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STRAIGHTENING OF THE ENDS OF GLASS CAPILLARY COLUMNS

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SUMMARY

After more than 10 years of controversy, it has become accepted that a capillary column should be directly connected to the injector and detector without the use of intermediate tubing. Although this is feasible with fused silica columns, with glass capillary columns it is necessary for the ends of the columns to be straightened. For most types of column, with moderate to high polarities, end straightening is simple and can be effected with a small flame followed by a Carbowax deactivation step, but for persilylated inert high-temperature columns this procedure leaves residual activity in the end sections. This activity, which cannot be eliminated, destroys the inertness of the whole column. A new electrical end-straightening device, which operates at far lower temperatures than those attained when flames are used, has been developed. At these lower temperatures no carbonization of apolar silicone stationary phases occurs. Consequently, there is no reactivation of the persilanized glass surface. Straightening of a new column is accomplished simply by pushing the end through the straightening device (no previous removal of the liquid phase and no deactivation after straightening are necessary). Full inertness, even of very thick film columns, is preserved. The design and use of the new device are discussed.

HOW TO CONNECT A CAPILLARY COLUMN TO A GAS CHROMATOGRAPH

The important question of how to connect a capillary column to a gas chromatograph is as old as the capillary column itself, and it may be useful to review the conflicting arguments. The parts connecting the coiled column to the injector and detector may be (A) integral parts of the gas chromatograph or (B) the ends of the column.

In earlier discussions, band broadening by system A was considered. The problem has been solved by avoiding dead volumes and/or by using scavenger flows. Contributions to this solution were made, for example, by Hartigan and Ettre¹ for the basic column-gas chromatograph combination, by Kaiser² for the cassette system and by Schomburg *et al.*³ and Bertsch⁴ for multi-column systems.

A second problem is the influence of the internal surfaces of the system on the sample. This influence has been minimized by careful temperature control and by the use of glass or noble metal surfaces. As early as 1969⁵, and repeatedly thereafter, we have emphasized that this minimum is not low enough to render such connecting systems suitable for general use. Although the integral systems work perfectly with a wide range of samples, they cause errors with more sensitive samples⁶. Only connections of type B assure general applicability. As we have shown⁷, the "intermediate" solution of connecting freshly cut column ends to short transfer tubing with shrinkable PTFE causes contamination of the carrier gas with oxygen.

There has been continuing opposition to system B owing to the necessity for column end straightening. It is interesting that this opposition had almost vanished 1 year before the advent of fused silica columns⁸, a major attribute of which is the direct realization of system B because of the inherently straight nature of such columns.

At present, connections of type B are almost universally accepted, which means that straightening the ends of glass capillary columns has become a regular manipulation.

END STRAIGHTENING

Procedure for columns of moderate to high polarity

In general, stationary phases that are more polar than SE-54 or OV-73 have upper temperature limits around 250°C or below (the few exceptions include sulphones^{9,10}).

This means that the support surface coated with these stationary phases is not expected to retain its inertness above 250°C, so the well known Aue-type deactivation with Carbowax¹¹ is sufficient for these columns. Thus, a conventional straightening procedure is adequate.

Any straightening technique (with a flame or an electrical heater) can be used, provided that it produces a smooth, straight end without constriction. Different stationary phases show a varying, but generally considerable, tendency toward carbonization upon heating. This very troublesome effect is precluded when straightening is carried out with a slow flow of air leaving the end under treatment. Under these conditions the liquid film is thoroughly oxidized instead of being pyrolysed. Care must be taken not to warm up the air-filled bulk of the column, either with the straightening flame or in the column oven.

The freshly straightened ends are extremely active. Deactivation is easily achieved by rinsing the heated section with 0.1% Carbowax (*e.g.*, Carbowax 1000) solution in methylene chloride, introduced with a 50–100- μ l syringe against a low air pressure. During prolonged use we recommend rinsing periodically with the deactivating solution in order to obtain a combined cleaning and deactivation effect.

