New Developments in Silver Resin Chromatography of *cis* and *trans* Fatty Methyl Esters[1]

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ABSTRACT

Chromatographic separations of geometric isomers of methyl octadecenoates and octadecadienoates on a silver-treated macroreticular resin are much improved with newer resins of greater surface area and ground to a smaller particle size. *cis,cis*-Dienes can be recovered in practical elution volumes. With methanol as solvent small molecules including even radioactive methanol are retarded on the column relative to methyl esters of saturated acids.

INTRODUCTION

Some years ago a series of papers described the use of silver-treated macroreticular cation exchange resins for the chromatographic separation of *cis* and *trans* isomers of fatty methyl esters (1,2,3). The method has not been generally used probably for two reasons: first, the unavailability of the resin—an experimental macroreticular resin with greater surface area than commercial resins; and second, the development of alternative methods of separation on silver nitrate-silicic acid columns, many variations of which have been used since they were first proposed by deVries (4).

Houx et al. (5) and Warthen (6) have used similar procedures with different resins to separate geometric isomers of alken-1-ol acetates, and other columns which can be used repeatedly have been reported with silver nitrate and silver halides on silica (7,8). Also, reverse-phase chromatographic systems have been reported which separate *cis* and *trans* fatty acids and esters on the basis of difference in partition coefficients rather than the complexing ability of their double bonds (9,10,11,12).

We have used the resin column technique frequently and believe it does have advantages; including, stability of the column which can be used many times; the fact that silver ion is not present in the eluant; and the fact that since solvent programming is not necessary the elution of components from the column can be monitored by a differential refractometer.

A macroreticular resin of greater surface area than the original experimental resin and giving even better separations has since been obtained, and this paper describes some of our results with this improved resin.

EXPERIMENTAL PROCEDURES

The apparatus was like that described previously (1) except that an improved differential refractometer (Water Associates Model R403) was used. The resin, Rohm and Haas Amberlite XE-284, was washed in a beaker with toluene, acetone, and water; then it was transferred to a 3.5-cm ID column and treated with 0.4 M silver nitrate solution. In this process effluent from the column is first acid and free of silver ion, then silver ion breaks through; finally when the resin is saturated, the effluent becomes neutral and silver ion is still present. Excess silver ion is washed out with water and the water is followed by methanol. The treated resin was transferred with methanol to a 185 cm x 1.1 cm ID column for use.

The Amberlite XE-284 resin as received was 20-40 mesh. To obtain a smaller particle size, a portion of the resin was ground in a mortar, and a larger portion was ground in a ball mill. Both were sieved and about half was recovered in the 100-200 mesh range while most of the rest was lost as fines. These resins were treated with silver nitrate as before. Resin from the mortar was used to fill a 65 cm x 1 cm ID column and that from the ball mill to fill a 55 cm x 2.2 cm ID column.

In the experiments with radioactive methanol, microcurie amounts of ^{3}H-labeled methanol alone and in mixture with ^{14}C-labeled methanol were added to the solution of methyl esters in unlabeled methanol. The radioactive solutions were chromatographed on the 185 cm x 1.1 cm ID column. ^{3}H-[Specific activity (S.A.) = 128 mCi/mmol)] and ^{14}C[(S.A. = 9.2 mCi/mmol) labeled methanol was obtained from Amersham/Searle Corporation (Chicago, IL). Methyl esters were monitored as usual by the refractometer. The eluate was collected serially in a fraction collector; aliquots from each fraction were mixed with 10 ml of scintillation solution (7.0 g of 2,5 diphenyloxazole per liter of toluene). Single isotope and dual isotope assays were performed using a Beckman three-channel LS-250 liquid scintillation counter. Discriminator window iso-sets were adjusted to give a counting efficiency of 80% for ^{14}C and 40% for ^{3}H with less than 8% overlap of the ^{14}C-channel into the ^{3}H-channel. The counter was interfaced to a Mod Comp real time computer programmed to calculate the disintegrations per minute (DPM) based on duplicate sample counting.

