SOLVENT COMPOSITION EFFECTS IN THE NORMAL-PHASE LIQUID CHROMATOGRAPHY OF NITROGEN HETEROCYCLES AND HYDROXYL AROMATICS ON A NITROPHENYL STATIONARY PHASE

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SUMMARY

  The normal-phase liquid chromatographic model of Snyder was considered for a high-performance Nucleosil NO_2 stationary phase. Various chloroform–*n*-heptane mobile phases were used to evaluate the ability of the Snyder model to approximately describe retention on the Nucleosil NO_2 column. It was found that log capacity factor (k') *vs.* solvent strength plots were linear for nitrogen heterocycle and hydroxyl aromatic solutes for a wide range of mobile phase compositions. It was concluded that retention behavior could be approximated for nitrogen and hydroxyl substituted aromatics by using plots of log k' *vs.* solvent strength for chloroform–*n*-heptane mobile phases ranging from 40% to 100% chloroform.

INTRODUCTION

  The composition of the mobile phase in normal-phase liquid chromatography (LC) is an easily adjustable parameter which plays a major role in the retention and selectivity in LC systems[1–3]. Several authors have presented models considering the mechanistic roles of the solvent, solute, and stationary phase in normal-phase systems[4–15]. If a model is applicable to a particular chromatographic system, it can then be used to predict the retention of solutes at various mobile phase compositions. This kind of information is useful in determining the mobile phase composition which will yield the best selectivity for a given separation.
  Scott and co-workers[10–15] have studied solute interactions in mobile and stationary phases with emphasis on silica gel. Snyder and co-workers[1–3] have developed an adsorption model based upon the displacement of solvent molecules by solute molecules from stationary phase sites. This model has been successfully used to predict the retention of solutes in a number of normal-phase systems. Soczewiński[4–6] has developed a similar model for adsorption chromatography. Snyder[16] and Snyder and Poppe[8] have discussed in considerable depth various aspects of the Snyder, Soczewiński, and Scott models. Slaats *et al.*[17] investigated the influence of solute–solvent competition on retention in liquid–solid chromatography by measurement of activity coefficients in mobile phases. Jaroniec and co-workers[18–22] have discussed the

theory of adsorption chromatography and derived equations for the capacity factor, k', based upon various solute, solvent and stationary phase interactions. Snyder and Schunk[23] were able to describe the behavior of solutes on an amino-bonded phase using a displacement model for mobile phases without alcohols. Snyder's displacement model was also successfully applied to an amino-bonded phase by Hammers et al.[24] who concluded that the stationary phase was similar to silica which was partially deactivated. Solutes displayed the same general elution order but exhibited diminished k' values compared to silica. Weiser et al.[25] successfully applied the Snyder model to a cyanopropyl bonded phase with weak mobile phases and also concluded that the column functioned as a deactivated silica. Hara et al.[26] was able to apply the Snyder model to the chromatography of steroid derivatives on silica. The slopes and intercepts from linear relationships between the logarithms of capacity factors and the solvent concentrations were calculated and discussed in terms of solute and solvent characteristics.

Lochmüller et al.[27,28] investigated various bonded nitrofluorenimino stationary phases for their ability to separate polycyclic aromatic hydrocarbons (PAHs). Hemetsberger et al.[29] studied donor–acceptor complex chromatography of a tetranitrofluorenone stationary phase for several solutes and mobile phases. Mikeš et al.[30] investigated various alkyl substituted nitrofluorenimino packings for their ability to resolve large racemic carbohelicenes. Nondek and co-workers[31,32] used a dinitroanilino stationary phase to separate PAHs. Nondek and Chvalovský[33] were able to use this stationary phase to separaste azaarenes from PAHs and pyrrolic nitrogen compounds in gasoline, kerosene, and diesel fuel. Hammers et al.[34] used the simple Snyder adsorption model to describe the retention of monosubstituted benzenes on dinitroanilino, bis(3-nitrophenyl)sulphone, and trinitrofluorenimine bonded silicas.

