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PREPARATION AND EVALUATION OF 3-AMINOPROPYLTRIETHOXYSILANE-TREATED SILICA FOR HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF LOW-MOLECULAR-WEIGHT ALDEHYDES AS THEIR LUTIDINE DERIVATIVES

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SUMMARY

Formaldehyde and other low-molecular-weight aldehydes were studied as lutidine derivatives by high-performance liquid chromatography using an NH_2-chemically bonded stationary phase, prepared from silica gel treated with benzene solutions containing 5–30% 3-aminopropyltriethoxysilane. From elemental analysis data for nitrogen and carbon, the maximum number of accessible NH_2 surface groups per gram of silica gel was estimated to be 0.45×10^{21}.

INTRODUCTION

Free formaldehyde (HCHO) remaining in underwear has been reported to be one cause of skin trouble[1,2]. Acetylacetone (AA) reacts with HCHO under mild conditions to form the corresponding lutidine, and colorimetric[3,4] and fluorometric methods[5] using this reaction have been widely applied for the determination of free HCHO in underwear. However, AA reacts similarly with other aldehydes, necessitating the chromatographic separation of lutidines.

On the other hand, Papa and Turner[6] and Carey and Persinger[7] reported on the high-performance liquid chromatography (HPLC) of carbonyl compounds as their 2,4-dinitrophenylhydrazone derivatives. This method was not successful in the determination of trace amounts of HCHO in underwear, because the high temperatures and high concentrations of acid required to transform low-molecular-weight aldehydes into hydrazones, also caused the decomposition of resins contained in underwear producing an error in the HCHO determination.

I have examined the separation of HCHO–AA and lutidine derivatives by HPLC using various columns such as ODS, styrene–divinylbenzene copolymer, silica gel ion-exchange resin and silica gel treated with 3-aminopropyltriethoxysilane (3APTS), but could not obtain satisfactory results except with the last column. Thus, I have now studied the optimal conditions for the HPLC determination and separation of HCHO in underwear as the lutidine derivative on silica gel treated with various 3APTS–benzene solutions. The separation of other lutidine derivatives of various aldehydes was also examined.

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EXPERIMENTAL

Reagents

HCHO (aqueous), acetaldehyde, propionaldehyde, *n*-butyraldehyde, *n*-valeraldehyde, *n*-capronaldehyde, *n*-heptanal and AA were obtained from Wako (Osaka, Japan). 3APTS was purchased from Aldrich (Milwaukee, WI, U.S.A.). A highly microporous spherical silica gel [mean pore diameter 95 Å, surface area (BET) 380 m^2/g, particle size distribution 5.5 μm] was obtained from Fuji-Davison (Aichi, Japan). Hexane and ethanol were used after distillation. The other reagents and organic solvents were reagent grade.

Apparatus

Two liquid chromatographs were employed: a Hitachi 635 T equipped with a visible spectrophotometer and a Hitachi 204 S fluorescence spectrophotometer; and a Type KHU 16 of Kyowa Seimitsu Mini Micro Pump equipped with a Type KLC-200 Kyowa Seimitsu variable-wavelength detector.

Stationary phase and elemental analysis

A 5-g amount of dried silica gel was added to 50 ml of a 0.5, 1.0, 1.5, 2.0, 2.5 or 3.0% benzene solution of 3APTS. After stirring for 24 h at room temperature, the silica gel was filtered with a glass filter, washed several times with benzene and acetone and then dried *in vacuo* at 70°C for 2 days. Nitrogen and carbon contained in the silica gel treated with 3APTS (3APTS-5 to 3APTS-30, shown in Table I) were determined by elemental analysis using a Perkin-Elmer Type 240 Elemental Analyzer.

TABLE I

SURFACE TREATMENTS AND ELEMENTAL ANALYSES

Mono = Monofunctional; Bi = bifunctional; for the meaning of these terms, see text.

Sample	*Concn. of 3APTS in benzene (%)*	*N Calc. (%)*		*N Found (%)*	*C Calc. (%)*		*C Found (%)*
		Mono	*Bi*		*Mono*	*Bi*	
SiO_2	0 (original)	0	0	0	0	0	0
3APTS-5	0.5	0.30	0.31	0.27	1.82	1.36	2.79
3APTS-10	1.0	0.58	0.60	0.58	3.52	2.56	3.66
3APTS-15	1.5	0.84	0.87	0.82	5.09	3.74	5.43
3APTS-20	2.0	1.09	1.13	1.03	6.55	4.85	6.85
3APTS-25	2.5	1.32	1.38	1.06	7.91	5.91	7.69
3APTS-30	3.0	1.53	1.61	1.13	9.19	6.91	6.56

Column preparation

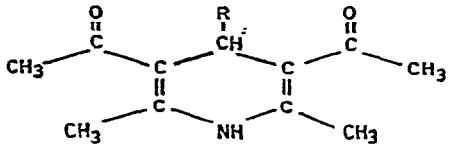
Silica gel treated with 3APTS was packed was packed into stainless-steel columns (50, 75, 125, 150, 200, 225, 250, 300, 400, 450 or 475 mm × 4 mm I.D.) using a balance density method and a 10-ml stainless-steel packer at a rate of 500 kg/cm^2 (Kyowa Seimitsu Type KHW-20 Ultra High Pressure Pump).

