ELSEVIER

Journal of Chromatography A, 753 (1996) 37–45

JOURNAL OF CHROMATOGRAPHY A

Synthesis and characterization of extended length alkyl stationary phases for liquid chromatography with application to the separation of carotenoid isomers

Christine M. Bell[a,*], Lane C. Sander[a], John C. Fetzer[b], Stephen A. Wise[a]

[a]*Analytical Chemistry Division, National Institute of Standards and Technology, Gaithersburg, MD 20899, USA*

[b]*Chevron Research and Technology Company, Richmond, CA 94802, USA*

Received 19 March 1996; revised 5 June 1996; accepted 5 June 1996

Abstract

A new, long-chain (~C_{34}) alkyl-bonded stationary phase for liquid chromatography (LC) was synthesized and utilized for the separation of carotenoid mixtures. This stationary phase exhibited increased selectivity for certain *cis* isomers of α- and β-carotene as compared to the recently reported C_{30} stationary phase. The new stationary phase was synthesized using two bonding strategies: traditional polymeric synthesis and a surface-polymerization synthesis, with the latter yielding columns with improved resolution for carotenoids. A natural extract with a high degree of *cis* isomerization was well separated with this long-chain stationary phase.

Keywords: ; Stationary phases, LC; Carotenoids; Carotenes

1. Introduction

The study of carotenoids, xanthophylls and associated polyenes has intensified recently due to their nutritional and biological importance [1–10]. This class of compounds consists of numerous related compounds, including isomer sets (some differing by as little as the location of double bonds or *cis* substitution), that can be quite difficult to separate in natural samples. Separation of the individual components of such mixtures is necessary for evaluation of their respective biochemical properties. Typically, these separations have been achieved in normal-phase liquid chromatography (LC) through the use of polar adsorbent stationary phases such as calcium hydroxide [11–13] and in reversed-phase LC using nonpolar C_{18} stationary phases [14–16]. Recently, a C_{30} stationary phase was developed that gives dramatically improved resolution of carotenoids and their isomers [17–20].

This improvement in column selectivity has been attributed to the increased chain length of the C_{30} stationary phase with which the large, rigid carotenoid molecules can more completely interact. Molecular models of carotenoids and small-angle-neutron-scattering measurements indicate that the length of the phase is approximately equal to the length of β-carotene [21]. In contrast, improved column selectivity is not observed for other large, rigid molecules such as polycyclic aromatic hydrocarbons (PAHs) with dimensions less than the length of the C_{30}

*Corresponding author. Bldg. 222, Rm. B208, NIST, Gaithersburg, MD 20899, USA.

0021-9673/96/$15.00 Published by Elsevier Science B.V.
PII S0021-9673(96)00562-6

stationary phase [22]. Although the C_{30} column enables significantly improved separation of complex carotenoid mixtures compared with more conventional C_{18} columns, additional improvements were anticipated from the use of even longer alkyl ligands. To this end, we have prepared and characterized the first alkyl stationary phase with a mean length near 34 carbons.

This increase of the stationary phase length was expected to result in increased selectivity for carotenoids. In fact, this new stationary phase has shown improved separations for some carotenoid isomer sets and has shown potential in the separation of extracts of natural samples. In addition, two distinct bonding strategies – traditional polymeric and so-called "surface polymerized" – were employed in the synthesis of these stationary phases. The latter proved to provide some increased resolution even for lower coverage phases and may offer significant utility in the manufacture of these stationary phases.

2. Experimental

2.1. Materials

The long-chain α-olefin mixture was obtained from Chevron Chemical (Kingwood, TX, USA). Trichlorosilane and hydrogen hexachloroplatinate(IV) hydrate (chloroplatinic acid) were purchased from Aldrich (Milwuakee, WI, USA). Five μm particle size YMC SIL200 silica (YMC, Wilmington, NC, USA) was used in the production of bonded stationary phases. This silica has a pore size of 20 nm and a surface area of approximately 200 m^2/g. Preparation of the C_{30} column with the same silica has been previously reported [17]. Comparisons to a commercial polymeric C_{18} column were made with a Hypersil Green PAH column (Shandon HPLC, Cheshire, UK). All-*trans* α- and β-carotene were obtained from Sigma (St. Louis, MO, USA), and 15- and 13-*cis*-β-carotene were obtained from Hoffman-LaRoche (Nutley, NJ, USA and Basel, Switzerland). A β-carotene preparation containing a high percentage of *cis* isomers was obtained from Betatene Ltd. (Melbourne, Australia). HPLC-grade solvents were used in all chromatographic separations. All other materials were obtained from commercial sources.

2.2. Synthesis of C_{34} bonded phases

Silica was pretreated in two fashions. For solution-polymerized stationary phases, the silica was dried at 150°C overnight. For surface-polymerized stationary phases, dry silica was equilibrated with humid air in a procedure described previously [23].