RESULTS AND DISCUSSION

All of the resins with which we have obtained useful separations have been macroreticular arylsulfonic acid resins. Gel type resins have given too little resolution for practical purposes. In the previous publication (1) it was concluded that improved separations with Amberlyst XN-1005 as compared with Amberlyst 15 were associated with the greater surface area of the former, i.e., 122 m^2/g compared to 42.5 m^2/g for Amberlyst 15. For Amberlite XE-284, the resin used here, a value of 570 m^2/g has been

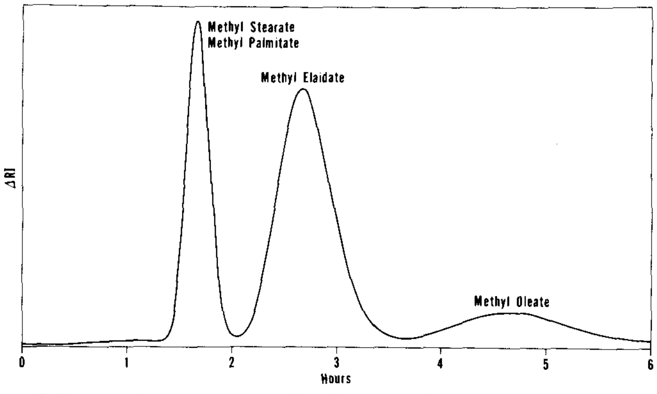

FIG. 1. Chromatogram of 100 μl methyl stearate, palmitate, elaidate, oleate mixture on silver XE-184 185 cm x 1.1 cm ID column. 0.98 ml methanol per minute. Refractometer attenuation 32X.

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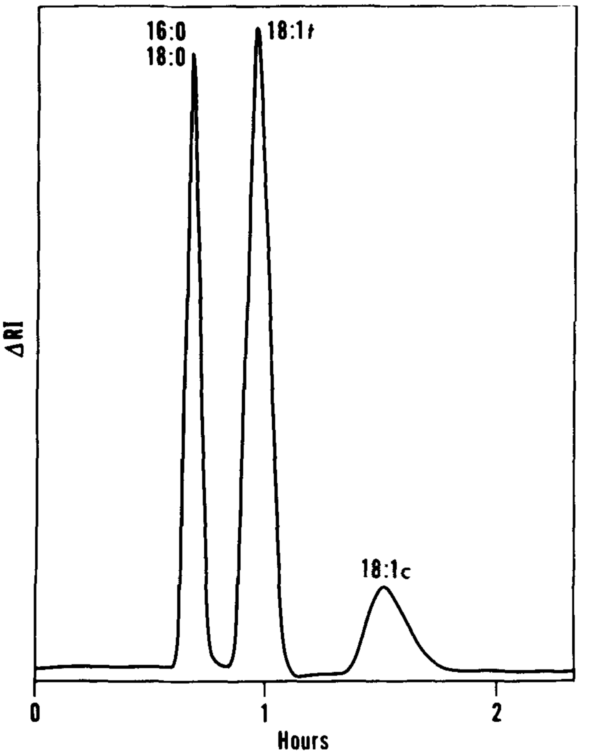

FIG. 2. Chromatogram of 25-μl methyl stearate, palmitate, elaidate, oleate mixture on 100-200 mesh silver XE-284 65 cm x 1 cm ID column. 0.98 ml methanol per minute. Refractometer attenuation 32X.

published (13). This is over 4.5 times the area for XN-1005. Amberlyst XN-1010 is said to be a dehydrated form of the same resin. For our use with methanol as solvent we would expect them to be equivalent.

Methyl Ester Separations

Figure 1 shows a separation on the original unground resin for 100 μl of a methyl stearate, palmitate, elaidate, and oleate mixture. The improved separation as compared with XN-1005 (1) is evident, especially between saturates and elaidate where a resolution of 1.2 was obtained. Samples as large as 0.4 ml have been chromatographed with little loss of resolution.

Good separations were also obtained of unconjugated *cis,trans* and *trans,trans* methyl octadecadienoates. On the 185 cm x 1.1 cm ID column, the *trans*-9,*trans*-12 isomer eluted as 325 ml and *cis*-9*trans*-12 at 675 ml while some oxidized or polymerized material came off at 140 ml.

Conjugated esters are well separated with methyl *trans*-9,*trans*-11 octadecadienoate eluted at 167 ml, the *cis*-9,*trans*-11 isomer at 250 ml, and the *cis*-9,*cis*-11 isomer at 380 ml.

