

Polar bonded phase with the zwitterionic sulfobetaine functional group

Comparison to silica

WALTER G. TRAMPOSCH^a and STEPHEN G. WEBER*

Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260 (U.S.A.)

ABSTRACT

A new bonded phase has been prepared by the reaction of *n*-propylsultone with dimethylaminopropylsilane-modified silica. The resulting functional group is the zwitterionic ammonium propane sulfonate. Chromatographic solvents based on three strong solvents, methylene chloride, diisopropyl ether and acetonitrile, were prepared in hexane. Solutes, including substituted benzenes, polycyclic aromatic hydrocarbons and low-molecular-weight species commonly used as solvents, were chromatographed on the zwitterionic phase and on silica from the same supplier of the dimethylamino phase. The zwitterionic phase is a weaker adsorbent than silica and retentions are less influenced by the type of strong solvent employed, compared to silica. The retention ($\log k'$) of solutes on the zwitterionic phase is highly correlated with the free energy of transfer of solute from the vapor phase to water.

INTRODUCTION

New bonded phases are useful because they allow for more flexibility in designing separations, especially those separations of relatively simple mixtures. There are also cases in which a particular stationary phase has an unanticipated advantage in certain situations. The use of cyano bonded phases in micellar chromatography to prevent the adsorption of the surfactant and the concomitant loss in selectivity caused by the similarity of the mobile and stationary phases [1] is one such example.

We have employed the zwitterionic surfactants *N*-alkyl-*N,N*-dimethylammonium propanesulfonates (sulfobetaines) as probes of silica chemistry [2], as modifiers of a reversed-phase [3] and as a micellar mobile phase [4]. They impart to separations a selectivity which is difficult to rationalize in simple terms, but that is different than reversed-phase chromatography, and therefore is useful. In this report, the sulfobetaine functional group is covalently attached to silica by means of the reaction between a commercially available *N,N*-dimethylaminopropyl silane column and *n*-propylsultone. The resulting column is studied as a normal-phase material with three

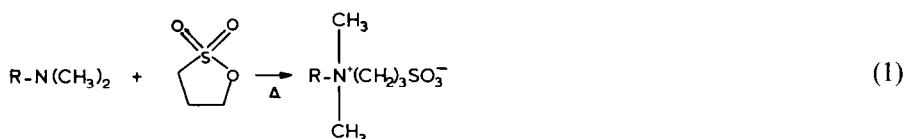
^a Present address: Calgon Carbon Corp., P.O. Box 717, Pittsburgh, PA 15230-0717, U.S.A.

solvent systems representing three solvent classes from the selectivity triangle, and the results are compared to those on silica. There is indirect evidence that the new stationary phase operates by imbibing water, and that retention is achieved by partitioning.

EXPERIMENTAL

Column preparation

A 10- μm Nucleosil column covalently modified with the N,N-dimethylamino-propyl group (Macherey-Nagel) was purchased from HPLC Technology. At the same time an unmodified Nucleosil column was obtained from the same source. The reaction (1) between the cyclic sultone and the amine proceeds to high yields (>95%) in homogeneous syntheses which occur in refluxing acetone [2].



We wished to carry out the synthesis at the same temperature as boiling acetone on the already packed column, but did not want to use acetone because the bubbles that might have formed would have interfered with homogeneous distribution of the reagent solution on the column. Therefore, toluene was used as the solvent. A 2.0- M solution of the propylsultone (Aldrich) was prepared by dissolving 25 g of the sultone in 100 ml toluene. Past experience had indicated that 1.5 h was enough for the reaction to go to completion, so the 100 ml were pumped into the column at 0.6 ml/min. The effluent was observed with a refractive index detector. There was a breakthrough at 12 min, which indicates that 24 mmol of sultone reacted immediately. After the retention experiments were done the column was sacrificed and the contents microanalyzed (Galbraith). The composition was found to be C:4.71%; H:1.15%; N: <0.5%; S:1.05%.