Inert high-temperature columns

The situation changes drastically with persilylated¹² columns. These columns have two properties that make the preservation of their inertness imperative but impossible by conventional methods. On the one hand, their inertness is so great that even the slightest activity of a fresh end becomes evident in the behaviour of the whole

column. On the other hand, Carbowax deactivation of newly straightened ends is useless as the columns themselves keep their inertness at temperatures far above the temperature limit of the deactivating layer obtained with Carbowax. The practical importance of the problem is best demonstrated by an example (Fig. 1).

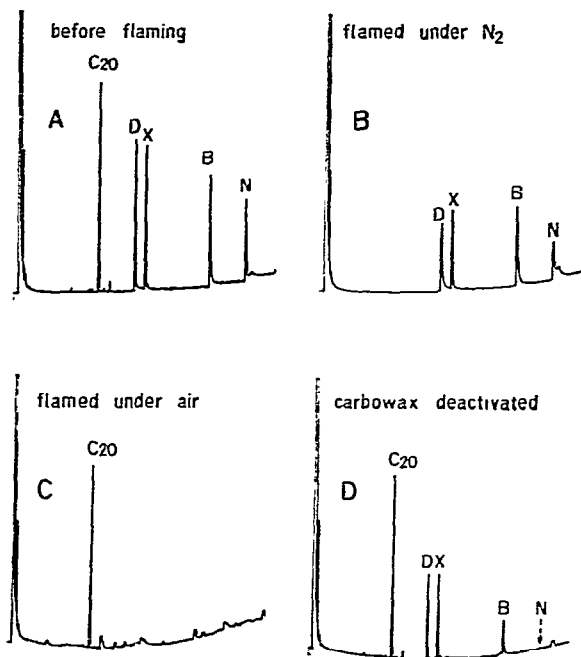


Fig. 1. Separation of silylated pterins on a persilylated Pyrex column (15 m \times 0.3 mm I.D., 0.12- μ m OV-73). Injection at 150°C, then temperature programmed at 6°C/min to 240°C. Substances: C₂₀ = *n*-eicosane (internal standard); D = dimethylpterin; X = xanthopterin; B = biopterin; N = neopterin. The differences between the four chromatograms are due to the different conditions in the first 12 cm of the column. For details see text.

The sample consisted of eicosane as an internal standard and of four silylated pterins¹³ which are known to be extremely sensitive to catalytic decomposition. Chromatograms A–D in Fig. 1 illustrate consecutive runs on the same persilylated Pyrex column coated with OV-73. Approximately 10 ng of each substance reached the column. The column had its original, persilanized ends in run A. Run B was carried out after preparing a new inlet end, straightened in a gas flame, with nitrogen flowing through the column. As expected, the stationary phase was carbonized. The alkane was totally adsorbed on the carbon layer. Note, however, that heating under an inert gas partly preserved the inertness of the column, as indicated by a relatively mild effect on the pterins. The same end was flamed again but under an air flow to remove the carbon. This treatment totally restored ideal elution of the alkane, but at the same time it caused total loss of the pterins (run C). This means that carbonization is avoided only at the expense of inertness. Run D was carried out after rinsing the same end with Carbowax solution. Significant deactivation is evident, but it was not possible to restore the original inertness.

The combined information conveyed by Fig. 1 is embarrassing, as one might infer that the inertness of the whole column is irrevocably destroyed upon straightening a new end!

Next we checked the situation with soft glass (Fig. 2). Chromatogram A shows the expected severe reactivation due to end straightening by flaming under an air flow. The result of run B was surprising. The oxidized end had been discarded, and a new end had been flamed under a flow of nitrogen. Unexpectedly, the column showed no carbonization (ideal elution of alkanes). Similar to the result shown in Fig. 1B, reactivation was relatively mild. Acceptably high inertness was restored by Carbowax deactivation (C). Note that the problem has not been solved, as the inertness demonstrated in run C would be lost upon heating the column above 250°C.

