Viscosity-Structure Relationship of Dilute Triglycerides' Solutions. Correlation to Retention Time in Reversed-Phase Liquid Chromatography

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The dynamic viscosity (η) of dilute solutions of the triglycerides triolein, trilinolein, trimyristin, tristearin and tripalmitin in benzene at temperatures in the range of 25–37°C can be expressed in terms of the viscosity of the solvent (η_0), the triglycerides' concentration (C) and structural characteristics, such as the length of the carbon chains (CN) and the number of double bonds (DB). The simple empirical equation $\ln \eta = k_0 + k_1 \ln \eta_0 + k_2\ CN + k_3\ DB + k_4\ C$ satisfactorily describes (within the experimental error of 0.002 cp) the solution viscosity of triglycerides in *p*-xylene when using the coefficients derived from the benzene solutions. In addition, a relation is derived extending the application of the above-mentioned empirical equation to multicomponent dilute solutions. This last one describes the dilute solution viscosity of natural oils in benzene and agrees with the experimental values. Furthermore, the triglycerides that have equal partition numbers in reversed-phase liquid chromatography (RPLC) exhibit equal values for the solution viscosity. This relationship is similar to the equation expressing the retention time of RPLC in terms of the structure of the solute. Hence, it is suggested that the shape of the solute, which is a significant factor for the solution viscosity of triglycerides, also plays an important role in the retention mechanism of RPLC.

KEY WORDS: Dilute solution, mechanism of retention, reversed phase, structure, triglycerides, viscosity.

The viscosity of oils in the pure state has been the subject of many studies, particularly in the older literature (1). The solution viscosity of fatty acid esters of glycerol has received attention in the literature mainly in conjunction with solution viscosity of oils and fats (2–6).

The effect of composition on low-temperature liquid viscosity cannot be estimated with any accuracy if only the pure-component properties are available (7). The solution viscosity is quite sensitive to the structure of the liquid, which depends on composition. For a solution following McAllister's approach (8) (which adopted the semitheoretical Eyring approach), a solute molecule may interact predominantly with a solvent molecule, less frequently with another solute molecule and rarely with a combination of a solute and a solvent molecule. Moreover, the interaction could be taken as a binary type or, more realistically, as a three-body type, four-body type, etc. It is inferred from McAllister's analysis that the only applicable possibilities for dilute solutions up to 10 g/L is the solvent-solvent and solute-solvent interactions. The dilute-solution viscosity has, in general, been used rather extensively to study changes in size and structure in polymeric systems *via* the measurement of intrinsic viscosity, which is a measure of the hydrodynamic volume (or size) of a molecule. The intrinsic viscosity $[\eta]$ is linked (9) to the radius of gyration, R, by the relation $[\eta] = kR^A$. So the intrinsic viscosity includes size and shape information about solute molecules.

It is generally believed that in reversed-phase high-performance liquid chromatography a significant part of the driving force for solute transfer arises from the nature of the pair interactions among neighboring solute and solvent molecules (10). Due to the weak interaction of solute with the stationary phase, the stationary phase historically has been given less attention than the mobile phase in describing reversed-phase retention (11,12). Any attempt to establish a mechanism for solute retention and selectivity in reversed-phase liquid chromatography (RPLC) requires consideration of the nature and of the role of the stationary phase, the mobile phase and their interface. Such an understanding is crucial for informed control and manipulation of liquid chromatography (LC) separations (13).

Many investigators (14–17) have reported that the elution of glyceride molecular species on RPLC is controlled by a structural parameter called partition number (PN). This is defined as PN = CN − 2DB, where CN is the total acyl carbon number and DB is the total number of double bonds in the molecule. PN is restricted only under certain defined analytical conditions. The basic relation between the logarithms of the retention times (t_R) and PN of the saturated triglycerides (by definition equal to the carbon number) is linear (18),

$$\ln t_{P_{sat}} = kCN + a \qquad [1]$$

where k and a are constants. Subsequent advances in column and instrument technology have enhanced LC performance in separating triglycerides with the same PN according to equivalent carbon number (ECN) (18–21).

