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PREPARATION AND EVALUATION OF N-(2-AMINOETHYL)-γ-AMINOPROPYLTRIMETHOXYSILANE-TREATED SILICA COLUMNS FOR THE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHIC ANALYSIS OF SOME AROMATIC AMINES

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SUMMARY

The retention and selectivity behaviour of some aromatic amines were studied by high-performance liquid chromatography using amino-chemically bonded stationary phases (column gel), prepared from silica gel treated with benzene solution containing 5–50% of N-(2-aminoethyl)-γ-aminopropyltrimethoxysilane (N2AAPTS). The N2AAPTS gel was compared with the 3-aminopropyltriethoxysilane (3APTS)-treated silica gel. From elemental analysis data for nitrogen and carbon, the maximum number of accessible amino surface groups per 100 Å^2 of silica gel (mean pore diameter 85 Å, particle size distribution 5.8 μm) in N2AAPTS gel was estimated to be 1.81. The N2AAPTS gel provided better resolution of some amines than the 3APTS gel. The effect of the kind of component in the mobile phase on the capacity factor was studied using various alkanes (basic component) and normal alcohols (additive component).

INTRODUCTION

Because of the carcinogenic properties of certain aromatic amines, their analysis in the environment has received a great deal of attention. Amino chemically bonded stationary phases were selected for the high-performance liquid chromatographic (HPLC) analysis of aromatic amines owing to their weakly basic properties. Although separations were obtained using various reversed-phase chemically bonded chain lengths[1–13], such as C_2, C_3, C_8 or C_{18}, there have been few reports on the HPLC analysis of aromatic amines using amino chemically bonded stationary phases or the influence of the length of the amino chemically bonded chain. Therefore, the preparation and evaluation of amino chemically bonded stationary phases and the separation of aromatic amines by HPLC using these columns were studied.

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In a previous paper[14] we considered the use of several 3-aminopropyltriethoxysilane (3APTS) stationary phases in HPLC. Therefore, in this work we compared the retention behaviour of aromatic amines on the length of the aminomethylene bonded chain using N-(2-aminoethyl)-γ-aminopropyltrimethoxysilane (N2AAPTS) and 3APTS[14], and considered the number of accessible N2AAPTS surface groups per 100 Å^2 of silica gels with several pore sizes and particle sizes, *i.e.*, 85 Å, 5.8 μm; 70 Å, 10 μm; 153 Å, 8.4 μm; and 180 Å, 8.4 μm. We also studied the effect of the mobile phase composition on the chromatographic behaviour of some aromatic amines.

EXPERIMENTAL

Reagents

o-, *m*- and *p*-nitroanilines and 3,4-, 2,6- and 2,4-dinitrotoluenes were obtained from Wako (Osaka, Japan). N2AAPTS was purchased from Tokyo Kasei (Tokyo, Japan) and four kinds of highly microporous spherical silica gels differing in mean particle size and mean pore diameter were purchased from Fuji-Davison (Nagoya, Aichi, Japan) (Table I). *n*-Pentane, *n*-hexane, *n*-heptane, methanol, ethanol, 1-propanol, 1-butanol and 1-pentanol from Wako were used after distillation. All chemicals were of analytical-reagent grade.

TABLE I

SILICA GELS USED

*Silica gel**	*Mean particle size (μm)*	*Mean pore diameter (Å)*	*Specific surface area (m^2/g)*	*Pore volume (ml/g)*
Fuji-Davison 1	10.0	70	490	0.87
Fuji-Davison 2	8.4	153	204	0.78
Fuji-Davison 3	8.4	180	167	0.75
Fuji-Davison 4	5.8	85	400	0.85

* These names and serial numbers were assigned by the authors for convenience and are not commercial names.

Apparatus

The HPLC measurements were carried out using a KHU 16 Kyowa Seimitsu Mini Pump equipped with a Uvidec 100-II Jasco variable-wavelength detector.

