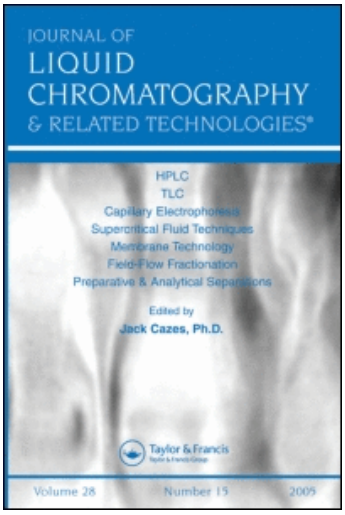

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

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To cite this Article Akapo, S. O. , Scott, R. P. W. and Simpson, C. F.(1991) 'The Production of Stable, Uniform Reverse Phases for Liquid Chromatography by Oligomeric Synthesis', Journal of Liquid Chromatography & Related Technologies, 14: 2, 217 — 236

To link to this Article: DOI: 10.1080/01483919108049610

URL: http://dx.doi.org/10.1080/01483919108049610

JOURNAL OF LIQUID CHROMATOGRAPHY, 14(2), 217-236 (1991)

THE PRODUCTION OF STABLE, UNIFORM REVERSE PHASES FOR LIQUID CHROMATOGRAPHY BY OLIGOMERIC SYNTHESIS

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Abstract

The use of oligomeric synthesis for the preparation of reverse phases by a fluidized bed technique is described. Reverse phases comprising of one to ten oligomers were synthesized and the physical and chromatographic properties of each phase examined and the results reported. The nature of the interactions were investigated by measuring the excess free enthalpies of absorption of a homologous series of aromatic hydrocarbons and aromatic esters from retention measurements taken at different temperatures. It is shown that a reverse phase comprising at least five oligomers behaves as a true dispersive phase, has consistent and predictable retentive properties and is far more stable to acid conditions than the normal type of reverse phase.

Introduction

The production of oligomeric stationary phase has been recently described by Akapo *et al* (1,2) and, in fact, the products synthesized in the original work described by the authors were employed in the work described in this paper. Briefly, oligomeric synthesis is carried out by employing a dichlorosilane as the silanizing reagent as opposed to a monochlorosilane or a trichlorosilane which are normally used in the production of reverse phases. As a consequence, a *monochloro* silane is bonded to the hydroxyl groups of the silica with the usual evolution of hydrochloric acid. Subsequent treatment with water hydrolyses the monochlorosilane to *monohydroxy* silane with more evolution of hydrochloric acid. The *monohydroxy* silane can then be further reacted with another molecule of the dichlorosilane, again with the evolution of hydrochloric acid, to link another monochlorosilane to the previous silane residue. Thus, by alternately treating the bonded phase with water and dichlorosilane a series of silane residues can be linked together building up the oligomeric reverse phase. The procedure is illustrated in the diagram shown in Figure 1. After the final oligomeric synthesis the last silanol groups are capped with trimethyl chlorosilane or hexamethyltrisilazane.

The synthesis was found to be carried out most efficiently by employing the fluidized bed technique that has been described by Khong and Simpson (2,4) and Khong (5). In this paper the products from the oligomeric synthesis are examined in order to determine the chemical nature of the oligomeric phases and the nature of the retentive surface. Retention measurements for a series of aromatic hydrocarbons and aromatic esters are made at different temperatures to determine the enthalpies of absorption and thus, identify the nature of interactions involved between the solutes and the stationary phase.

