

Reversed-phase liquid chromatographic study of excess and absolute sorption isotherms of acetonitrile–water mixtures

A. ALVAREZ-ZEPEDA and D. E. MARTIRE*

Department of Chemistry, Georgetown University, Washington, DC 20057 (USA)

ABSTRACT

The retention volumes of the disturbance peak, $V_{r,(1,2)}$, and the isotopically labeled counterparts, $V_{R,1}$ and $V_{R,2}$, of the components of acetonitrile–water mixtures were determined over a wide composition range at 25, 35, 45 and 55°C. An explicit, model-independent, self-consistency equation relating $V_{R,(1,2)}$ and the composition derivatives of $V_{R,1}$ and $V_{R,2}$ derived and successfully tested. Also derived were equations that lead to the evaluation (from the same data set) and linkage of the excess and absolute sorption isotherms of the components. The latter isotherms are based on an internally consistent model which permits the determination of the mobile phase volume.

INTRODUCTION

In an attempt to understand the solute retention mechanism(s) in reversed-phase liquid chromatography (RPLC), a large number of papers have been devoted to the surface characterization of *n*-alkyl-bonded phases [1,2]. Sander and Wise [2] reviewed various types of substrates, stationary bonded phases, their methods of characterization and chromatographic properties. In addition to the characterization of the *n*-alkyl-bonded chain and the residual silanols on the silica surface, Knox and Pryde [3] were among the first to propose the importance of the uptake of the organic modifier in the stationary phase. Soon thereafter, this was experimentally demonstrated by Scott and Kucera [4,5] and others [6,7].

Introduction in the column of a very small amount of an isotopically labelled solute (ILS) (*e.g.*, $^2\text{H}_2\text{O}$) in a binary system causes a finite “minor disturbance peak” or disturbance peak (DP) [8,9], in addition to the elution peak of the ILS [10–16]. The elution data of the ILS have been used to investigate the distribution of organic modifiers in reversed-phase columns [11,12,16,17], as a method for the determination of surface excess isotherms [12–14,17–19] and the determination and interpretation of the column dead volume [11,13–16].

We review here some aspects of previous work relevant to this paper. McCormick and Karger [11] studied the elution behavior of the ILS in three organic modifiers mixed with water: methanol, acetonitrile (ACN) and tetrahydrofuran (THF). The interpretation of the elution data of the ILS and the DP was based on

Helferich's equation for the finite band migration in liquid chromatography [20,21]. A generalized distribution isotherm was proposed to explain the elution behavior of the ILS and DP or "vacancy band". The enrichment of the stationary phase by the organic modifier was also considered. Slaats *et al.* [12] used various experimental methods to measure the surface excess isotherms of ACN and CH₃OH with aqueous binary mixtures. Two models were proposed to calculate the volume of the adsorbed layer in the stationary phase. Zhu [16], using the ILS elution data in ACN-water mixtures, determined an absolute adsorption isotherm of ACN and defined the column dead volume as the difference between the maximum column hold-up volume and the volume of the adsorbed solvent (ACN). Based on his absolute isotherm of ACN, Zhu attempted to explain the chromatographic behavior of the DP, ²H₂O and deuterated acetonitrile (ACN-d₃).

The work of Riedo and Kováts [13] is particularly noteworthy. From the differential equation of the material balance in the column, they found an analytical solution for the retention volume of a solute in isocratic, isothermal liquid chromatography. This equation, which has general validity for a binary mixture and is independent of any specific model, determines the retention volume of a solute introduced in the column as an infinitesimal perturbation of the eluent mixture composition or "the concentration vector" [13]. The retention volume of the solute depends on the changes occurring in the column eluent composition caused by the introduction of the solute(s). This equation can be expressed in terms of mass, moles or volumes [14,19].

The purpose of this paper is two-fold. First, we shall derive a set of equations that describe quantitatively the elution behavior of the ILS and DP in terms of absolute quantities (*i.e.*, absolute sorption isotherms). An explicit, self-consistency relationship is derived that relates the retention volumes of the ILS to the retention volume of the DP. The retention of the DP can be determined in terms of absolute quantities or excess quantities. We show that they are equivalent and that the surface excess isotherm can be calculated from the absolute sorption isotherms. A second purpose is to propose and test a simple model for the sorbed water ($V_{1,s}$) and the sorbed ACN ($V_{2,s}$) in the stationary phase (*i.e.*, the absolute sorption isotherms). We have investigated this problem because of the need for a model of the stationary phase in RPLC, *i.e.*, its volume, composition and structure [22]. A consequence of this model is the determination of the mobile phase volume, V_m .

EXPERIMENTAL

Equipment and materials

The liquid chromatograph consisted of a Beckman Model 112 pump, a Valco injector with a 0.5- μ l internal sample loop, a differential refractive index detector (Model R401, Waters Assoc., Milford, MA, USA) and a Perkin-Elmer Model 56 strip-chart recorder. The column used was a C₁₈ chemically bonded phase, purchased from ES Industries (Marlton, NJ, USA). The column (25 cm \times 4.6 mm I.D.) was packed with 4.7 g of *n*-octadecyl-bonded amorphous silica. The packing specifications reported by the manufacturer were mean particle diameter 5 μ m, nominal pore diameter 300 Å, surface area 100 m²/g and carbon content 5.5%. The high-performance liquid chromatographic-grade eluents, acetonitrile and water, were obtained

from Fisher Scientific (Fair Lawn, NJ, USA). Deuterium oxide and deuterated ACN were obtained from MSD Isotopes, a division of Merck Frosst Canada (Montreal, Canada).