Stationary phase and elemental analysis

According to the previous method[14], 5 g of dried Fuji-Davison 4 silica gel were added to 50 ml of a 0, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 or 5.0% benzene solution of N2AAPTS. After stirring for 24 h at room temperature, the silica gel suspension was filtered with a glass filter (1 μm), washed several times with benzene and methanol and then dried *in vacuo* at 70°C for 2 days, finally producing the silica gels for HPLC, which are listed as N2AAPTS 4–0 to 4–50, respectively, in Table II. Also, after 5 g of dried Fuji-Davison 1, 2 or 3 had been added to 50 ml of a 5.0% benzene solution of N2AAPTS, the same procedure as with the N2AAPTS 4 series in Table II was carried

TABLE II

SURFACE TREATMENTS AND ELEMENTAL ANALYSES FOR GELS PREPARED FROM FUJI-DAVIDSON SILICA GEL USING DIFFERENT CONCENTRATIONS OF N2AAPTS IN BENZENE

Mono = Monofunctional, Bi = bifunctional (see text).

Treated gel	*Concentration of N2AAPTS in benzene (%)*	*Specific surface area (m^2/g)*	*N calculated (%)*		*N found (%)*	*C calculated (%)*		*C found (%)*
			Mono	*Bi*		*Mono*	*Bi*	
N2AAPTS 4-0	0	400	0	0	0	0	0	0
N2AAPTS 4-5	0.5	357	0.51	0.51	0.62	1.53	1.32	2.23
N2AAPTS 4-10	1.0	324	0.98	0.99	1.13	2.93	2.54	3.84
N2AAPTS 4-15	1.5	302	1.41	1.43	1.59	4.23	3.68	5.86
N2AAPTS 4-20	2.0	296	1.81	1.84	2.04	5.43	4.75	6.75
N2AAPTS 4-25	2.5	292	2.18	2.23	2.34	6.54	5.74	7.72
N2AAPTS 4-30	3.0	274	2.52	2.59	2.42	7.57	6.68	7.38
N2AAPTS 4-50	5.0	287	3.69	3.85	2.61	11.07	9.90	7.81

out, producing N2AAPTS 1–50, 2–50 and 3–50, which are shown in Table III together with N2AAPTS 4–50. Hereafter, N2AAPTS 4–0 to 4–50 and 1–50 to 3–50 will be abbreviated to "column gel".

The nitrogen and carbon contents of each column gel were determined by elemental analysis using an MT-3 Yanagimoto CHN elemental analyser, giving the data indicated as "Found" in Tables II and III. The specific surface areas of the column gels were determined with an SA-1000 Shibata surface area pore volume analyser, giving the data in Tables II and III.

TABLE III

SURFACE TREATMENTS AND ELEMENTAL ANALYSES FOR GELS PREPARED FROM FUJI-DAVISON 1, 2, 3 AND 4 SILICA GELS

Concentration of N2AAPTS in benzene: 5.0%.

Treated gel	*Specific surface area (m^2/g)*	*N found (%)*	*C found (%)*
N2AAPTS 1-50	330	2.85	7.98
N2AAPTS 2-50	166	1.55	4.21
N2AAPTS 3-50	125	1.31	3.35
N2AAPTS 4-50	287	2.61	7.81

Column preparation

The column gels were packed into stainless-steel columns (250 × 4 mm I.D.) using a balanced density method through a 10-ml stainless-steel packer at a rate of 500 kg/cm^2 with a Kyowa Seimitsu KHW-20 ultra-high-pressure pump.

RESULTS AND DISCUSSION

Figs. 1A and 2A show the correlations between the capacity factors (k') of nitroaniline or diaminotoluene and the concentration of N2AAPTS solution with

which the silica gel was treated. Saturation of log k' was observed at least from N2AAPTS 4–20 to 4–30, almost all of the N2AAPTS-reactive hydroxyl groups on the silica gel surface having been replaced with N2AAPTS.

Figs. 1B and 2B show the corresponding curves obtained with 3APTS stationary phase, which has a shorter aminomethylene bonded chain than N2AAPTS.

