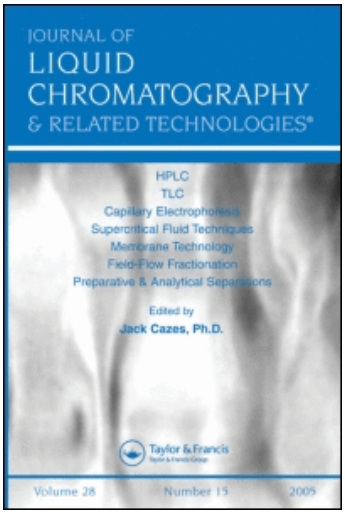

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TECHNIQUE FOR IN SITU COATING OF Ag^+ ONTO SILICA GEL IN HPLC COLUMNS FOR THE SEPARATION OF GEOMETRICAL ISOMERS

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ABSTRACT

A method is described for the in situ coating of silver nitrate onto silica gel in high performance liquid chromatography columns. The columns effectively separated a series of geometrical isomers.

INTRODUCTION

The use of Ag^+ complexes to separate the geometrical isomers of olefinic compounds is well known (1-3). Heath et al. (4) showed that many olefinic isomers could be rapidly analyzed and purified, using HPLC columns packed with silver nitrate-coated silica gel. Although such columns are highly efficient, their use has been limited, for they are not commercially available and few laboratories are equipped with a packing apparatus required to pack HPLC columns. Because the HPLC technique with silver nitrate-coated silica gel is potentially useful in our work on insect pheromones, many of which are isomeric olefins that are difficult to separate, we sought a simple method of preparing the required columns. We now report an in situ method of coating $AgNO_3$ onto silica gel, prepacked into HPLC columns. We applied the method both to columns we had prepared and to commercial columns; then we compared the coated columns in their ability to separate the positional and geometrical isomers of decenes, octadecenes, and tetradecen-1-ol

acetates. All the 10-cm long columns we tested completely resolved most of the geometrical isomers with retention times of less than 6 minutes.

MATERIALS

We packed 1.25-cm (OD) x 10-cm columns and 0.63-cm (OD) x 25-cm columns with 5 μ irregular silica (Lichrosorb® Si60 - E. M. Merck), using a high-flow air-driven pump (Haskel Engineering) at ca. 9000 psi as previously described (4). The commercial columns (Waters Associates RM-100®), 1.25 cm (OD) x 10 cm, were packed with 10 μ spherical silica by radial compression. A reciprocating pump (Laboratory Data Control Constametric II®) was used for solvent delivery. Samples were injected with a modified Precision Sampling Injector® (4) and sample components were detected with a UV detector (Schoeffel #7122®) at 254λ or with a refractive index (RI) detector (Waters Associates 401®). When required, data were recorded by computer (Nicolet 1180®) at a rate in excess of 10 data points per second. The reservoir used for the *in situ* coating procedure consisted of a 1/2-in. (OD) x 30-in. length of s.s. tubing fitted with 1/2-in. to 1/4-in. reducing unions (Swagelok®) at each end. We placed a 0.5 μ frit at the outlet end, using a previously described column terminator (5). The solvents -- hexane, benzene, and acetonitrile (J. T. Baker) -- were used without purification. Silver nitrate was reagent grade (Fisher Scientific Co.). The positional and geometrical isomers of decene were purchased from Chemical Samples Co. Octadecenes and the tetradecen-1-ol acetate isomers were prepared in our laboratory as part of another project, and their structures confirmed with ^{13}C nuclear magnetic resonance spectroscopy.

METHODS

Prior to the *in situ* coating the activity and efficiency of each silica column were determined with a mixture of benzene and naphthalene using hexane as the mobile phase. The HETP values were

determined and plotted against solvent flow rate; and the slope of the plot calculated. After the initial series of chromatograms had been obtained, the injector was removed, the column disconnected from the UV detector, and the reservoir attached to the column in a vertical position. With the top fitting of the reservoir removed, a solution of 15 ml of acetonitrile and 0.35 g of $AgNO_3$ was poured into the reservoir; the remaining void was filled with hexane. The reservoir top was connected to the solvent delivery system, and ca. 30 ml of hexane was pumped through the column at 1 ml/min. The reservoir was removed, the injector reconnected to the column, and the solvent changed to benzene. The column was equilibrated with 30 ml of benzene at a flow rate of 3 ml/min, and then connected to the RI detector. A series of chromatograms for the separation of the (Z)- and (E)-9-tetradecen-1-ol acetates by the coated column, with benzene at different flow rates, were then obtained; the HETP values and slopes were determined. The ability of the column to resolve geometrical isomers was determined using the analogous series of decene, octadecene and tetradecen-1-ol acetates.

RESULTS

Table 1 shows the HETP values for a commercially obtained column and a 10-cm column we prepared. The respective slopes for the uncoated columns using napthalene were 0.48 and 0.38. The slopes for the *in situ*-coated commercial columns in separating the (E)- and (Z)-9-tetradecen-1-ol acetates were 9.38 and 11.48, respectively; and the corresponding slopes for the coated laboratory-packed columns were 9.88 and 11.42. Because the majority of commercial columns now in use are 25-cm x 0.63-cm (OD), we include data obtained from such a column that we prepared and then coated with Ag^+: the slope for the column was 0.57 as determined with napthalene, and the slope for the coated column was 9.2 and 13.4, as determined respectively with the (E)- and (Z)- isomers of 9-tetradecen-1-ol acetate.