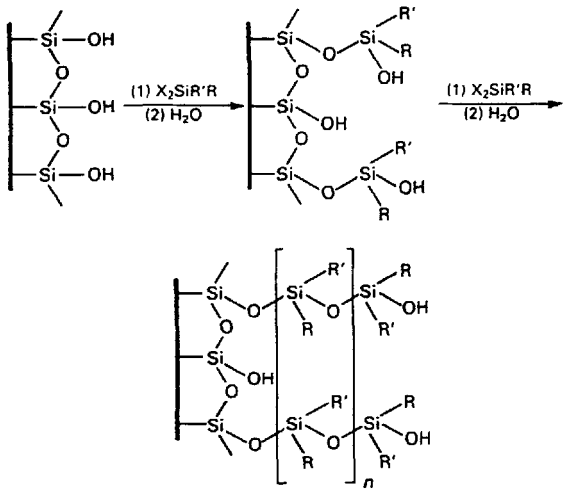

Figure 1

Diagramatic Representation of Oligomeric Synthesis

Experimental

Details of the original synthesis were given by Akapo *et al* (1) but for clarity will be briefly reiterated here. The materials employed were spherical silica (Research sample from Professor Unger's laboratory, Johannes Gutenberg Universitat,Mainz, F. R. G.) particle diameter 20μm, surface area 250m^2/g. Octylmethyldichlorosilane was prepared by the hydrosilation (6) of distilled octene (Aldrich) with methyl dichlorosilane (Fluka). The reaction was catalyzed by chloroplatinic acid (Johnson Mathey Chemicals, London U.K.). The chemical composition of the octylmethyl-dichlorosilane was confirmed by elemental analysis.

The fluidized bed method used was that already described (3,4 and 5). About 50 g of silica was fluidized in a tower using nitrogen as the

fluidizing gas and hydrothermally heated at 200°C for 6 hours to remove all physically adsorbed water. The treated silica was then silanized with octylmethyl-dichlorosilane at 200°C for 4 hours, continuing to employ dry nitrogen as the fluidizing gas. The bonded material was again hydrolyzed by steam and this procedure repeated nine times. Sufficient material was removed from the bulk after each step for chemical analysis and to provide a well packed column. The % w/w carbon obtained by micro analysis for each oligomeric phase is given in table 1. The values were confirmed by oxidizing differential scanning calorimetry.

Each bonded phase was slurry packed into a 250 X 4.6 mm I. D. stainless-steel columns, fitted with appropriate frits, using a Haskel pump, Model DSTV-122 at 8000 p.s.i.. The slurry mixture consisted of isopropyl alcohol-cyclohexanol-chloroform mixture (2:1:1v/v) and methanol was used as the displacement solvent.

The apparatus used for the chromatographic examination of the columns was a Shimadzu LC-5A pump and an SPD 2AM variable-wavelength UV

Table 1
Carbon Content of Bonded Phases

Reaction Step Number	% w/w Carbon
0	0
1	7.58
2	9.69
3	11.14
4	12.18
5	12.69
6	14.43
7	14.90
8	15.80
9	16.79
10	17.45

detector operated at 254n. The solutes employed were a series of aromatic hydrocarbons, benzene, naphthalene, biphenyl, anthracene and pyrene and a series of aromatic esters, methyl, ethyl, propyl, butyl and pentyl benzoates. Samples were injected onto the column as a 1% v/v solutions of each solute in the mobile phase by means of a Rheodyne sample valve (Model 7143) fitted with a 5µl loop. The column temperature was controlled by enclosing each column in a glass jacket through which water was circulated from a thermostated water bath (Grant Instruments Ltd, Cambridge, U. K.). Prior to entrance to the Rheodyne valve, the mobile phase (70%v/v methanol-30% v/v water) was preheated in a 1m coil, also situated in the thermostat. After any temperature change, columns were allowed to come into thermal equilibrium for one hour before any measurements were taken. Measurements at different temperatures were taken over the range of 30°C to 50°C (+/- 1°C) and all retention data was taken as the average of three replicates. The dead volume of a column was taken as the product of the

Table 2

Retention Volumes of Pyrene and Pentyl Acetate Taken on each Column at 30°C

% w/w Carbon	Corrected Retention Volume (ml)	
	Pyrene	Pentyl Acetate
7.58	12.76	8.82
9.69	15.07	10.38
11.14	15.70	11.03
12.18	13.81	9.57
12.69	13.89	9.92
14.43	14.38	8.65
14.90	13.99	9.79
15.80	13.14	9.91
16.79	15.14	10.61
17.45	13.30	9.58

average retention time of potassium nitrate and the mobile phase flow-rate. The flow-rate was measured by taking the time for a defined volume of mobile phase to pass through the column and was continuously checked throughout all retention measurements. Retention data for pyrene and pentyl benzoate on all columns taken at 30°C are shown in table 2.