Synthesis of the C_{34} silane mixture was as follows: 7.0 g (approximately 13 mmol) of the α-olefin mixture was melted at about 75°C. A crystal of chloroplatinic acid (~1 mg) was added and the solution stirred under N_2 for 15 min. Maintaining a N_2 purge, 1.31 ml (1.76 g, 13 mmol) of trichlorosilane was then added dropwise over 15 min and the solution stirred for an additional 25 min. Because trichlorosilane is easily hydrolyzed and water was not rigorously excluded, some HCl was generated which occasionally had to be vented when its production outpaced the ability of the nitrogen purge to remove it. The solution was then cooled, and any unreacted trichlorosilane was removed by evaporation under reduced pressure.

Next, the silane mixture was dissolved in 100 ml of xylene at 75°C. Activated carbon was added to remove most of the residual Pt catalyst. The efficacy of this procedure was not assessed, but its utility has been reported previously [24]. X-Ray fluorescence measurements indicated that some Pt was present in the final stationary phase, but it was not quantified. Based on the amount of catalyst employed, it is unlikely that enough Pt would be present in the phase to significantly affect selectivity. Trace quantities of Pt may, however, affect carotenoid recoveries (not studied in these experiments).

The carbon particles were allowed to settle and the hot solution decanted onto a slurry of silica (~2.8 g) and xylene (50 ml). For solution-polymerized stationary phases, 0.5 ml of water was then added and the solution heated to reflux under N_2 for 30 min. For surface-polymerized stationary phases, where the silica had been pre-equilibrated with water, no additional water was added. This silane-silica solution was refluxed gently under N_2 for 4 h. Following the heating period, the silica was filtered hot, washed sequentially with xylene, ethanol, water,

ethanol and pentane, and dried in air. Samples were analyzed for carbon content by Atlantic Microlab (Norcross, GA, USA), and surface coverages were calculated from equations derived by Berendsen and de Galan [25], with appropriate considerations for the polymeric nature of the phases [26]. Stationary phases were slurry packed at 62 MPa into standard 250 mm×4.6 mm I.D. columns.

2.3. Gas chromatography

Gas chromatography–mass spectrometry (GC–MS) of the olefin mixture was performed on a 30 m×0.25 mm DB5 column (film thickness 0.25 μm) with the following temperature program: initial temperature, 100°C; ramped at 5°C/min to 290°C; and held at 290°C for 120 min. The carrier gas, He, was maintained at an inlet pressure of 103 kPa. The total ion count was monitored over the 50 to 550 amu range. Identification of α-olefin peaks was achieved by comparison with standard chromatograms provided by Chevron Research and Technology.

2.4. Liquid chromatography

Separations of carotenoids were carried out using an isocratic mobile phase consisting of methanol–methyl-*tert.*-butyl ether (95:5, v/v) at a flow-rate of 2 ml/min. Columns were thermostatted at 24°C using a circulating water jacket. Carotenoids were dissolved in ethanol or hexane, and injection volumes were 5 or 10 μl depending on the concentration of the particular sample. Detection was at 450 nm. Void volumes were determined by injecting acetone, with detection at 254 nm. Resolutions were determined by measuring the peak widths at half height.

2.5. Preparation of α-carotene isomers

Solutions of isomerized α-carotene were prepared using a procedure based on that of Zechmeister [27]. A crystal of iodine (~2 mg) was dissolved in 2 ml of hexane. Two drops of this solution were added to a solution of approximately 300 μg of α-carotene in 1 ml of hexane. The resulting solution was exposed to laboratory light for 1 h. Other researchers have reported more careful controls of the catalyst concentration [11,18,28], but numerous repetitions of this procedure in our laboratory have yielded no noticeable differences in the isomer distribution.

3. Results and discussion

The stationary phases reported here were synthesized with silanes prepared from a mixture of long-chain α-olefins. A GC–MS separation of this mixture is shown in Fig. 1 along with the distribution of the major α-olefins. It is probable that there are additional higher molecular-mass components that did not elute under the GC conditions employed. The α-olefins contained in the mixture range from 28 to 42 carbons in length with a mean length of just over 34 carbons, and only even-carbon-number olefins are present. It is assumed that the silane mixture synthesized from these olefins maintains this distribution. In addition, as all of these silanes probably have similar reactivities, the resulting bonded phases are also assumed to reflect the distribution of the original mixture. These stationary phases will henceforth be referred to as C_{34}, although it should be understood that the actual phase consists of a range of lengths, the exact distribution of which is unknown. Attempts to further purify the α-olefin mixture to obtain the longer chains failed due to the high temperatures necessary for vacuum distillation and the tendency of the mixture to "crack" resulting in an enrichment of the shorter fractions. It should also be noted that direct synthesis of a longer chain silane ($\geq C_{36}$) was also pursued, however, while some progress was made, extremely poor solubility and low reactivity ultimately caused abandonment of this approach.