The effect of different parameters (length of hydrocarbon, type of stationary phase, polarity of solvent, temperature, sample diluent) in triglyceride separation by RPLC was studied by several researchers (18,20,22,23). Various mechanisms for triglyceride solute retention (19,23) on reverse-phase columns have been proposed in conjunction with the various parameters of mobile and stationary phases by using the framework of partitioning, adsorption and solvophobic theories.

The present investigation proposes two generalized empirical relationships for calculating viscosities of dilute solutions of triglycerides and oils in benzene and *p*-xylene from molecular characteristics. To our knowledge, there is no equation relating viscosities of dilute solutions of triglycerides to molecular structure of the solute. A generalized empirical relationship has been produced by Duff and Prasad (24) describing the viscosity of fatty oils (expressed as $\ln \eta$) in terms of the iodine value, the saponification value and the temperature. In an effort to gain further insight into the mechanism of solute retention, an attempt was made to correlate the viscosity of selected triglycerides with the retention time in RPLC. Because the binary solute-solvent viscosimetric studies convey information about size and shape of solute into this particular solvent, these can be useful for quantifying triglyceride-solvent interactions in RPLC: hence the correlation between viscosity of dilute solutions of triglycerides and the retention time of high-performance liquid chromatography (HPLC).

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EXPERIMENTAL PROCEDURES

Materials. Analytical-grade triglycerides at better than 99% purity were as follows: tristearin, tripalmitin (Sigma Chemical Co., St. Louis, MO), trimyristin (Roth GMBH, Karlsruhe, Germany) and triolein (Fluka, Buchs, Switzerland). The triglycerides trilinolein (Fluka) and tripalmitolein (Sigma) were of 98% quality. Cottonseed, olive and corn oils were purchased locally. Solvents used for the viscosity measurements were benzene (Ferak GMBH, Berlin, Germany; purity > 99.7) and *p*-xylene (Fluka; purity > 99.5) without further purification.

Equipment. An automated Schott Geräte Viscosimeter (Model Series AVS 300) equipped with an Ubbelohde-type microcuvette (k = 0.009533) of capillary diameter of 0.40 mm was used for the viscosity measurements. The temperature bath was a Schott Geräte (Model CT1150) (BMGH, Hofheim, Germany) connected to a Schott Geräte (Model CK100) cooling device. The temperature was maintained constant to 0.01°C. Density measurements were made with an Anton Paar Densitometer (model DMA 40) (KG, Graz, Austria).

The preparation of methyl esters of fatty acids of oils was carried out by the Morrison method (25). The analysis of methyl esters was carried out on a Varian 3700 gas chromatograph equipped with a dual-flame ionization detector (Varian Associates, Palo Alto, CA). The column was a DEGS 15% on Chromosorb W 80–100% mesh.

RESULTS AND DISCUSSION

In a previous study (26) the following functional relationship between dynamic viscosity (η), temperature (T) and the molar fraction of each solute triglyceride (x_1) was assumed:

$$\eta(T,x_1)/cP = F_2(x_1)e^{F_1(x_1)/(T/K)} \qquad [2]$$

$F_1(x)$ and $F_2(x)$ are defined as follows:

$$F_1(x_1) = 10^{-6}\sum_{i=0}^{4}(a_i\, x_1^{\,i}) \qquad [3]$$

$$F_2(x_1) = \sum_{i=0}^{4}(b_i\, x_1^{\,i}) \qquad [4]$$

Hence, for the five triglycerides studied, 50 parameters were required for a description of the solution viscosity to reproduce the experimental level of accuracy for a particular solvent.