Preparation of lutidine derivatives

A 2-ml volume of HCHO (aq.) or other aldehydes (Table II), 2 ml of AA, 3 ml of acetic acid and 15 g of ammonium acetate were added to 100 ml of water. For HCHO, the mixture was warmed to 40°C for 60 min and for other aldehydes to 60°C for 30 min. After cooling, the product was filtered off, washed with cold water and then cold ethanol, dried at room temperature and recrystallized from diethyl ether.

TABLE II

PREPARATION OF HCHO–AA AND OTHER LUTIDINE DERIVATIVES OF ALDEHYDES



Parent compound	*R*	*M.p. (°C)*	*Formula*	*Analysis: Calc. (Found) (%)*		
				C	*H*	*N*
HCHO	H	200–204	$C_{11}H_{15}NO_2$ (HCHO–AA)	68.35 (68.02)	7.83 (7.85)	7.25 (6.97)
CH_3CHO	CH_3	157–159	$C_{12}H_{17}NO_2$	69.53 (69.47)	8.27 (8.51)	6.76 (6.73)
CH_3CH_2CHO	CH_3CH_2	162–164	$C_{13}H_{19}NO_2$	70.54 (70.42)	8.66 (8.82)	6.33 (6.37)
$CH_3(CH_2)_2CHO$	$CH_3(CH_2)_2$	130–132	$C_{14}H_{21}NO_2$	71.44 (71.69)	9.00 (9.25)	5.96 (6.14)
$CH_3(CH_2)_3CHO$	$CH_3(CH_2)_3$	110–113	$C_{15}H_{23}NO_2$	72.24 (72.12)	9.30 (9.25)	5.62 (5.40)
$CH_3(CH_2)_4CHO$	$CH_3(CH_2)_4$	120–122	$C_{16}H_{25}NO_2$	72.95 (72.76)	9.57 (9.86)	5.32 (5.16)
$CH_3(CH_2)_5CHO$	$CH_3(CH_2)_5$	141–143.5	$C_{17}H_{27}NO_2$	73.60 (73.80)	9.82 (10.01)	5.05 (5.01)

Procedure

A 1-g amount of chopped sample (underwear) was immersed in 100 ml of water for 60 min at 40 °C, and the extract was filtered through a glass filter (G2) by the method of Kojima and Ohba[4]. To a 5-ml aliquot of the filtrate, a mixture of 5 ml of 2 *M* ammonium acetate, 0.05 *M* acetic acid and 0.02 *M* AA was added. The reaction mixture was warmed in a water-bath at 40°C for 30 min as described by Kojima and Ohba[4]. After cooling, 5 ml of internal standard solution (30 μg of the lutidine derivative of propionaldehyde in 1 ml of chloroform) were added. The mixture was shaken well and allowed to stand for some minutes. The aqueous phase was then discarded and the organic phase dried over anhydrous sodium sulphate. A 50-μl volume of the resulting solution was subjected to HPLC. The operating conditions are given in the legend to Fig. 3.

RESULTS AND DISCUSSION

Fig. 1 shows the correlation between the capacity factor (k') of each lutidine derivative and the concentration of the 3APTS solution with which the silica gel was treated. A plateau in k' was observed from 3APTS-15 to 3APTS-30, which means that almost all reactive OH groups on the silica gel surface were substituted with 3APTS.

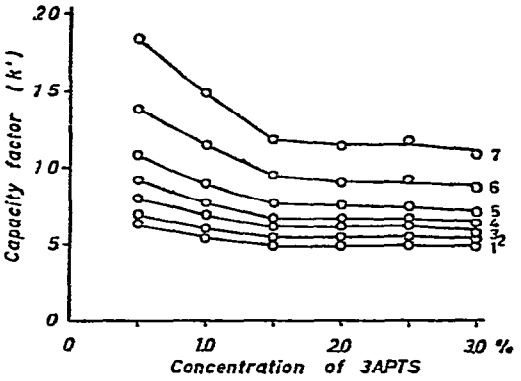
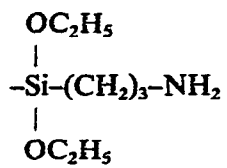


Fig. 1. Relationship between the capacity factor, k', of lutidine derivatives and the concentration of 3APTS solution. Column: 250 × 4 mm I.D. Mobile phase: hexane–ethanol (25:1). Curves: 1 = formaldehyde; 2 = acetaldehyde; 3 = propionaldehyde; 4 = *n*-butyraldehyde; 5 = *n*-valeraldehyde; 6 = *n*-capronaldehyde; 7 = heptanal.

