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Effect of Film Thickness on the Selectivity
of Cyanosilicone Capillary Columns

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Abstract. Data presented herein show that the film thickness of a polar stationary phase, SP-2380, affects column selectivity under isothermal analysis conditions. The expected linear relationship between film thickness and solute capacity factors (k), as predicted by the partition coefficient equation, was confirmed experimentally. However, selectivity, as determined by ECL values for the fatty acid methyl esters, does not remain constant. As the film thickness is increased from 0.10 μm to 0.20 μm, 0.30 μm, and 0.50 μm, there is an increase in effective column polarity. This increase mimics the polarity shift seen when comparing a 90% *bis*-cyanopropyl/10% cyanopropyl phenyl polysiloxane capillary column to a 100% *bis*-cyanopropyl polysiloxane column of the same film thickness (0.20 μm) under isothermal conditions. The increase in selectivity over the film thickness range studied is revealed by ECL values for an unsaturated C18:3 ester relative to an unsaturated C20:1 ester and saturated esters, C18:0(methyl stearate), C20:)(methyl arachidate), and C22:0(methyl behenate). As the film thickness increased, the C18:3 ester eluted farther from the C20:0 ester and changed from eluting before the C20:1 ester to eluting after the C20:1 ester and closer to the C22:0 ester.

The effective polarity increase also reduced the retention time for all of the saturated FAMEs, compared to their respective unsaturated counterparts. Possible causes of the differences in selectivity among these columns include a lessening of the surface adsorption and increased partitioning with the thicker stationary phase film.

Key words: *capillary gas chromatography, cyanosilicone stationary phase, stationary phase film thickness, selectivity, fatty acid methyl ester analysis, equivalent chain length values*

INTRODUCTION

Increasing the stationary phase film thickness in a capillary column provides the analyst with a method of increasing the resolution of components. Columns coated with nonpolar and intermediate polarity phases have been widely used to demonstrate this benefit. The increased resolution has been associated with minimal to no apparent changes in column selectivity. There is concern, however, about the effects on column selectivity with increased film thickness of a highly polar stationary phase, such as a cyanosilicone. Some authors have addressed this topic. Supina noted that the change of effective polarity with change in concentration of a stationary phase is important, but has received little attention [1]. This effect is more pronounced with the more polar stationary phases. Ackman discussed the effects of both temperature and stationary phase concentration on peak shifting and overlap [2]. Grob also stated, when discussing the capillary column film thick-

ness/varying retention relationship that a column with a different film thickness of the same stationary phase is basically a different column [3].

Recent work by Berezkin and Korolev [4], examining SP-2380 capillary columns, and Bemgard and Blomberg [5,6], from their work on other cyanosilicone capillary columns, addressed the influence of adsorption phenomena on the retention index values of various nonpolar and polar compounds. However, these reports did not discuss the selectivity of the columns for various saturated and unsaturated fatty acid methyl esters, a common sample type routinely analyzed on these highly polar columns.

We present data to show the effect of increasing film thickness of a highly polar cyanosilicone stationary phase, SP-2380, on the analysis of fatty acid methyl esters (FAMEs). Columns with phase ratios of 625, 313, 208, and 125 were prepared and evaluated using a rapeseed oil FAME sample under isothermal temperature conditions. Selectivity differences for the columns, based on the elution pattern and equivalent chain length (ECL) values for the FAMEs, are discussed to demonstrate the effect of film thickness.

EXPERIMENTAL

Columns. Capillary columns coated with 0.10, 0.20, 0.30, and 0.50 μm films of the SP-2380 phase, a proprietary cyanosilicone phase from Supelco, Inc. [7], were prepared using standard methodology. All columns were 15 meters long with internal diameters of 0.25 mm. Eight columns of each film thickness were evaluated.