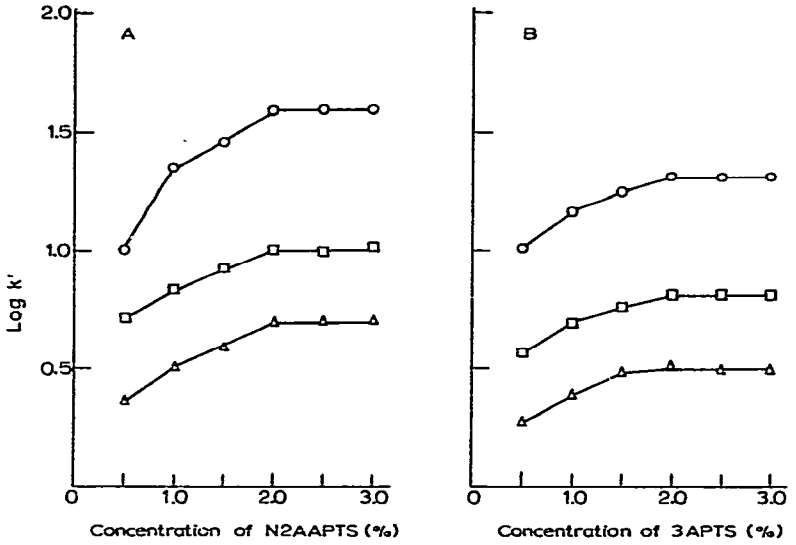


Fig. 1. Relationships between capacity factors (log k') of nitroanilines and the concentrations of (A) N2AAPTS and (B) 3APTS in benzene solution. Column: 250 × 4 mm I.D. Mobile phase: *n*-hexane–ethanol (25:1). Flow-rate: 2.0 ml/min. Detection: UV, 254 nm. ○, *p*-Nitroaniline; □, *m*-nitroaniline; △, *o*-nitroaniline.

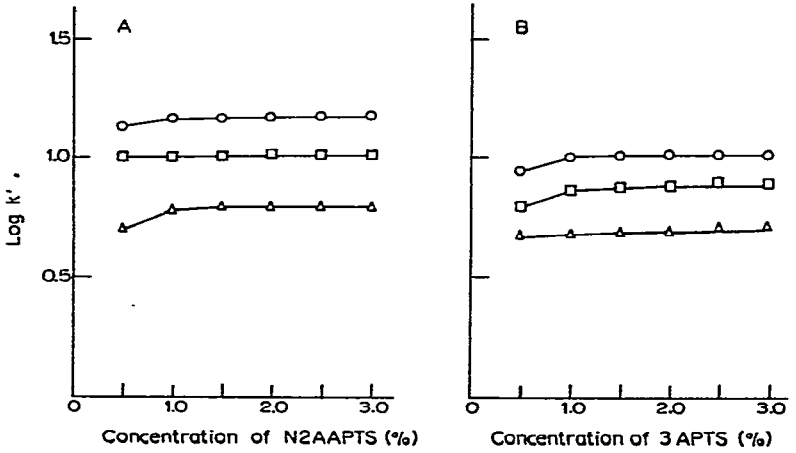
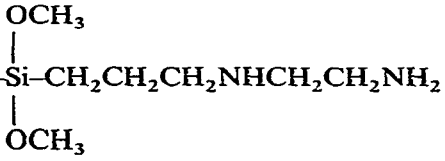


Fig. 2. Relationships between capacity factors (log k') of diaminotoluenes and the concentrations of (A) N2AAPTS and (B) 3APTS in benzene solution. Conditions as in Fig. 1. ○, 2,4-Diaminotoluene; □, 2,6-diaminotoluene; △, 3,4-diaminotoluene.

From the elemental analysis of silica gel treated with various concentrations of N2AAPTS, the number of accessible amino surface groups per 100 Å^2 of silica gel surface can be estimated as follows.

If N2AAPTS is substituted monofunctionally on silica gel, the surface structure of the saturated column gel can be written as



The number of accessible amino surface groups per 100 Å^2 of silica gel surface is then given by

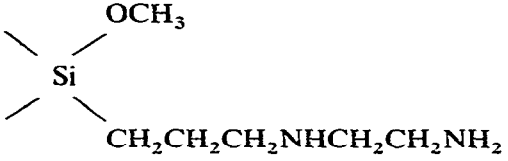
$$[(N/100)/14.0067 \cdot 2] \cdot 6.022 \cdot 10^{23}/S \cdot 10^{18} \quad (1)$$

or

$$[(C/100)/12.011 \cdot 7] \cdot 6.022 \cdot 10^{23}/S \cdot 10^{18} \quad (2)$$

where N = weight percentage of nitrogen, C = weight percentage of carbon, $6.022 \cdot 10^{23}$ = Avogadro's number and S = specific surface area (m^2/g) of treated silica gel.

