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Study of Chromatographic Retention in Phosphate Solution on the Basis of Linear Solvation Energy Relationships

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Two kinds of phosphates *i.e.*, sodium dihydrogen phosphate (NaH_2PO_4) and disodium hydrogen phosphate (Na_2HPO_4) were used as additives in linear solvation energy relationships (LSER) model to investigate the fundamental chemical interactions governing the retentions of 7 aromatic compounds. The effects of different concentrations of the buffer and modifier on the retentions were discussed by using acetonitrile/water (v/v) mobile phases on a C_{18} column. Linear solvation energy relationships model demonstrated high potential to predict retention factors with very high correlation coefficients ($r^2 > 0.99$ in NaH_2PO_4 and $r^2 > 0.96$ in Na_2HPO_4). A comparison of predicted and experimental retention factors suggests that LSER formalism is able to reproduce the experimental retention factors of the solutes studied in the different experimental conditions adequately.

Key Words: Descriptors, Phosphates, Chromatographic retention, Linear solvation energy relationships.

INTRODUCTION

Reversed-phase high-performance liquid chromatography (RP-HPLC) is now one of the most widespread chromatographic techniques for the separation and determination of various classes of organic compounds. The retention behaviors of solutes for RP-HPLC mainly depend on many physical and chemical parameters of the system, such as temperature, solute molecules and compositions of the mobile and stationary phases. Predicting and explaining the chromatographic retention behavior in different mobile phase has been the subject of study for the last few decades.

Several empirical retention models are used for the purpose, such as linear solvent strength (LSS) model and linear solvation energy relationships (LSER) model. The fundamental conceptual definition of the LSER model, known as the solvatochromic model, was first introduced by Kamlet and Taft[1,2] and then it was developed by Abraham[3,4]. Linear solvation energy relationships model has more recently been proposed as the method of choice to describe and/or predict the partitioning behaviour of neutral organic compounds over a large range of environmental matrices and for a broad variety of compounds in a consistent manner. It has been successfully applied to systems in which a solute is transferred between two phases such as those found in GC, LC, micellar electrokinetic chromatography and supercritical fluid chromatography[5-11].

Recently, LSER model has been extensively used for the characterization of the quantitative structure-retention relationship (QSRR) and selectivity in micellar liquid chromatography using surfactant such as cationic surfactant cetyltrimethyl ammonium bromide (CTAB) and anionic surfactant, sodium dodecyl sulfate (SDS)[12,13], but there is little research on the chromatographic retention as the inorganic salt buffers in the mobile phase. The aim of this work is to investigate the fundamental chemical interactions responsible for retention in phosphate solution systems modified by acetonitrile. The variations of these interactions are studied as a function of the nature and concentration of NaH_2PO_4 and Na_2HPO_4 and organic modifier. For this study, we have used LSER model to explain retention in phosphate solution system containing NaH_2PO_4 and Na_2HPO_4 in the presence of 30, 40 and 50 % (v/v) acetonitrile.

EXPERIMENTAL

All experiments were performed on a Younglin M930 (Korea) equipped with a spectrophotometer (M 7200 absorbance detector, Young-In Scientific Co., Korea) and a Rheodyne injector (Hamilton Company, USA) valve with a 20 mL sample loop. The software Chromate (Ver. 3.0 Interface Eng., Korea) was used for system control and data handling. The detector was operated at 254 nm for LSER test solutes. Experiments were performed with a commercially available C_{18} column (Optimapak, Korea, 4.6 mm × 150 mm, 5 μm). All procedures were carried out at 30 ºC.

All of the LSER test solutes were purchased from C-TRI (Korea), and had a purity of 99 %. Sodium dihydrogen phosphate (NaH_2PO_4, 99.8 %), disodium hydrogen phosphate (Na_2HPO_4, 99.5 %) and acetonitrile (ACN, HPLC grade) were purchased from Duksan (Korea). Deionized water was obtained *via* a water purification system from Millipore Corp. (Milford, MA).