The olefin mixture was hydrosilylated with trichlorosilane in the presence of a trace amount of chloroplatinic acid catalyst [29–31]. After evaporation under reduced pressure of any excess trichlorosilane, the silane mixture was bonded to 5 μm silica in two fashions. In one procedure, dry silica was employed, and a measured amount of water (0.5 ml) was added to the reaction mixture in xylene resulting in a "solution"-polymerized phase, i.e., a traditional polymeric phase [26]. In the second procedure, dry silica was equilibrated with humid air to form a surface monolayer of water prior to reaction. The

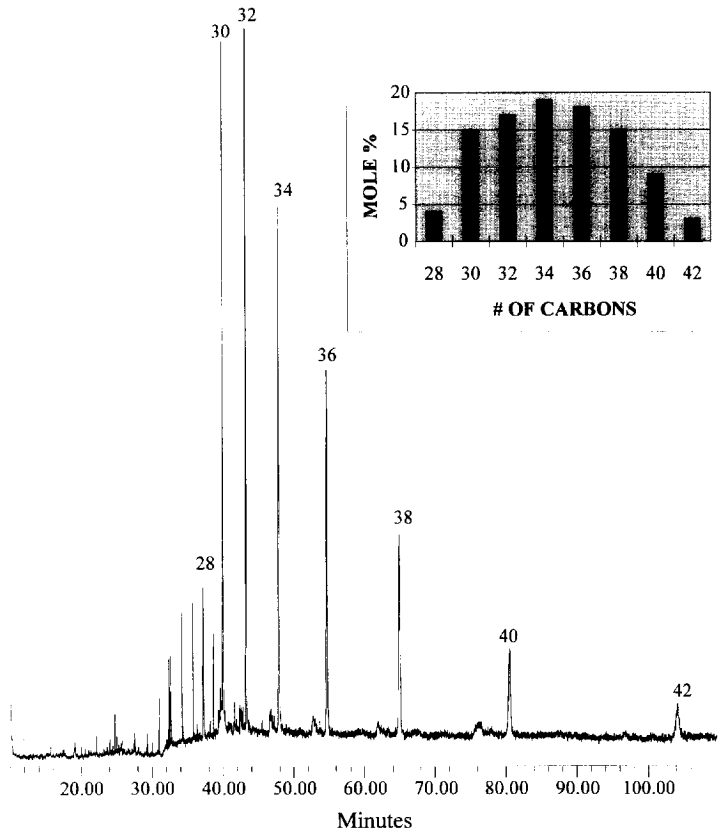

Fig. 1. Chromatogram from GC–MS analysis of α-olefin mixture. Insert shows percent distribution of carbon chain lengths.

silane mixture was then added resulting in a phase with crosslinking of the reactive silanes only at the surface. This bonding technique, referred to as "surface" or "horizontal" polymerization, is based on the early work of Sagiv [32–35], Whitesides [36,37] and others who grew similar "self-assembled" monolayers on planar surfaces as analogs of Langmuir–Blodgett films. Extensive work has been done in this field delineating the remarkable order and stability that can be realized for these films [38–41]. Wirth and Fatunmbi [42–44] have used this procedure with mixed monolayers of three and eighteen carbons on silica to make bonded phases with monomeric-like selectivity and increased hydrolytic stability, and Sander and Wise [23] have reported C_{18} phases synthesized by this approach which possess polymeric-like selectivity. The latter report showed that the surface-polymerized stationary phases could give increased shape selectivity for PAHs, but that peak shape was sometimes degraded for highly loaded phases.

Seven stationary phases were prepared for investigation (Table 1). The surface coverages for these phases were calculated using carbon chain lengths based on the distribution of the olefins in the original mixture. Columns were prepared from these materials, and changes in selectivity were observed for certain carotenoid mixtures compared with C_{30} and C_{18} columns. Fig. 2 shows the separations of β-carotene and its 15-*cis* and 13-*cis* isomers on C_{34}, C_{30} and C_{18} columns. These isomers differ only in the position of the *cis* substitution, yet their biological properties may be quite different [45]. The C_{30} phase reported earlier is a marked improvement on the C_{18} phase, but even better separation is afforded by the thicker C_{34} phase (Fig. 2). It is believed that the improved selectivity of the long-chain phases is a consequence of greater shape

Table 1
Properties of stationary phases

Column	Synthesis	Particle size (μm)	% Carbon	Coverage (μmol/m^2)	α/Resl[a] (13/15-*cis*-β-carotene)
1	Solution polym. C_{34}	5	19.21	3.1	1.124/1.84
2	Solution polym. C_{34}	5	16.03	2.4	1.122/1.41
3	Surface polym. C_{34}	5	17.02	2.6	1.119/2.16
4	Surface polym. C_{34}	5	20.90	3.5	1.127/1.60
5	Surface polym. C_{34}	5	16.39	2.5	1.120/1.72
6	Surface polym. C_{34}	3	16.22	2.5	1.128/2.25
7	Solution polym. C_{30}	5	18.08	3.3	1.096/1.75

[a] Selectivity and resoultion are for mobile phase conditions, methanol–methyl-*tert.*-butyl ether (95:5, v/v) and column temperature 24°C.