Procedures

The solvent mixtures were prepared by measuring separately and then mixing known volumes of ACN and water. Every mixture was degassed prior to use by applying a vacuum for at least 10 min while stirring with a magnetic bar. The column was thermostated by putting it inside a water-jacket, the temperature of which was controlled to within $\pm 0.1^\circ\text{C}$. The nominal flow-rate was 1 ml/min. The actual flow-rate of (collected) eluent under ambient conditions was measured with a timer and a 5-ml calibrated volumetric flask. The correction for isothermal compressibility was found to be insignificant. However, a necessary correction from ambient temperature was made using the equation

$$F_c = F_a(\rho_a/\rho_c)$$

where F_c and F_a are the corrected and measured (ambient) flow-rates and ρ_a and ρ_c are the densities of the eluent at ambient temperature and at the column temperature. Retention volumes (V_R) were calculated using the equation

$$V_R = F_c t_r$$

where t_r is the retention time. The retention volumes were corrected for the extra-column volume. Each retention time was recorded at least three times. Retention times were obtained by injecting pure ILS or eluent (water or ACN). Injection of an ILS generally generates two peaks, one for the elution of the ILS and the other corresponding to the DP. The retention time of the DP was separately corroborated by injecting pure water or ACN. Four temperatures were investigated, 25, 35, 45 and 55°C .

THEORY

We start with the Riedo-Kováts basic equation [13,19]. The retention volume of a solute ($V_{R,i}$), in a binary solvent mixture, under isothermal and isocratic conditions, when an infinitesimal amount of solute i is injected, is given by

$$V_{R,i} = \left(\frac{\partial V_i}{\partial \theta_i} \right)_{\theta^0} - \theta_i^0 \left(\frac{\partial V_t}{\partial \theta_i} \right)_{\theta^0} \quad (1)$$

where V_i represents the volume of component i in the column (*i.e.*, its partial volume in the column), θ_i is the volume fraction of component i in the column, V_t is the total volume of the liquid mixture in the column, θ^0 is the isocratic composition of the equilibrated mixture prior to the injection of solute i and θ_i^0 is the composition of i in the column prior to the injection of solute i . If the solute i is not present in the column liquid mixture, its retention volume is given by

$$V_{R,i} = \left(\frac{\partial V_i}{\partial \theta_i} \right)_{\theta^0} \quad (1a)$$

From material balance, V_i can be expressed as

$$V_i = V_{i,s} + \theta_i V_m \quad (2)$$

where $V_{i,s}$ represents the volume of i sorbed on the modified surface (*i.e.*, the stationary phase) and $\theta_i V_m$ represents the volume of i in the mobile phase with a volume V_m .

Retention volume of the disturbance peak (DP)

The retention volume of the DP in a binary mixture is analyzed as follows. Consider a mobile phase consisting of water (component 1) and ACN (component 2) (*i.e.*, i is 1 or 2). If a very small perturbation in the composition of the mobile phase is caused by injecting an infinitesimal amount of component 1 or 2, the overall column liquid composition remains the same. The total liquid volume in the column (V_t) in this case is

$$V_t = V_1 + V_2 \quad (3)$$

Using eqn. 2 in eqn. 3, we obtain

$$V_t = V_m + V_{1,s} + V_{2,s} \quad (4)$$

where s refers to the stationary phase and m refers to the mobile phase. The substitution of the derivatives of eqns. 2 ($i = 1$) and 4 with respect to θ_1 into eqn. 1 gives the retention volume of component 1 as

$$V_{R,1} = V_m + \left(\frac{\partial V_{1,s}}{\partial \theta_1} \right) + \theta_1 \left(\frac{\partial V_m}{\partial \theta_1} \right) - \theta_1^0 \left[\left(\frac{\partial V_m}{\partial \theta_1} \right) + \left(\frac{\partial V_{1,s}}{\partial \theta_1} \right) + \left(\frac{\partial V_{2,s}}{\partial \theta_1} \right) \right] \quad (5)$$

where we have dropped the subscript θ^0 in the partial derivative notation. As the amount of component 1 injected is very small, $\theta_1 = \theta_1^0$, and eqn. 5 reduces to

$$V_{R,1} = V_m + \left(\frac{\partial V_{1,s}}{\partial \theta_1} \right) - \theta_1 \left[\left(\frac{\partial V_{1,s}}{\partial \theta_1} \right) + \left(\frac{\partial V_{2,s}}{\partial \theta_1} \right) \right]$$

Similarly for component 2, we obtain

$$V_{R,2} = V_m + \left(\frac{\partial V_{2,s}}{\partial \theta_2} \right) - \theta_2 \left[\left(\frac{\partial V_{1,s}}{\partial \theta_2} \right) + \left(\frac{\partial V_{2,s}}{\partial \theta_2} \right) \right]$$

and using the relationship $\partial \theta_1 = -\partial \theta_2$, we obtain

$$V_{R,1} = V_{R,2} = V_{R,(1,2)} = V_m + \theta_1 \left(\frac{\partial V_{2,s}}{\partial \theta_2} \right) + \theta_2 \left(\frac{\partial V_{1,s}}{\partial \theta_1} \right) \quad (6)$$

where $V_{R,1}$, $V_{R,2}$ and $V_{R,(1,2)}$ are the retention volumes of component 1, component 2 and the DP, respectively. Elution volumes of the DP are described by eqn. 6, in terms of the absolute quantities V_m , $V_{1,s}$ and $V_{2,s}$; however, the evaluation of eqn. 6 requires a model that relates these quantities to the mobile phase composition.