Solutes were purchased from Aldrich, solvents were MCB Omnisolve.

The pump used was a Waters M45, the detectors were a Gilson Holochrome variable-wavelength UV-VIS detector and a Waters 401 refractive index detector. The injector was from Valco, and 20- μl injections were made. Data acquisition was by a DEC LSI-11 microcomputer, and in-house-written software was used to determine the first moments of the peaks. Redundant data collection was done by recorder (Heath SR206).

RESULTS

The solvents and their compositions are shown in Table I. They represent three different solvent classes, and the acetonitrile, as a strong solvent, will be localized [5]. The Kamlet-Taft π^* , α_{KT} and β_{KT} values are indicative of polarity/dipolarity (π^*), hydrogen bond donor ability (α_{KT}), hydrogen bond acceptor ability (β_{KT}). The differences among the solvents are in all three parameters, which explains their different positions in the selectivity triangle [6,7].

TABLE I
B SOLVENTS USED (A = HEXANE)

Solvent	ϵ^0 ^a	π^* ^b	α_{KT} ^b	β_{KT} ^b	% (v) ^d	Class ^a
Methylene chloride (MC)	0.32	0.82	0.30	0	3	7 ^c
Diisopropyl ether (DIE)	0.34	0.27	0	0.49	14.5	1
Acetonitrile (ACN)	0.50	0.75	0.19	0.31	1	6

^a Taken from ref. 5.

^b Taken from ref. 6 and references therein.

^c Recently reclassified [7].

^d Composition of chromatographic solvent system, %B in hexane by volume.

The retention data for a variety of solutes (Tables II and III) have been treated using the Snyder formalism shown in eqn. 2 [5]

$$\log k' = \log \varphi + \alpha_a(S^0 - A\epsilon^0) \quad (2)$$

where k' is the capacity factor, φ is the phase ratio, α_a is the adsorbent activity, S^0 is the adsorption free energy of the solvent, A is the solute molecular area and ϵ^0 is the adsorption free energy per area of the solvent. If one assumes that the phase ratio remains the same when solvents are changed, then the ratio of k' for a solute in one

TABLE II
SOLUTES AND k' VALUES FOR SULFOBETAINE ZWITTERIONIC PHASE

	k'				k'		
	MC	DIE	ACN		MC	DIE	ACN
<i>Non-polar benzenes</i>				<i>Polycyclic aromatics</i>			
Benzene	0.32	0.27	0.18	Anthracene	2.68	2.51	0.76
Bromobenzene	0.41	0.41	0.23	2-Methylantracene	2.73	2.77	0.75
Chlorobenzene	0.38	0.40	0.22	9-Methylantracene	2.61	2.67	0.79
Toluene	0.33	0.26	0.16	1-Chloroanthracene	2.00	2.64	0.73
<i>m</i> -Xylene	0.32	0.30	0.11	2-Chloroanthracene	1.95	2.32	0.78
<i>Polar benzenes</i>				Pyrene	3.43	3.66	2.04
Nitrobenzene	4.27	2.84	1.65	Fluoranthene	4.38	3.67	1.10
Benzonitrile	8.07	3.85	2.29	Chrysene	6.56	5.33	1.47
Benzyl alcohol	2.70	2.70	21.8	Benz[<i>a</i>]anthracene	6.16	4.69	1.48
<i>Non-aromatic</i>				Perylene	10.6	8.49	2.04
Carbon tetrachloride	0.07	0.15	0.04	Benzo[<i>a</i>]pyrene	9.27	7.65	1.82
Carbon disulfide	0.04	0.12	0.05	Coronene	16.1	5.07	— ^a
Chloroform	0.30	0.52	0.44	Dibenz[<i>a,h</i>]anthracene	6.16	— ^a	2.85
Methylene chloride	0.32	0.72	0.51				
1,2-Dichloroethane	0.65	0.92	0.57				
Bromoethane	0.27	0.39	0.21				
Methylacetate	6.92	2.13	1.12				
Ethylacetate	6.77	1.65	0.91				
Diisopropyl ether	2.52	0.12	0.18				

^a Not done.