Retention data taken for each solute at different temperatures on columns (1) and (10) are given in tables 3 and 4.

Finally, columns (1), (3), (5) and (10) were treated continuously with a 0.1%v/v of TFA (trifluoroacetic acid) in 70% v/v-30% v/v water mixture and the retention volume of aniline and benzene measured after different volumes of the mobile phase mixture had passed through the column. The results obtained are shown in table 5.

Discussion of Results

The sequential linking of methyloctylsilanyl groups to the silica results in a progressive increase in carbon content of the stationary phase as given in Table 1. In Figure 2 the percentage carbon on the silica is plotted against the synthesis step number.

It is seen from Figure 2 that there is an initial rapid increase in carbon content as the layers of hydrocarbon chains are laid down on the surface of the silica gel. During the first 5 steps, previously unreacted silanol groups on the surface are silanized as well as the previously deposited oligomer and thus, the increase in carbon content with synthesis step is not linear. After the fifth step, however, either all the surface silanols are reacted or they are now buried beneath the methyloctyl silyl layers and are no longer available. Consequently, the carbon content increases linearly during the last five steps at about 0.79%w/w per synthesis step. It is interesting to note that, if it is assumed that the column is packed with about 3g of

Table 3

Retention Data Taken for Two Homologous Series of Solutes at Four Different Temperatures on Column 1.

	Retention Volume (ml)			
Solute	303°K	313°K	323°K	333°K
Methyl Benzoate	2.12	1.92	1.70	1.59
Ethyl Benzoate	2.98	2.66	2.35	2.14
Propyl Benzoate	4.21	3.71	3.14	2.91
Butyl Benzoate	6.13	5.29	4.55	4.03
Pentyl Benzoate	8.82	7.50	6.38	5.53
Benzene	2.07	1.89	1.79	1.63
Naphthalene	4.13	3.63	3.27	2.88
Biphenyl	6.05	5.21	4.58	4.00
Anthracene	8.85	7.46	6.36	5.45
Pyrene	12.76	10.48	8.78	7.35

Table 4

Retention Data Taken for Two Homologous Series of Solutes at Four Different Temperatures on Column 10.

	Retention Volume (ml)			
Solute	303°K	313°K	323°K	333°K
Methyl Benzoate	1.87	1.76	1.55	1.34
Ethyl Benzoate	2.74	2.49	2.18	1.92
Propyl Benzoate	4.14	3.65	3.13	2.75
Butyl Benzoate	6.28	5.41	4.64	3.97
Pentyl Benzoate	9.58	8.07	6.68	5.69
Benzene	2.50	2.23	2.02	1.81
Naphthalene	4.67	4.05	3.53	3.06
Biphenyl	7.11	6.00	5.12	4.40
Anthracene	9.74	7.99	6.60	5.54
Pyrene	13.30	10.66	8.58	7.10

Table 5

Stability Data on Columns 1, 3, 5, and 10

Column	k' aniline	k' benzene	Volume Passed Through Column (ml)
1	0.35	0.68	0
	0.37	0.64	500
	0.41	0.59	1000
	0.62	0.57	1500
	1.10	0.56	2000
3	0.35	0.80	0
	0.36	0.78	500
	0.37	0.77	1000
	0.39	0.76	1500
	0.43	0.75	2000
5	0.27	0.83	0
	0.28	0.81	500
	0.28(5)	0.79	1000
	0.29	0.78	1500
	0.32	0.76	2000
10	0.26	0.84	0
	0.26	0.83	500
	0.26(5)	0.82 (5)	1000
	0.27	0.81	1500
	0.29	0.79	2000