Retention volume of the isotopically labeled solutes (ILS)

The elution volumes of the ILS, deuterium oxide (²H₂O or 1*) and deuterated ACN (ACN-d₃ or 2*) are found as follows. As the ILS are not present in the column prior to the injection of either i^* , eqn. 1a applies and substitution of eqn. 2 in eqn. 1a gives

$$V_{R,i^*} = V_m + \theta_{i^*} \left(\frac{\partial V_m}{\partial \theta_{i^*}} \right) + \left(\frac{\partial V_{i^*,s}}{\partial \theta_{i^*}} \right)$$

Also, as a very small amount of the ILS is injected, $\theta_{i^*} \approx 0$ and the previous equation reduces to

$$V_{R,i^*} = V_m + \left(\frac{\partial V_{i^*,s}}{\partial \theta_{i^*}} \right) \quad (7)$$

where V_{R,i^*} is the retention volume of ILS component i .

If isotopic exchange is ignored [23], and one considers only the thermodynamic distribution (*i.e.*, partition by dilution) of the ILS into the corresponding non-labeled eluent (water or ACN) between the mobile phase and the stationary phase, the dilution partition constant (K_*) is

$$K_* = \frac{C_{1^*,s}}{C_{1^*,m}} = \frac{v_1^* n_{1^*,s} V_{1,m}}{v_1^* n_{1^*,m} V_{1,s}} = 1 \quad (8a)$$

or

$$V_{1^*,s} = V_{1^*,m} \left(\frac{V_{1,s}}{V_{1,m}} \right) \quad (8b)$$

where $C_{1^*,s}$ and $C_{1^*,m}$ are the concentrations of 1* in the stationary and mobile phases, respectively, v and n represent the molar volume and number of moles of the ILS, respectively, and V represents volume. The substitution of $V_{1,m} = \theta_1 V_m$ in eqn. 8b gives

$$V_{1^*,s} = \theta_{1^*,m} \left(\frac{V_{1,s}}{\theta_1} \right) = \theta_{1^*} \left(\frac{V_{1,s}}{\theta_1} \right) \quad (9)$$

where $\theta_{1^*,m}$ is the volume fraction of 1* in the mobile phase, which is equal to θ_{1^*} . Taking the derivative of eqn. 9 with respect to θ_{1^*} and substituting the result into eqn. 7, we obtain

$$V_{R,1^*} = V_m + \frac{V_{1,s}}{\theta_1} \quad (10a)$$

Similarly,

$$V_{R,2*} = V_m + \frac{V_{2,s}}{\theta_2} \quad (10b)$$

where $V_{R,1*}$ and $V_{R,2*}$ are the retention volumes of $^2\text{H}_2\text{O}$ and ACN-d_3 , respectively. Eqns. 10 can be derived in an alternative manner [16,22].

The substitution of eqns. 10a and 10b into eqn. 4 gives

$$V_t = \theta_1 V_{R,1*} + \theta_2 V_{R,2*} = \langle V_{R,(1*,2*)} \rangle \quad (11)$$

which is the total or "hold-up" column volume of the liquid solvent mixture [13,19,22].

The derivative of $V_{1,s}$ with respect to θ_1 in eqn. 10a is

$$\left(\frac{\partial V_{1,s}}{\partial \theta_1}\right) = V_{R,1*} + \theta_1 \left(\frac{\partial V_{R,1*}}{\partial \theta_1}\right) - V_m - \theta_1 \left(\frac{\partial V_m}{\partial \theta_1}\right) \quad (12)$$

The derivative of V_m with respect to θ_2 in eqn. 10b is

$$-\left(\frac{\partial V_m}{\partial \theta_2}\right) = \left(\frac{\partial V_m}{\partial \theta_1}\right) = -\left(\frac{\partial V_{R,2*}}{\partial \theta_2}\right) + \frac{1}{\theta_2} \left(\frac{\partial V_{2,s}}{\partial \theta_2}\right) - \frac{V_{2,s}}{(\theta_2)^2} \quad (13)$$

Utilizing eqns. 6 and 10–13 we obtain the following equivalent forms:

$$V_{R,(1,2)} = \theta_1 V_{R,2*} + \theta_2 V_{R,1*} + \theta_1 \theta_2 \left[\frac{\partial (V_{R,2*} - V_{R,1*})}{\partial \theta_2} \right] \quad (14a)$$

$$V_{R,(1,2)} = \theta_2 \left[\frac{\partial (\theta_1 V_{R,1*})}{\partial \theta_1} \right] + \theta_1 \left[\frac{\partial (\theta_2 V_{R,2*})}{\partial \theta_2} \right] \quad (14b)$$

$$V_{R,(1,2)} = \langle V_{R,(1*,2*)} \rangle + \left\{ \frac{\partial [\theta_1 \theta_2 (V_{R,2*} - V_{R,1*})]}{\partial \theta_2} \right\} \quad (14c)$$

Eqn. 14, which relates the retention volumes of the DP and ILS peaks, is an important, model-independent, self-consistency equation (see below).

