[3] J. Samuelsson, T. Fornstedt, J. Chromatogr. A 1203 (2008) 177.
[4] J. Samuelsson, A. Franz, B.J. Stanley, T. Fornstedt, J. Chromatogr. A 1163 (2007) 177.
[5] J. Lindholm, P. Forssen, T. Fornstedt, Anal. Chem. 76 (2004) 4856.
[6] J. Lindholm, P. Forssen, T. Fornstedt, Anal. Chem. 76 (2004) 5472.
[7] R. Arnell, P. Forssen, T. Fornstedt, J. Chromatogr. A 1099 (2005) 167.
[8] R. Arnell, T. Fornstedt, Anal. Chem. 78 (2006) 4615.
[9] T. Fornstedt, J. Chromatogr. A 1217 (2010) 792.
[10] J. Samuelsson, R. Arnell, J.S. Diesen, J. Tibbelin, A. Paptchikhine, T. Fornstedt, P.J.R. Sjoberg, Anal. Chem. 80 (2008) 2105.
[11] G. Guiochon, A. Felinger, A. Katti, D. Shirazi, Fundamentals of Preparative and Nonlinear Chromatography, Academic Press, Boston, MA, 2006.

[12] P.G. Boswell, J.R. Schellenberg, P.W. Carr, J.D. Cohen, A.D. Hege, J. Chromatogr. A 1218 (2011) 6732.
[13] P.G. Boswell, J.R. Schellenberg, P.W. Carr, J.D. Cohen, A.D. Hege, J. Chromatogr. A 1218 (2011) 6742.
[14] G. Schay, Surface and Colloid Science, Wiley-Interscience, New York, 1969, p. 180.
[15] F. Riedo, E. Kovats, J. Chromatogr. 239 (1982) 1.
[16] D.H. Everett, Pure Appl. Chem. 58 (1986) 967.
[17] J.H. Knox, R. Kaliszan, J. Chromatogr. 349 (1985) 211.
[18] Y.V. Kazakevich, H.M. McNair, J. Chromatogr. Sci. 31 (1993) 317.
[19] C.A. Rimmer, C.R. Simmons, J.G. Dorsey, J. Chromatogr. A 965 (2002) 219.
[20] P. Sajonz, J. Chromatogr. A 1050 (2004) 129.
[21] F. Gritti, Y.V. Kazakevich, G. Guiochon, J. Chromatogr. A 1161 (2007) 157.
[22] J. Mallette, M. Wang, J.F. Parcher, Anal. Chem. 82 (2010) 3329.
[23] M. Wang, J. Mallette, J.F. Parcher, Anal. Chem. 80 (2008) 6708.
[24] M. Wang, J. Mallette, J.F. Parcher, Anal. Chem. 81 (2009) 984.
[25] M. Wang, J. Mallette, J.F. Parcher, J. Chromatogr. A 1213 (2008) 105.