Capillary GLC has finally 'arrived'

The following article focuses on the development of capillary gas liquid chromatography and the advantages it offers to lipid chemists. It was prepared by Robert G. Ackman of the Canadian Institute of Fisheries Technology, Technical University of Nova Scotia, Halifax, Nova Scotia, Canada.

In 1980 in this journal, I quoted from **Julius Caesar** (Act IV) to the effect that, "Good reason must, of force, give place to better." Nowhere is this more true than in the field of gas liquid chromatography (GLC), which has advanced more in lurches, with occasional stumbles, than by the smooth evolutionary process usually imagined for science. This irregular process reflects the inputs of individuals in many countries. Associated with GLC since 1955, I would like to comment on the importance to lipid chemists of one of the current forward lurches, that of the sudden popularity of "capillary" GLC.

In 1978, an elegant little volume was published by the Perkin-Elmer Corp., authored by L.S. Ettre who may fondly be thought of as the "Godfather" of capillary GLC. It took only 69 pages to summarize the principles of capillary (open-tubular) columns. This brings up the question, "Why 'open-tubular?'" This name is derived from the original theoretically "pure" concept proposed by Golay in 1957, in which the liquid phase was distributed in a thin film on the wall of a long open tube. By 1962, it was proposed that a porous layer be put on the inside of the tube and this support coated with liquid phase. The more modern terms of wall-coated open-tubular (WCOT) and support-coated open-tubular (SCOT) replaced "capillary" to differentiate these two types of columns. In general usage, the term "capillary columns" refers to WCOT, and this is the term I will use.

It may seem curious to some modern fats and oils chemists that it took nearly 30 years for capil-

lary columns to become popular. Figure 1 provides a partial answer. The markings include a Perkin-Elmer U.S. patent number, and this patent was sufficient to deter other GLC equipment manufacturers from encouraging the use of such columns. Instead, the other GLC-oriented companies promoted features such as temperature programming of packed columns to provide sharper peaks, or very long (20 feet) metal one-eighth inch o.d. packed columns, instead of the common six- or eight-foot lengths of one-quarter inch o.d., but these concepts seldom improved component resolution to the same extent as capillary columns.

Most laboratories in the fats and oils industry were perfectly satisfied with packed columns. If they could measure palmitic, stearic, oleic, linoleic and linolenic acids, they were happy. If a column failed after a few months, it could be repacked and replaced in a few hours. Some columns lasted longer than others. Two decades ago, I asked a large Canadian food company about the type of liquid phase in use in a packed column in its laboratory and caused consternation, since the laboratory had been using the same column for over 10 years and nobody could remember what it contained!

Although excellent glass capillary columns were prepared in individual laboratories, especially in Europe, these were not readily available on a retail basis. People were nervous about the fragility of glass, whereas thin glass in coil form is in fact very flexible. A well written book by Jennings on that subject appeared in 1978, with a second edition in 1980. The same author also has written about glass capillary columns in flavor research, an area where they were found extremely useful.

Commercial metal capillary columns had two drawbacks: that of expense (about $300 circa 1965) and a limited lifespan. Glass capillary columns, to some extent, had similar problems but generally were homemade. In our research laboratory, cost was less of a factor provided it yielded information on unusual fatty acids, such as the *cis*-vaccenic acid of Figure 2. Our satisfactory compromise was to operate Perkin-Elmer metal WCOT columns with butanediol succinate polyester (BDS) coatings at 175-188°C and 40-60 psig He. Under these conditions, a common vegetable oil could be analyzed in 12-15 minutes, rapeseed oil (to 22:1) in 25-30 minutes and a fish oil (to 22:6n-3) in about 60 minutes. The life of such a column in daily use was at least three months and usually somewhat longer. Later, the cyanosilicone SILAR-5CP as a liquid phase provided equivalent service and the benefit of useful, if incomplete, separations of many *trans* and *cis* fatty acids.

