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PREPARATION OF INERT GLASS CAPILLARY COLUMNS FOR GAS CHROMATOGRAPHY

A REVISED, COMPREHENSIVE DESCRIPTION

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SUMMARY

A critical comparison of commercial with laboratory-made columns has revealed important new facets. Whereas commercial columns are now preferentially made of fused silica, there are strong arguments for retaining glass for laboratory-made capillary columns. In parallel with the intense development of commercial columns there has been a considerable increase in interest in making individual columns. A primary reason may be that the study and optimization of new analytical applications require rapid and flexible column tailoring, which is not available from commercial manufacturers.

The laboratory preparation of inert columns has recently been facilitated. Simpler and safer preparation techniques have become available, which permit non-specialized laboratories to include column preparation in their regular analytical activities. To support such laboratories, a detailed and comprehensive description of recent column preparation techniques is presented.

THE ROLE OF COLUMN TYPES

The success of packed columns is based on balancing a relatively modest separation efficiency with selectivity, so that it is important to have a wide choice of stationary phases. Automatically, associated thinking has been extended to capillary columns. In the past it was difficult to convince an experienced gas chromatographer that selecting a good (*i.e.*, inert, thermostable, efficient) column is more successful than searching for a column coated with a particular stationary phase. The same experts had trouble in realizing that not every stationary phase with a high reputation for use of packed columns is suitable for making good capillary columns.

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Unexpectedly, the advent of fused-silica columns has greatly supported the spread of more appropriate thinking. Originally, only two types of stationary phases (non-polar silicone; Carbowax) were suitable for coating these columns with high quality. For packed columns, this would have been an unacceptable limitation, whereas it caused almost no problems with capillary columns. In particular, the almost universal services rendered by persilylated, non-polar columns have greatly helped to emphasize that equally valuable columns with more polar coatings were not available.

Today an additional fact is increasingly accepted, namely that combining quality aspects such as inertness, thermal stability, separation efficiency and phase immobilization is feasible only with non-polar silicone phases. An increasing number of chromatographers have realized that a perfect SE-54 column (which is easy to make) does a far better job, in most applications, than the best, but still modest, OV-17 column. Thus, in capillary gas chromatography (GC), column selection means first looking for a high-quality column, and only second selecting a specific stationary phase.

The problem limiting the quality of polar capillary columns is film stabilization. With increasing polarity, surface tension also increases, which means that in order to keep the stationary phase spread additional measures are required (suitable groups on the support as a basis for intermolecular attraction, or reactive sites to produce covalent bonding). Up to present there remains the unfortunate rule that all these measures create additional column activity. Consequently, the inert, polar column remains unrealized.

COMMERCIAL OR LABORATORY-MADE COLUMNS?

The above question has been asked and discussed repeatedly in the past[1]. It may be answered again against the background of substantial modifications in column technology. Two recent modifications are prevalent.

First, the advent of fused-silica columns[2] suddenly broadened the application of capillary columns, including work in a large number of non-expert laboratories. The breakthrough was primarily due to the circumvention of end straightening. In addition, it is supported by increased confidence in the quality of commercial columns. A fact that is not scientifically related to the two column types is that fused-silica columns were better than the average commercial glass capillary columns.

The second modification has the opposite effect. The preparation of glass capillary columns has recently been developed and simplified to such an extent that making capillary columns in the laboratory has become substantially more attractive. In an average GC laboratory, columns of equal or higher quality than that of fused-silica columns may easily be prepared.

We see the effect of this controversial situation on future developments as follows.

Commercial columns are now a reliable tool for laboratories excluding *a priori* any activity in column technology. They may also be suitable for known applications, which means that experience with the proposed analytical task leaves little doubt concerning the optimum column to be ordered. When, as in these applications, there is no intention of touching a column more than necessary for connecting the injector and detector, then the preferential material will be fused silica.

Laboratory-made columns are hardly replaceable in research work, *i.e.*, when the technique to be used for the analysis of a particular sample is not known from the literature or from previous experience. Selecting a presumably suitable column from a manufacturer's list is then largely a matter of guesswork. When, possibly after several months, the ordered column arrives, there is no way of learning how well or how poorly it will solve the given problem. Optimization is based on variation and comparison. Variation has to be broad (not limited to commercially available columns), and rapid (a modified column is ready in a matter of days instead of months). These requirements are easily fulfilled by laboratory-made columns. These columns will continue to be preferentially made of glass. When considering the availability and cost of the raw material, as well as the working convenience, this decision leaves no alternative.

In addition to this general distinction of the two sources of capillary columns, there are more specific considerations.

We know of laboratories that rely only on their own columns even for routine work. The reason is very stringent standardization of analytical procedures to be maintained over a number of years. As a matter of experience, commercial columns are not sufficiently constant in their characteristics. This is not surprising, as manufacturers will naturally improve their procedures as soon as it becomes practicable. In certain applications, however, constant characteristics are more important than any improvement. Another laboratory working with difficult samples of biological origin has previously spent much effort on reducing the harmful effects of the sample on the column. However, a totally different policy has proved to be more successful. The required optimum columns are now prepared as required as a simple operation. No sample clean-up is applied, and the columns are replaced after a few days' use.