From the elemental analysis of silica gel treated with 3APTS solutions of various concentrations, the number of accessible NH_2 surface groups per gram of silica gel can be estimated by the following procedure. If 3APTS is substituted monofunctionally on silica gel, the surface structure of silica gel can be written as:



The number of accessible NH_2 surface groups per gram is then given by

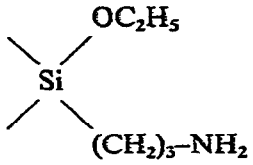
$$[(N/100)/14.0067 \times 1] \times 6.022 \times 10^{23} \quad (1)$$

or

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

where N = nitrogen weight percentage, C = carbon weight percentage and 6.022×10^{23} = Avogadro's number.

If 3APTS is substituted bifunctionally on silica gel, the surface structure of silica gel can be written as:



The number of accessible NH_2 surface groups per gram is then given by:

$$[(N/100)/14.0067 \times 1] \times 6.022 \times 10^{23} \quad (3)$$

or

$$[(C/100)/12.011 \times 5] \times 6.022 \times 10^{23} \quad (4)$$

Substitution of the values of *N* and *C* found by elemental analysis (Table I) into eqns. 1, 2, 3 or 4 gives the number of accessible NH_2 surface groups per gram of silica gel (Table III). As is seen from the data in Tables I and III, increase of the 3APTS concentration in benzene increases the surface modification of silica gel, but approaches saturation at greater than 1.5% 3APTS. This tendency explains well the plateau in k' from 3APTS-15 to 3APTS-30 in Fig. 1. The agreement between the values calculated according to the monofunctional reaction mechanism seems to be better than that for those according to the bifunctional reaction mechanism. It is therefore suggested that the reaction between silica gel and 3APTS takes place monofunctionally. The nitrogen and carbon percentages and the number of accessible NH_2 surface groups according to eqns. 1, 2, 3 or 4 were also calculated with the assumption that all 3APTS molecules react with silica gel (calc. values in Tables I and III). The comparison between these values and the corresponding "found" values again shows that saturation of 3APTS on silica gel takes place with about 1.5% 3APTS in benzene. Using the data for 3APTS-15 to 3APTS-30 in Table III, the number of accessible NH_2 surface groups per gram of silica gel was 0.45×10^{21}.

TABLE III

SURFACE TREATMENTS AND THE NUMBER OF ACCESSIBLE NH_2 SURFACE GROUPS PER GRAM

Sample	*No. of accessible NH_2 surface groups per gram ($\times 10^{21}$)*							
	Calc. Mono		*Found Mono*		*Calc. Bi*		*Found Bi*	
	eqn. 1	*eqn. 2*	*eqn. 1*	*eqn. 2*	*eqn. 3*	*eqn. 4*	*eqn. 3*	*eqn. 4*
SiO_2	0	0	0	0	0	0	0	0
3APTS-5	0.13	0.13	0.12	0.20	0.13	0.14	0.12	0.28
3APTS-10	0.25	0.25	0.25	0.26	0.26	0.26	0.25	0.37
3APTS-15	0.36	0.37	0.35	0.39	0.37	0.38	0.35	0.55
3APTS-20	0.47	0.47	0.44	0.49	0.49	0.49	0.44	0.69
3APTS-25	0.57	0.57	0.46	0.55	0.59	0.59	0.46	0.77
3APTS-30	0.66	0.66	0.49	0.47	0.69	0.69	0.49	0.66

It was found that HCHO–AA could be easily extracted with organic solvents, such as dichloromethane, chloroform and ethyl acetate. Of these solvents, chloroform was chosen as the most suitable since it can be clearly separated from the aqueous

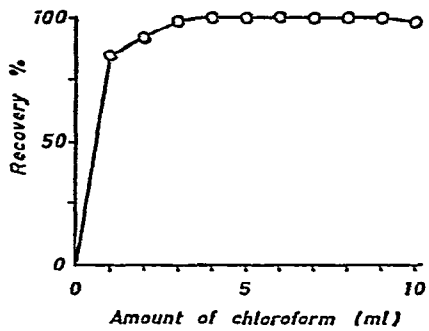


Fig. 2. Effect of chloroform on the extraction of HCHO-AA (20 μg).

phase as lower layer, which is convenient for the separation procedure. Fig. 2 shows the efficiencies of HCHO–AA (20 μg) extraction with chloroform from aqueous solvents. A 5-ml volume of chloroform was required.