Preparation of mobile phases and standard solutions: NaH_2PO_4 and Na_2HPO_4 were added into the acetonitrile/water solution directly. The molar concentrations were adjusted to 0.01, 0.02 and 0.03 M, respectively. The mixed mobile phase containing 30, 40 and 50 % (v/v) acetonitrile modifiers for the mixed solution. After thorough mixing in a sonicator for 0.5 h, the final running eluents were filtered through a syringe filter (HA-0.45, Division of Millipore, Waters, USA) and then sonicated for more 0.5 h prior to the experiments. All stock solute solutions were prepared at concentrations of 1 mg/mL each. All of the seven solute samples were dissolved in methanol. It should be emphasized that the working solutions were re-prepared every 3 days so as to avoid potential errors arising from decomposition.

Theoretical backgrounds: The general LSER equation used in this work is [4]:

$$\log k = \log k_0 + m(V_x/100) + s\pi_2^H + a\Sigma\alpha_2^H + b\Sigma\beta_2^H + rR_2 \quad (1)$$

where log k_0 the regression intercept, the V_x, π_2^H, $\Sigma\alpha_2^H$, $\Sigma\beta_2^H$ and R_2 terms are solute descriptors, where V_x represents the solute's size/polarizability, π_2^H is the dipolarity/polarizability ($\Sigma\alpha_2^H$) is the hydrogen bond (HB) donating ability, $\Sigma\beta_2^H$ is the HB accepting ability and R_2 is the excess molar refraction. The subscript "2" simply signifies that these parameters are solute descriptors. The regression coefficients, m, s, a, b and r, quantitatively describe the contribution to retention for each of these various intermolecular interactions. Because there are intermolecular interactions in both the mobile and stationary phases, the fitting coefficients can be either positive or negative indicating an increase or decrease in retention, respectively, upon increases in the solute parameters under consideration. The m is the difference in the cohesivity/dispersive ability of the two bulk phases. A quite small positive 'm' value shows that the endoergic cavity formation term does not have the most important effect on retention. A large positive 's' coefficient, therefore, indicates that the solute/stationary phase dipolarity/polarizability interactions dominate over the solute/mobile phase dipolarity/polarizability interactions. A large, negative 'b' coefficient indicates that the analyte HB accepting interactions with a HB donating mobile phase is dominant over the analyte HB accepting interactions with a hydrogen bond donating stationary phase.

Retention factor estimation: The retention factor (k) of each solute was measured according to the following formula:

$$k = (t_R - t_M)/t_M \quad (2)$$

where t_R and t_M are the retention times of the retained analyte and the retention times of the unretained analyte (also known as dead time), respectively. Sodium nitrite was used as a t_M marker and was measured from the time of injection to the first deviation from the baseline following a 2 mL injection of 1 % sodium nitrite solution. The retention factors reported in this study are the averages of at least three determinations. Evaluation of the results of the chromatographic experiments was carried out using mathematical statistic techniques. The relative error of a single measurement did not exceed 5 %.

Linear solvation energy relationship estimations: Retention factors were determined for the 7 compounds used in this study and the system constants were calculated by multiple linear regressions using Origin Pro 6.0 software (Microcal Software Inc., MA, USA). The statistical validity of the LSER models was evaluated through a F test, squared correlation coefficient (r^2) and root mean square error in the estimate (SD). The differences in LSER coefficients indicate the variations in the types of interactions between stationary phases and solutes.