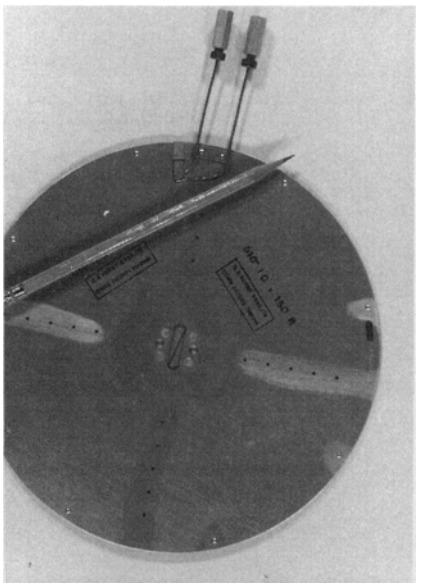

FIG. 1. A Perkin-Elmer "pancake" capillary column (circa 1965) for use in the Model 226 GLC, with pencil for scale. Note patent number and caps on 1/16 inch "zero volume" Swagelok fittings. Stainless steel tubing, 0.01 inch i.d. and 150 ft in length (0.25 mm × 47 m).

With such capillary columns, we were able to obtain a great deal of information of interest to biochemists. Although I published one of our first capillary GLC analyses (of methyl esters of rapeseed oil fatty acids) in this journal in 1966, Figure 2 is from 1974, when Perkin-Elmer metal columns had graduated to the modern form of a coil in a metal mandrel, and instead of the clumsy Model 226, the elegant toroidal oven of the Model 900 was available for rapid heating and/or programming. This original chart shows, for example, the resolution of *cis*-vaccenic (18:1n-7) acid from oleic (18:1n-9). Two minor 18:1 isomers (18:1n-11 and 18:1n-5) are also visible. One of our reasons for sticking to the low polarity BDS or SILAR-5CP liquid phases is also apparent. All of the C_{16} fatty acids (to 16:4n-1) elute before the C_{18} acids (18:0) begin. This property of BDS and SILAR-5CP led to the absence of chain-length overlap for C_{18}-C_{20}, C_{20}-C_{22} and usually for C_{22}-C_{24}. This was a common problem with packed columns even in the edible vegetable oils, where linolenic acid (18:3n-3) often coincided with 20:0 or 20:1 on more polar liquid phases. We thus had, from theoretical considerations and from the superior separations provided by these columns, absolute confidence in identifying arachidonic and other biochemically important acids. For years, any fish oil feeding study published using high polarity packed columns usually confused 22:1 with 20:4n-6. Ironically, other scientists feeding radiolabeled 18:3n-3 (linolenic acid) to rats were often baffled by the apparent appearance of the label in their GLC peak for 20:4n-6 (arachidonic acid), whereas we could easily see the 20:3n-3 derived from 18:3n-3 when it resolved from 20:4n-6 on our capillary columns.

The revolution in capillary GLC took place in 1979-1980 with the introduction of flexible fused silica columns. This simple concept, pioneered by the Hewlett-Packard Co., produced the perfect column, uniform in bore and with a chemically inert wall, and also flexible and easily installed in or adapted to modern GLC units. One problem lay in the limited availability of liquid phases and the necessity of purchasing these columns, a situation almost identical to the status of Perkin-Elmer metal capillary columns some 20 years earlier. However, most prominent GLC suppliers were soon offering methyl silicone and at least one polar liquid

phase, Carbowax-20M, in flexible fused silica column (ffsc) format.

From our point of view, the principal early problem was that Carbowax-20M, the only polar liquid phase available in 1980, was apparently unstable, although it had a long history of use in glass capillary columns. Gradually, it emerged that batch-to-batch variations in the chemical properties of Carbowax-20M, which was produced on a large scale as an industrial chemical, were responsible for the frequent instability and shortened life of some columns. There was one other serious "noise" problem, which we called "spiking" and Schomberg called the "Christmas tree effect." In GLC, there is an inevitable tendency to blame any problem on the column. I wrestled with spiking for several months. One day a flash of insight occurred; I scrounged a disposable aluminum pie plate from the faculty club kitchen and put it under the column. Most of the spiking vanished. In another 10 minutes, a second pie plate was added to cover the column and the problem was solved. This effect was thus shown to be due principally to localized oven overheating and devices comparable to the two disposable aluminum pie plates also were devised elsewhere. Proper column enclosures such as Perkin-Elmer part 0332-4048 were eventually introduced in May of 1984.

The final improvement in polar flexible fused silica columns was the introduction of "bonded" liquid phases. In *Chemistry & Industry* in 1981, I showed how the quartz column wall could be coated with a superpolymer of degraded Carbowax-20M, and this concept has been extended to cross-linking of most molecules in the operating film of this liquid phase. These liquid phases, for example SUPELCOWAX-10, can be so inert that columns can be periodically rinsed with a few ml of methylene chloride, a cleaning process that usually restores performance and extends the useful life.