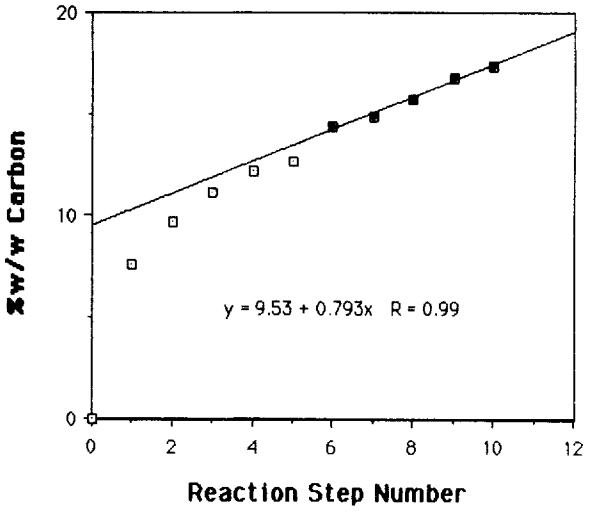

Figure 2

Graph of %w/w Carbon on Bonded Phase against Synthesis Step

material, then this represents about 23.7 mg of bonded phase per step. Assuming a mean molecular weight for each oligomer of 156 this would be equivalent to the addition of 9.14×10^{19} chains per synthesis step. In Figure 3 the retention volume of pyrene and pentyl benzoate is plotted against carbon content of the bonded phases and the results are a little surprising.

It is seen that within experimental error the corrected retention times of the solutes are independent of the carbon load on the stationary phase. The scatter is to be expected, as the retention volume will vary as the quantity of packing in the column, which, of course, will differ from column to column. Nevertheless, the slopes of the curves are very close to zero indicating that as each oligomer is laid down no new interactive surface is produced and the oligomeric phase is, in fact, a multilayer system. Thus the

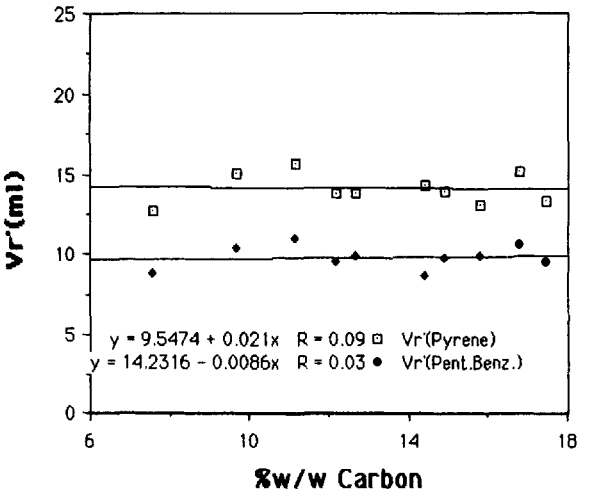

Figure 3

Graph of Vr' of Pyrene and Pentyl Benzoate against %w/w Carbon

chromatographically interactive surface is moved further and further away from the silica gel surface with each synthetic step which, as will be shown later, accounts for the stability of the phase and its consistent retentive properties.

It is of interest to examine the thermodynamic properties of the oligomeric phase. The corrected retention volume (V_r') of a solute is given by,

$$V_r' = KV_S$$

where K is the distribution coefficient of the solute between the stationary and mobile phase,
and V_S is the volume of mobile phase in the column.

Consequently, $K = V_r'/V_S$ ---------- (1)

The Gibbs free energy change, ΔG^0, associated with the reversible interaction of the solute with the stationary phase is related to the distribution coefficient, (K), by

$$\Delta G^0 = -RT\ln K \quad \text{---------- (2)}$$

Where (R) is the gas constant and (T) is the absolute temperature.

Substituting for (K) in equation (2) from equation (1)

$$\Delta G^0 = -RT\ln(V_r'/V_S)$$

$$= -RT(\ln V_r' - \ln V_S) \quad \text{---------- (3)}$$

Employing the Gibbs-Helmholtz relationship, $\Delta G^0 = \Delta H^0 + T\Delta S^0$ in equation (3), (where ΔH^0 and ΔS^0 are the excess free enthalpy and entropy of solute transfer between the two phases respectively) the corrected retention volume of a solute can be expressed in the form,

$$\ln V_r' = -\Delta H^0/RT + \Delta S^0/R + \ln V_S \quad \text{---------- (4)}$$

Employing equation (4) in conjunction with the data given in tables (3) and (4) the excess free enthalpy for each solute on both columns 1 and 10 can be calculated from the slope of the curves relating ($\ln V_r'$) with (1/T). Unfortunately due to the uncertainty of the magnitude of (V_S) for the two columns a reliable value for the excess free entropy of each solute can not be determined in the same way from the intercept of the ($\ln V_r'$) / (1/T) curves.