If N2AAPTS is substituted bifunctionally on silica gel, the surface structure of saturated column gel can be written as



The number of accessible amino surface groups per 100 Å^2 of silica gel surface is then given by

$$[(N/100)/14.0067 \cdot 2] \cdot 6.022 \cdot 10^{23}/S \cdot 10^{18} \quad (3)$$

or

$$[(C/100)/12.011 \cdot 6] \cdot 6.022 \cdot 10^{23}/S \cdot 10^{18} \quad (4)$$

Substitution of the values of N and C found by elemental analysis into eqns. 1–4 gives the number of accessible amino surface groups per 100 Å^2 of silica gel surface, indicated as "Found" in Table IV. As can be seen from the "Found" data in Tables II and Table IV, an increase in the N2AAPTS concentration in benzene increases the surface modification of the silica gel, but approaches saturation over about 2.0% of

N2AAPTS. This tendency explains well the saturated curves of log k' from N2AAPTS 4–20 to 4–30 in Fig. 1A. The log k' curves obtained with 3APTS, reported previously[14], show the same pattern as those obtained with N2AAPTS in this work. As the agreement between the values ("Found") for accessible amino surface groups in Table IV according to the monofunctional reaction mechanism (eqns. 1 and 2) seems to be better than that between the values according to the bifunctional reaction mechanism (eqns. 3 and 4), it is suggested that the reaction between silica gel and N2AAPTS, and also 3APTS[14], takes place monofunctionally. We calculated the nitrogen and carbon percentages and the number of accessible amino surface groups according to eqn. 1, 2, 3 or 4 with the assumption that all N2AAPTS molecules react with silica gel. Both the former and the latter values are shown as "Calculated" in Tables II and IV, respectively.

TABLE IV

SURFACE TREATMENTS AND NUMBER OF ACCESSIBILE AMINO SURFACE GROUPS PER 100 Å^2

Mono = Monofunctional; Bi = bifunctional (see text).

Column gel	*No. of accessible NH_2 surface groups per 100 Å^2*							
	Calc. Mono		*Found Mono*		*Calc. Bi*		*Found Bi*	
	Eqn. 1	*Eqn. 2*	*Eqn. 1*	*Eqn. 2*	*Eqn. 3*	*Eqn. 4*	*Eqn. 3*	*Eqn. 4*
N2AAPTS 4-0	0	0	0	0	0	0	0	0
N2AAPTS 4-5	0.31	0.31	0.37	0.44	0.31	0.31	0.37	0.52
N2AAPTS 4-10	0.65	0.64	0.75	0.84	0.66	0.66	0.75	0.99
N2AAPTS 4-15	1.00	1.00	1.13	1.38	1.02	1.02	1.13	1.62
N2AAPTS 4-20	1.31	1.31	1.48	1.62	1.33	1.34	1.48	1.90
N2AAPTS 4-25	1.60	1.60	1.72	1.89	1.64	1.64	1.72	2.21
N2AAPTS 4-30	1.98	1.98	1.95	1.93	2.03	2.04	1.95	2.15
N2AAPTS 4-50	2.75	2.75	1.95	1.95	2.87	2.87	1.95	2.27

The comparison between these calculated values and the corresponding "Found" values shows again that saturation of N2AAPTS on silica gel taken place at about 2.0% of N2AAPTS in benzene. Using the data from N2AAPTS 4–20 to 4–50 in Table IV, the number of accessible amino surface groups per 100 Å^2 of silica gel was calculated to be 1.81.

On the other hand, from the data given in Tables I and III, the important parameters of silica gel with respect to the number of accessible N2AAPTS groups per 100 Å^2 of silica gel were considered to be the pore diameter and the surface area.

n-Pentane, *n*-hexane and *n*-heptane were used as basic components of the mobile phase. Taking into consideration that lower viscosities lead to higher column efficiencies, *n*-pentane was the best component (the viscosities of *n*-pentane, *n*-hexane and *n*-heptane are 0.22, 0.31 and 0.40 cP, respectively, at 22°C). However, the low boiling point of *n*-pentane[15] may cause bubble formation in the detector, so *n*-hexane seemed to be the optimal basic component. The addition of an alcohol to a mobile phase can be used to adjust the retention volumes of aromatic amines, because of its

competitive hydrogen bonding with amines against the amino-bonded stationary phase on the column gel. The k' values of nitroaniline and diaminotoluene were measured using various kinds of mobile phase containing an *n*-alkanol and a basic component. As can be seen in Fig. 3, the k' values generally increased with increasing carbon numbers or decreasing polarity[16] of the *n*-alkanols, reflecting the decreasing solubility in the mobile phase.