The stability of bonded Carbowax-20M in ffsc form is now such that temperature programming with little or no baseline rise is possible. Marine lipids, with a fatty acid range from 14:0 to 22:6n-3, can be determined in as little as 20 minutes. Figure 3 gives a detailed analysis of fatty acids from a somewhat unusual marine fat. Notice that the peaks for arachidonic acid (20:4n-6, peak 8) and EPA (20:5n-3, peak 9) are equal in area, a feature often found in fatty acids of tropical organisms. The special feature to be noted is that the baseline did not rise during programming, and there was no special increase in noise. The analysis was completed in scarcely more time than that needed to give the C_{18} fatty acids in Figure 2. This range of fatty acids corresponds to those of most rat and human organ lipids, so these columns could be made the standard method for GLC analyses of fatty acids in most biochemical laboratories.

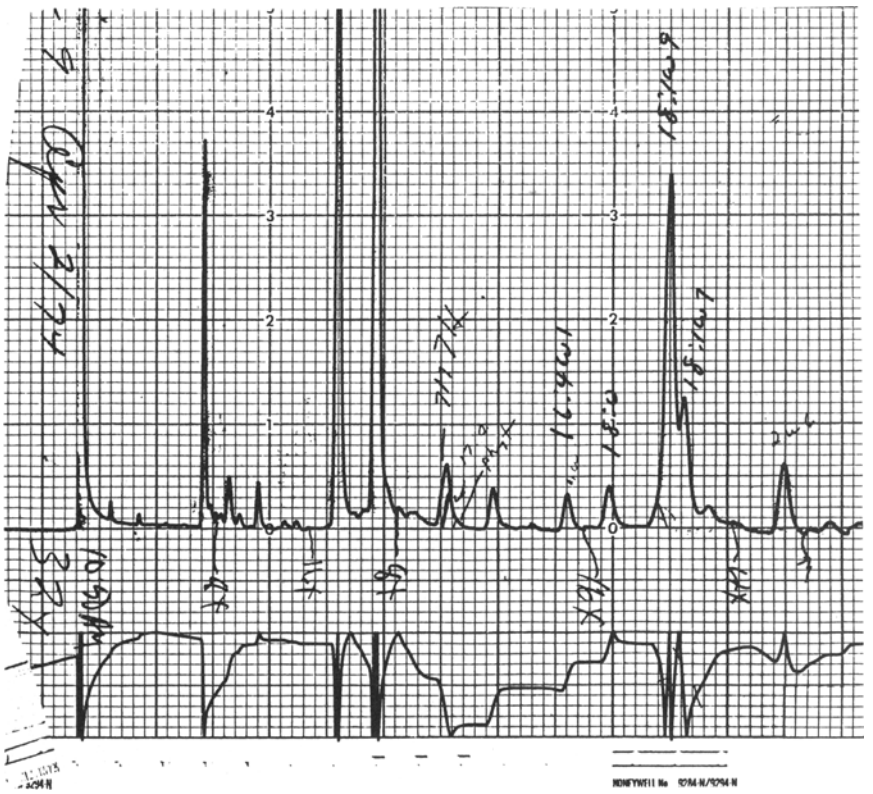

FIG. 2. The original early part of a chromatogram of the analysis in 1974 of the methyl esters of fatty acids from a fish (capelin) oil on a Perkin-Elmer stainless steel column, 0.01 inch i.d. × 150 ft operated at 180°C with He carrier gas at 60 psig. The first three large peaks are 14:0, 16:0 and 16:1n-7. Note shoulder for 18:1n-11 before 18:1n-9 and small 18:1n-5 peak after 18:1n-7. Time to 18:2n-6 (linoleic acid) peak was 17 minutes.

The low bleed rate of "bonded" Carbowax-20M columns makes their application to GC-MS feasible for materials of moderate molecular weight, whereas formerly the involatile methyl silicones were preferred. The narrow bore of ffsc columns means that the column end can be passed directly into the mass spectrometer inlet, eliminating many interface problems. GC-MS papers also are now common in the literature, partly for this reason.

It would be nice to say that all problems relating to the stability of the Carbowax-20M type columns are solved. However, cross-linking can be achieved in different ways and this feature probably has a minor effect on column polarity. Silicone-based nonpolar columns

should also be uniformly of the same polarity if of pure methyl polysiloxane, and cross-linking permits high temperature analyses of triglycerides, etc., on ffsc. Sometimes it is not clear if mixed polysiloxanes such as SE-54 have different properties from the pure liquid phase because of the cross-linking agent or because of variations in the component ratios.