An example of a set of curves relating ($\ln V_r'$) with (1/T) for the aromatic ester series chromatographed on column 10 is given in Figure 4. It is seen that excellent linear curves are obtained giving precise values for the

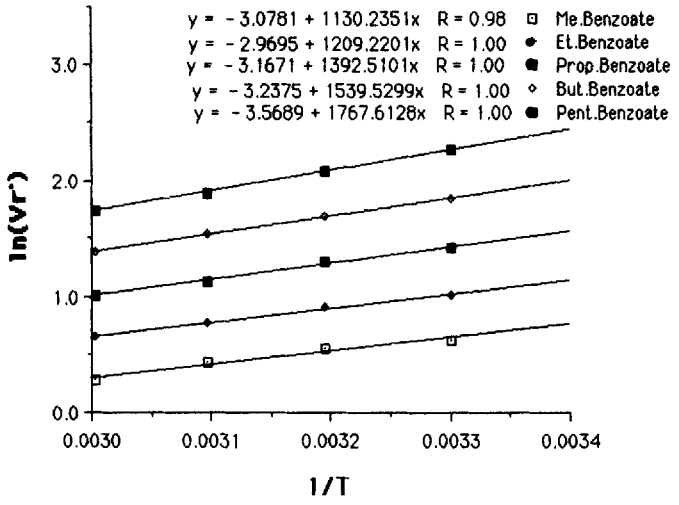

Figure 4

Graph of Ln(Vr') against 1/T for the Aromatic Esters on Column 10

A summary of the values for the slopes and intercepts together with the excess free enthalpy values calculated from the product of the slopes and the gas constant (R) are given in table 6.

slopes and intercepts. Similar curves were constructed for both solute series on both columns and the slopes and intercepts obtained using a similar curve fitting procedure. Curves relating (lnV_r') and (1/T) for the two solute series were also obtained for the intermediate columns and in all cases excellent linear correlation was obtained. However, as the values for the slopes and intercepts fell regularly between those given for column 1 and 10, the data did not contribute any further significant information and thus,for the sake of brevity, are not reported here.

Table 6

Excess Free Enthalpy Values for the Different Solutes Chromatographed on Columns 1 and 10

Column 1	Solute	Intercept	Slope	Excess Free Enthalpy (cal/mol.)
	Methyl Benzoate	-2.52	992	8280
	Ethyl Benzoate	-2.63	1128	9380
	Propyl Benzoate	-2.70	1254	10430
	Butyl Benzoate	-2.88	1422	11820
	Pentyl Benzoate	-3.03	1577	13110
	Benzene	-1.84	778	6470
	Naphthalene	-2.52	1194	9930
	Biphenyl	-2.77	1383	11500
	Anthracene	-3.19	1626	13520
	Pyrene	-3.55	1847	15360

Column 10	Solute	Intercept	Slope	Excess Free Enthalpy (cal/mol.)
	Methyl Benzoate	-3.08	1130	9400
	Ethyl Benzoate	-2.97	1209	10050
	Propyl Benzoate	-3.17	1392	11570
	Butyl Benzoate	-3.24	1539	12800
	Pentyl Benzoate	-3.57	1768	14700
	Benzene	-2.64	1077	8950
	Naphthalene	-3.14	1419	11800
	Biphenyl	-3.36	1614	13420
	Anthracene	-4.00	1900	15800
	Pyrene	-4.41	2121	17630

A graph relating the excess free enthalpy of each solute chromatographed on column 1 against the excess free enthalpy for each solute when chromatographed on column 10 is shown in Figure 5.