Hurtubise et al.[35] and Hussain et al.[36] considered the models of Snyder[7], Soczewiński[4], and Scott and Kucera[10], using various high-performance columns. Hurtubise et al.[35] concluded that the Snyder and Soczewiński models approximately described the behavior of alkylphenols on μBondapak NH$_2$. Hussain et al.[36] concluded that the simple Snyder[7] and Soczewiński[4] models also approximated the retention of alkylphenols on high-performance cyano and silica gel stationary phases over certain organic modifier concentrations.

Although a variety of solutes have been used to investigate the different adsorption models, most are of relatively low molecular weight compared to species expected to be present in complex organic mixtures such as coal related liquids, tar sands or crude oils. Also, these adsorption models have been applied to relatively few bonded phases.

In this work, the normal-phase chromatographic adsorption model developed by Snyder[7] was investigated for its ability to approximate solute retention on a nitrophenyl-bonded phase for polar nitrogen heterocycle and hydroxyl aromatic standards of relatively high molecular weight over various mobile phase concentration ranges. Although charge transfer stationary phases have been investigated by others[27-34], these bonded phases were not of the simple mononitrophenyl type. This particular stationary phase has received little attention in the literature and apparently no reports have appeared investigating this packing with any chromatographic adsorption models. An approach similar to the one discussed by Hammers et al.[34] was used in this work in applying the Snyder model.

TABLE I

COMPOUNDS INVESTIGATED ON NUCLEOSIL NO₂

Compound	Structure
Nitrogen heterocycles	
1,2-Bis(2-pyridyl)ethylene	
7-Azaindole	
Amino substituted compound	
3-Aminofluoranthene	
Monohydroxyl aromatic compounds	
4-Phenylphenol	
3-Hydroxybenzo[c]phenanthrene	
1-Naphthol	
1-(Hydroxymethyl)-benzo[a]pyrene	
2-Hydroxypyrene	
13-Hydroxypicene	

EXPERIMENTAL

For the solutes investigated, experimental equipment, conditions, procedures and k' values for chloroform–*n*-heptane mobile phases 40:60, 50:50, 60:40 and 70:30 have been previously reported[37]. In this work, experimental conditions were identical as reported earlier for calculating capacity factors for the chloroform–*n*-heptane 80:20, 90:10 and 100:0 mobile phases. Table I gives the names and structures of the compounds investigated.

Programmable calculator

Linear regression calculations were carried out using as TI-59 programmable calculator.

RESULTS AND DISCUSSION

Theoretical considerations

The following equation is used in the Snyder model.

$$X_m + nS_a \rightleftharpoons X_a + nS_m \tag{1}$$

The subscripts m and a refer to molecules in the mobile and adsorbed phases, respectively. The adsorption of a solute molecule X results in the displacement of n molecules of adsorbed solvent. From the simple model of Snyder[1,7,34] it can be shown that

$$\log (V_N/W) = \log V_A + \alpha(S^0 - \varepsilon^0 A_s) \tag{2}$$

where V_N (cm^3) is the net retention volume, W (g) is the weight of adsorbent in the column, α is the absorbent activity, S^0 is the adsorption energy of the solute, ε^0 is the mean eluent strength, and A_s is the effective surface area of the adsorbed solute molecule. V_A is related to the specific surface area of the asdsorbent. S^0 can be calculated for unsubstituted aromatic hydrocarbons on silica according to Snyder[38] from

$$S^0 = \sum^i Q_i^0 - F(Q_k^0)(n - 6)Q_i^0 \tag{3}$$

where Q_i^0 is the relative adsorption energy of group i, $F(Q_k^0)$ is the solute localization function (equals 0.43 for narrow pore silicas), and n is the number of aromatic carbon atoms in the molecule. If one plots $\log (V_N/W)$ *vs.* S^0 for PAH solutes using hexane or heptane as the eluent, α and $\log V_A$ are easily determined from the slope and intercept, respectively. If a linear relationship is observed between $\log (V_N/W)$ and S^0, it can be stated that the adsorption energies (S^0) on the bonded phase are proportional to those on bare silica[34]. Once α is known, the solvent strength of binary eluents on the bonded phase can be calculated according to Snyder[23,39] from