It is especially important to note that scientists experienced with packed columns but nervous about capillary columns can often move to "wide-bore" capillary columns with a minimum of changes in operations or modifications to older GLC units. These satisfy most of the needs of the industrial chemist and merge with the shorter ffsc columns required for some high temperature analyses. The popular cyanosilicone liquid phases resisted cross-linking or bonding for a long time. Part of their popularity was due to the stability and long life of these phases in packed columns, permitting high temperatures and rapid analyses even if chain-length overlap among natural *cis* polyunsaturated fatty acid was a problem. Their application to the separation of *trans* fatty acids from their *cis* analogs is an almost unique advantage of these liquid phases, as shown in Figure 4. A partially cross-linked cyanosilicone phase, SP-2380, has recently been described and is temperature-stable to at least 275°C.

It is fair to say that the analysis of red blood cell phosphatidylethanolamine *sn*-1,2-diacylglycerides as TMS ethers on RTx-2330, a partially cross-linked cyanosilicone coated in flexible fused silica (Fig. 5), is an example of the leading edge of ffsc analytical technology. The average biochemist may not need to identify such complex molecular species, and the eventual benefits to our understanding of human health are difficult to grasp at this time. Exactly the same thoughts must have been expressed in the early 1950s when GLC was invented by Janes and

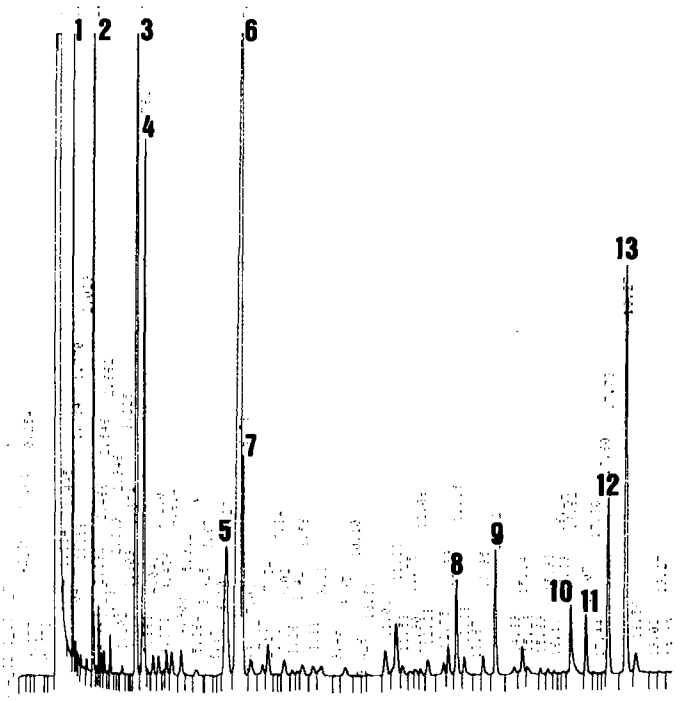

FIG. 3. Chromatogram of fatty acids from fats of a marine Pacific green turtle *Chelonia mydas* on a modern SUPELCOWAX-10 ffsc, 0.25 mm × 30 m in length, with hydrogen as carrier gas at 0.56 kg/cm². Peaks of interest are: [1] 12:0, [2] 14:0, [3] 16:0, [4] 16:1n-7, [5] 18:0, [6] 18:1n-9, [7] 18:1n-7, [8] 20:4n-6, [9] 20:5n-3, [10] 22:4n-6, [11] 22:5n-6, [12] 22:5n-3, [13] 22:6n-3. Operating temperature was 172°C for eight minutes (to after 18:1 group), then 6°/min to 220°C, hold. Total analysis time approximately 20 minutes.