TABLE 1

Comparison of column efficiencies before and after *in situ* coating as determined with naphthalene and the isomers of (*E*)- and (*Z*)-9-tetradecen-1-ol acetate.

LFV mm/sec	HETP in microns: Before *in situ* Naphthalene	After *in situ* *E* isomers	After *in situ* *Z* isomers
Commercial column 10 cm x 1.25 (10 μ)			
0.72	20.1	40.6	48.0
1.45	19.2	54.6	69.6
2.90	20.2	66.6	83.3
5.80	22.1	90.9	111.1
Laboratory column 10 cm x 1.25 (5 μ)			
0.69	17.0	36.0	42.0
1.38	16.4	47.0	58.3
2.76	15.9	60.1	80.1
5.52	18.6	83.4	99.8

Table 2 shows the chromatographic data for all the isomers tested. As it indicates, the 10-cm commercial and laboratory-packed column did not differ appreciably in resolving ability. Figure 1 illustrates the speed and resolution of the *in situ* coated columns in resolving the isomers of 9-tetradecen-1-ol acetate.

DISCUSSION

Two types of columns were tested (commercial and laboratory-packed) because they differed considerably in particle size of the silica and in the packing procedure. The results showed, however, that the deficiencies of the 2 types of columns were similar both before and after *in situ* coating with Ag^+. The coating decreased column efficiency by ca. 50%. Similarly, Heath et al. (4) reported that HPLC columns packed with silica that had been coated

TABLE 2

Chromatographic properties of geometrical isomers for separations by silver nitrate-coated silica columns eluted with benzene at 2 ml/min.

	10 cm x 1.25 columns (10 & 5 μ)[a]			25 cm x 0.63 columns (5 μ)		
	k'[b] (Z isomer)	α[c]	R[d]	k' (Z isomer)	α	R
Decene						
Δ2	0.50	2.9	3.8	0.31	3.5	6.1
Δ3	0.46	3.1	3.2	0.27	3.0	5.7
Δ4	0.39	2.6	2.7	0.24	2.8	4.6
Δ5	0.39	2.6	2.6	0.24	2.8	4.6
Octadecene						
Δ2	0.26	2.6	2.6	0.20	2.0	2.2
Δ5	0.19	1.9	1.8	0.17	1.7	1.5
Δ7	0.15	1.5	1.6	0.12	1.2	1.3
Δ9	0.14	1.4	1.1	0.12	1.2	1.2
C-14 Acetates						
Δ3	1.22	1.8	3.4	1.6	1.6	5.7
Δ5	2.36	2.2	4.5	2.8	2.1	7.2
Δ7	2.53	2.4	4.9	2.9	2.2	7.1
Δ9	2.20	2.1	5.4	2.7	1.8	6.8
Δ11	2.35	2.2	5.6	2.8	2.9	7.0

[a] No significant differences in chromatographic properties noted between 10-cm columns packed with 5 and 10 μ silica.

[b] $k' = (Ts - To)/To$, where Ts = elution time of sample and To = elution time of an unretained solute.

[c] $\alpha = k'_2/k'_1$, where k'_2 = second isomer Z and k'_1 = first isomer E.

[d] $R = 2\Delta T/(Wb1 + Wb2)$, where ΔT = distance between isomers and Wb = width at base of peak.

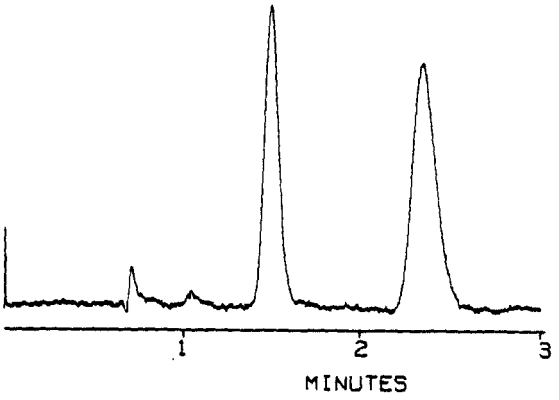

FIGURE 1

Separation of (E)- and (Z)-9-tetradecen-1-ol acetate on a Waters RM-100 10-cm column coated in situ with Ag^+. Mobile phase, benzene; flow, 4.0 ml/min; detector, refractive index.

with Ag were less efficient than those packed with uncoated silica. They attributed the loss in efficiency to the formation of the Ag^+ ion complex with the pi electrons of the double bond in the olefin. In the plots of HETP vs. flow rate for geometric isomers, the increased slope (loss in efficiency) for the cis isomer indicated that it formed a stronger complex with Ag^+ than the trans isomer. We employed benzene as the solvent to demonstrate that the strength of the Ag^+/olefinic bond complex decreases with increasing chain length. Additional treatment of the columns with Ag^+ solution did not result in an increase in partition ratio (k'), separation factor (α), or resolution (R). A less competitive solvent (i.e., benzene-hexane) could be used to increase the capacity factors and resolution of the olefinic paraffins. Although we have been able to coat and pack 25-cm x 0.94-cm (ID) Ag^+ ion columns resulting in theoretical plates >10,000 and a resultant resolution of >10 for the tetradecen-1-ol acetates, most separations do not warrant this type of column. By combining the resolving power of Ag^+ complexes with the short efficient silica columns that are now

available, researchers should be able to separate many geometrical isomers with minimal effort.

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