Finally, we relate surface excess volumes (V_1^E) to the absolute volumes $V_{1,s}$ and $V_{2,s}$. We adopt the convention "nothing is adsorbed in terms of volumes, VNA" [14,19]. From the relationships

$$V_2^E = V_{2,s} - \theta_2 (V_{1,s} + V_{2,s}) = \theta_1 V_{2,s} - \theta_2 V_{1,s} \quad (15)$$

and

$$V_1^E = V_{1,s} - \theta_1 (V_{1,s} + V_{2,s}) = \theta_2 V_{1,s} - \theta_1 V_{2,s} \quad (16)$$

one obtains

$$V_2^E = -V_1^E \quad (17)$$

From eqns. 10a, 10b, 15 and 17, we obtain

$$V_2^E = -V_1^E = \theta_1 \theta_2 (V_{R,2*} - V_{R,1*}) \quad (18)$$

The substitution of eqn. 18 in eqn. 14c gives

$$V_{R,(1,2)} = \langle V_{R,(1*,2*)} \rangle + \left(\frac{\partial V_2^E}{\partial \theta_2} \right) = \langle V_{R,(1*,2*)} \rangle + \left(\frac{\partial V_1^E}{\partial \theta_1} \right) \quad (19)$$

which is equivalent to eqn. 7 in ref. 19.

RESULTS AND DISCUSSION

Temperature dependence

Four temperatures were investigated, 25, 35, 45 and 55°C. Elution volumes of the ILS ($V_{R,1*}$ and $V_{R,2*}$) and the DP [$V_{R,(1,2)}$] are listed in Tables I-III. $V_{R,(1,2)}$ is calculated as the average of the retention volumes of water and ACN. The average difference between the retention volumes of water ($V_{R,1}$) and ACN ($V_{R,2}$) is 0.005 ml, which is within the experimental error. Tables I-III show temperature effects, which

TABLE I
RETENTION VOLUMES OF ²H₂O

Volume fraction of ACN	$V_{R,1*}$ (ml)			
	25°C	35°C	45°C	55°C
0.15	3.066	3.050	3.049	3.040
0.20	3.041	3.011	3.020	3.017
0.25	3.001	2.990	2.989	2.994
0.30	2.967	2.965	2.975	2.985
0.35	2.945	2.943	2.970	2.967
0.40	2.925	2.927	2.958	2.957
0.45	2.906	2.904	2.934	2.942
0.50	2.893	2.887	2.915	2.931
0.55	—	2.896	2.916	2.936
0.60	2.921	2.930	2.937	2.957
0.65	—	2.964	2.986	2.980
0.70	2.987	2.992	3.006	3.006
0.75	—	3.018	3.034	3.040
0.80	3.054	3.037	3.047	3.062
0.85	—	3.093	3.123	3.126
0.90	3.214	3.182	3.199	3.222
0.95	—	3.255	3.252	3.271

TABLE II
RETENTION VOLUMES OF ACN-d₃

Volume fraction of ACN	$V_{R,2*}$ (ml)			
	25°C	35°C	45°C	55°C
0.15	3.835	3.788	3.767	3.752
0.20	3.777	3.692	3.663	3.622
0.25	3.708	3.639	3.593	3.573
0.30	3.652	3.562	3.528	3.493
0.35	3.590	3.512	3.480	3.463
0.40	3.500	3.443	3.421	3.381
0.45	3.461	3.393	3.379	3.360
0.50	3.408	3.354	3.329	3.319
0.55	—	3.316	3.285	3.275
0.60	3.323	3.288	3.256	3.257
0.65	—	3.249	3.235	3.226
0.70	3.267	3.232	3.220	3.204
0.75	—	3.213	3.205	3.197
0.80	3.205	3.177	3.169	3.177
0.85	—	3.172	3.170	3.160
0.90	3.208	3.160	3.169	3.162
0.95	—	3.153	3.150	3.148

TABLE III
RETENTION VOLUMES OF THE SOLVENT DISTURBANCE PEAK

Volume fraction of ACN	$V_{R,(1,2)}$ (ml)			
	25°C	35°C	45°C	55°C
0.15	3.536	—	3.458	3.407
0.20	3.517	3.427	3.383	3.353
0.25	3.445	3.362	3.309	3.270
0.30	3.358	3.274	3.233	3.212
0.35	3.273	3.199	3.184	3.142
0.40	3.143	3.080	3.047	3.053
0.45	3.027	2.995	2.991	2.975
0.50	2.928	2.917	2.917	2.932
0.55	—	2.846	2.865	2.896
0.60	2.810	2.814	2.840	2.865
0.65	—	2.813	2.837	2.860
0.70	2.801	2.836	2.865	2.883
0.75	—	2.857	2.889	2.904
0.80	2.889	2.892	2.925	2.932
0.85	—	2.955	2.989	2.967
0.90	3.030	3.000	3.067	3.023
0.95	—	3.054	3.081	3.066