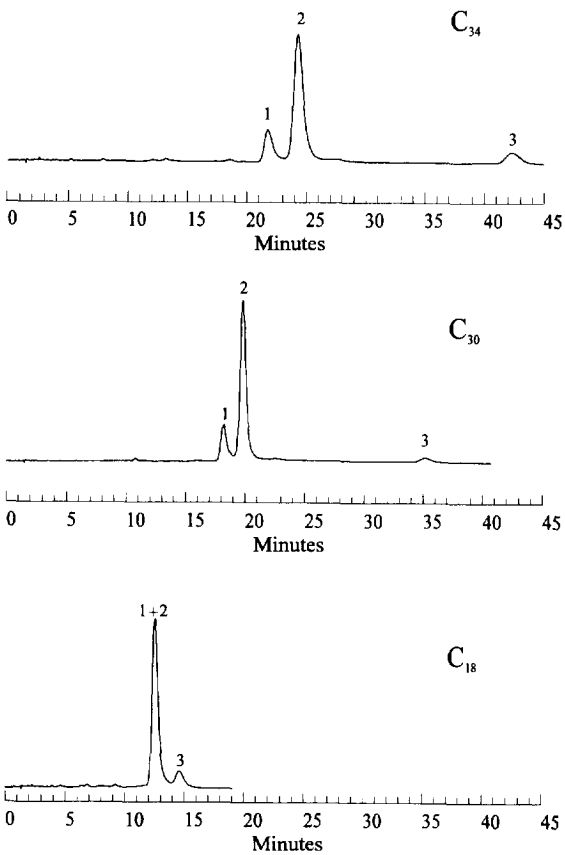

Fig. 2. Liquid chromatographic separations of β-carotene isomers on a C_{34} phase (column 1), a C_{30} phase (column 7) and a commercial polymeric C_{18} column. Peaks are (1) 15-*cis*-β-carotene, (2) 13-*cis*-β-carotene and (3) *trans*-β-carotene. Conditions are described in Section 2.4.

recognition. Numerous studies of alkyl-bonded stationary phases have shown that several properties lead to increased shape recognition ability. Among these are high ligand density [26,46,47], rigid chain conformations [46,48,49], low ligand mobility [49], long ligand chain length [17,22,46] and polymeric phase bonding [16,17,26,46–48,50]. Because the C_{34} stationary phases have ligand chain densities close to but less than that of the C_{30} stationary phase and the polymeric bonding and chain conformations are comparable, the slight increase in carotenoid selectivity for the C_{34} column is attributed to its greater ligand chain length. In addition, the ligands of longer chain phases are more rigid than those of C_{18} stationary phases with similar coverages [51,52] which may also contribute to their increased selectivity. The polymeric C_{18} stationary phase has a much higher surface coverage ($\geq$4.5 μmol/m^2), yet no separation of the closely related 13- and 15-*cis* isomers of β-carotene is possible on this phase.

Based on thicknesses determined by small-angle-neutron-scatttering for monomeric C_8, C_{18} and C_{30} phases [21,53] and the fact that polymeric C_{18} phases are approximately 25% thicker than their monomeric analogs, it is estimated that a polymeric C_{34} phase would have an average length near 35 Å, or approximately 13% longer than that estimated for a polymeric C_{30} phase (31 Å). It must be emphasized also that the C_{34} phase actually consists of a distribution of chain lengths some potentially as long as 42 Å, and that the conformation of the chains is unknown. The commercial C_{30} silane also contains some shorter and longer chain impurities, but the distribution is quite tight with C_{30} clearly as the major component [54]. Baseline resolution of the

15-*cis* and 13-*cis* isomers was achieved with the C_{34} phase (column 1), whereas slightly inferior separation and reduced retention were obtained with the C_{30} phase (column 7). This is despite the fact that the coverage for the C_{30} stationary phase is greater than that of column 1 (3.3 μmol/m^2 vs. 3.1 μmol/m^2, respectively). In fact, the coverages for the C_{34} stationary phases are consistently below those of C_{30} and C_{18} phases. This may be due to the greater space requirements for longer chains bonded to a curved surface (pore interiors). Berendsen and coworkers [55] described this effect when they showed that ligand-bonding density decreased with increasing chain length for a series of monomeric stationary phases.

As can be seen in Table 1, there is some variation in the ligand surface coverages among the C_{34} stationary phases (even among those prepared in the same manner). This variation is believed to be related to batch-to-batch differences in the yield of the hydrosilylation reaction. Because of problems with cracking resulting from high distillation temperatures, the silanes were not distilled prior to bonding. Efforts were made to remove unreacted trichlorosilane, however, some low volatility oligomers of trichlorosilane may have been present to bond with the silica, leading to some scatter in the coverages obtained. Columns 5 and 6 were bonded from the same batch of silane onto 5 μm and 3 μm silica with the same surface areas, and these stationary phases give the same carbon loadings and bonding densities. In spite of the variation in coverages for the C_{34} stationary phases, selectivity of these phases toward carotenoid isomers is consistently greater than that of C_{30} stationary phases. While these selectivity differences are small, they are easily reproduced, and they suggest that longer chain ligands can improve selectivity for carotenoid isomers, but that stationary phases much beyond the length of the solute of interest may offer only limited advantages.