TABLE III
SOLUTES AND k' VALUES FOR SILICA PHASE

	k'				k'		
	MC	DIE	ACN		MC	DIE	ACN
<i>Non-polar benzenes</i>				<i>Polycyclic aromatics</i>			
Benzene	0.20	0.01	0.18	Anthracene	0.72	0.08	0.38
Bromobenzene	0.18	0.01	0.21	2-Methylanthracene	0.78	0.08	0.37
Chlorobenzene	0.16	0.01	0.40	9-Methylanthracene	0.75	0.08	0.35
Toluene	0.16	0.01	0.27	1-Chloroanthracene	0.51	0.13	0.36
<i>m</i> -Xylene	0.25	0.01	0.07	2-Chloroanthracene	0.55	0.10	0.38
<i>Polar benzenes</i>				<i>Pyrene</i>			
Nitrobenzene	5.53	0.66	1.43	Fluoranthene	0.95	0.13	0.55
Benzonitrile	20.9	1.17	2.63	Chrysene	1.27	0.18	0.67
Benzyl alcohol	23.1	9.02	2.13	Benz[<i>a</i>]anthracene	1.21	0.17	0.80
<i>Non-aromatic</i>				<i>Perylene</i>			
Carbon tetrachloride	0.40	0.01	0.22	Benzo[<i>a</i>]pyrene	1.32	0.23	3.47
Carbon disulfide	0.30	0.01	0.08	Coronene	1.52	0.35	— ^a
Chloroform	0.40	0.01	0.25	Dibenz[<i>a,h</i>]anthracene	2.01	— ^a	0.98
Methylene chloride	0.50	0.05	0.60				
1,2-Dichloroethane	0.80	0.20	0.70				
Bromoethane	0.65	0.02	0.28				
Methylacetate	0.40	1.30	2.20				
Ethylacetate	0.30	1.00	1.70				
Diisopropyl ether	0.30	0.01	0.60				

^a Not done.

solvent compared to k' in another will be related to the difference in solvent strengths as shown in eqn. 3. Regression of $\log \alpha$ vs. molecular area [8] for the non-polar benzene compounds and the polycyclic aromatics yielded the slopes shown in Table IV.

$$\log \alpha = \log (k'_1/k'_2) = -(\alpha_1 \varepsilon_1^0 - \alpha_2 \varepsilon_2^0) A \quad (3)$$

TABLE IV
SLOPES OF $\log (k'_1/k'_2)$ VS. SOLUTE AREA

Stationary phase	Slope \pm S.D.		
	Solvents (1-2)		
	MC-DIE	DIE-ACN	ACN-MC
Sulfobetaine	0.020 \pm 0.011 (<i>n</i> = 17)	0.050 \pm 0.009 (<i>n</i> = 16)	-0.054 \pm 0.010 (<i>n</i> = 17)
Silica	-0.081 \pm 0.011 (<i>n</i> = 17)	0.105 \pm 0.028 (<i>n</i> = 16)	-0.027 \pm 0.024 (<i>n</i> = 17)

If the log k' values themselves are regressed on area, then the slopes shown in Table V are obtained, which are equal to the term $\alpha_a(S/A - \epsilon^0)$ as seen in eqn. 2. If the term S/A , that is the adsorption energy of an aromatic hydrocarbon per unit area on a surface of unit activity, is constant for each column, then the differences in Table IV should be reflected in Table V.