Chromatographic conditions. All analyses were performed isothermally at 180°C. Helium carrier gas was used at a linear velocity of 25 cm s^{-1}. Detection was by flame ionization, and the injector and detector temperatures were 250°C and 260°C, respectively. Split injection was used with a 100:1 split ratio. Duplicate 0.5 μL injections of the rapeseed oil standard (25 mg mL^{-1}, Supelco) were made on each column. Rapeseed oil was chosen as the standard because it contains a homologous series of even-numbered fatty acids ranging from C14:0 to C24:0, along with various unsaturated fatty acids ranging from the monoenoic, C18:1, to the trienoic, C18:3. This range of fatty acids is fairly typical of many lipid-containing samples.

Equivalent chain length calculation. ECL values for the unsaturated fatty acid methyl esters were calculated according to the following formula [8-10]:

$$ECL\,(x) = z + 2\,\frac{\log t_r\,(x) - \log t_r\,(z)}{\log t_r\,(z+1) - \log t_r\,(z)} \quad (1)$$

where ECL(x) is the equivalent chain length of the FAME of interest, x; z is the carbon number of the normal saturated FAME eluting prior to x; log t_r(x) is the log of the adjusted retention time of x; log t_r(z) is the log of the adjusted retention time of FAME z; log t_r(z + 1) is the log of the adjusted retention time of normal saturated FAME z + 1, the next member of the homologous series after z and which elutes after x. Retention time differences are multiplied by 2 because the homologous series of FAMEs in rapeseed oil is an n + 2 series (C14, C16, *etc.*).

RESULTS AND DISCUSSION

Film thickness effects of partition coefficient. Chromatographic theory predicts that the relationship between increases in stationary phase film thickness (for a given column diameter) and component capacity factors is linear under isothermal conditions. This relationship is defined by the partition coefficient, which is a constant for a solute analyzed on one stationary phase at one analysis temperature [11]. In mathematical terms

$$K = k\beta \quad (2)$$

where K is the partition coefficient, k is the capacity factor, and β is the phase ratio. Therefore, at one temperature, k for a component will change proportionally with a change in β, because K is a constant.

The data presented in Table I and Figure 1 demonstrate the linear relationship between stationary phase film thickness and the capacity factor for a solute (C24:0, methyl tetracosanoate), as predicted by the partition coefficient equation. The coefficient of determination (R^2) for these data was 0.9999. Similar values were calculated for correlations between film thickness and capacity factor for other FAMEs in the rapeseed oil test mix. These data show that as the film thickness is increased, capacity factors increase linearly. Therefore, the data fit well with chromatographic theory.

Although the partition coefficient, K, is based on a linear relationship between film thickness and the capacity factor, the K value for the columns is not constant. The products of kβ for the 0.10, 0.20, 0.30, and 0.50 d$_f$ columns are 3,425, 2,570, 2,317, and 2,119, respectively, as determined using the nominal column diameters and film thicknesses,

and the absolute k values listed in Table I. These values indicate that more than one retention mechanism might be operating within these columns. Furthermore, the line in Figure 1 does not pass through the origin, also possibly indicating that other retention mechanisms in addition to partitioning are active in these columns.

Retention in these columns may reflect both partitioning and adsorption. Partitioning occurs in the bulk of the stationary phase, while adsorption occurs at the gas/liquid interface. The relative contribution of partitioning is proportional to the volume of the stationary phase and decreases with decreases in film thicknesses. This is the relationship demonstrated in Table I. The effect of surface adsorption, however, is proportional to the surface area of the stationary phase. Therefore, as the stationary phase film thickness is changed, adsorption will remain fairly constant. Thus, with changing film thickness the relative contribution of adsorption to the total retention mechanism changes. With thinner films of stationary phase, the relative contribution of adsorption is greater than with thicker film columns. This would explain some of the shifts in the kβ products measured for these columns.