TABLE IV
SOLVENT HOLD-UP VOLUMES

Volume fraction of ACN	V_i^a (ml)			
	25°C	35°C	45°C	55°C
0.15	3.181	3.161	3.157	3.147
0.20	3.188	3.148	3.149	3.138
0.25	3.178	3.152	3.140	3.139
0.30	3.172	3.144	3.141	3.138
0.35	3.171	3.142	3.148	3.141
0.40	3.155	3.133	3.143	3.127
0.45	3.155	3.124	3.134	3.130
0.50	3.150	3.120	3.122	3.125
0.55	—	3.127	3.119	3.122
0.60	3.162	3.145	3.128	3.137
0.65	—	3.149	3.148	3.140
0.70	3.183	3.160	3.156	3.145
0.75	—	3.164	3.162	3.158
0.80	3.175	3.149	3.145	3.154
0.85	—	3.160	3.163	3.155
0.90	3.209	3.162	3.172	3.168
0.95	—	3.158	3.155	3.154

^a Calculated from eqn. 11 and the results in Tables I and II.

appear to be relatively small; however, the effect of temperature is clearly noticeable in the absolute and excess quantities to be discussed. Also, listed in Table IV are the solvent hold-up volumes, which, as expected, are virtually independent of temperature.

Retention of the disturbance peak

The retention volume of the disturbance or "vacancy" peak has an interesting behavior as a function of mobile phase composition (Fig. 1). It passes through

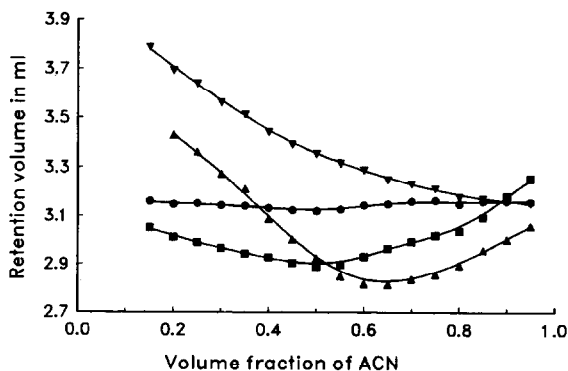


Fig. 1. Dependence of (■) $V_{R,1^*}$, (▼) $V_{R,2^*}$, (▲) $V_{R,(1,2)}$ and (●) V_{total} on θ_2 at 35°C.

a minimum, and both the water-enriched band and the ACN-enriched band essentially co-elute. Similar behavior has been observed in other binary systems. McCormick and Karger [11] explained this behavior by proposing a convex-type distribution isotherm, which has a maximum, with positive and negative slopes. Depending on the sense of the slopes (positive or negative), the vacancy velocity would decrease or increase with respect to the mobile phase velocity [21]. Zhu [16] proposed an absolute Langmuir-type adsorption isotherm for ACN, which we believe is essentially correct. However, it only partially explains the overall retention behavior of the DP. It does not consider the water adsorption isotherm contribution to the retention of the DP.

Eqn. 6 describes the retention volume of the DP as function of V_m , mobile phase composition and the derivatives (slopes) of the absolute isotherms of the solvent components. This relationship clearly indicates that the overall retention of the DP depends on the slopes of both isotherms (components 1 and 2). Eqn. 6 involves no approximations and any model that one proposes has to be consistent with it. We use eqn. 6 (with eqns. 10) to test our model for the absolute sorption isotherms, which provide insight into the changes occurring in the stationary phase due to changes in the mobile phase composition. Retention of the DP can also be expressed in terms of the hold-up volume and the derivative of the surface excess isotherm by eqn. 19. This relationship is exact and model independent, but does not provide a means of determining the individual contributions of components 1 and 2 to the overall retention of the DP. Note that eqns. 6 and 19 are essentially equivalent, though, in that they are related through the self-consistency relationship, eqn. 14, and eqns. 10 and 15–18.

Retention of the isotopically labeled solutes

Retention of the ILS is described by eqns. 10a and 10b, and their validity can be tested through eqns. 14. If $V_{R,(1,2)}$ can be reproduced by means of eqns. 14, then retention of the ILS is correctly described by eqns. 10 (in fact, the assumed absence of isotopic effects in the derivation of eqns. 10 also applies to eqns. 14 and 18, which derive from eqns. 10). Similarly, the reproduction of $V_{R,(1,2)}$ by means of the surface excess isotherm [19] is an equivalent way of testing eqns. 10. Accordingly, $V_{R,(1,2)}$ values were calculated using eqn. 14a. The derivative of the difference, $V_{R,2*} - V_{R,1*}$, in eqn. 14a was evaluated by first fitting this difference to a third-order polynomial in θ_2 . The coefficients of the equation and the correlation coefficients are displayed in Table V. The absolute average differences of the $V_{R,(1,2)}$ values calculated using