An alternative approach for describing the solution viscosity (η) of a triglyceride with reference to the solvent viscosity (η_0) and the structural formula of the solute triglyceride can be used. A simple empirical equation is derived to relate the logarithm of the solution viscosity (ln η) of a triglyceride to its molecular formula. Only five parameters are used for describing the dilute-solution viscosity for all triglycerides in one particular solvent.

Table 1 contains the results of the measurements of dilute solutions in benzene for trimyristin (MMM), tristearin (StStSt), tripalmitin (PPP), triolein (OOO), trilinolein (LLL) at 25, 30, 32, 35 and 37°C, respectively. The values for 25, 30 and 35°C were taken from previous work (26). The contents of Table 1 were used to produce, by means of a multiple linear regression analysis, the following equation:

$$\ln \eta = k_0 + k_1 \ln \eta_0 + k_2\,\mathrm{CN} + k_3\,\mathrm{DB} + k_4\,\mathrm{C} \qquad [5]$$

TABLE 1

Dynamic Viscosities (cp) of Dilute Solutions of a Number of Triglycerides at Various Temperatures

Triglyceride	Concentration (g/L)	CN	DB	η 25°C^a	30°C^a	32°C	35°C^a	37°C
MMM	4	42	0	0.6117	0.5706	0.5550	0.5331	0.5191
MMM	5	42	0	0.6137	0.5724	0.5568	0.5348	0.5208
MMM	6	42	0	0.6158	0.5743	0.5587	0.5367	0.5225
MMM	8	42	0	0.6199	0.5783	0.5625	0.5405	0.5262
MMM	10	42	0	0.6236	0.5819	0.5661	0.5439	0.5297
PPP	4	48	0	0.6147	0.5733	0.5577	0.5357	0.5216
PPP	5	48	0	0.6169	0.5753	0.5596	0.5375	0.5234
PPP	6	48	0	0.6192	0.5775	0.5617	0.5395	0.5253
PPP	8	48	0	0.6240	0.5820	0.5661	0.5439	0.5296
PPP	10	48	0	0.6286	0.5865	0.5706	0.5482	0.5339
StStSt	4	54	0	0.6181	0.5770	0.5616	0.5397	0.5257
StStSt	5	54	0	0.6213	0.5799	0.5642	0.5422	0.5281
StStSt	6	54	0	0.6237	0.5819	0.5662	0.5440	0.5298
StStSt	8	54	0	0.6268	0.5846	0.5686	0.5461	0.5318
StStSt	10	54	0	0.6323	0.5897	0.5736	0.5510	0.5364
OOO	4	54	3	0.6137	0.5726	0.5573	0.5355	0.5216
OOO	5	54	3	0.6157	0.5745	0.5591	0.5372	0.5233
OOO	6	54	3	0.6178	0.5764	0.5610	0.5389	0.5250
OOO	8	54	3	0.6220	0.5803	0.5647	0.5425	0.5285
OOO	10	54	3	0.6262	0.5842	0.5685	0.5462	0.5320
LLL	4	54	6	0.6132	0.5720	0.5566	0.5346	0.5208
LLL	5	54	6	0.6152	0.5738	0.5584	0.5364	0.5224
LLL	6	54	6	0.6172	0.5756	0.5602	0.5381	0.5241
LLL	8	54	6	0.6212	0.5794	0.5638	0.5416	0.5275
LLL	10	54	6	0.6254	0.5833	0.5676	0.5452	0.5311
Benzene				0.6060	0.5651	0.5497	0.5284	0.5147

^{a}Values for the dilute dynamic viscosities taken from Reference 26.