$$\varepsilon_{AB} = \varepsilon_A + \frac{\log [N_B 10^{\alpha n_b(\varepsilon_A - \varepsilon_B)} + 1 - N_B]}{\alpha n_b} \tag{4}$$

where ε_{AB} is the solvent strength of the binary eluent, ε_A is the solvent strength of the weak solvent, ε_B is the solvent strength of the polar organic modifier, N_B is the mole fraction of solvent B in the eluent, and n_b is the relative area of a molecule of solvent B. Snyder and Schunk[23] have stated that ε_B can vary with N_B due to "restricted-access delocalization" of strong solvent molecules on the silica. However, due to the flexible nature of the nitrophenyl group which is attached to the silica by a propyl group, restricted access delocalization should be minimized. Snyder and Schunk[23] stated that the flexible nature of bonded NH$_2$ stationary phases works against the occurrence of restricted access delocalization and therefore ε_B in eqn. 3 should be

constant. Because the nitrophenyl group is also flexible, we also assume the constancy for ε_B with different N_B values.

The variation in retention for a solute chromatographed with two different mobile phases is given by the simple Snyder[16] model as

$$\log(k_2/k_1) = \alpha A_s(\varepsilon_1 - \varepsilon_2) \tag{5}$$

where the capacity factor k_1 corresponds to a solute eluted in mobile phase 1 of solvent strength ε_1 and k_2 corresponds to the solute eluted in mobile phase 2 with solvent strength ε_2. For a n-heptane mobile phase, $\varepsilon_1 = 0$. If one then plots log k' *vs.* ε_{AB} for chloroform–n-heptane eluents, the slope can yield experimental A_s values simply by dividing the slope (αA_s) by α. If linear log k' *vs.* ε_{AB} plots can be obtained, the displacement model of Snyder should be able to generally describe retention on the bonded phase. Experimental A_s values are expected to be larger than calculated A_s values for polar solutes because, firstly polar solutes can localize onto adsorbent sites and secondly site-competition delocalization of the solute is expected for nitrophenyl columns due to the exposed nature of the nitrophenyl group[23]. This argument is analogous to Snyder and Schunk's[23] discussion of NH_2 columns.

The calculated A_s values for the solutes investigated, assuming a bare silica surface, were determined according to Snyder[40] using the following formula for substituted aromatic hydrocarbons:

$$A_s = 6 + 0.80(h - 6) + 0.25(c - h) \tag{6}$$

where h is the number of aromatic hydrogens in the solute molecule and c is the number of aromatic carbons. For nitrogen heterocycles, when $=N-$ replaces $=CH-$, A_s increases by *ca.* 8 units for SiO_2 (ref. 40). For hydroxyl aromatics, each $-OH$ increases A_s by 7.6 units. For other functionality, $-NH$ increases A_s by 8.4 units, and aromatic NH_2 increases A_s by 8.7 units.

Dependence of retention on mobile phase composition

Table II lists capacity factor values for the six PAHs chromatographed with a 100% n-heptane mobile phase at 2 ml/min on the Nucleosil NO_2 column. Also included in Table II are the calculated adsorption energies (S^0) for the PAH compounds assuming a bare silica stationary phase. The plot of log k' *vs.* S^0 for the

TABLE II

CAPACITY FACTORS AND CALCULATED ADSORPTION ENERGIES (S^0) OF PAHs ELUTED FROM A NUCLEOSIL NO_2 COLUMN WITH 100% n-HEPTANE MOBILE PHASE

Flow-rate: 2 ml/min. Capacity factor values taken from ref. 47.