TABLE V

COEFFICIENTS OF THE THIRD-ORDER POLYNOMIAL FIT OF $V_{R,2*} - V_{R,1*}$ TO θ_2

$$V_{R,2*} - V_{R,1*} = a + b\theta_2 + c\theta_2^2 + d\theta_2^3.$$

Temperature (°C)	<i>a</i>	<i>b</i>	<i>c</i>	<i>d</i>	<i>R</i> ²
25	0.839	-0.401	-0.443	-0.169	0.999
35	0.885	-1.154	1.035	-0.960	0.998
45	0.912	-1.600	1.781	-1.289	0.998
55	0.946	-2.069	2.769	-1.879	0.997

eqn. 14a and the results in Tables I, II and V, and the experimental values of $V_{R,(1,2)}$ are 0.040, 0.034, 0.028 and 0.036 ml at 25, 35, 45 and 55°C, respectively, or an overall average difference of about 1%. Fig. 2 compares the calculated and experimental values at 35°C, revealing both negative and positive deviations. We believe that these differences can be further minimized with a more elaborate curve fitting of $V_{R,2*} - V_{R,1*}$ to the mobile phase composition [19]. These results indicate that eqns. 10, 11 and 18 are essentially valid for the ACN-water mixtures in RPLC.

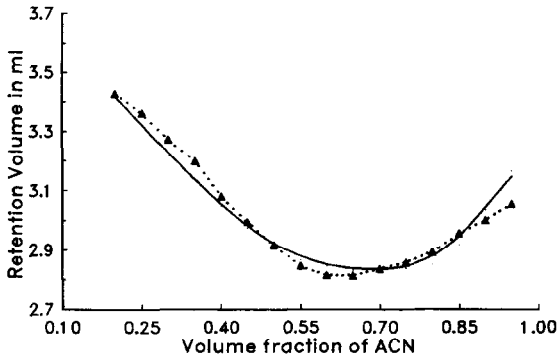


Fig. 2. Comparison of (\blacktriangle) experimental $V_{R,(1,2)}$ values and (solid line) calculated $V_{R,(1,2)}$ values from eqn. 14a at 35°C.

Model for the absolute isotherms

Here we develop a model for the absolute volumes of ACN ($V_{2,s}$) and water ($V_{1,s}$) sorbed on the stationary phase, based on two experimental observations. Water adsorption from the gas phase on trimethylsilylated surfaces is weak and completely reversible at low pressures [24]. The wettability of reversed-phase packing materials is a simple way to test their hydrophilic character. Wettability is defined as the organic composition (v/v) in an aqueous-organic mixture at which floating particles in the mixture sink to the bottom of the vessel [25,26]; non-wettable particles float on the surface of the solution. It has been observed that an RP-C₁₈ packing is wetted if the ACN concentration is greater than 40% (v/v) [25]. Wettability has been demonstrated to be highly dependent on the characteristics of the RP materials [26], *i.e.*, the length and population of alkyl chains, the amount of residual silanols, etc.

We begin by first assuming that water is not sorbed below 0.40 volume fraction of ACN. From eqns. 10, if $V_{1,s} = 0$, then $V_m = V_{R,1*}$ and $V_{2,s} = (V_{R,2*} - V_{R,1*})\theta_2$. $V_{2,s}$ is then fitted to a Langmuir-type form:

$$V_{2,s} = (a\theta_2)/(1 + b\theta_2) \quad (20)$$

From eqn. 20, $V_{2,s}$ values are generated for all eluent compositions and V_m is then calculated from eqn. 10b and $V_{1,s}$ from eqn. 10a. Through successive iterations, the best fit to eqn. 20 is eventually found by assuming that there is no sorption of water for $\theta_2 \leq 0.50$. Table VI shows the values of the parameters a and b for the temperatures studied here.

TABLE VI
COEFFICIENTS OF THE CURVE FITTING OF EQN. 20

It is assumed that $V_{1,s}$ is zero for $\theta_2 \leq 0.50$.

Temperature (°C)	<i>a</i>	Standard error	<i>b</i>	Standard error
25	1.063	0.066	2.041	0.293
35	1.016	0.031	2.358	0.155
45	1.058	0.030	3.102	0.170
55	1.065	0.079	3.518	0.471

Fig. 3 illustrates that effect of temperature on the absolute isotherm of ACN and shows the absolute isotherm of water at 35°C, as obtained from the model. As expected, the volume of sorbed ACN decreases with increasing temperature at all compositions. The calculated $V_{2,s}$ values at $\theta_2 = 1$, for example, are 0.350, 0.303, 0.258 and 0.236 ml at 25, 35, 35 and 55°C, respectively. The volume of sorbed water, which is

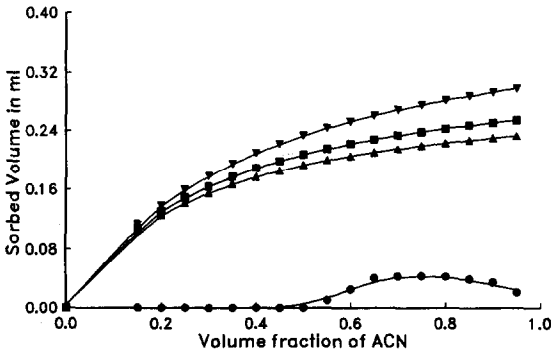


Fig. 3. Absolute sorption isotherms of ACN at (▼) 35, (■) 45 and (▲) 55°C and (●) absolute sorption isotherm of H₂O at 35°C.