Film thickness effects on effective column polarity. The selectivity of a capillary column for analysis of fatty acid methyl esters can be expressed numerically by calculating equivalent chain length (ECL) values for a variety of unsaturated FAMEs. The effect of film thickness on the selectivity of a particular stationary phase can be examined by comparing the changes in the ECL values with increasing film thickness. Table II lists mean ECL values for various unsaturated FAMEs determined on the SP-2380 phase at each of the four film thicknesses.

The increase in the ECL values for each of the unsaturated FAMEs in Table II indicates that as the film thickness of the SP-2380 phase is increased,

Table I. *Linear relationship between stationary phase film thickness and solute capacity factor.*

Film thickness (μm)	Mean k value (C24:0 FAME)
0.10	5.48
0.20	8.21
0.30	11.14
0.50	16.95
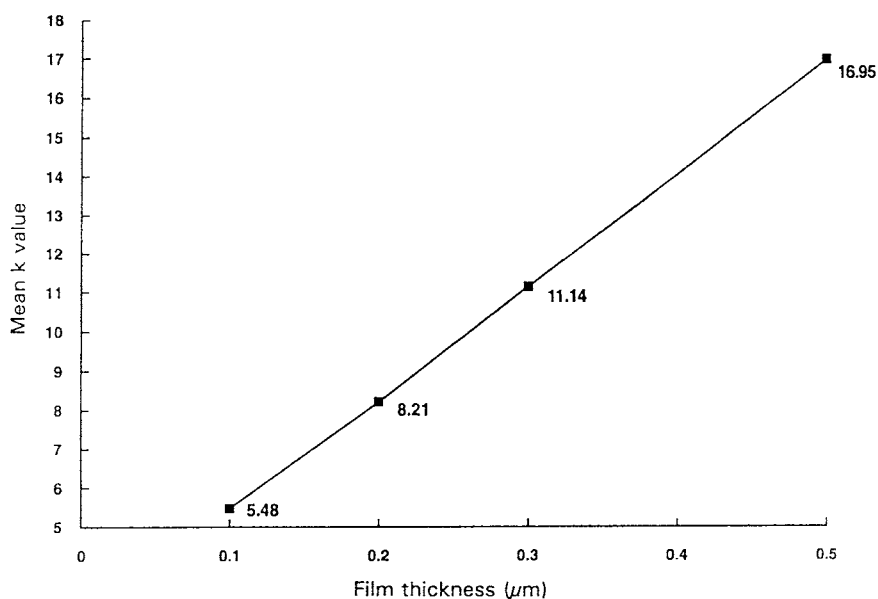
Figure 1. *Linear relationship between stationary phase film thickness and solute capacity factor.*

there is an increase in the selectivity of the capillary column for FAMEs analysis. As film thickness is increased, under the given set of analytical conditions, the magnitude of the ECL values would shift, but similar changes in the selectivity of the columns would be expected. The effect of analysis temperature on the ECL values for highly polar SP-2380 capillary columns was presented in a previous report [12].

Two key probes which demonstrate the increase in column selectivity are the C18:3 (methyl linolenate) and C20:1 (methyl eicosenoate) FAMEs. On the 0.10 μm columns, the C18:3 FAME eluted prior to the C20:1 FAME. Mean ECL values for these FAMEs were 20.17 and 20.43, respectively. As the film thickness increased, mean ECL values for each of these FAMEs also increased, but the increase was greater for the C18:3 FAME. At a film thickness

of 0.30 μm, mean ECL values for the two FAMEs were identical, indicating that the FAMEs coelute under this set of analytical conditions. On columns with a 0.50 μm phase film, the mean ECL value for the C18:3 FAME exceeded that for the C20:1 FAME. The elution order of the two probes was reversed, with the C18:3 FAME now eluting last.

Figure 2 presents plots of the stationary phase film thickness *vs.* mean ECL values for the C20:1, C18:2 (methyl linoleate), and C18:3 FAMEs. The shape of each line is characteristic. Mono-enoic, dienoic, and trienoic FAMEs of other carbon numbers fit the same patterns. The cross-over of the C18:3 and C20:1 plots also demonstrates graphically the changes in selectivity with increasing phase film thickness.