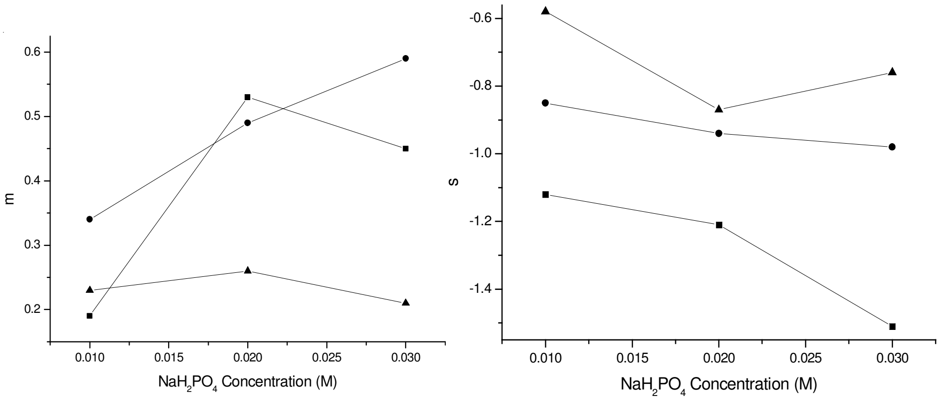
RESULTS AND DISCUSSION

The retention behaviours of the 7 test solutes (caffeine, pyridine, phenol, methylparaben, aniline, acetophenone and benzene) in each phosphate system were examined and compared using LSER model, which is described in eqn. 1. The test solutes and their descriptors used in this study are given in Table-1.

TABLE-1
TEST SOLUTES AND THEIR DESCRIPTORS FOR
THE SOLVATION PARAMETER MODEL

Solutes	Descriptors				
	V_X (cm^3/mol^{-1})	π_2^H	α_2^H	β_2^H	R_2 ($cm^3/10$)
Caffeine	1.500	1.60	0	1.35	1.3630
Pyridine	0.631	0.84	0	0.52	0.6753
Phenol	0.805	0.89	0.60	0.30	0.7751
Methylparaben	0.900	1.37	0.69	0.45	1.1310
Aniline	0.955	0.96	0.26	0.50	0.8162
Acetophenone	0.818	1.01	0	0.48	1.0139
Benzene	0.610	0.52	0	0.14	0.7164

The coefficients for the LSER equations obtained for NaH_2PO_4 and Na_2HPO_4 used in this work are grouped in Tables-2 and 3. In all the systems investigated the coefficients of π_2^H, $\Sigma\alpha_2^H$ and $\Sigma\beta_2^H$ (s, a and b) were negative, *i.e.*, an increase in the solute dipolarity/polarizability, the HB donating and the HB basicity ability decreases the overall retention of the molecule. Furthermore, the coefficients of VX (m) and R_2 (r) were positive in all the systems studied, indicating that increasing in the solute volume and excess molar refractivity lead to increases in retention. The 'm' values indicate that retention in the different mobile phases increased with increasing solute size. Furthermore, the relatively small change of 'm' value shows that the endoergic cavity formation term did not have the strongest effect on retention. The small 'm' values also suggest that retention was not influenced by the solute size. In the NaH_2PO_4 system, the 'a' value had a small change (0.32-0.59), except in the 30 % v/v of acetonitrile with 0.01 M NaH_2PO_4 system, as shown in Fig. 1. This suggests that increasing the concentration of NaH_2PO_4 and acetonitrile had no effect on the size of the solute size. The same trend was observed in Na_2HPO_4, as shown in Fig. 2, but the 'a' value was larger than in the NaH_2PO_4 system.