TABLE V
SLOPES OF LOG k' VS. SOLUTE AREA

Stationary phase	Slope \pm S.D.		
	Solvent		
	MC	DIE	ACN
Sulfobetaine	0.21 \pm 0.006 ($n=18$)	0.20 \pm 0.011 ($n=17$)	0.16 \pm 0.011 ($n=17$)
Silica	0.13 \pm 0.006 ($n=18$)	0.21 \pm 0.009 ($n=17$)	0.10 \pm 0.22 ($n=17$)

For the silica column, but not for the sulfobetaine column, an estimate of the value of S/A can be determined in the following way. If α_a can be determined, then the solvent strength can be calculated. If α_a and ϵ^0 are both known, then from the values in Table V, S/A will be revealed. The activity is determined by regressing, for each solvent, values of log $(k'_{\phi R}/k'_{\phi H})$ for the substituted benzenes on $Q_{R,SIL}^0$, the substituent adsorption energies on unit activity silica (SIL) [8]. The values of α_a shown in Table VI, are the slopes of that regression. With these values of α_a , ϵ^0 can be estimated from the formula given by Snyder [8] and Snyder and Kirkland [5] (but note that the equation in the latter reference, eqn. 9.1, is missing a parenthesis on the far right hand side of the numerator), and these values are also given in Table VI. Table VII shows the resulting calculation of S/A .

The same quantitative analysis of the data from the sulfobetaine (SB) column is precluded because values of functional group adsorption energies on unit activity sulfobetaine, $Q_{R,SB}^0$ and S/A , are not in the literature. However, some inferences can be made. Consider the experiments using the same solvent but different columns. If the stationary phase surface area is the same for each, then a comparison of log α values

TABLE VI
ESTIMATE OF SILICA ACTIVITY AND SOLVENT STRENGTH

Solvent	$\alpha_a \pm$ S.D. ($n=8$)	ϵ^0
MC	0.53 \pm 0.029	0.045
DIE	0.66 \pm 0.027	0.13
ACN	0.27 \pm 0.058	0.070

TABLE VII
CALCULATION OF *S/A* SILICA COLUMN

Solvent	$\alpha_a(S/A - \epsilon^0)$	$S/A - \epsilon^0$	ϵ_{calc}^0	$S/A \pm \text{S.D.}$
MC	0.13	0.24	0.045	0.29 ± 0.04
DIE	0.21	0.32	0.13	0.45 ± 0.05
ACN	0.10	0.37	0.07	0.44 ± 0.13

(referenced to benzene on each column) with $Q_{R,SIL}^0$ will be related to the term shown in eqn. 4. It can be seen that if the activity is the same for both columns, and if $Q_{R,SB}^0$ is directly proportional to $Q_{R,SIL}^0$, then the values of $\Delta \log \alpha$ will be proportional to Q as shown in eqn. 5.

$$\log \alpha_{SB} - \log \alpha_{SIL} = \alpha_{a,SB} (Q_{R,SB}^0 - \epsilon_{SB}^0) - \alpha_{a,SIL} (Q_{R,SIL}^0 - \epsilon_{SIL}^0) \quad (4)$$

$$\log \alpha_{SB} - \log \alpha_{SIL} = \alpha_a (Q_{R,SB}^0 - Q_{R,SIL}^0) - \alpha_a \Delta \epsilon^0 = \alpha_a (\beta - 1) Q_{R,SIL}^0 - \alpha_a \Delta \epsilon^0 \quad (5)$$

In eqn. 5, β is a proportionality constant relating $Q_{R,SB}^0$ to $Q_{R,SIL}^0$. Fig. 1 shows the plots of the data, and Table VIII shows the results of the regressions. Fig. 2 shows plots of all of the data for 17 organic liquids as solute (all but the polycyclic aromatic hydrocarbons) against the water solubility of the compounds [9], and Table IX shows the related correlations.