Based on the relationship between k' and the carbon number of *n*-alkanols, methanol is the most suitable. However, methanol has a very low solubility in *n*-

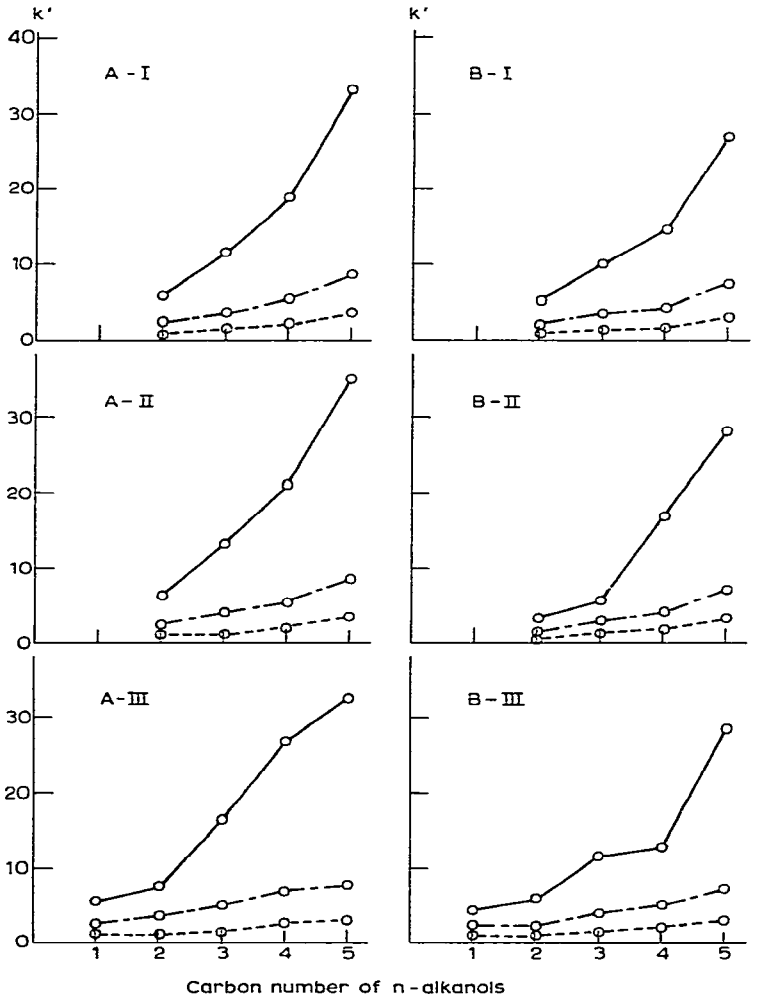


Fig. 3. Relationships between capacity factors (k') of nitroaniline and the *n*-alkanol added to the mobile phase. Column: 250 × 4 mm I.D. Stationary phase: A, silica gel treated with 2.5% N2AAPTS; B, silica gel treated with 2.5% 3APTS. Mobile phase: basic components–aliphatic alcohols (9:1) (basic components: I = *n*-heptane, II = *n*-hexane, III = *n*-pentane; aliphatic alcohols: 1 = methanol, 2 = ethanol, 3 = 1-propanol, 4 = 1-butanol, 5 = 1-pentanol). Flow-rate: 1.49 ml/min. Detection: UV, 254 nm. O——O, *p*-Nitroaniline; O– – –O, *m*-nitroaniline; O– – –O, *o*-nitroaniline.