TABLE 2

Comparison Between Predicted and Experimentally Measured Dynamic Viscosities (cp) of a Number of Triglycerides at 28°C in Dilute Solutions of Benzene

	C = 5 (g/L)		C = 10 (g/L)	
	Experimental	Calculated	Experimental	Calculated
MMM	0.5884	0.5883	0.5980	0.5986
LLL	0.5898	0.5890	0.5996	0.5993
PoPoPo	0.5889	0.5886	0.5985	0.5989
PPP	0.5914	0.5915	0.6028	0.6018
OOO	0.5905	0.5919	0.6005	0.6023
StStSt	0.5958	0.5948	0.6062	0.6052
Benzene	0.5809			

The parameters k_i ($i = 0, \ldots, 4$) have the following values: $k_0 = -0.04430 \pm 0.00236$, $k_1 = 1.00043 \pm 0.00251$, $k_2 \times 10^3 = 0.94159 \pm 0.03611$, $k_3 \times 10^3 = -1.63523 \pm 0.07156$, $k_4 \times 10^3 = 3.47420 \pm 0.06603$; η_0 is the dynamic viscosity of the solvent at the corresponding temperature, CN is the total number of carbon atoms in the three fatty chains, DB is the total number of double bonds and C is the concentration of the triglyceride, ranging from 4 to 10 g/L. The correlation coefficient of Equation 5 is 0.99962.

In Figure 1 a small set of the values contained in Table 1 is shown. This figure relates the logarithm of solution viscosity of triolein in benzene to changes in temperature and concentration. The concentration dependence of solution viscosity is described by parallel lines for all temperatures.

Table 2 carries a comparison at another temperature, 28°C, of the above-mentioned triglycerides MMM, StStSt, OOO, PPP and LLL, as well as palmitolein (PoPoPo) for the concentrations 5 and 10 g/L between experimentally measured solution viscosities and predicted values from Equation 5. There is an agreement to better than 0.002 cp, which is the limit of the experimental accuracy.

A simple model based on the binary description of Equation 5 was used to provide the solution viscosity (η) values of an arbitrary mixture of a number, r, of triglycerides.

$$\eta = \sum_{i=0}^{r} x_i \eta_i \qquad [6]$$

where η_i is defined as:

$$\eta_i = \frac{[\eta_{ib} - x_{sb}\eta_s]}{[1 - x_{sb}]} \qquad [7]$$

and η is the solution viscosity of the oil in cp, x_i is the molar fraction of the i constituent triglyceride of the oil, η_{ib} is the binary solution viscosity of the i constituent triglyceride as it is calculated from Eqation 5, η_s is the solvent viscosity in cp and x_{sb} is the molar fraction of the solvent for the binary solution. Equation 6 assumes that the components of the mixture form an ideal solution. The range of applicability of Equation 6 is for very dilute solutions, usually less than 10 g/L. Table 3 contains the computed values of the solution viscosity η for cotton, corn and olive oil from Equation 5, along with the experimental values. The computed and the experimental values agree within the error of the experiment, 0.002 cp.

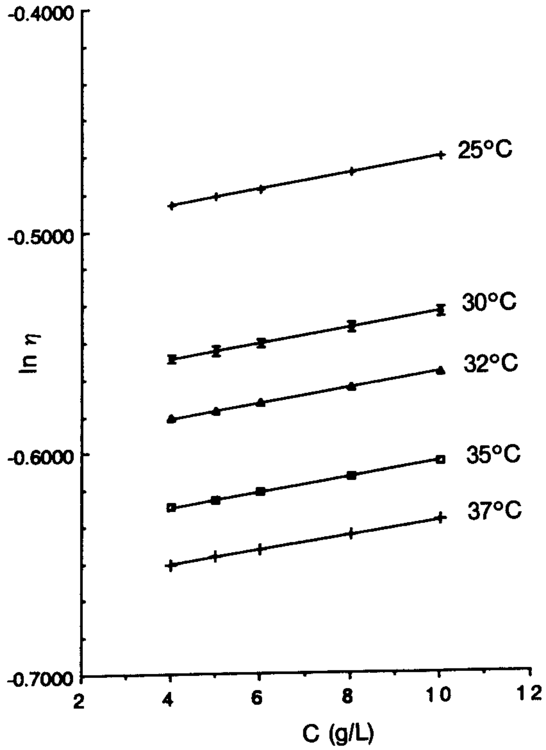

FIG. 1. The temperatures and concentrations of ln η dependence on triolein. Experimental error bars are shown for the isotherm of the 30°C.