TABLE VIII
SULFOBETAINE COLUMN

Solvent	$\Delta \log \alpha$ vs. $Q_{R,SIL}^0$	
	Slope \pm S.D. ($n=8$)	Intercept \pm S.D. ($n=8$)
MC	-0.24 ± 0.33	0.09 ± 0.07
DIE	-0.40 ± 0.046	0.1 ± 0.1
ACN	0.12 ± 0.06	-0.08 ± 0.13

TABLE IX
CORRELATION COEFFICIENTS

Stationary phase	Correlation coefficients, $\log k'$ vs. \log (molal solubility in water)		
	Solvent		
	MC	DIE	ACN
Sulfobetaine	0.604	0.614	0.611
Silica	0.392	0.370	0.739

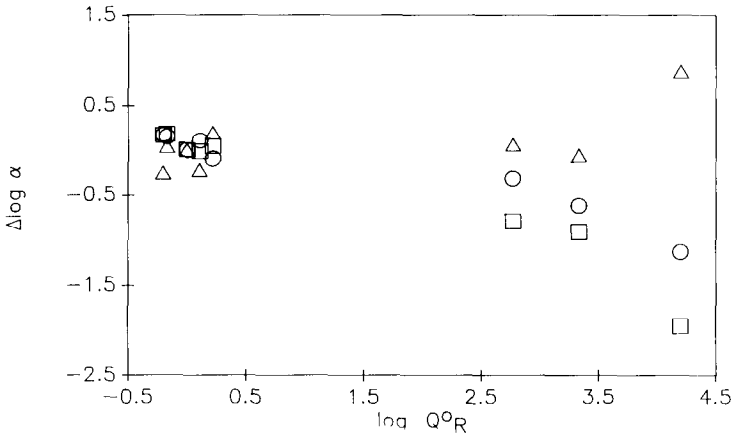


Fig. 1. $\log \alpha_{SB} - \log \alpha_{SIL}$ vs. the log of the functional group adsorption energy for functionalized benzenes. Solvents: \circ = MC; \square = DIE; \triangle = ACN.

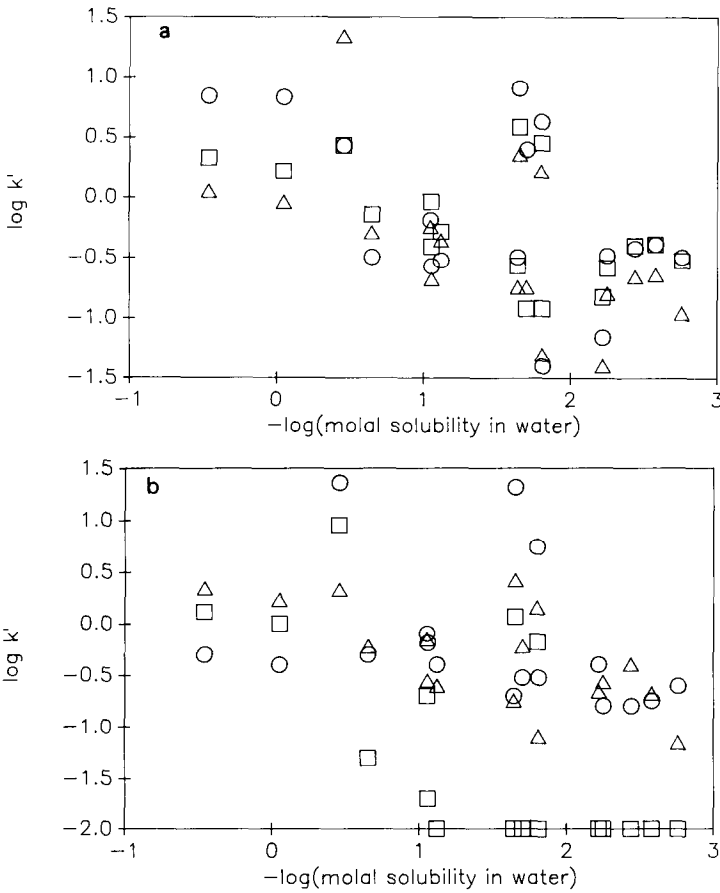


Fig. 2. (a) Sulfobetaine phase: $\log k'$ vs. $-\log(\text{molal water solubility})$ for liquid solutes. Solubilities taken from ref. 9. Solvents: \circ = MC; \square = DIE; \triangle = ACN. (b) Same for silica.