The chromatographic behaviour of HCHO–AA was studied on columns of various lengths of silica gel treated with 3APTS in mixed solvents such as hexane–methanol, –ethanol, –*n*-propanol, –isopropanol and –*n*-butanol. The hexane–ethanol solvent (25:1) and 250 × 4 mm I.D. column gave the best separation, and with a suitable flow-rate owing to the low viscosity. Fig. 3 shows a liquid chromatogram of

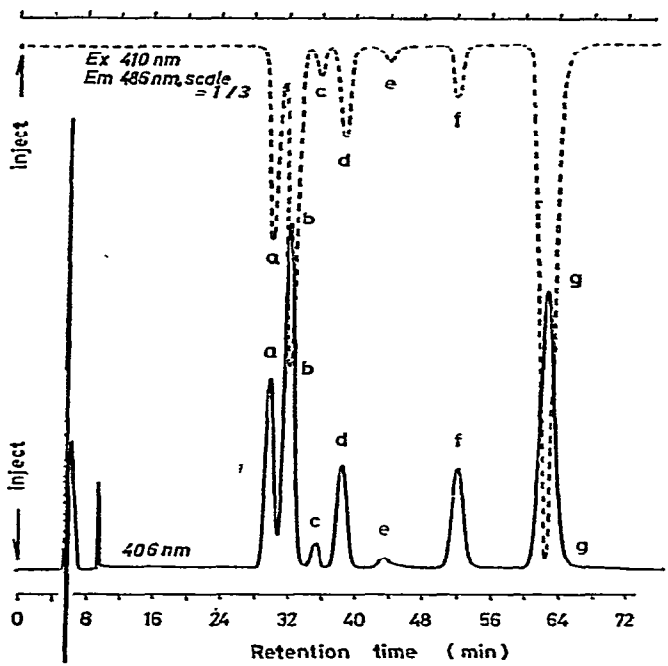


Fig. 3. Separation of HCHO-AA and other lutidine derivatives. Stationary phase: silica gel treated with 2.0% 3APTS. Mobile phase: hexane–ethanol (25:1), flow-rate 0.8 ml/min. Detection: ———, 406 nm, 0.04 a.u.f.s.; - - -, excitation 410 nm (slit 10 nm), emission 486 nm (slit 10 nm). Peaks: a = *n*-heptanal (6.1 μg); b = *n*-capronaldehyde (9.2 μg); c = *n*-valderaldehyde (1.3 μg); d = *n*-butyraldehyde (5.2 μg); e = propionaldehyde (0.2 μg); f = acetaldehyde (2.1 μg); g = formaldehyde (3.6 μg).

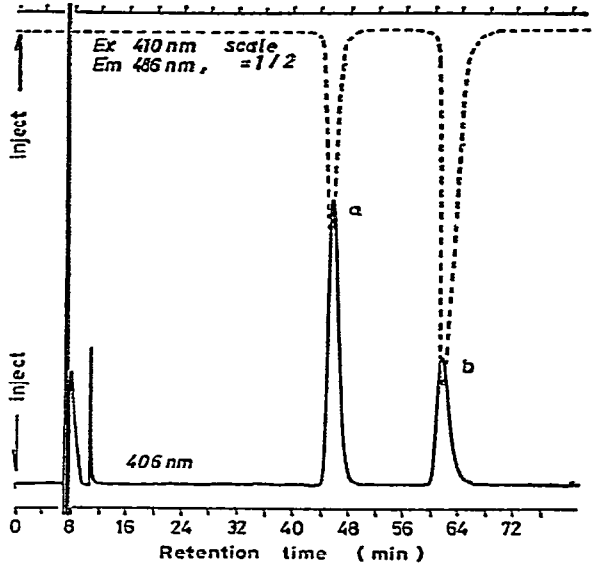


Fig. 4. Liquid chromatogram of formaldehyde (b) in underwear and propionaldehyde as internal standard (a, 1.5 μg). Stationary phase: silica gel treated with 2.5% APTS. Other conditions as in Fig. 3.

HCHO–AA and lutidine derivatives of some parent compounds in hexane–ethanol (25:1). From this result, propionaldehyde was chosen as internal standard for HCHO.

Fig. 4 shows a typical liquid chromatogram obtained from HCHO in underwear and propionaldehyde as internal standard.

A calibration graph constructed by plotting the ratio of the peak area of HCHO to that of the internal standard was linear and passed through the origin for HCHO in the range, 0.02–1.0 μg and 0.05–6.0 μg in 1 ml of aqueous solution, using fluorescence and visible spectrophotometers respectively. Nine replicate determinations on a test solution containing HCHO (1 μg) gave a standard deviation of 1.83%, and the limit of detection was 100 pg.

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