Two bonding strategies were employed in the synthesis of the C_{34} stationary phases. Solution-polymerized phases are formed when addition of water to the reaction mixture leads to formation of solution oligomers that then bond with the surface. Surface-polymerized stationary phases involve the reaction of silanes with surface water such that cross-linking between silanes occurs only at the surface. Because of differences in the ligand densities obtained using either method, it is difficult to isolate the true effects of the two bonding methods. As indicated in Table 1, better separations of 15-*cis*-β-carotene and 13-*cis*-β-carotene were observed with a surface-polymerized C_{34} stationary phase (column 3; 2.6 μmol/m^2) compared with solution-polymerized C_{34} phases of comparable (column 2; 2.4 μmol/m^2) or higher bonding densities (column 1; 3.1 μmol/m^2). The improved resolution of column 3 may be due to a more uniform distribution of the chains on the silica. Recently, Pursch and coworkers [52] reported NMR data of solution- and surface-polymerized C_{18} stationary phases that support this model of ligand chain distribution. They showed that for solution-polymerized and surface-polymerized phases of the same ligand density, the ligand chains of the former were more rigid and less mobile. The authors concluded that there are patches of high ligand density on the solution-polymerized phases, while the surface-polymerized phases exist with more regular ligand spacing. It should be noted that at high bonding densities, peak tailing can be significant with surface-polymerized long-chain stationary phases as reported for C_{18} phases [23]. For example, column 4 is a higher loaded surface-polymerized C_{34} stationary phase that has high selectivity for 13-*cis*- and 15-*cis*-β-carotene, yet gives decreased resolution due to peak tailing.

To a large extent, the motivation for improving separations of carotenoids is a consequence of the different antioxidant, and pro-vitamin A properties exhibited by *cis* and *trans* isomers. Isomerization of all-*trans* carotenoids is known to occur during thermal food processing, altering nutritional benefits [56–58]. Emenhiser and coworkers have used C_{30} columns to separate isomers of a variety of carotenoids [18]. Fig. 3 shows the separations on C_{34} and C_{30} columns of an α-carotene isomer mixture prepared by catalytic photoisomerization of an α-carotene standard. This procedure is based on the work of Zechmeister [27]. As an asymmetrical molecule, α-carotene can adopt numerous *cis* configurations. The photoisomerization of α-carotene results in an equilibrium mixture after only a short irradiation period ($\leq$1 h in laboratory light). This behavior is typical of the photoisomerization of polyene compounds [11,28]. Consequently, it is very easy to reproduce this mixture (even without strictly

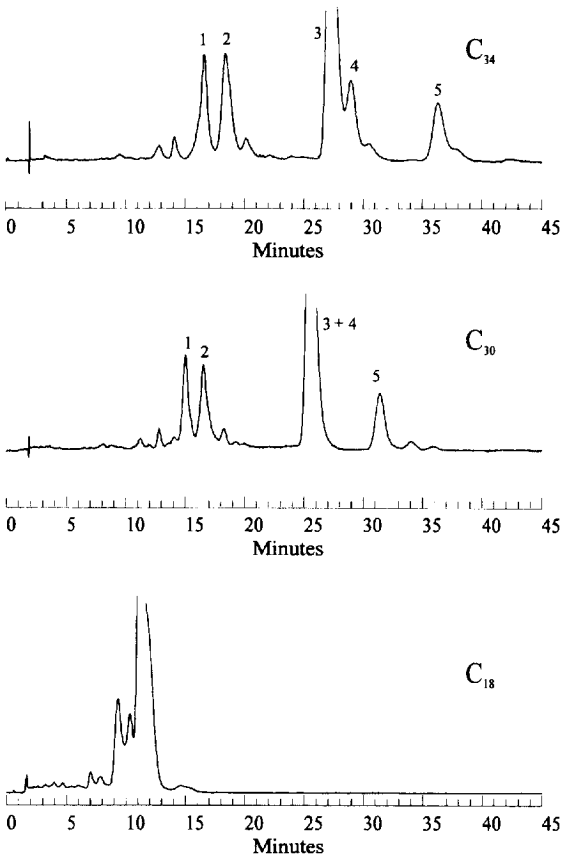

Fig. 3. Liquid chromatographic separations of α-carotene isomers on a C_{34} phase (column 3), a C_{30} phase (column 7) and a commercial polymeric C_{18} column. Peaks are (1) 13-*cis*-α-carotene, (2) 13′-*cis*-α-carotene, (3) *trans*-α-carotene, (4) 9-*cis*-α-carotene and (5) 9′-*cis*-α-carotene. Conditions are described in Section 2.4.

controlling the catalyst concentration), thereby making it an ideal test mixture as well as producing many molecules of interest that are not available commercially. This procedure works similarly for other carotenoids [11,18].