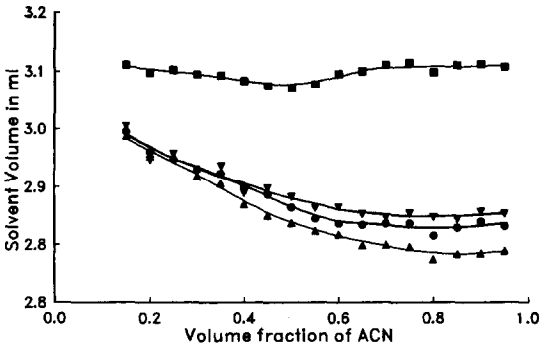


Fig. 4. Comparison of (■) the total solvent volume, V_t , at 35°C and the mobile phase volume, V_m , which is illustrated at (▲) 35, (●) 45 and (▼) 55°C.

much smaller than that of ACN, has a parabolic-like dependence on θ_2 for $\theta_2 \geq 0.50$. At 35°C a maximum value of $V_{1,s} = 0.043$ ml is found at $\theta_2 \approx 0.75$, decreasing to 0.037 and 0.034 ml at 45 and 55°C, respectively, and increasing to 0.050 ml at 25°C. We believe that, at high θ_2 , the water is initially driven onto the stationary phase by the already sorbed ACN, which renders the C₁₈ stationary phase somewhat hydrophilic. Then, consistent with the wetting studies [25,26], the volume of sorbed water starts to decrease with decreasing θ_2 and reaches negligible levels below $\theta_2 \approx 0.50$. This also suggests that the structure of the stationary phase may be altered by the sorbed solvent [22].

Fig. 4 and Table IV show that the hold-up volume (V_t) is only slightly dependent on the composition of the eluent. V_t has been shown to be equivalent to the column void volume [14,19,22]. A consequence of this model is the determination of the mobile phase volume, which, according to eqn. 4, is the difference between the total or hold-up volume and the volume of the solvent sorbed in the stationary phase (*i.e.*, water + ACN). Fig. 4 shows that the values of V_m obtained from this model vary with mobile phase composition and temperature, in the opposite direction of the corresponding variation of $V_{2,s}$ with the same variables (Fig. 3).

Test of the model

As was mentioned before, this model or any other model of absolute isotherms for a binary solvent mixture in liquid chromatography has to satisfy eqn. 6. Therefore, we tested our model by generating the values of $V_{R,(1,2)}$ from the V_m values and the absolute isotherms, utilizing eqn. 6. We evaluated the derivatives in eqn. 6 by first fitting the values of $V_{2,s}$, in terms of θ_2 , to a third-order polynomial (correlation coefficient in excess of 0.999) and $V_{1,s}$, in terms of θ_1 , to a fourth-order polynomial, in the composition range $0.50 \leq \theta_2 \leq 0.95$ (correlation coefficient 0.99). The evaluation of the derivative of $V_{1,s}$ with respect to θ_1 is especially difficult in the vicinity of $\theta_2 = 0.95$ and $\theta_2 = 0.50$, because the slopes are changing rapidly in these regions. In the vicinity of $\theta_2 = 0.60$, there appears to be an inflection point (see Fig. 3). We assume that the rate of change of this function in the vicinity of this point is symmetrical with respect to the inflection point. Fig. 5 shows a comparison of the experimental $V_{R,(1,2)}$

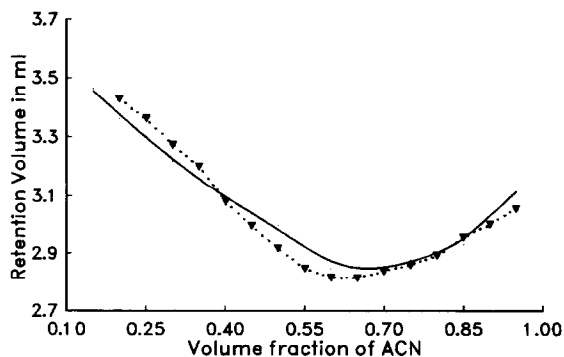


Fig. 5. Comparison of (\blacktriangledown) the experimental $V_{R,(1,2)}$ values and (solid line) calculated $V_{R,(1,2)}$ values from the absolute isotherms and eqn. 6 at 35°C.