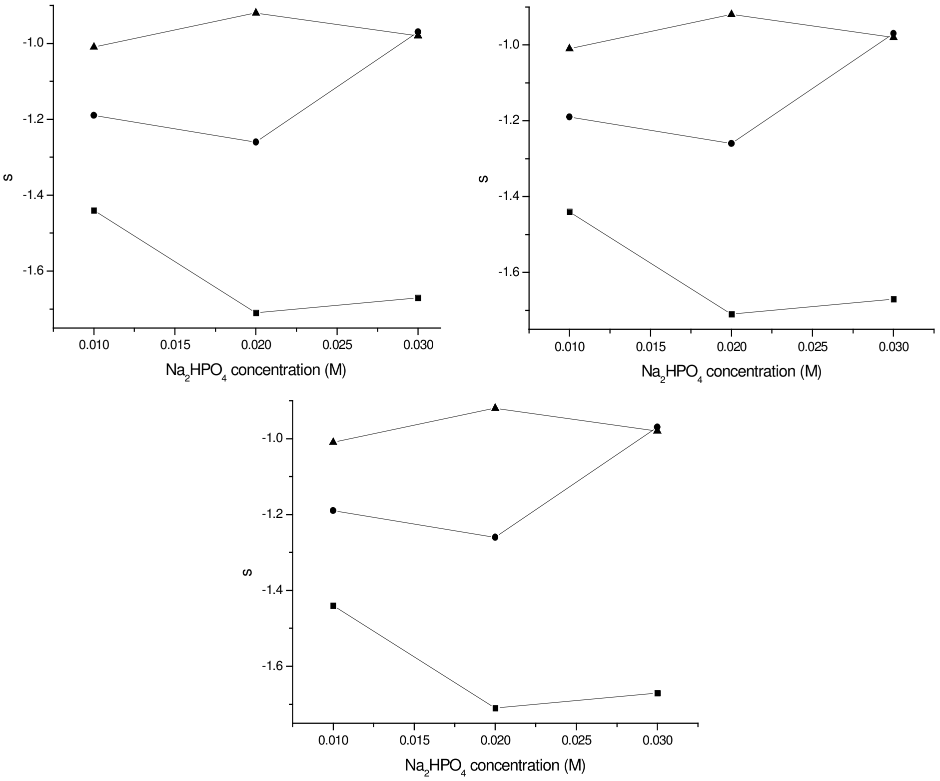


Fig. 1. LSER coefficients as a function of NaH_2PO_4 concentrations. Modifiers are: ■30 % acetonitrile, ●40 % acetonitrile and ▲50 % acetonitrile error bars have been omitted for clarity

The difference in dipolarity/polarizability is represented by the coefficient ‘s’. A negative sign for this coefficient indicates that the solutes experience a microenvironment that has smaller dipolar/polarizable characteristics than the aqueous phase. On the contrary, a positive ‘s’ value indicates that the solutes experience a more dipolar microenvironment in the mobile phases. In all the studied systems, the ‘r’ value almost exhibited no change with increase phosphate concentration, but a larger change was induced when the acetonitrile concentration in the NaH_2PO_4 system was changed. Increasing the acetonitrile concentration can change the polarity of the mobile phase, which fits with the observed change of ‘s’.

The coefficient ‘a’ is one of the important factors in the LSER model. A positive coefficient meant that the hydrogen bond accepting ability of the mobile phase was greater. As shown in Tables 2 and 3, the coefficient ‘a’ was quite small (less than -0.33) and statistically insignificant, which meant that the solute's hydrogen-bond-donating ability had only a small or zero effect on retention. In other words, the smaller values of the coefficient ‘a’ for these three different concentrations of phosphates indicate that their hydrogen bond accepting strength was not significantly different from that of the mobile phase without additives.