The major *cis* isomers in Fig. 3 are identified based on comparisons of retention profiles with the reports of Emenhiser et al. who used a C_{30} column along with NMR and absorption data to assign peaks [18,20]. Schmitz et al. [13], working in the same group, have also used a calcium hydroxide stationary phase to resolve these isomers. While this latter work gave excellent results, such stationary phases are not available commercially and separations using them can be difficult to reproduce due to extreme sensitivities to mobile phase composition and temperature. Reproducibility of retention times on reversed-phase C_{30} and C_{34} columns is also improved by temperature control, but small changes in temperature or mobile phase do not significantly affect selectivity. Large temperature changes, however, do cause notable retention and selectivity differences for carotenoids on long-chain stationary phases [59]. Peaks not identified in Fig. 3 are likely di-*cis* isomers which are known products of the photoisomerization of carotenoids [11], or oxidation products of the isomers, although the latter is less likely as oxygenated products generally elute very early under these conditions. While the separations in Fig. 3 are similar, it is clear that there are some selectivity differences. For example, the C_{30} column gives one peak equivalent to the all-*trans* α-carotene retention time, whereas the C_{34} column gives three peaks in sufficient amounts as to be major isomer components. This behavior is observed for all the C_{34} columns tested, and the separation factor, α, is the same for each despite the slight variation in ligand coverage. The separation factor for 13- and 13′-*cis*-α-carotene is also consistently greater for the C_{34} columns as compared to the C_{30} column. The C_{30} column does separate later eluting peaks more completely, an observation whose explanation is hampered by the lack of complete peak identification.

Finally, the ability of the C_{30} stationary phase to separate extracts of natural samples has been shown by Sander et al. [17] and by Emenhiser et al. [19]. Fig. 4 shows the separation on a C_{34} column and a C_{30} column of a sample derived from *Dunaliella* algae. This sample contains a high percentage of *cis* isomers, many of which are tentatively identified in Fig. 4 by comparison of retention times with standards and known isomer mixtures. The peaks prior to 12 min are probably polar carotenoids or oxidation products of the various carotenoids. Like the previous examples, these separations are similar, yet show clear distinctions. For example, the 9-*cis* (peak 12) and all-*trans* (peak 11) isomers of β-carotene are completely resolved on the C_{34} stationary phase but not on the C_{30} stationary phase. Conversely, all-*trans*-β-carotene (peak 11) is better separated from another unknown *cis* isomer (peak 9) on the C_{30} column. It is possible that better resolution of one pair of isomers obscures the separation of others. These differences may be exploited to better separate

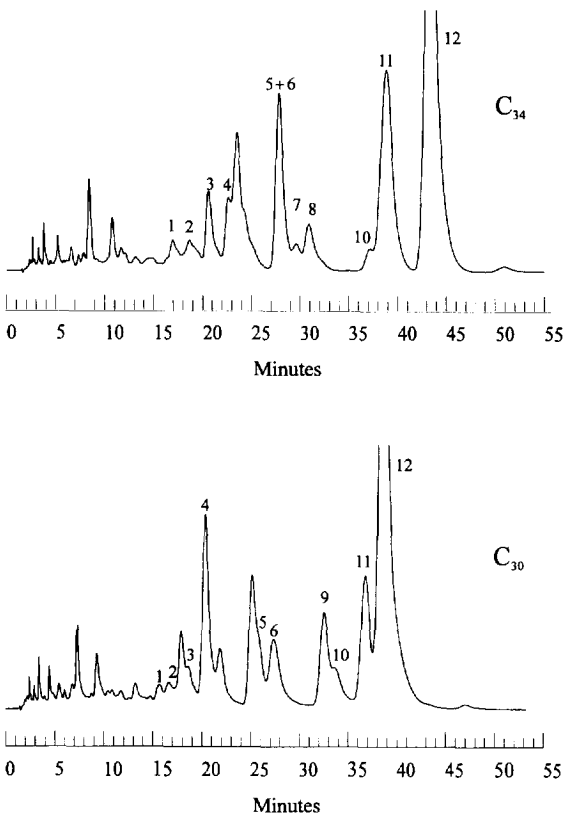

Fig. 4. Liquid chromatographic separation on a C_{34} phase (column 3) and a C_{30} phase (column 7) of a natural carotene mixture containing high percentages of *cis* isomers. Tentative peak identifications are as follows: (1) 13-*cis*-α-carotene, (2) 13′-*cis*-α-carotene, (3) 15-*cis*-β-carotene, (4) 13-*cis*-β-carotene, (5) *trans*-α-carotene, (6) *cis*-β-carotene, (7) *cis*-α-carotene, (8) *cis*-α-carotene, (9) *cis*-β-carotene, (10) 9′-*cis*-α-carotene, (11) *trans*-β-carotene and (12) 9-*cis*-β-carotene. Because many of the compounds are identified by comparisons with known isomer mixtures and not individual standards, certain isomers are not identified on one or the other of these chromatograms as they were not resolved on that column. Conditions are described in Section 2.4.

similar complicated samples, and suggest that the C_{34} stationary phase may complement, and not replace, the C_{30} phase for carotenoid separations.