A similar relationship for oils and constituent triglycerides is found in the work of McClements and Povey (27). They examined the ultrasonic velocity of oils and reported a reasonable agreement between the experimental and predicted values. They suggest that the derived equations appear to be suitable for relating the ultrasonic velocity of vegetable oils to their triglyceride composition. Solution viscosimetry has the added flexibility of the solvent parameter, which can be tuned so that the differentiation of the physicochemical property of viscosity can be at the practically required level. The results suggest that the solution viscosities of vegetable oils can be related to their chemical composition by simple empirical formulae. Dilute viscosimetric measurements may therefore prove useful

TABLE 3

Comparison Between Predicted and Experimentally Measured Dynamic Viscosities (cp) of Cottonseed, Corn and Olive Oils in Solution (benzene, 10 g/L) at Various Temperatures^a

	Cottonseed oil		Corn oil		Olive oil	
T (°C)	Experimental	Calculated	Experimental	Calculated	Experimental	Calculated
32	0.5694	0.5690	0.5679	0.5672	0.5689	0.5683
34	0.5545	0.5541^a	0.5530	0.5528^a	0.5538	0.5529^a
35	0.5473	0.5464	0.5458	0.5464	0.5465	0.5462
37	0.5331	0.5334	0.5316	0.5311	0.5323	0.5320

	Fatty acid composition of oils (%)^b						
	M	P	Po	St	O	L	Ln
Oil	14:0	16:0	16:1	18:0	18:1	18:2	18:3
Cottonseed	0.06	22.27	0.14	2.33	18.36	56.38	0.0
Corn	0.10	13.72	0.12	3.03	24.09	58.13	0.0
Olive	0.0	10.02	1.29	2.76	77.18	7.38	0.97

$^a\eta_{(\mathrm{Benzene})}$ 0.5354 at 34°C.

^{b}The constituent triglycerides of the oils are assumed to be simple.

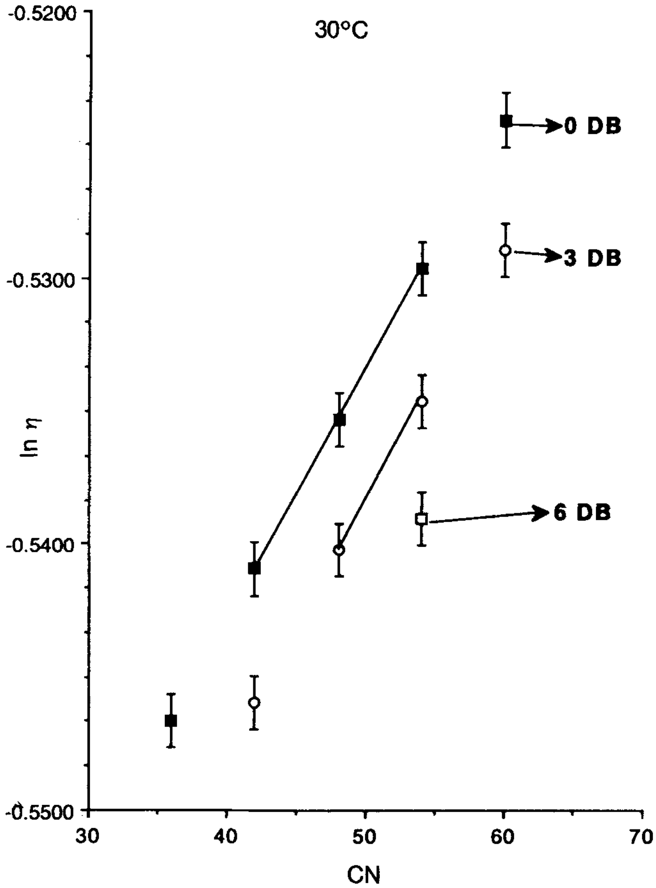

FIG. 2. The carbon number (CN) and the logarithm of the solution viscosity diagram at 30°C for the concentration of 10 g/L triglycerides in benzene, for saturated, for one double bond per chain and for two double bonds per chain.

for characterizing oils or for monitoring changes in the degree of unsaturation or chainlength of the fatty acids present.