DISCUSSION

The solvents were chosen to represent different classes so that contrasts between the newly prepared zwitterionic sulfobetaine phase and silica could be seen. If only one solvent were used, say methylene chloride, then if the difference in the two phases was primarily their hydrogen bond donating ability, it would not be seen easily. However, by using a hydrogen bond accepting solvent, like diisopropyl ether, these differences could be perceived. Note that acetonitrile is a localizing solvent on silica, and agreement with eqn. 1 is not expected [8] especially for polar solvents.

Tables IV and V show evidence that the Snyder formalism holds for non-polar solutes over a wide range of areas, from benzene (six carbons) to coronene (twenty four carbons). If the differences of the slopes in Table V are taken, the values in Table IV are obtained. This internal agreement means that the value of S/A , the energy of adsorption of an aromatic hydrocarbon of unit area on an adsorbent of unit activity, is constant for the silica and zwitterionic sulfobetaine column, though not necessarily the same constant for each. If the term S/A is constant, then the relative differences among the entries in Table V for a given stationary phase reflect the difference in energy of adsorption of the solvent and an aromatic hydrocarbon of equivalent area. These values are more homogeneous with the zwitterionic phase than with silica. It probably reflects the lesser importance of hydrogen bonding with the zwitterion phase.

Through the use of literature values for the adsorption energy of a functional group R on unit activity silica, $Q_{R,SIL}^{\circ}$, the activity, α_a , can be estimated (see Table VI). For the two less polar solvents the statistics are good, and the values of the activity are in reasonable agreement. For acetonitrile, the fit is poor, and the activity is very low. This again reflects the importance of specific interaction in the case of acetonitrile [5]. Further calculations using acetonitrile data will be made, but it is likely that they are quantitatively inaccurate. In principle, then, one ought to be able to infer the value for S/A as described above, from the values of α_a , $\alpha_a (S/A - \epsilon^0)$ and ϵ^0 . These values are shown in Table VII. The literature [8] value for silica is 0.25. The diisopropyl ether calculation is significantly different from this value. This may be experimental error; the diisopropyl ether solvent was stronger than the other two, and retentions for the monosubstituted benzenes were small, so that an error in the determination of t_0 , the retention time for a solute with a k' of 0, could have a significant influence on the outcome. Recalculation using only the polycyclic aromatic hydrocarbon data in the area regression leads to $S/A \approx 0.37$, a value that is still significantly higher than expected.

The same analysis for the zwitterion phase is precluded because values for $Q_{R,SB}^{\circ}$, the adsorption energy of a functional group at unit activity, are not known. It can be asked, though, whether or not the values of $Q_{R,SIL}^{\circ}$ and $Q_{R,Z}^{\circ}$ are correlated, as shown above (eqns. 4 and 5). The data are shown in Fig. 1 and Table VIII. Good correlations for methylene chloride and diisopropyl ether solvents are seen, but for acetonitrile, there is none. Clearly the interaction energy of the functional groups is considerably lower with the zwitterion column. This may be due to the nature of the stationary phase itself, or may reflect a poor assumption. Recall that a constant α_a was assumed. This may not be true; the adsorbent activity of the zwitterionic sulfobetaine surface may be much lower than that of the silica.