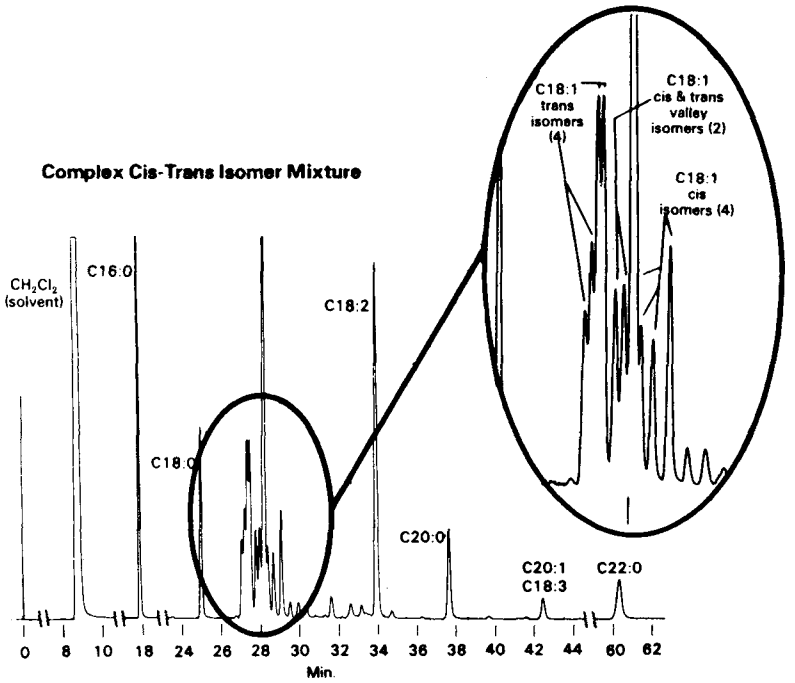

FIG. 4. Detail of the separations among methyl esters of C_{18} *trans* and *cis* monoethylenic acid isomers on a flexible fused silica column coated with the cyanosilicone phase SP-2560. The column was 100 m in length and 0.25 mm i.d., 0.20 μm film thickness, and operated at 175° with He carrier gas at a linear velocity of 20 cm/sec. Reproduced through the courtesy of Supelco Inc., Bellefonte, PA.

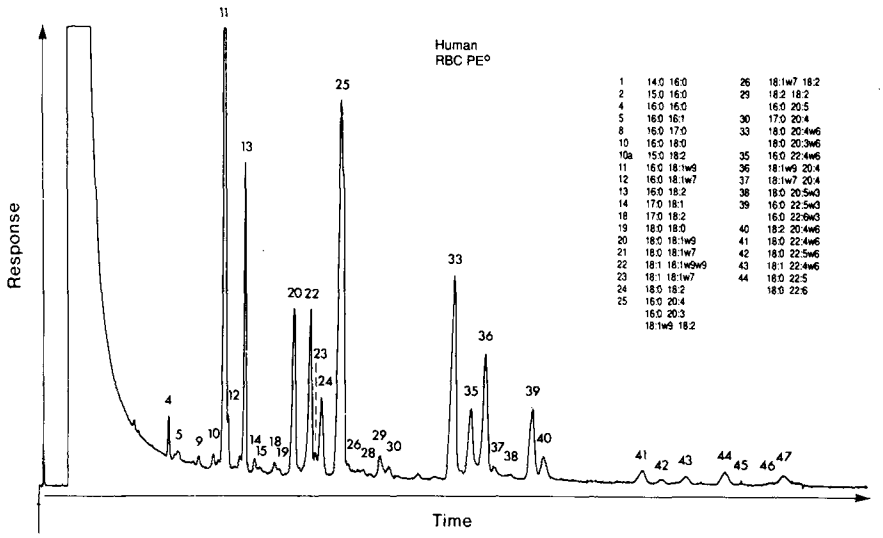

FIG. 5. Chromatogram of the TMS ethers of the *sn*-1,2-diacylglycerols of human red blood cell phosphatidylethanolamine. The column was 15 m × 0.32 mm i.d., flexible fused silica, with the bonded phase RT_x-2330 (Restek Corp., Bellefonte, PA) operated at 250° with hydrogen carrier as at 3 psig head pressure (From Myher et al., *Lipids 24*, 1989, in press, by permission).

Martin to solve a biomedical problem.

In 1961, *Chemical Engineering News* asked the rhetorical question, "Gas liquid chromatography is versatile, sensitive, and fast; and its principle is simple...or is it?" Life is made much simpler for the fats and oils chemist by eliminating the variable of the type of support and that of most of the effects of the liquid phase concentration on the support, and in fact 99% of the plethora of liquid phases in the literature and in catalogs. The flexible fused silica (capillary) column has arrived at a stage where it truly measures up to a title published in 1987 in *Analytical Chemistry*: "New Promises for An Old Technique."