Another interesting observation is that MMM-LLL-PoPoPo and PPP-OOO at the same concentration (g/L) and temperature have close solution viscosities in the experimental and calculated columns of Table 2, which refers to the PN equivalency of these groups of triglycerides. These findings are visualized in Figure 2, where the CN and the logarithm of the solution viscosity are shown at 30°C for 10 g/L triglycerides in benzene for saturated (DB = 0), one double bond per chain and two double bonds per chain. Experimental error bars are included. The points that are not line-connected are calculated from Equation 5, except for the value of LLL, which is experimental. Additional pairs are predicted to have the same value for ln η.

To test these observations, a set of experimental data of solution viscosities of the triglycerides MMM and LLL in another solvent is compared with the solution viscosities derived from Equation 5. Table 4 contains the solution viscosities of triglycerides MMM and LLL in another solvent, namely *p*-xylene. The experimental and the calculated [η] are in accordance within 0.002 cp. The pairs MMM-LLL showed similar behavior as far as the PN equivalence of the solution viscosity. Equation 5 can be analyzed so that the term $k_1 \ln \eta_0$ takes care of solvent-solvent interactions and the k_2 CN, k_3 DB and k_4 C terms take care of solute-solvent interactions. So, the solute-solvent interactions for benzene and *p*-xylene are almost identical, because the coefficients of the CN, DB and C are equal. The similarity of Equation 5 and the relationship between retention time-structure of triglycerides in RPLC (Eq. 1) requires some further investigation into the mechanism of retention. Dorsey and Dill (13) have reviewed the current understanding of the molecular mechanism of retention in RPLC, and their conclusion was that retention is a process of transfer of solute from a mobile-phase environment into a stationary-phase environment and, hence, that it depends on the nature of both the mobile and stationary phases. The association of the solute with the stationary phase can involve partitioning, adsorption or both. They concluded that solute partitioning should generally be the dominant mechanism for RPLC.

Moreover, under certain chromatographic conditions (very polar mobile-phase solvent and relatively long carbon-bonded-phase *n*-alkyl chains), solute distribution in RPLC appears to approach that of partitioning between two bulk liquid phases, suggesting quasi-liquid behavior

TABLE 4

Comparison Between Predicted and Experimentally Measured Dynamic Viscosities (cp) of MMM and LLL at Various Temperatures in Dilute Solutions of *p*-Xylene[a]

	C = 5 (g/L)				C = 10 (g/L)			
	LLL		MMM		LLL		MMM	
T (°C)	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.	Meas.	Calc.
25	0.6156	0.6135	0.6138	0.6126	0.6263	0.6242	0.6250	0.6233
28	0.5937	0.5918	0.5921	0.5909	0.6040	0.6021	0.6027	0.6012
30	0.5799	0.5782	0.5785	0.5773	0.5898	0.5883	0.5888	0.5874
32	0.5663	0.5647	0.5651	0.5638	0.5760	0.5746	0.5750	0.5737
35	0.5471	0.5456	0.5460	0.5448	0.5565	0.5552	0.5555	0.5543
37	0.5349	0.5334	0.5338	0.5328	0.5440	0.5428	0.5430	0.5421

[a] MMM, trimyristin, LLL, trilinolein.

at unswollen, chemically-bonded-phase *n*-alkyl chains (28). Mobility of the stationary phase is dependent upon column temperature. The phase transition due to change from "solid-like" to "liquid-like" behavior with increasing temperature has been clearly demonstrated by nuclear magnetic resonance (NMR) (29).