What is, then, the chemical means by which the zwitterion column retains

solutes? No certain answer can come from the data provided, but some tendencies can be inferred. For example, among the series of halomethanes, CH_2Cl_2 , CHCl_3 , CCl_4 , it is always the CH_2Cl_2 that is more retained than the other two on silica, but the CCl_4 that is less retained on the zwitterion. In fact, on the zwitterion the retention order is always $\text{CH}_2\text{Cl}_2 > \text{CHCl}_3 > \text{CCl}_4$. In Table X are some useful parameters of these solutes. Note that the water solubility (which is correlated with \bar{V} , π^* and β_{KT} [9]) is in the same order, and grouping, as the sulfobetaine k' value. The same grouping is also seen for α_{KT} , but this can be ruled out as the dominant factor because benzyl alcohol, which also possesses a high α_{KT} , is not particularly strongly retained. Also, for the methylene chloride solvent especially, the acetate esters are strongly retained, but the α_{KT} of the functional group is negligible. It makes sense, and it is our laboratory experience, that the zwitterionic group has a high affinity for water. Thus perhaps the polar phase is wet, and the chromatographic process on the phase is based on water solubility. The correlation of $\log k'$ vs. $\log 1/S$ plots for both stationary phases are shown in Fig. 2. The correlation coefficients (Table IX) are rather low, but higher for the zwitterion column than the silica column. For the latter, except in the case of acetonitrile solvent which is localizing, there is virtually no correlation between water solubility and retention.

TABLE X
PARAMETERS OF THE HALOMETHANE SOLUTES [9]

Solute	$\bar{V}/100^a$	$\log S_w^b$	π^*	α_{KT}	β_{KT}
CH_2Cl_2	0.624	-0.65	0.82	0.30	0.10
CHCl_3	0.805	-1.12	0.58	0.44	0.10
CCl_4	0.968	-2.22	0.29	0	0.10

^a Molar volume/100.

^b Log molal solubility in water.

The solubility of a liquid in water involves the solute leaving the self-solvation environment [10,11] as well as its entering the solvent-solvation environment. The former portion of the solubility is not relevant to this chromatographic process, but it is a part of the measured solubility. The poor correlations mentioned may reflect this influence of self-solvation. While solubility data from the vapor phase would be ideal, extensive tabulations of these have not been found by the authors. An acceptable approach is to correct the \log (solubility) by some measure of the heat of vaporization, like the boiling point. The boiling point is, for solutes of similar size, correlated with this energy. Gas chromatographers will recall using the fact that the boiling point of a liquid increases 30 K with the addition of a methyl or methylene group to a compound. Thus, a regression for each solvent system and each stationary phase, has been done for $\log k'$ against \log (molal solubility in water) and boiling point. Table XI shows the regression parameters, and Fig. 3 shows the correlation plot of predicted vs. actual $\log k'$ for the sulfobetaine data. The overall correlation coefficient displayed by Fig. 3 is 0.91.

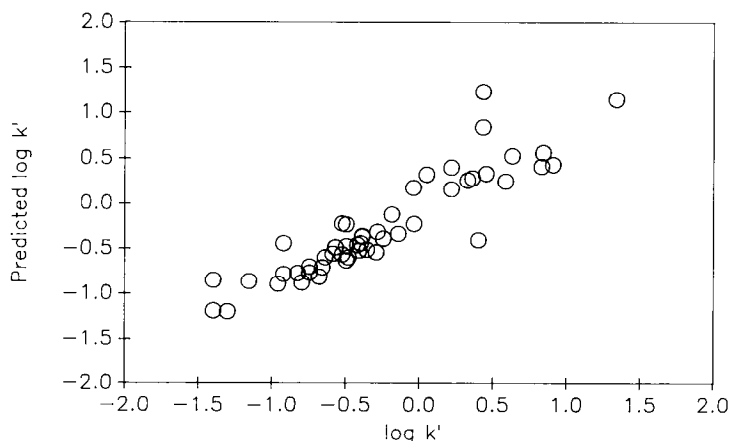
Caution must be used when interpreting correlations. Certainly the same properties of a molecule that cause it to be well solvated by itself contribute to its

TABLE XI

REGRESSION OF $\log k'$ ON \log (MOLAL SOLUBILITY IN WATER) AND $10^{-3} \times$ BOILING POINT (K) \pm S.D.; $n = 17$ for each set of conditions.