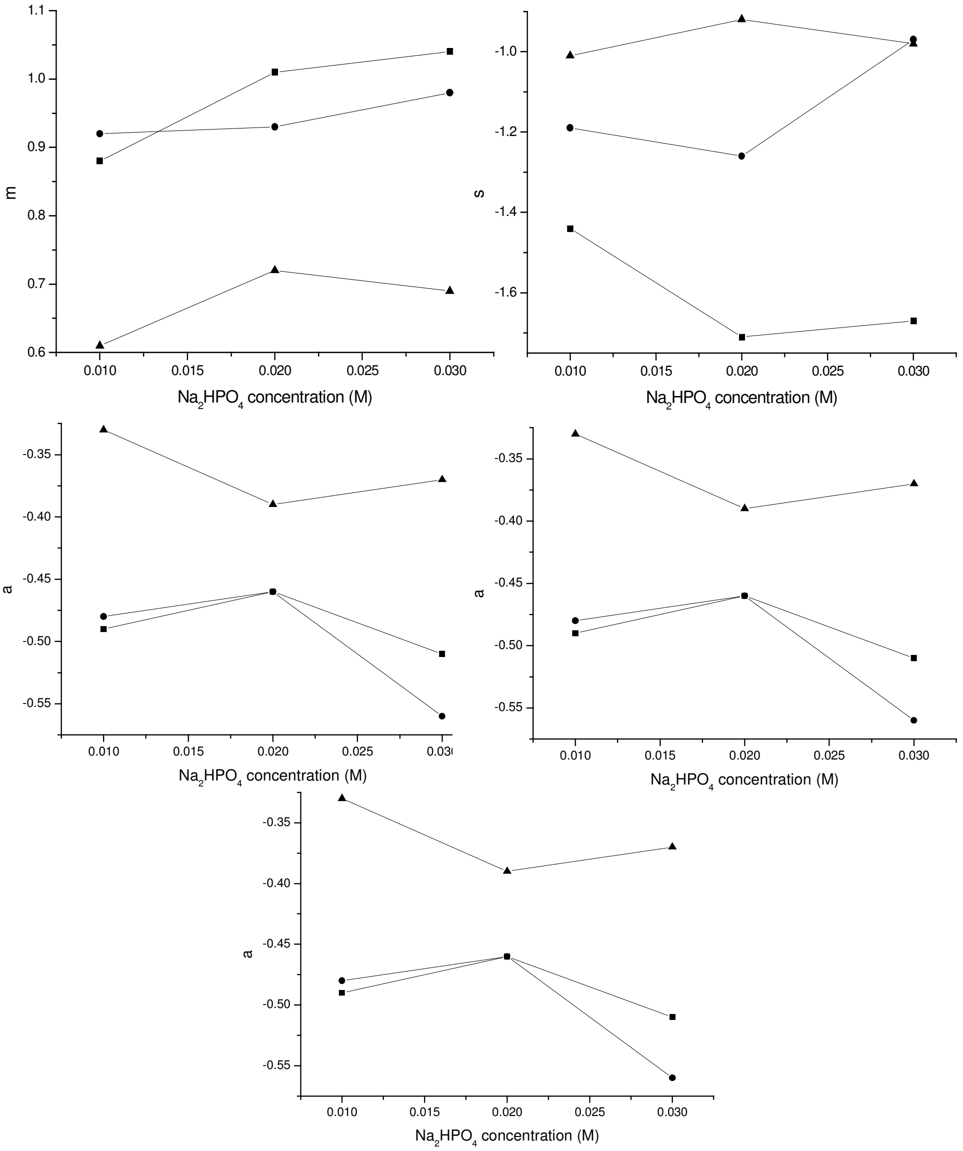


Fig. 2. LSER coefficients as a function of NaH_2PO_4 concentrations. Modifiers are: ■ 30 % acetonitrile, ● 40 % acetonitrile and ▲ 50 % acetonitrile error bars have been omitted for clarity

The coefficient 'b' is the second most important factor in the LSER solvation parameter model in different systems used in this study. A comparison of the coefficients for additive concentration reveals that 'b' had the largest absolute value among all the coefficients for all the concentrations studied here. The 'b' coefficient in Tables 2 and 3 plays the most important roles in LSER model and is proportional to the difference in the hydrogen bond donating ability of the mobile phase and that of the aqueous phase. The larger (or less negative) 'b' coefficient is, the higher is the hydrogen bond donating ability strength of the mobile phase. The relative hydrogen bond donating strength of the acetonitrile-contained phases used in this study was in the order 0.03 M > 0.02 M > 0.01 M. The pH of NaH_2PO_4 was lower than that of Na_2HPO_4, indicating that the hydrogen bond donating strength of Na_2HPO_4 must be higher, which agreed with the results in Tables 2 and 3.