Compound	k'	S^0
Acenaphthene	0.80	2.21
3,4-Benzofluoranthene	7.66	3.49
Perylene	9.60	3.49
Coronene	12.3	4.06
1,2,3,4-Dibenzanthracene	13.1	3.78
1,2-Benzperylene	15.3	4.06

TABLE III

SOLVENT STRENGTH VALUES OBTAINED FROM EQN. 4 FOR CHLOROFORM-n-HEPTANE MOBILE PHASES

Mobile phase (chloroform–n–heptane)	Solvent strength, ε_{AB}
40:60	0.438
50:50	0.459
60:40	0.476
70:30	0.487
80:20	0.497
90:10	0.506
100:0	0.514

solutes in Table II yielded a correlation coefficient of 0.977 and a slope of 0.68. The approximate linear relationship observed between log k' and S^0 indicated that the adsorption energies of the PAHs on the nitrophenyl column were proportional to S^0 values for these solutes on silica. The adsorbent activity (slope value) of 0.68 is less than the value of 1.12 reported by Snyder[41] for activated small pore silicas.

The polar nitrogen heterocycle and hydroxyl aromatic solutes which were investigated appear in Table I along with their respective structures. Solvent strength values for chloroform–n-heptane mobile phases 40:60, 50:50, 60:40, 70:30, 80:20, 90:10, and 100:0 were calculated using eqn. 4. Table III lists the solvent strength values obtained for the various chloroform–n-heptane mobile phases. For these calculations a n_B value of 5 was chosen for chloroform[42]. The capacity factors for the

TABLE IV

CAPACITY FACTORS FOR SOLUTES WITH CHLOROFORM-n-HEPTANE MOBILE PHASES

The capacity factor values for 40:60, 50:50, 60:40, and 70:30 have been reported previously[37].

Compound	Chloroform–n–heptane						
	40:60	50:50	60:40	70:30	80:20	90:10	100:0
Nitrogen heterocycles							
3-Aminofluoranthene	4.00	2.77	2.16	1.40	0.80	0.70	0.53
2,2-Bis(2-pyridyl)-ethylene	7.64	5.61	4.53	3.49	2.82	2.40	1:82
7-Azaindole	19.1	13.0	9.20	7.53	5.53	4.86	4.13
Hydroxyl aromatics							
1-Naphthol	15.2	9.84	7.83	6.30	4.93	4.06	3.44
1-(Hydroxymethyl)-benzo[a]pyrene	16.0	8.80	6.16	4.13	2.83	2.07	1.57
4-Phenylphenol	18.3	12.0	9.34	6.91	4.93	4.06	3.43
2-Hydroxypyrene	40.7	36.4	23.2	18.9	14.1	11.7	8.77
13-Hydroxypicene	48.3	28.7	22.9	17.0	10.7	7.73	6.07
3-Hydroxybenzo[c]-phenanthrene	51.5	31.5	22.5	16.0	11.2	8.43	6.87

TABLE V

CHROMATOGRAPHIC PARAMETERS OBTAINED FROM THE SNYDER MODEL

Compound	$A_s(calc)$	$A_s(exp)$	ΔA_s	Slope	Correlation coefficient
Basic nitrogen heterocycles					
7-Azaindole	25.3	13.0	12.3	8.83	0.998
2,2-Bis(2-pyridyl)ethylene	30.0	11.7	18.3	7.98	0.989
Amino substituted					
3-Aminofluoranthene	19.4	17.7	1.7	12.01	0.978
Hydroxyl aromatics					
1-Naphthol	15.7	12.2	3.5	8.32	0.997
2-Hydroxypyrene	18.3	13.3	5.0	9.04	0.980
4-Phenylphenol	15.7	14.4	1.3	9.77	0.994
3-Hydroxybenzo[c]phenanthrene	20.4	17.1	3.3	11.62	0.996
13-Hydroxypicene	22.0	17.3	4.7	11.81	0.984
1-(Hydroxymethyl)-benzo[a]pyrene	23.2	19.3	3.9	13.14	0.995

nitrogen heterocycles and hydroxyl aromatics eluted from the Nucleosil NO_2 column with each of the chloroform–*n*-heptane mobile phases appears in Table IV.