Figure 3 shows representative chromatograms for the rapeseed oil FAME analysis on columns of each film thickness. These chromatograms effectively depict how column selectivity for the FAMEs analysis increased with increasing film thickness. Interestingly, the increase in selectivity mimics the shift in polarity found when increasing the percentage of the cyanopropyl functionality in the stationary phase polymer backbone [7]. In a previous paper, we showed that an SP-2330 stationary phase capillary column (β = 313), which contains 90% *bis*-cyanopropyl phenyl polysiloxane substitution in its polymer backbone, elutes the rapeseed oil FAMEs in a pattern similar to that of the 0.10 μm SP-2380 columns (β = 625) studied in this report. A column coated with a 0.20 μm film of a 100% *bis*-cyanopropyl polysiloxane stationary phase, SP-2340 (β = 313), elutes the FAMEs in a pattern similar to the 0.50 μm SP-2380 columns (β = 125) studied here. Potential causes of selectivity shift include increased dipole effects between the stationary phase and the solute, and reduced surface adsorption in the columns with a thicker stationary phase.

Table II. *Mean ECL values increase with increases in phase film thickness (SP-2380 cyanosilicone phase).*

Film thickness (μm)	FAME and mean ECL value				
	C18:1	C18:2	C18:3	C20:1	C22:1
0.10	18.60	19.29	20.17	20.43	22.39
0.20	18.70	19.51	20.51	20.52	22.52
0.30	18.71	19.61	20.65	20.65	22.59
0.50	18.75	19.70	20.79	20.68	22.66
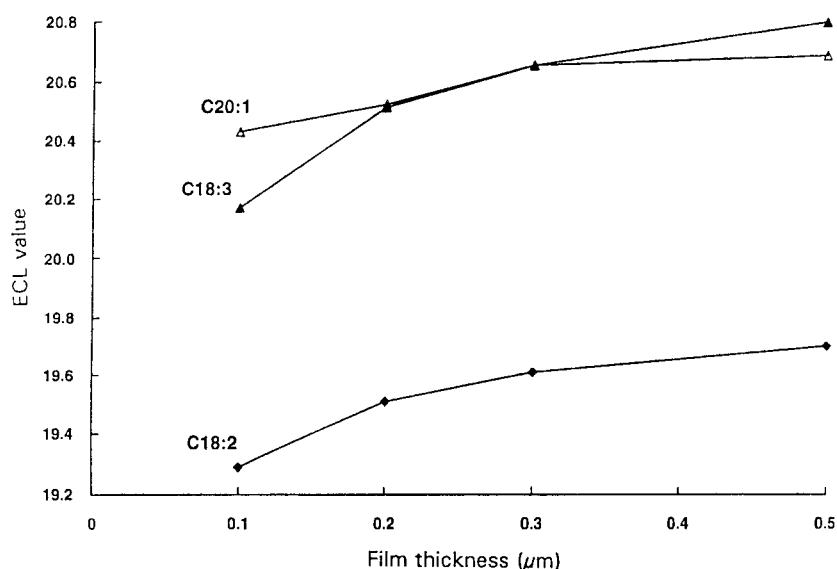
Figure 2. *Selectivity changes with increasing film thickness.*