TABLE-2
CALCULATED RESULTS OF THE PARAMETERS IN NaH_2PO_4

NaH_2PO_4 concentration, M		0.01	0.02	0.03
Acetonitrile concentration, % v/v		30		
Constants	log k_0	0.49 (0.28)	0.26 (0.12)	0.19 (0.20)
	m	0.19 (0.70)	0.53 (0.29)	0.45 (0.49)
	s	-1.12 (0.94)	-1.21 (0.39)	-1.51 (0.67)
	a	-0.24 (0.5)	-0.32 (0.21)	-0.17 (0.36)
	b	-1.10 (0.86)	-1.55 (0.36)	-1.28 (0.61)
	r	1.93 (0.86)	2.21 (0.36)	2.54 (0.61)
Statistics	r^2	0.9870	0.9980	0.9950
	SD	0.0998	0.0417	0.0708
	F	15.7530	123.3456	38.3653
Acetonitrile concentration, % v/v		40		
Constants	log k_0	0.33 (0.22)	0.26 (0.19)	0.20 (0.20)
	m	0.34 (0.541)	0.49 (0.48)	0.59 (0.49)
	s	-0.85 (0.73)	-0.94 (0.65)	-0.98 (0.66)
	a	-0.30 (0.39)	-0.29 (0.35)	-0.30 (0.35)
	b	-1.09 (0.66)	-1.16 (0.59)	-1.24 (0.60)
	r	1.48(0.66)	1.56 (0.59)	1.64 (0.60)
Statistics	r^2	0.9900	0.9920	0.9930
	SD	0.0771	0.0689	0.0698
	F	19.9916	25.4865	26.5466
Acetonitrile concentration, % v/v		50		
Constants	log k_0	0.23 (0.12)	0.26 (0.15)	0.21 (0.14)
	m	0.41 (0.29)	0.32 (0.39)	0.39 (0.36)
	s	-0.58 (0.39)	-0.87(0.52)	-0.76 (0.49)
	a	-0.36(0.21)	-0.21 (0.28)	-0.27 (0.26)
	b	-0.98 (0.36)	-0.74 (0.48)	-0.91 (0.44)
	r	1.01 (0.36)	1.23 (0.48)	1.29 (0.44)
Statistics	r^2	0.9960	0.9920	0.9930
	SD	0.0416	0.0553	0.0515
	F	45.6633	23.6178	30.5439

The 'r' coefficient represents the excess molar refraction of the solute. All mobile phases had a positive coefficient 'r' (Tables 2 and 3). According to the data, the polarity of the mobile phases is ranked as: 0.01 M > 0.02 M > 0.03 M of the mobile phase additives.

Estimation of LSER equations: Calculated (or predicted) log k values of the test solutes were computed for each mobile phase using eqn. 1. The solvation parameter model provided statistically verifiable and chemically useful results. This was demonstrated by comparing the statistics (*i.e.*, r^2 and SD) of the solvation parameter model results in Tables 2 and 3). The correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from acetonitrile 40 % (v/v) with different concentrations of NaH_2PO_4 and NaH_2PO_4 demonstrated in Figs. 3 and 4. It indicated that good correlations were obtained for the experimental log k values *versus* predicted log k values, *i.e.*, LSER model is able to approximately reproduce the experimental log k values for the solutes studied in the different mobile phases.

TABLE-3
CALCULATED RESULTS OF THE PARAMETERS IN Na_2HPO_4

NaH_2PO_4 concentration, M		0.01	0.02	0.03
Acetonitrile concentration, % v/v			30	
Constants	$\log k_0$	0.52 (0.15)	0.59 (0.27)	0.58 (0.30)
	m	0.88(0.37)	1.01 (0.67)	1.04 (0.75)
	s	-1.44 (0.50)	-1.71 (0.90)	-1.67 (1.02)
	a	-0.49 (0.26)	-0.46(0.48)	-0.51 (0.54)
	b	-1.30(0.45)	-1.18 (0.82)	-1.23(0.92)
	r	1.72 (0.45)	1.72 (0.82)	1.70 (0.92)
Statistics	r^2	0.998	0.992	0.990
	SD	0.0526	0.0953	0.1073
	F	73.2512	24.3999	19.5815
Acetonitrile concentration, % v/v			40	
Constants	$\log k_0$	0.40 (0.15)	0.44 (0.18)	0.42 (0.27)
	m	0.92 (0.37)	0.93 (0.45)	0.98 (0.67)
	s	-1.19 (0.50)	-1.26 (0.61)	-0.97(0.90)
	a	-0.48(0.27)	-0.46 (0.33)	-0.56 (0.48)
	b	-1.11 (0.46)	-1.07 (0.56)	-1.16 (0.82)
	r	1.21(0.46)	1.22 (0.56)	0.94 (0.82)
Statistics	r^2	0.9960	0.9940	0.9860
	SD	0.0529	0.0649	0.0954
	F	51.8665	36.0658	14.5421
Acetonitrile concentration, % v/v			50	
Constants	$\log k_0$	0.34 (0.07)	0.33(0.09)	0.38 (0.13)
	m	0.61 (0.15)	0.72 (0.23)	0.69 (0.32)
	s	-1.01(0.20)	-0.92(0.31)	-0.90 (0.44)
	a	-0.33(0.11)	-0.39 (0.16)	-0.37 (0.23)
	b	-0.80 (0.19)	-0.88(0.28)	-0.81 (0.40)
	r	1.02 (0.19)	0.89 (0.28)	0.90 (0.40)
Statistics	r^2	0.9990	0.9980	0.9950
	SD	0.0216	0.0325	0.0463
	F	206.0066	89.7307	44.0728