Plots of log k' *vs.* ε_{AB} for the solutes in Table I yielded linear correlation coefficients ranging from 0.978 to 0.998. The correlation coefficient, slope, experimental A_s, calculated A_s, and ΔA_s (calculated A_s − experimental A_s) values appear in Table V. The high correlation coefficient values for the solutes were reflected in the linearity of the log k' *vs.* ε_{AB} plots. Although 3-aminofluoranthene yielded a relatively low correlation coefficient of 0.978, the other solutes generally yielded much
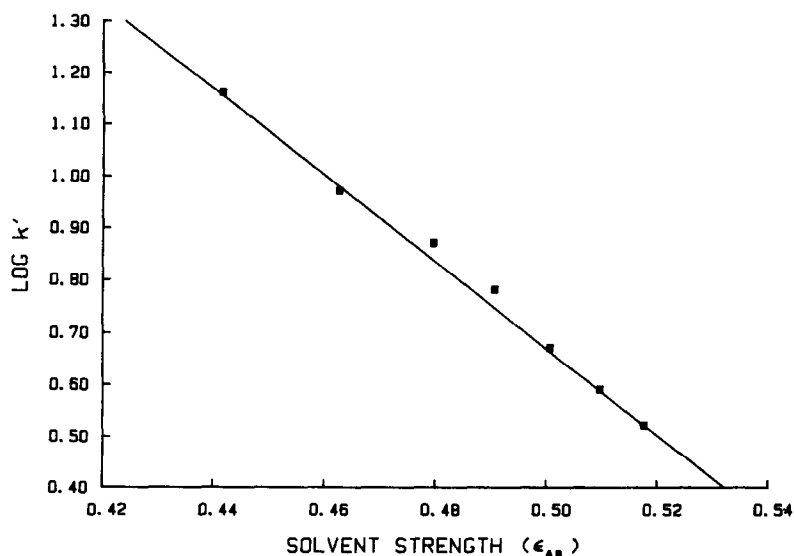

Fig. 1. Plot of log k' *vs.* solvent strength (ε_{AB}) for 1-naphthol.

higher values with the average correlation coefficient value of 0.990. Slope values in Table V are absolute values of slope because these values are all negative for log k' vs. ε_{AB} plots. An illustration of the linearity of the log k' vs. ε_{AB} plots is given in Fig. 1 for 1-naphthol.

The slopes of the log k' vs. ε_{AB} plots can be divided by α, according to eqn. 5, to yield experimental A_s values (see Table V). The difference in the calculated and experimental A_s values is given as ΔA_s. Due to the polar nature of the solutes in Table I, localization of these solutes on stationary phase sites is expected, especially when one considers that the Nucleosil NO_2 packing contains uncapped silanol groups[43]. If localization occurs, experimental A_s values should be larger than the calculated A_s values[3,44]. It can be seen from Table V that the experimental A_s values are smaller than the calculated A_s values for the Nucleosil NO_2–chloroform–n-heptane system. This could be due to the charge transfer nature of the nitrophenyl group[45,46], which may decrease solute–stationary phase localization effects in favor of a weaker electron donor–acceptor interaction. Further work is needed to elucidate the reason for the small experimental A_s values obtained for these polar solutes in the rather complicated chromatographic system which was investigated.

CONCLUSIONS

The experimental data presented in this report indicate that the simple model of Snyder approximately describes the retention behavior of large molecular weight, polar nitrogen heterocycles and hydroxyl aromatics over a wide range of chloroform–n-heptane mobile phase compositions.

Due to the different types of solutes, the presence of uncapped silanol groups, and the character of the nitrophenyl group, the actual chromatographic process is undoubtedly complex. Considering the scope of this study, statements concerning the specific mechanism of interaction cannot be made. However, in practical applications the linear relationships between log k' and solvent strength over relatively wide mobile phase composition ranges will be useful in predicting optimum mobile phases for the separation of nitrogen heterocycle and hydroxyl aromatic mixtures.

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