ACKNOWLEDGMENT

REFERENCES

1. W.R. Supina, *The Packed Column in Gas Chromatography* (Supelco, Bellefonte, PA, 1974), p. 85.
2. R.G. Ackman, *Lipids* **2**, 502 (1967).
3. K. Grob and G. Grob, *Chromatographia* **17**, 484 (1983).
4. V.G. Berezkin and A.A. Korolev, *J. High Resolut. Chromatogr.* **12**, 37 (1989).
5. A. Bemgard and L. Blomberg, *J. Chromatogr.* **437**, 37 (1989).
6. A. Bemgard and L. Blomberg, *J. Chromatogr.* **502**, 1 (1990).
7. L.M. Sidisky, P.L. Stormer, L. Nolan, M.J. Keeler, and R.J. Bartram, *J. Chromatogr. Sci.* **26**, 320 (1988).
8. J. Krupcik, J. Hrivaak, and J. Janak, *J. Chromatr. Sci.* **14**, 4 (1976).
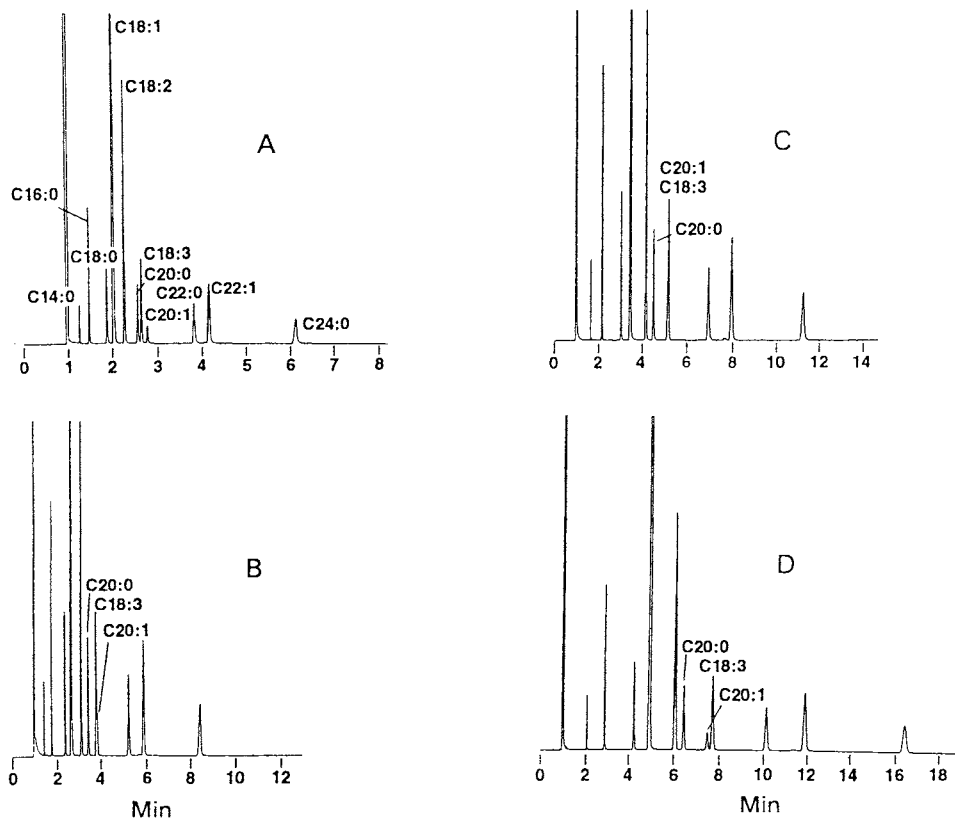
Figure 3. *Column selectivity for FAMEs analysis changes with increasing phase film thickness. (A) 0.1 μm dᵧ, (B) 0.2 μm dᵧ, (C) 0.3 μm dᵧ, (D) 0.5 μm dᵧ*

9. F.P. Woodford and C.M. van Gent, *J. Lipid Res.* **1**, 188 (1960).
10. T.K. Miwa, K.L. Mikolajczak, F.R. Earle, and I.A. Wolfe, *Anal. Chem.* **32**, 1739 (1960).
11. L.S. Ettre, *Basic Relationships of Gas Chromatography* (Perkin Elmer, Norwalk, CT, 1977), p. 14.
12. L.M. Sidisky and H.J. Ridley, *J. High Resolut. Chromatogr.* **3**, 191 (1991).