The assumption that RPLC is a pure, bulk-phase partitioning model is not necessarily true. Separations between polyaromatic hydrocarbon isomer molecules with the same partition coefficient often can be obtained. These experiments offer strong evidence that the bulk-phase partitioning model does not provide a fully satisfactory description of retention. Similar evidence in other systems shows that selectivity is partially dependent upon the shapes of solute molecules (30). In brief, molecules that can most effectively align with the grafted chains, normal to interface, are those that are most effectively retained. It costs more free energy to insert each solute substructure that lies parallel to the interface than each substructure that aligns with the chains normal to the interface; hence, the shape selectivity, which relates to characteristics such as length-to-width ratio and planarity of the solute.

Jinno *et al.* (31) reported a structural relationship of the solute to the mobile phase composition, specifically that a change in mobile phase composition from pure methanol to pure dichloromethane induces further nonplanarity in nonplanar solutes. This type of selectivity, *i.e.*, shape selectivity, is dependent upon the characteristics of the chemically-bonded phases. It has been found that steric considerations were crucial in the retention of rigid or planar molecules. As octadecyl phases consist of lengthy alkyl chains bonded to the silica surface, the conformation of the bonded-phase plays an important role in retention (32,33). Sander and Wise (34) studied the effect of column temperature on shape selectivity.

From the above discussion of polyaromatics, it seems that a consensus is emerging that retention in RPLC is sensitive to size and shape differences between the solute molecules. The shape of solute is subject to changes related to variations in the chromatographic conditions. Therefore, the retention time includes information about topological solute properties.

Because the binary solute-solvent viscosimetric studies include information about size and shape of solute in a particular solvent, it can be seen as a useful tool for quantifying triglyceride-solvent interactions in RPLC. "Solvent" can be considered both the mobile phase and the chemically-bonded stationary phase (28). Hence, it is proposed that a correlation exists between the viscosity of dilute solutions of triglycerides and the retention time of RPLC. We refer to Plattner's work (20), which evaluated columns for RPLC for triglyceride separation of m-Bondapak-phenyl (the stationary phase is similar to the solvent used in the present work). He found that the retentiveness of the m-Bondapak-phenyl column was not sufficient to elute triglycerides according to the ECN (20). So, retention is controlled by the PN. Plattner's reverse-phase research (20) showed that triglycerides that belong to the same class of equal PN simultaneously exhibit similar retention times. It can be assumed that similar shapes are likely obtained under these particular chromatographic conditions, in the light of the dilute-solution viscosity measurements. Plattner and Payne-Wahl (35) suspect that it might be possible to separate triolein and tripalmitin on silica columns (normal phase) with benzene, toluene or xylene in the solvent system. They found that a mixture of benzene and acetic acid (99.9:0.1) eluted triolein slightly before tristearin and tripalmitin, whereas with xylenes and acetic acid (99.9:0.1), triolein, tristearin and tripalmitin had retention volumes of 9, 10 and 11 mL, respectively. Therefore, by using these particular solvents, the retention order of triglycerides OOO and StStSt has been reversed when compared to isooctane as eluent solvent. It can be assumed that the triglycerides are sensitive to solvent influence and/or solvent changes so that they respond by modifying their shape, which is expressed as variation of the retention time. Hence, shape selectivity can be used to explain the retention mechanism of triglycerides in RPLC. In addition to the solvophobic theory, partitioning and adsorption, which have been proposed previously, shape selectivity also contributes to retention. Further work is needed to investigate the kind of relationship that exists between solution viscosity of triglycerides and polarity of the solvents, as well as the solution viscosity correlation to "flexible" solvents, namely molecules with substantial rotational degrees of freedom.

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