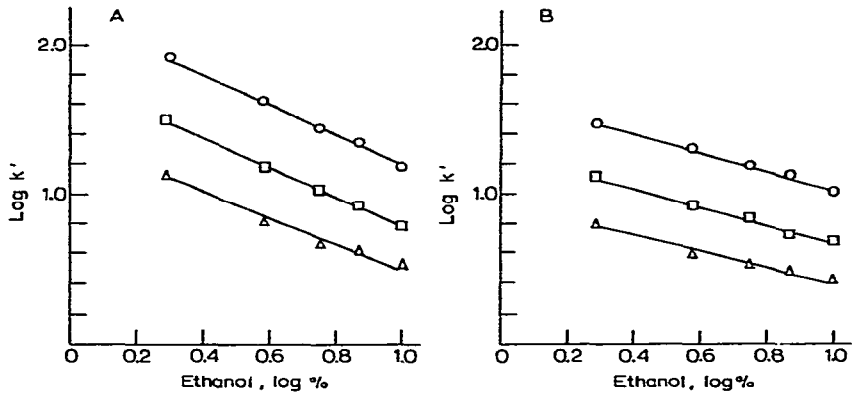


Fig. 4. Relationship between capacity factors (log k') of nitroanilines and concentration of ethanol in *n*-hexane. Column: 250 × 4 mm I.D. Stationary phase: A, silica gel treated with 2.5% N2AAPTS; B, silica gel treated with 2.5% 3APTS. Flow-rate: 1.49 ml/min. Detection: UV, 254 nm. ○, *p*-Nitroaniline; □, *m*-nitroaniline; △, *o*-nitroaniline.

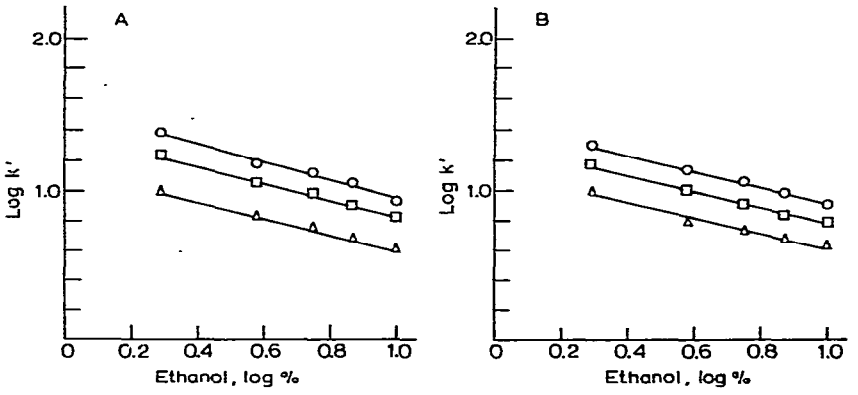


Fig. 5. Relationship between capacity factors (log k') of diaminotoluenes and concentration of ethanol in *n*-hexane. Conditions as in Fig. 4. ○, 2,4-Diaminotoluene; □, 2,6-diaminotoluene; △, 3,4-diaminotoluene.

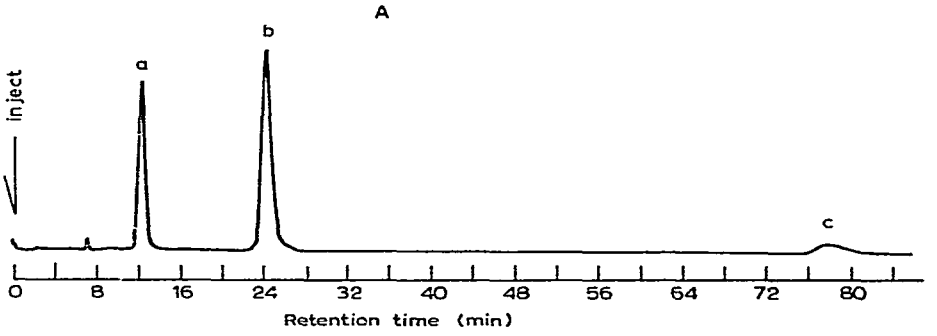


Fig. 6.