TABLE VII

COMPARISON OF EXPERIMENTAL AND CALCULATED $V_{R,(1,2)}$ VALUES AT 35°C

Volume fraction of ACN	Experimental $V_{R,(1,2)}$ (ml)	V_m	$\theta_1(\partial V_{2,s}/\partial \theta_2)$	$\theta_2(\partial V_{1,s}/\partial \theta_1)$	Calculated $V_{R,(1,2)}^a$ (ml)
0.15	—	3.038	0.425	0.000	3.463
0.20	3.427	3.002	0.359	0.000	3.361
0.25	3.362	3.000	0.300	0.000	3.300
0.30	3.274	2.967	0.249	0.000	3.216
0.35	3.199	2.956	0.205	0.000	3.161
0.40	3.080	2.920	0.167	0.000	3.087
0.45	2.995	2.900	0.135	0.000	3.035
0.50	2.917	2.887	0.108	0.000	2.995
0.55	2.846	2.873	0.086	-0.037	2.922
0.60	2.814	2.867	0.068	-0.089	2.846
0.65	2.813	2.848	0.053	-0.089	2.812
0.70	2.836	2.849	0.041	-0.037	2.853
0.75	2.857	2.846	0.032	-0.001	2.877
0.80	2.892	2.825	0.024	0.032	2.880
0.85	2.995	2.834	0.018	0.078	2.929
0.90	3.000	2.834	0.012	0.157	3.003
0.95	3.054	2.839	0.006	0.295	3.140

^a Calculated from eqn. 6.

TABLE VIII

MODEL-INDEPENDENT SURFACE EXCESS VOLUMES OF ACN

 V_2^E was calculated from eqn. 18 and the results in Tables I and II.

Volume fraction of ACN	V_2^E (ml)			
	25°C	35°C	45°C	55°C
0.00	0.000	0.000	0.000	0.000
0.15	0.098	0.094	0.092	0.091
0.20	0.118	0.109	0.103	0.097
0.25	0.132	0.122	0.113	0.109
0.30	0.144	0.125	0.116	0.106
0.35	0.147	0.130	0.116	0.113
0.40	0.138	0.124	0.111	0.102
0.45	0.137	0.121	0.110	0.103
0.50	0.129	0.117	0.104	0.097
0.55	—	0.104	0.091	0.084
0.60	0.096	0.086	0.077	0.072
0.65	—	0.065	0.056	0.056
0.70	0.059	0.050	0.045	0.042
0.75	—	0.036	0.032	0.029
0.80	0.024	0.022	0.019	0.018
0.85	—	0.010	0.006	0.004
0.90	0.000	-0.002	-0.003	-0.005
0.95	—	-0.005	-0.005	-0.006

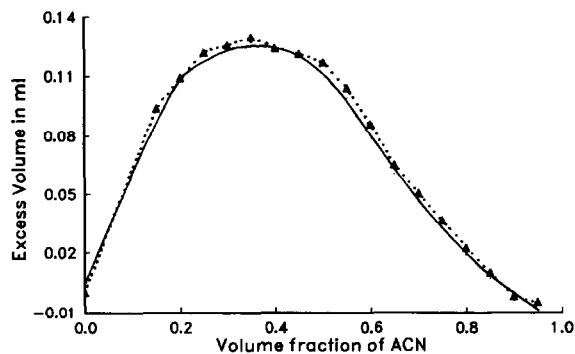


Fig. 6. Comparison of (\blacktriangle) the surface excess isotherm determined from eqn. 18 and (solid line) the surface excess isotherm determined from the absolute isotherms and eqn. 15 at 35°C.

and calculated $V_{R,(1,2)}$ values at 35°C. It can be seen that the greatest differences occur around $\theta_2 = 0.50$ and $\theta_2 = 0.95$, where $V_{1,s}$ is changing rapidly with solvent composition. The average difference between the experimental and calculated $V_{R,(1,2)}$ values is 0.037 ml, which is about 1%. Table VII shows the partial contributions to the calculated $V_{R,(1,2)}$ values. From Table VII it can be seen that this model explains and predicts the behavior of the DP.

The absolute isotherms can be tested in a second way. The surface excess volumes can be directly determined from the results in Tables I and II, and eqn. 18, which is model independent. The values of V_2^E , so determined, are listed in Table VIII. These results can then be compared with those obtained from eqn. 15, using the absolute volumes, $V_{1,s}$ and $V_{2,s}$, previously determined from our model. Fig. 6 illustrates this comparison at 35°C. The average difference between the two sets of V_2^E results is about 0.002 ml at 25 and 35°C and about 0.001 ml at 45 and 55°C. This clearly demonstrates the consistency of our model.

An independent check of the physical reasonableness of the results for $V_{1,s}$ and $V_{2,s}$ can be achieved by determining the maximum volume available to the solvent within the chain structure of the chemically bonded phase (CBP), *i.e.*, the volume which would be obtained if the alkyl chains were fully extended [22]. From the quoted total mass and carbon content of the packing material, and from available densities [27], the volume of the CBP chains, *per se*, is estimated to be about 0.39 ml. In addition, using the quoted specific surface area of the packing, a surface coverage of 2.47 $\mu\text{mol}/\text{m}^2$ is calculated, corresponding to a specific occupancy of one CBP chain per 66.7 \AA^2 . With a close-packed arrangement of the CBP, each chain would occupy about 25 \AA^2 . Therefore, the fractional chain coverage is about 0.375, leaving the remaining 62.5% of potential space, or *ca.* 0.65 ml, available for sorbed solvent. Accordingly, there is sufficient volume available for the sorbed solvent to be accommodated wholly within the milieu of the CBP chains.

We plan to use the results for the ACN-water mixtures and the same general model to analyze other organic modifier-water mixtures and to investigate the solute retention mechanism in RPLC in more quantitative and molecular-level detail [22].

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