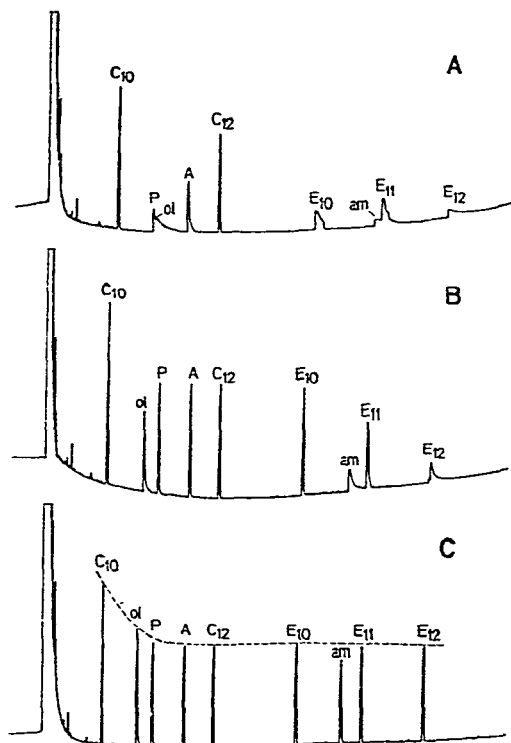


Fig. 2. Grob test¹⁴ on a persilylated soft glass column (15 m × 0.31 mm I.D., 0.15- μ m SE-52). Injection at 25°C, then programmed from 40°C at 3.3°C/min. A, Inlet end flamed under air flow; no carbonization, but severe adsorption of polar substances. B, Fresh end flamed under N₂ flow; adsorption strongly reduced; no carbonization in contrast to Fig. 1B; the behaviour of the esters (E₁₀-E₁₂) is typical of catalytic activity. C, Same end deactivated with Carbowax; perfect inertness (substances eluted 100%). ol = 1-octanol; P = dimethylphenol; A = dimethylaniline; E₁₀-E₁₂ = methyl esters of fatty acids C₁₀-C₁₂; am = dicyclohexylamine.

Role of the deformation temperature

The surprising result produced by flaming soft glass under nitrogen was caused by the lower deformation temperature of soft glass compared with that of Pyrex. When a soft glass capillary is straightened its temperature may not reach the level at which

apolar silicone phases are carbonized. As under these low-temperature conditions the inertness is not drastically affected, the hope arose that a further reduction in the deformation temperature might eliminate both undesirable consequences of flaming, namely carbonization and reactivation. Such a temperature reduction is feasible.

During flaming very weak mechanical forces, caused only by the weight of the 10–30-cm long column end (10–25 mg), are effective and deform only glass of very low viscosity. The forces can be significantly increased, *e.g.*, by fixing a weight (*e.g.*, a septum of *ca.* 100 mg) to the end of the broken column. Under these mechanical conditions deformation occurs at a higher viscosity, *i.e.*, at a lower temperature. In fact, this procedure caused a further reduction in, but still not the complete elimination of, carbonization and reactivation. Increasing the weight hanging at the column end proved to be impractical. Some additional improvement was obtained by replacing the gas flame with an alcohol flame, which resulted in a lower flame temperature over a longer capillary section.

Complete elimination of carbonization and reactivation proved to be feasible only by means of a specially designed electrical end-straightening device.

NEW ELECTRICAL END-STRAIGHTENING DEVICE

Design and function

Electrically heated straightening devices have been in use for many years and are commercially available. Because they do not exert mechanical forces much stronger than those which are effective during flaming, the characteristics of the column ends which they produce are similar to those of ends produced by flaming. Far stronger forces are exerted when the coiled capillary is moved through narrow hot tubing in which the glass capillary has little clearance. Minimal clearance between glass and metal produces maximal mechanical force, which allows the lowest possible deformation temperature. Compared with flaming, the practical deformation temperature is reduced by *ca.* 400°C for Pyrex and *ca.* 300°C for soft glass.