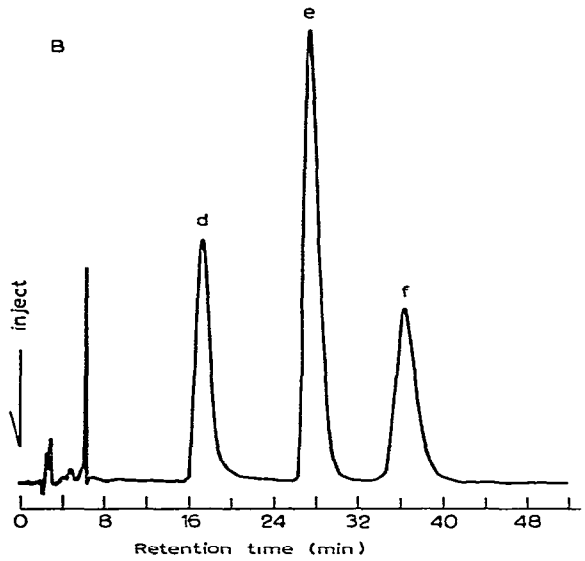


Fig. 6. Separation behaviour of nitroanilines (A) and diaminotoluenes (B) on N2AAPTS-treated silica gel column. Stationary phase: silica gel treated with 2.5% N2AAPTS. Mobile phase: *n*-hexane–ethanol (25:1); flow-rate, 2.0 ml/min. Detection: UV, 254 nm, 0.32 a.u.f.s. Peaks: a = *o*-nitroaniline (3.7 μg); b = *m*-nitroaniline (4.4 μg); c = *p*-nitroaniline (3.4 μg); d = 3,4-diaminotoluene (21.4 μg); e = 2,6-diaminotoluene (22.4 μg); f = 2,4-diaminotoluene (17.1 μg).

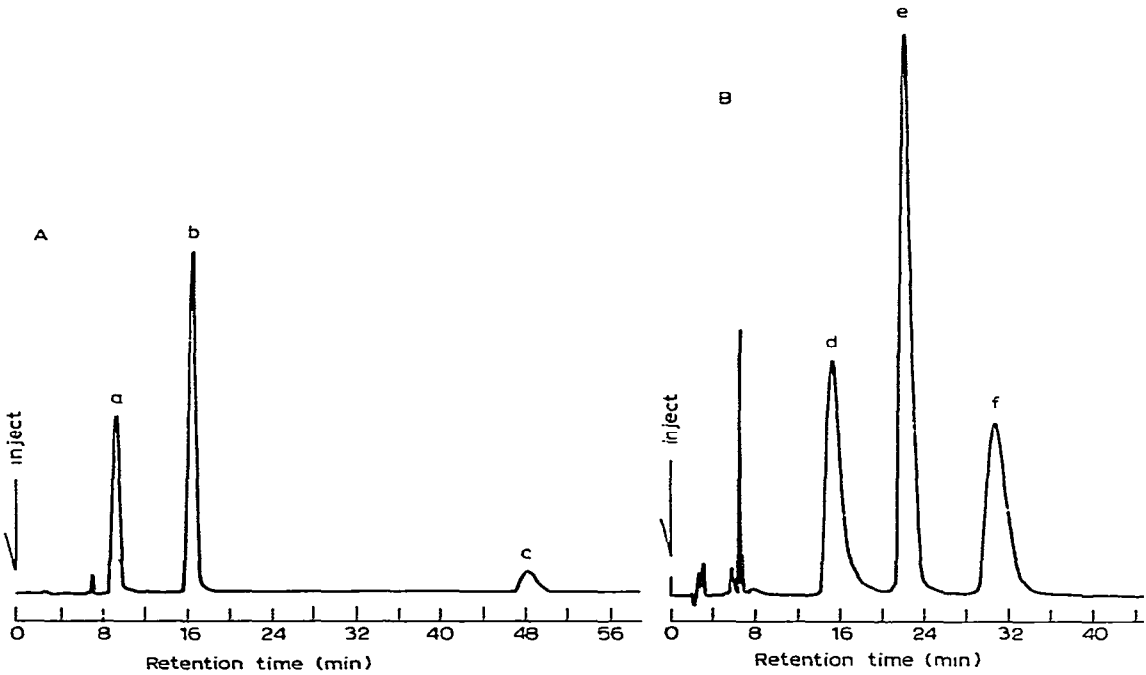


Fig. 7. Separation behaviour of nitroanilines (A) and diaminotoluenes (B) on 3APTS-treated silica gel column. Stationary phase: silica gel treated with 2.5% 3APTS. Other conditions and peaks as in Fig. 6.

hexane, so that ethanol seems to be optimal from the point of view of the separation. The dependence of k' on the alcohol concentration was studied with ethanol, and optimal separations of nitroanilines and diaminotoluenes were achieved with 25:1 *n*-hexane–ethanol (Figs. 4 and 5). Figs. 6 and 7 show typical liquid chromatograms obtained with nitroanilines and diaminotoluenes on N2AAPTS and 3APTS stationary phases.

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