4. Conclusions

The new, extended chain length bonded stationary phases presented here provide excellent separations of carotenoid isomers, with slight improvement compared with C_{30} stationary phases. This improvement is attributed to the ability of the large carotenoid molecules to more fully interact with the thicker C_{34} phase. It does appear, however, that a limit has been reached in the quest for increased selectivity of carotenoids through the use of longer chain length phases. This is not surprising as the same observation has been made with smaller solutes on shorter chain alkyl stationary phases [22]. The ability to prepare selective, efficient columns from silica that is bonded directly from a freshly synthesized, unpurified silane mixture has also been demonstrated. While this procedure needs refinement in order to improve the reproducibility of the resultant stationary phases, synthesis of other phases utilizing high-boiling silanes may require the use of similar bonding methods. The stationary phases reported here may eventually be of use for other large molecules, such as oligonucleotides [60] or very long chain fatty acid esters [61], whose separations have been improved by the use of C_{30} stationary phases.

Acknowledgments

The authors thank Robert Cooley (YMC, Wilmington, NC, USA) for the gift of the YMC SIL200 silica, Christophe Lawrie (Chevron Chemical Co., Kingwood, TX, USA) for the contribution of the α-olefin mixture, Hemmi Bhagavan and Willy Schüep (Hoffman-LaRoche, Nutley, NJ, USA and Basel, Switzerland) for providing carotenoid standards, and Peter Pella of NIST for performing X-ray fluorescence analysis. Certain commercial equipment, instruments, or materials may be identified in this report to specify adequately the experimental procedure. Such identification does not imply recommendation or endorsement by the National Institute of Standards and Technology or Chevron Research and Technology Company, nor does it imply that the materials or equipment identified are necessarily the best available for the purpose.

References

[1] A. Wahid and L. Gerber, Vitamin A, 2102 (1990) 771.
[2] K. Iwai, M. Kanamori, Y. Muto and T. Tanaka, Proc. 10th Int. Cong. of Nut., (1975) 201-212.