Conclusion

Phosphates were applied as additives in the mobile phases using acetonitrile as the modifier. The LSER model was successfully applied to investigate the effect of additives concentrations on retentions of 7 aromatic compounds in RP-HPLC. The results obtained from the solvation parameter model showed that most coefficients in compared information had a little change with the increasing concentration of NaH_2PO_4 and Na_2HPO_4, but in different buffers the coefficients 'b' had larger changes which meant that HB basicity generally played the largest role in determining the retentions of solutes in all the systems studied. Due to the excellent correlation between experimental and calculated retention across the mobile phase composition range used in this study ($r^2 > 0.99$ in NaH_2PO_4 and $r^2 > 0.96$ in Na_2HPO_4), the utility of the LSER model was feasible to describe retentions of the seven solutes in phosphates systems.

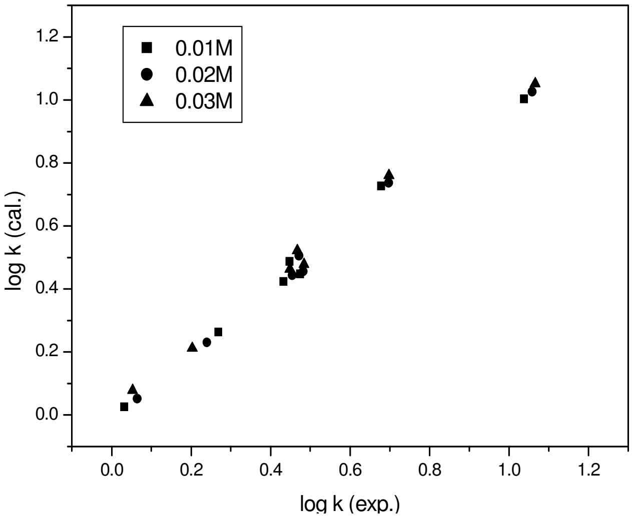


Fig. 3. Correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from acetonitrile 40 % (v/v) with different concentrations of NaH_2PO_4)

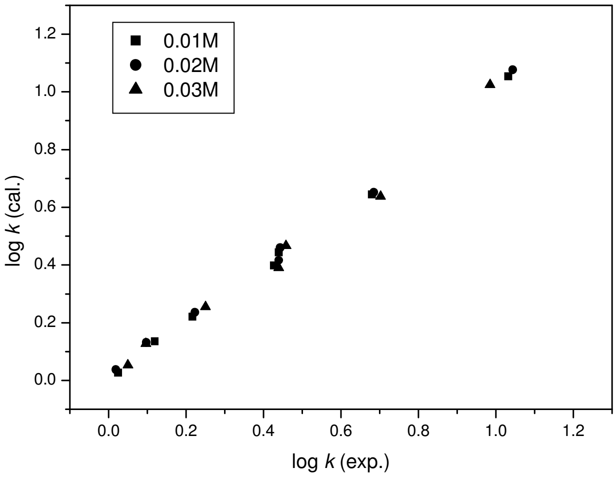


Fig. 4. Correlation between experimental (exp) and calculated (cal) log k (mobile phases composed from acetonitrile 40 % (v/v) with different concentrations of Na_2HPO_4)

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