Employing the data given in table 6 it is of interest to determine whether the excess free enthalpy of absorption changes as the oligomeric phase is built up. As each oligomer is basically a hydrocarbon chain the interaction between the solutes and the stationary phase should remain essentially the same irrespective of the amount of oligomer present on the silica. Thus, a

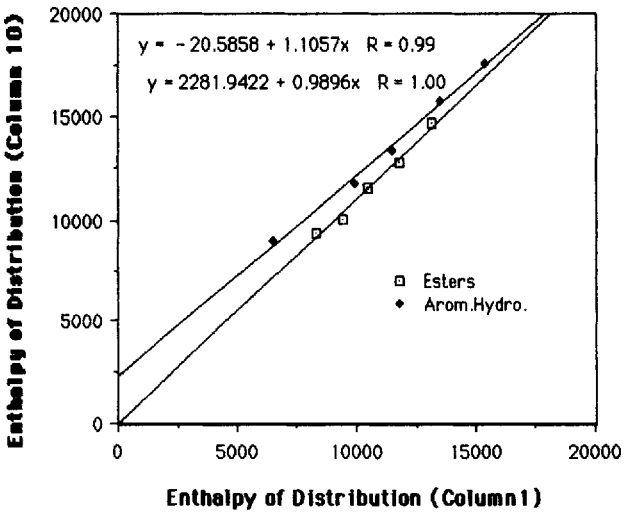

Figure 5

Graph Excess Free Enthalpies (Column 10) / those on (Column 1)

graph relating the excess free enthalpy of absorption for each solute on Column 1 to the excess free energy of the solutes on column 10 should provide a linear curve.

It is seen from Figure 5 that, indeed, an excellent linear relationship is realized for both the aromatic hydrocarbon series of solutes and the aromatic ester series when their enthalpy values obtained on column 10 are plotted against those obtained from column 1. It is obvious that the nature of the interactions is not significantly changed by the oligomeric synthesis. The slope of the curves for the esters is close to unity, whereas that for the aromatic hydrocarbons is slightly greater than unity (1.11). This is a small effect, but might result from the replacement of the polar interactions with

residual silanols on column 1 by the stronger dispersive interactions with the oligomers in column 10. This could occur as the residual silanols are eliminated or buried beneath the oligomers. As the polar interactions between the esters and the free silanols on the silica surface are stronger than with the aromatic hydrocarbons, the change to dispersive interactions as the silanol groups are removed or hidden is less significant for the series of esters and so the slope of the curve for the esters remains close to unity.

As the oligomeric phase carries a thick layer of hydrocarbon chains over its surface, it should only exhibit dispersive type interactions with each solute. If this is so then, as dispersive interactions are dependant on the molar volume of the solutes and, consequently, for a homologous series of solutes also on the molecular weight of the solutes, there should be linear correlation between the molecular weight of the solutes and their excess free enthalpy. Curves relating solute enthalpy with solute molecular weight for column 10 in Figure 6

It is seen that the expected linear relationship is indeed obtained demonstrating that retention on an oligomeric phase is controlled almost exclusively by dispersive interactions. It follows, that in contrast to other types of bonded phases, residual silanol groups are not present in an oligomeric phase. Consequently, provided sufficient oligomers are present, residual silanol groups do not effect retention to any significant extent .

Due to the thick covering of an oligomeric phase the silica surface is well screened from the mobile phase and thus should exhibit excellent stability. The relative stability of columns 1, 3, 5 and 10 to a mobile phase carrying 0.1% TFA is shown in Figure 7 the data being taken from table 5.

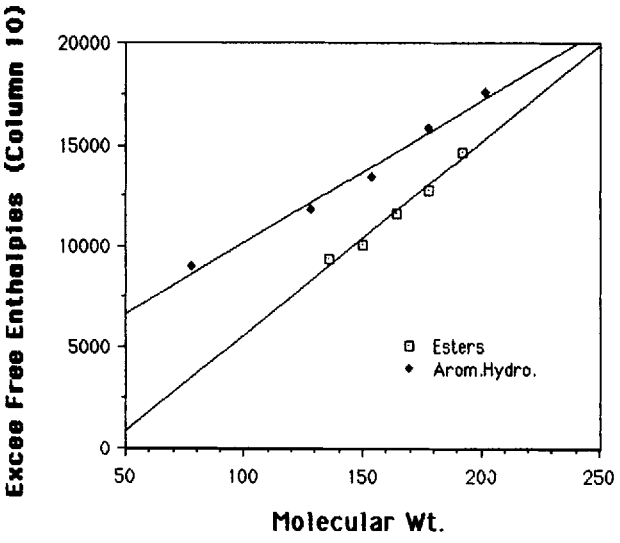

Figure 6

Graph of Excess free Enthalpy against Molecular Weight (Column 10)