The design of the straightener is shown in Figs. 3 and 4. The 1-mm I.D. tubing (suitable for 0.8–0.95 mm O.D. glass capillaries) is heated by electrical resistance (*ca.* 1.5 V, 30 A). Straightening occurs in a short section near the top of the tube where the heat is focused in the following way. From the upper to the lower end the wall thickness of the tubing increases, which causes a decreasing electrical resistance, *i.e.*, decreasing heat production. Consequently, the hottest point should be the upper end. However, this end is effectively cooled by the heavy metal ring that is needed to supply the relatively high electrical current. The opposing effects (heating and cooling) cause heat focusing, the location of which is controlled by the dimensions of the device. The short hot zone must be located at the lower end of the funnel-shaped entrance and the first section of the narrow tubing. Direct temperature measurement is difficult and expensive because of the small dimensions. Even an extremely thin thermocouple disturbs the equilibrium by diverting heat from the focusing point.

Practical application

In practice, the new end-straightening device is used in the following way. The current to the device is turned on and the voltage is adjusted so that the tube is heated to the required temperature. When the straightening tube has reached temperature

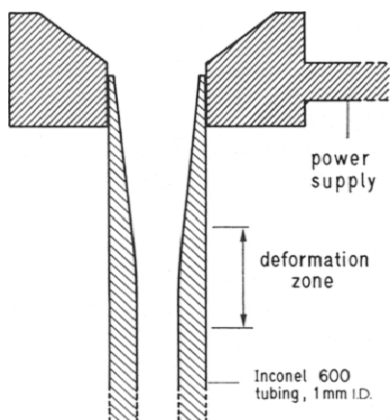


Fig. 3 Schematic diagram of the straightening tube. Short hot zone at junction between funnel-shaped entrance and cylindrical tubing, as a combined result of varying electrical resistance of the tubing wall and heat loss via the power supply connection.

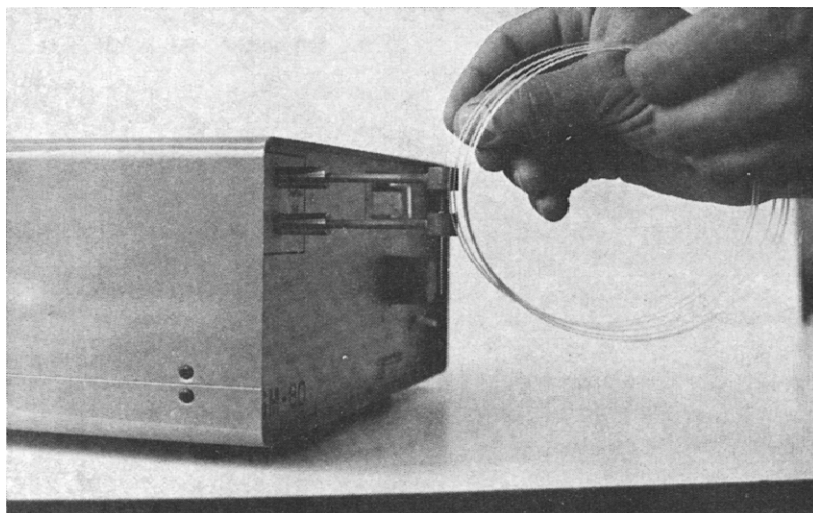


Fig. 4. The straightening device in operation. In the middle is the narrow straightening tube through which the broken end is pushed. The connection of the opposite end of the column to an inert gas supply is not shown. For details see under *Practical application*.

equilibrium, the end of the column to be straightened is inserted and pushed through the straightening tube at a rate of about 2 mm/sec. Because of the curvature of the column, the narrow clearance between the column and the straightening tube and the relatively low temperature of the straightening tube, the capillary cannot be fed at a uniform rate. The rate of 2 mm/sec is maintained discontinuously as short sections of the column pass through the hottest zone of the straightening tube. It is important that the force used to feed the capillary be applied along a tangent to the coil at the point of the upper end of the hottest zone. If this is not done, the likelihood of

breaking the column increases. The capillary should be held about 2–3 cm above the position where it enters the straightening tube.