	Sulfobetaine			Silica		
	Log (solubility) 10^{-3} b.p.	Intercept		Log (solubility) 10^{-3} b.p.	Intercept	
MC	0.57 ± 0.12	8.2 ± 1.9	-2.4 ± 0.7	0.4 ± 0.12	9.6 ± 1.9	-3.2 ± 0.7
DIE	0.42 ± 0.06	6.7 ± 1.0	-2.2 ± 0.4	0.98 ± 0.10	13.1 ± 1.5	-4.7 ± 0.6
ACN	0.61 ± 0.05	9.6 ± 0.8	-3.1 ± 0.3	0.46 ± 0.07	5.4 ± 1.1	-1.7 ± 0.4

adsorption on silica, and the same can be said for the factors that contribute to water solubility [9]. Thus, inferring cause and effect relationships from correlations is unwise. A profitable route to interpretation of the meaning of correlations is in understanding the degree to which the results make physical sense. This approach can be used in the current instance to interpret the intercept of the multiple regressions shown in Table XI. The intercept is the $\log k'$ value that a compound would have if it had no cohesive energy density (boiling point = 0 K), and its solubility in water was 1 molal. If there is no influence of mobile phase solvation on the retention, as is assumed here in accord with Snyder [8], then a change in k' accompanying a change in solvent must only represent a change in phase ratio. Thus, if the correlation is to be interpreted as implying that partitioning to water is the mechanism of the separation, then the intercepts from the multiple regressions ought to be similar. It can be seen that they are not similar in the case of silica, but are reasonably close to each other in the case of the sulfobetaine.

Fig. 3. Predicted $\log k'$ vs. experimental $\log k'$ from the multiple regression shown in Table XI. Sulfobetaine phase.

CONCLUSION

A new zwitterionic bonded phase has been prepared and compared to silica. It is a weaker adsorbent, and less influenced by the type of strong solvent used in the solvent system. A significant factor governing the retention of solutes is the solute's water solubility from the vapor phase.

ACKNOWLEDGEMENT

It is a pleasure to acknowledge the support of the NIGMS (grant 28112).

REFERENCES

- 1 M. Arunyanart and L. J. Cline Love, *Anal. Chem.*, 57 (1985) 2837.
- 2 W. G. Tramosch and S. G. Weber, *Anal. Chem.*, 56 (1984) 2567.
- 3 W. G. Tramosch and S. G. Weber, *Anal. Chem.*, 58 (1986) 3006.
- 4 J. P. Berry and S. G. Weber, *J. Chromatogr. Sci.*, 215 (1987) 307.
- 5 L. R. Snyder and J. J. Kirkland, *Introduction to Modern Liquid Chromatography*, Wiley-Interscience, New York, 2nd ed., 1979, Ch. 6, 9.
- 6 J. H. Park and P. W. Carr, *J. Chromatogr.*, 465 (1989) 123.
- 7 S. C. Rutan, P. W. Carr, W. J. Chcong, J. H. Park and L. R. Snyder, *J. Chromatogr.*, 463 (1989) 21.
- 8 L. R. Snyder, *Principles of Adsorption Chromatography*, Marcel Dekker, New York, 1968.
- 9 M. J. Kamlet, R. M. Doherty, M. H. Abraham, P. W. Carr, R. F. Doherty and R. W. Taft, *J. Phys. Chem.*, 91 (1987) 1996.
- 10 J. H. Hildebrand and R. L. Scott, *Regular Solutions*, Prentice-Hall, Englewood Cliffs, NJ, 1962.
- 11 B. L. Karger, L. R. Snyder, and Cs. Horváth, *An Introduction to Separation Science*, Wiley-Interscience, New York, 1973, Ch. 2.