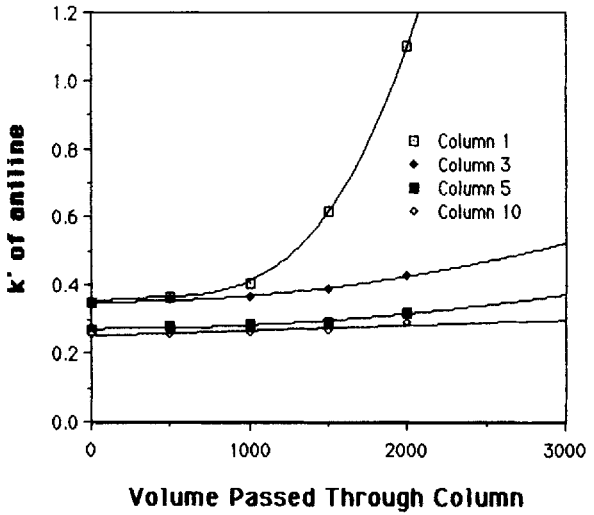

Figure 7

Graph of (k') against Volume of Mobile Phase Passed Through Column

The curves shown in Figure 7 are for aniline and relate k' of the solute to the volume of mobile phase passed through the column. It is seen that the mobile phase rapidly strips off the bonded phase from column 1. As the solute is basic and the free silanol groups are acidic the value of the k' rapidly rises. As the layers of the oligomeric phase becomes thicker the loss of bonded phase become progressively less and after five oligomers have been bonded on the silica the the material has become quite stable. After 10 oligomers have been bonded to the silica the material has become very stable. Such material would be extremely useful in the separation of proteins and polypeptides where TFA is often used to adjust the pH and achieve sample solubility. The usual reverse phase columns have notoriously short lives when operated under the low pH conditions.

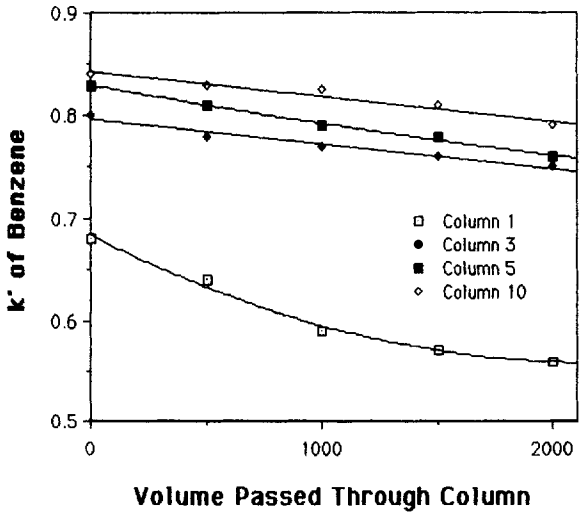

Figure 8

Graph of (k') against Volume of Mobile Phase Passed Through Column

In Figure 8 similar curves are shown for the solute benzene and the same results are indicated. In this case, however, the solute is not basic and thus, as the bonded phase is stripped off the surface, the value of the k' of the solute falls. Again the very significant difference between the stability of column 10 compared with column 1 is demonstrated.

Conclusions

Oligomeric reverse phases are relatively simple to produce employing the fluidized bed technique and have a number of advantages over the more common reverse phases. As the synthetic process produces a thick, multilayer of reverse phase material on the surface all the silanol groups are either fully reacted or are buried under the hydrocarbon moieties of the bonded phase. Thus, the silanol groups are no longer readily available to either solute or solvent and, as a consequence, there are virtually no polar interactions on the surface and the mechanism of retention is purely dispersive. It also follows, that an oligomeric reverse phase will provide more consistent retention behavior and could be used to provide standard reference columns. The unavailability of any silanol groups on the surface renders oligomeric phases extremely stable to harsh solvent environments and, in particular, stable to extensive exposure to mobile phases of low pH. This could be particularly valuble in biotechnological work where TFA is frequently employed as a component in the mobile phase for the separation of peptides and proteins. In principal the oligomeric method of stationary phase synthesis could be applied to other types stationary phase provided the appropriate dichlorosilane was available or could be synthesized.

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