As with the XN-1005 resin unconjugated *cis,cis* octadecadienoates are very strongly bound. We previously stated that *cis,cis* diene was not eluted from the XN-1005 resin (1). However, with the improved refractometer methyl linoleate has been seen to be eluted as a strongly retained broad low band. With the original XE-284 resin, 100 μl of methyl linoleate was eluted from the column as a broad band extending from about 1300 to 2400 ml with maximum at 1780 ml. Methyl *cis*-9,*cis*-15 octadecadienoate is retained even more strongly. It comes off as a broad band barely distinguishable from baseline between 2500 and 6000 ml. These elution volumes are much greater than we would predict from those of the monoenes. However, with smaller particle size XE-284 practical separations with *cis,cis* dienes were obtained.

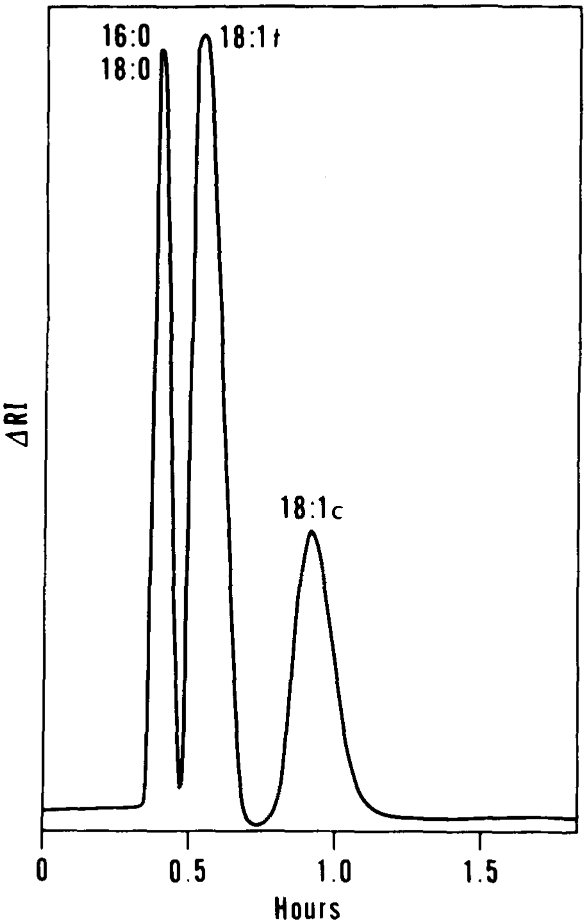

FIG. 3. Chromatogram of 957-mg methyl stearate, palmitate, elaidate, oleate, mixture on 100-200 mesh silver XE-284 55 cm x 2.2 cm ID column 5.4-ml methanol per minute. Refractometer attenuation 128X, nonlinear range.

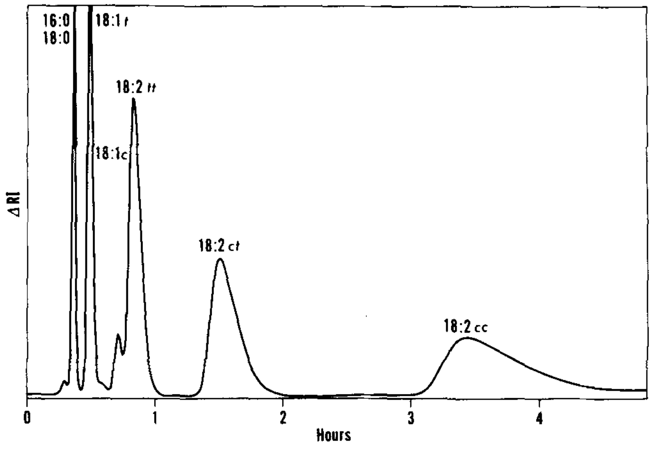

FIG. 4. Chromatogram of 20-μl methyl stearate, palmitate, elaidate, oleate mixture and 20-μl each of 9-*trans*, 12-*trans*, 9-*cis*,12-*trans*, and 9-*cis*,12-*cis* methyl octadecadienoates on 100-200 mesh silver XE-284 65 cm x 1 cm ID column. Methanol (1.94 ml) per minute. Refractometer attenuation 32X.

It is generally known that in high pressure liquid chromatography, height equivalent to a theoretical plate (HETP) is decreased and resolution in increased with smaller solid phase particles. The same principles hold here although with the lower pressures, particle size is more limited by pressure drop through the column. This is evident in columns prepared with a 100-200 mesh material from XE-284 as described in the experimental section.