The capillary that emerges from the straightening tube will probably have some curvature as a result of two factors. For a given rate of feed, a relatively high temperature causes the emerging capillary to have a curvature opposite to that of the original coil, whereas a relatively low temperature results in the same curvature as the original. If the temperature is constant, a relatively fast feed rate causes the emerging tubing to have the same curvature as the original and a relatively slow feed rate results in the converse curvature.

When the required length of capillary has been pushed through the straightening tube, the capillary is withdrawn at a uniform rate (*ca.* 1 cm/sec) using a force that is applied axially to the straightening tube. If the process did not produce a perfectly straight end, the capillary should be re-inserted in the straightening tube and withdrawn again.

Many chromatographs require the column to have straightened ends that are perpendicular to a tangent to the coil. The formation of the right-angled bend is most easily effected with a flame (nitrogen flowing through the column), but one must not heat the capillary more than is necessary. In order to keep the mechanical force high so that the temperature at which the capillary bends is at a minimum, it is necessary to rotate the column coil once or twice so that the straight end is at an angle between the horizontal and 45° from the vertical. After the right-angled bend has been formed, the straight end should be cut to the required length and the end fire-polished at as low a temperature as possible.

Role of the type of glass

We have shown that flaming under the mildest possible conditions affects an organic layer on soft glass much less than on borosilicate glass. In contrast, columns prepared from different varieties of glass behave almost identically when the ends are straightened with the new electrical device.

The explanation is easily given in terms of deformation temperature. A given straightening procedure requires an optimal viscosity of the glass, independent of the type of glass. As Fig. 5 shows, the low viscosity required for regular flaming is attained by borosilicate glass at *ca.* 950°C, whereas soft glass reaches the same viscosity at 800°C. The far higher viscosity which is sufficient for the new straightener corresponds to 530°C for borosilicate and 500°C for soft glass. Both temperatures are sufficiently low to preclude carbonization of apolar silicone stationary phases or reactivation of the support surface. This means that the end treatment becomes practically independent of the type of glass when low-temperature electrical straightening is applied.

Optimization of straightening conditions

The electrical straightener works reasonably over a temperature range of *ca.* 150°C. The lower temperature limit is characterized by glass breakage due to the excessively high viscosity. Above the upper limit the glass becomes compressed in the lowest part of the funnel-shaped entrance of the hot tubing. Obviously it is essential to test the influence of temperature within the above-mentioned range on the inertness of the straightened glass capillary. For apolar silicone stationary phases, even on borosilicate glass, we did not find differences in behaviour as a function of temperature

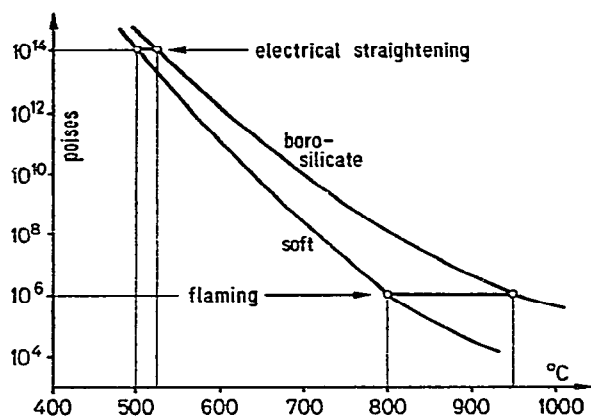


Fig. 5. Dependence of viscosity on temperature for soft (Schott 16¹¹¹) and borosilicate glass (Duran 50) according to Deeg and Richter¹⁵. Whereas the regular viscosity for glass blowing is *ca.* 10^4 P, flaming is presumably done at 10^6 P. The lowest viscosity sufficient for straightening under a strong mechanical influence is 10^{14} P. Note that different glasses reach this viscosity at very similar temperatures.

over the 150°C range. This means that the selection of the straightening temperature can be based purely on mechanical convenience and one need not be concerned about chemical effects.