[3] N. Orentreich, J. R. Matias, J. H. Vogelman, R. M. Salkeld, H. Bhagavan and G. D. Friedman, Nutr. Cancer, 16 (1991) 167.
[4] P. Di Mascio, S. Kaiser and H. Sies, Arch. Biochem. Biophys., 274 (1989) 532.
[5] M.S. Menkes, G.W. Comstock, J.P. Vuilleumeir, K.J. Helsing, A.R. Rider and R. Brookmeyer, N. Engl. J. Med., 20 (1986) 1250.
[6] G.A. Colditz, L.G. Branch, R.J. Lipnick, W. C. Willett, B. Rosner, B. M. Posner and C. H. Hennekens, Am. J. Clin. Nutr., 41 (1985) 32.
[7] W.C. Willett, B.F. Polk, B.A. Underwood, M.J. Stampfer, S. Pressel, B. Rosner, J.O. Taylor, K. Schneider and C.G. Hames, N. Engl. J. Med., 310 (1984) 430.
[8] R. Peto, R. Doll, J.D. Buckley and M.B. Sporn, Nature, 290 (1981) 201.
[9] R.G. Ziegler, T.J. Mason, A. Stemhagen, R. Hoover, J. B. Schoenberg, G. Gridley, P.W. Virgo and J.F. Fraumeni, Jr. Am. J. Epidemiol., 123 (1986) 1080.
[10] R.G. Ziegler, Am. J. Clin. Nutr,. 53 (1991) 251S.
[11] K. Tsukida, K. Saiki, T. Takii and Y. Koyama, J. Chromatogr., 245 (1982) 359.
[12] H. Hashimoto and Y. Koyama, J. Chromatogr., 448 (1988) 182.
[13] H.H. Schmitz, C. Emenhiser and S.J. Schwartz, J. Agric. Food Chem., 43 (1995) 1212.
[14] B.L. Lee, S.C. Chua, H.Y. Ong and C.N. Ong, J. Chromatogr., 581 (1992) 41.
[15] N.E. Craft, Methods Enzymol., 213 (1992) 185.
[16] E. Lesellier, A. Tchapla and A.M. Krstulovic, J. Chromatogr., 645 (1993) 29.
[17] L.C. Sander, K.E. Sharpless, N.E. Craft and S.A. Wise, Anal. Chem., 66 (1994) 1667.
[18] C. Emenhiser, L.C. Sander and S.J. Schwartz, J. Chromatogr. A, 707 (1995) 205.
[19] C. Emenhiser, L.C. Sander and S.J. Schwartz, J. Agric. Food Chem., (1996) in press.
[20] C. Emenhiser, G. Englert, L.C. Sander, B. Ludwig and S.J. Schwartz, J. Chromatogr. A, 719 (1996) 333.
[21] L.C. Sander, C.J. Glinka and S.A. Wise, Anal. Chem., 62 (1990) 1099.
[22] L.C. Sander and S.A. Wise, Anal. Chem., 59 (1987) 2309.
[23] L.C. Sander and S.A. Wise, Anal. Chem., 67 (1995) 3284.
[24] H. Shih-Hsien, S. Fazio, S.A. Tomellini, J.B. Crowther and R. Hartwick, Chromatographia, 20 (1985) 161.
[25] G.E. Berendsen and L. de Galan, J. Liq. Chromatogr., 1 (1978) 561.
[26] L.C. Sander and S.A. Wise, Anal. Chem., 56 (1984) 504.
[27] L. Zechmeister, Cis-Trans Isomeric Carotenoids, Vitamins A, and Arylpolyenes, Academic Press, New York, 1962.
[28] Y. Yamano, S. Sumiya and M. Ito, J. Chem. Soc, Perkin Trans. 1, (1995) 167.
[29] J.L. Speier, in F.G.A. Stone and R. West (Editors), Advances in Organometallic Chemistry, Vol. 17, Academic Press, New York, 1979, p. 407.
[30] R.K. Gilpin, J.A. Korpi and C.A. Janicki, Anal. Chem., 46 (1974) 1314.
[31] G. Felix, A. Thienpont, M. Emmelin and A. Faure, J. Chromatogr., 461 (1989) 347.
[32] J. Sagiv, J. Am. Chem. Soc., 102 (1980) 92.
[33] R. Maoz and J. Sagiv, J. Colloid Interface Sci., 100 (1984) 465.
[34] R. Maoz and J. Sagiv, Thin Solid Films, 132 (1985) 135.
[35] S. R. Cohen, R. Naaman and J. Sagiv, J. Phys. Chem., 90 (1986) 3054.
[36] S.R. Wasserman, Y.T. Tao and G.M. Whitesides, Langmuir, 5 (1989) 1074.
[37] S.R. Wasserman, G.M. Whitesides, I.M. Tidswell, B.M. Ocko, P.S. Pershan and J.D. Axe, J. Am. Chem. Soc., (1989) 5852.
[38] M.K. Chaudhury and G.M. Whitesides, Science, 256 (1992) 1539.
[39] A. Ulman, An Introduction to Ultrathin Organic Films: From Langnuir-Blodgett to Self-Assembly, Harcourt Brace Jovanovich, Boston, 1991.
[40] V. DePalma and N. Tillman, Langmuir, 5 (1989) 868.
[41] R. Maoz and J. Sagiv, Langmuir, 3 (1987) 1034.
[42] M.J. Wirth and H.O. Fatunmbi, Anal. Chem., 64 (1992) 2783.
[43] M.J. Wirth and H.O. Fatunmbi, Anal. Chem., 65 (1993) 822.
[44] M.J. Wirth and H.O. Fatunmbi, LC·GC, 12 (1994) 222.
[45] Y. Koyama, I. Takatsuka, M. Kanaji, T. Koichi, M. Kito, T. Shimamura, J. Tamashita, K. Saika and K. Tsukida, Photochem. Photobiol., 51 (1990) 119.
[46] L.C. Sander and S.A. Wise, LC·GC, 8 (1990) 378.
[47] S.A. Wise and L.C. Sander, J. High Resolut. Chromatogr., 8 (1985) 248.
[48] K. Jinno and Y. Lin, Chromatographia, 41 (1995) 311.
[49] L.C. Sander and S.A. Wise, Anal. Chem., 61 (1989) 1749.
[50] E. Lesellier, C. Marty, C. Berset and A. Tchapla, J. High Resolut. Chromatogr., 12 (1989) 447.
[51] M. Pursch, S. Strohschein, H. Handel and K. Albert, Anal. Chem., 68 (1996) 386.
[52] M. Pursch, L.C. Sander and K. Albert, in preparation.
[53] C.J. Glinka, L.C. Sander, S.A. Wise and N.F. Berk, Mat. Res. Soc. Symp. Proc., 166 (1990) 415.
[54] C.M. Bell and L.C. Sander, (1996) unpublished results.
[55] G.E. Berendsen, K.A. Pikaart and L. de Galan, J. Liq. Chromatogr., 3 (1980) 1437.
[56] F.W. Quackenbush, J. Liq. Chromatogr., 10 (1987) 643.
[57] L.A. Chandler and S.J. Schwartz, J. Agric. Food Chem., 36 (1988) 129.
[58] J.P. Sweeney and A.C. Marsh, J. Am. Diet. Ass., 59 (1971) 238.
[59] C.M. Bell, L.C. Sander and S.A. Wise, J. Chromatogr. A, in press.
[60] K. Makino, H. Ozaki, H. Imaishi, T. Takeuchi and T. Fukui. Chem. Lett., (1987) 1251.
[61] K. Takayama, H.C. Jordi and F. Benson, J. Liq. Chromatogr., 3 (1980) 61.