Figure 2 shows a separation of the stearate, palmitate, elaidate, oleate mixture on the 65 cm x 1 cm ID column under our optimum conditions. Better separation than with the original XE-284 resin was obtained in 2 hr instead of 6. Good separations are still obtained with as much as 200 μl sample and 2 ml/min methanol flow. Figure 3 shows separation of 957 mg of the mixture in a larger 2.2-cm ID column in slightly over 1 hr. Separation in Figure 3 may actually be better than is apparent because tops of high peaks trend to be shortened in the nonlinear range of the refractometer.

On columns of 100-200 mesh particle size elution of *cis,cis* dienes is also improved to the extent that useful separations are obtained. Figure 4 shows a separation of isomeric monoenes and dienes on the 65 cm x 1 cm ID column. Although methyl oleate and methyl *trans,trans* octadecadienoate overlap, methyl linoleate is eluted in 400 ml and good separation of *trans,trans, cis,trans*, and *cis,cis* dienes is obtained. On the same column methyl *cis*-9*cis*-15 octadecadienoate was eluted in 780 ml in a broad but still distinct band. Another comparison of the columns is their values for height equivalent to a theoretical plate (HETP). For the chromatogram in Figure 1 with the original resin for saturates HETP is 0.82 cm, for elaidate 1.68 cm. For the chromatogram in Figure 3 with 100-200 mesh resin for saturate HETP is 0.11 cm, for elaidate 0.17 cm. For the larger 55 cm x 2.2 cm ID column with 100-200 mesh resin a 50-μl sample with 3.82 ml per min solvent flow and with the refractometer in a linear range, 16X, HETP for saturates was 0.08 cm for saturate and 0.16 cm for elaidate.

Retention Volume of Nonretained Component

To attempt to better understand the large retention volumes for unconjugated *cis,cis* dienes, and because the value is necessary in any theoretical study of the behavior of a chromatographic system, chromatograms were run to determine the retention volume for a nonretained component, Vn, on this 185 cm x 1.1 cm ID column. The value is generally found by passing a sample closely related to the eluting solvent rhough the column and assuming it is not retained (14). However, with methanol as solvent, both water and ethanol were found to be retained longer than methyl stearate or palmitate. Because of this unexpected behavior, retention volumes for a number of compounds including short-chain alcohols and esters were determined and are listed in Table I.

Halásaz (15) has suggested the value may be obtained by measuring the retention of the radioactive labeled molecules of the eluent itself. Following this approach tritiated methanol together with the methyl stearate, palmitate, elaidate, oleate mixture was chromatographed. Contrary to expectations, the radioactive methanol was retained longer than methyl stearate or palmitate. To eliminate the possibility of an isotope effect, this result was confirmed by other runs using a mixture of ^{3}H- and ^{14}C-labeled methanol. Two radioactive peaks were obtained for both ^{3}H and ^{14}C methanol as listed in Table I. The effect was not investigated further and no explanation is offered other than that the larger retention volume for both ^{3}H and ^{14}C methanol may be caused by an adsorption or gel permeation effect.

TABLE I

Average Retention Volumes of Various Substances on 185 cm x 1.1 cm ID XE-284 Column with Methanol as Solvent

Substance	Retention volume (ml)
Water	160
^{3}H and ^{14}C methanol	123,212
Ethanol	121
Butanol	121
Octanol	114
Decanol	112
Tetradecanol	106
Hexane	108
Methyl formate	130
Methyl propionate	115
Methyl hexanoate	110
Methyl octanoate	106
Methyl nonanoate	107
Methyl decanoate	98
Methyl laurate	104
Methyl palmitate, stearate mixture	99
Methyl arachidate	102
Methyl elaidate	156
Methyl oleate	271

Greater retention volumes approaching a constant value as chain length increases are found for short chain methyl esters and alcohols. Hexane is also retarded on the column. Similar results for water and ethanol were found on a 100-200 mesh XE-284 and an Amberlyst 15 column. Water, ethanol, and hexane were also retarded on an XE-284 column with potassium ion instead of silver. The fact that the longer chain saturated methyl esters approach the same retention volume and that this is the smallest retention volume found suggests that it is the correct Vn.

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