Further, we wanted to compare the effect of electrical straightening with the effect of the mildest flaming procedure. Fig. 6 illustrates the comparison for borosilicate glass. Treatment A included careful removal of the stationary phase from the

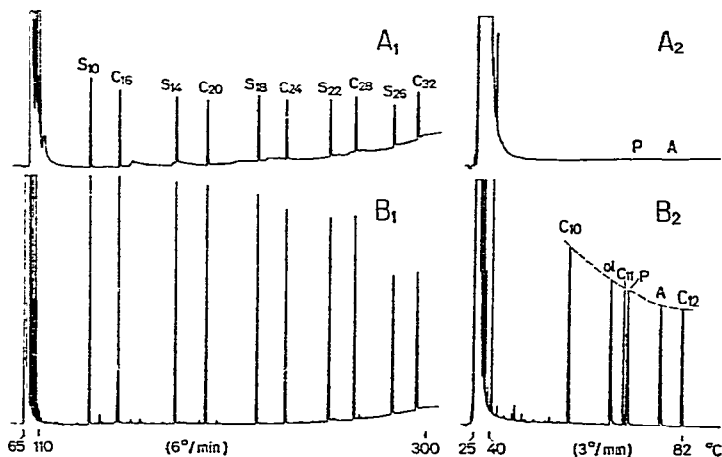


Fig. 6. Results obtained with persilylated borosilicate glass column ($15\text{ m} \times 0.30\text{ mm I.D.}$, $0.15\text{-}\mu\text{m OV-73}$). The mildest feasible flaming involving stationary phase removal, alcohol flame and 100-mg weight at the column end (A_1/A_2), compared with simple electrical straightening without stationary phase removal (B_1/B_2). On-column injection. Left: Donike¹⁶ activity test, 10 ng per peak. Substances: C = *n*-alkanes; S = silylated fatty acids; subscript numbers indicate number of carbon atoms in the main chain. Right: early section of Grob test¹⁴, 2 ng per peak. See text for interpretation.

end to be treated by washing three times with fresh *n*-hexane. Straightening was then carried out in the alcohol flame, with a weight of *ca.* 100 mg fixed to the end of the capillary. The activity test according to Donike (A_1) showed a severe, general reduction of all signals due to adsorption on carbon produced from residual, non-extractable stationary phase. Virtually no catalytic activity was introduced by mild flaming (signals of silyl esters not reduced relative to signals of alkanes). Except for traces of A and P, the substances of the Grob test disappeared completely (A_2) owing to adsorption. The striking difference between A_1 and A_2 is related to the much higher sensitivity and lower temperature used in A_2 , both of which make adsorption more visible. Treatment B consisted of the simplest electrical straightening (pushing the broken end through the straightener; no removal of stationary phase). The test results B_1 and B_2 are identical with those obtained from the original column. They show excellent inertness.

The situation with soft glass is considerably different (Fig. 7). Treatments A and B correspond exactly to those in Fig. 6. After mild flaming, no carbonization was detected (no difference in alkane signals in A and B), but a considerable loss of heavy silyl esters, indicating increased catalytic activity, was observed. The Grob tests are not shown, as they were identical after treatments A and B. This means that the results of mild flaming and of electrical straightening differ much less with soft glass than with borosilicate glass. One must remember, however, that the imperfect inertness obtained by mild flaming is the result of a series of careful manipulations, in contrast to the very simple electrical straightening.

All of the above conclusions are valid for apolar silicone stationary phases.

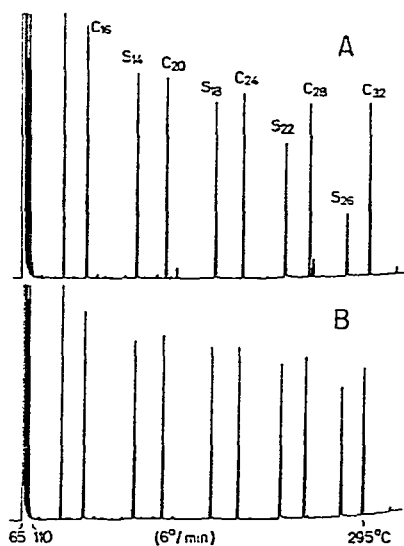


Fig. 7. Results obtained with persilylated soft glass column (18 m \times 0.29 mm I.D., 0.12- μ m SE-52). End treatments identical with those in Fig. 6. On-column injection. A, Donike test after mild flaming; B, same test after simple electrical straightening. Note the particular behaviour of soft glass after flaming under inert gas: no signs of carbonization, but significant catalytic reactivation, as observed also in Fig. 2B. Grob test not shown (see text).

With increasing polarity the carbonization temperature decreases. In Fig. 8 treatment A was the simple electrical straightening without solvent removal of the stationary phase (OV-17). Considerable carbonization occurred, causing severe adsorption effects (no catalytic reactivation). The problem was overcome by removing the stationary phase prior to electrical straightening (treatment B). Perfect inertness was obtained in this way.

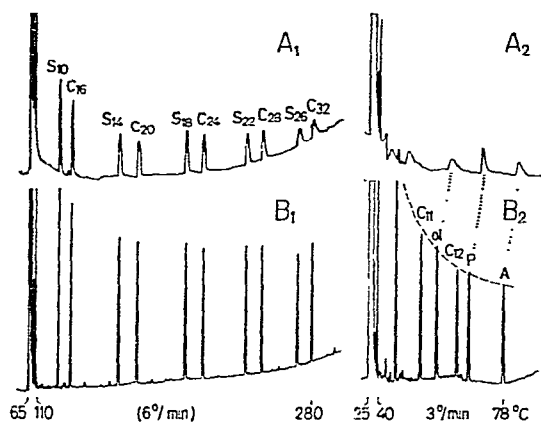


Fig. 8. Results obtained with a borosilicate glass column (14 m \times 0.31 mm I.D.) coated with OV-17 after persilylation with tetraphenyldimethyldisilazane¹⁷. Samples and sampling conditions as in Fig. 6. Treatment A, simple electrical straightening; B, electrical straightening after removal of stationary phase. Primary result: silicone stationary phases with considerable concentrations of functional groups are carbonized even at the low temperature (*ca.* 550°C) of electrical straightening. No reactivation in either treatment is observed.

SUMMARIZED RECOMMENDATIONS FOR END STRAIGHTENING

(1) Columns with a \pm active glass surface (surface covered with NaCl, BaCO₃, silica whiskers, Silanox). Two steps are required:

(a) Any straightening technique which does not produce carbonization.

- flaming under air flow;
 - electrical straightening under air flow;
 - electrical straightening under inert gas;
 - electrical straightening under inert gas after removal stationary phase
- } to be
} selected
} empirically.

(b) Deactivation of straightened section with 0.1% Carbowax in CH₂Cl₂.

(2) Columns with very inert (persilanized) glass surface. Treatment depends on the coating:

- apolar stationary phase (SE-30, SE-52, SE-54, OV-1, OV-73): electrical straightening without removal of stationary phase;
- more polar stationary phase (OV-3 and higher content of functional groups): electrical straightening after removal of stationary phase.

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