In summary, it should be noted that being confined to commercial columns means limitations to and possibly a substantial reduction in the flexibility and efficiency of research work. We wonder whether all laboratories consider this fact when making decisions about column selection.

THE PURPOSE OF THIS PAPER

The increasing interest in laboratory-made columns is concentrated on inert, high-temperature, multi-purpose columns. It is natural that a column type is preferred on which most types of samples can be analysed, thus freeing the chromatographer from becoming involved in the quite different column technology of other types. Fortunately, the preparation of a particular column type has reached a level of simplicity and safety that permits column technology to become a regular activity of many laboratories that may previously have hesitated to initiate column preparation. Top quality is easily and constantly attained, as shown by Fig. 1, which originates from a non-specialized industrial laboratory.

The preparation of inert columns includes a series of steps which, owing to incomplete understanding, had to be studied empirically. The major problem proved to be the strong interrelation of the single treatments. There is hardly any useful optimization of a single step; the combined procedure has to be optimized. Therefore, it is not surprising that comparable final results for the overall procedure may be achieved using different parameters for the individual steps. This is probably why, in recent years, confusing recommendations have been published.

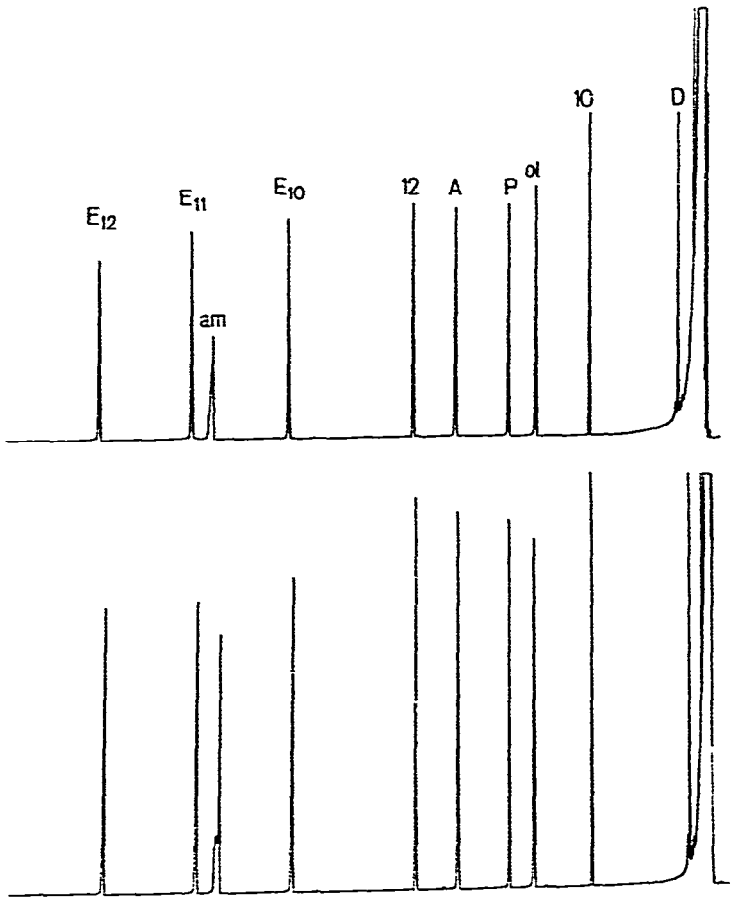

Fig. 1. Example demonstrating the durability of a persilylated column under industrial routine conditions. Column: 19 m × 0.32 mm I.D., persilylated HMDS–DPTMDS (1:1), 0.3 μm SE-54, 0.2% DCUP. During 9 months of continuous use, 1800 runs with temperature programming to 300°C, 500 runs to 325°C and 200 runs to 350°C were carried out. The column was washed repeatedly with methylene chloride. Upper chromatogram: test according to ref. 18, but without ethylcaproic acid, run after column preparation. The chromatogram shows excellent quality. Lower chromatogram: test at the end of observation period (after 2500 runs). Except for a weak alcohol, phenol and aniline adsorption, the column shows no degradation; the overall quality is still good. For substances see ref. 18.

A systematic optimization programme is best carried out in several laboratories. Parallel work may reveal that apparently negligible working differences may have important consequences; a detail emphasized by one worker may, under slightly different conditions, be revealed as avoidable by another worker.

The purpose of optimization is to produce top-quality columns by the simplest procedure, and we think that our work has reached a level that merits description. In the interest of potential users, the complete preparation procedure for inert non-polar columns is described. Consequently, details are repeated unchanged from previous reports. In the first part we present our experiences and ideas concerning individual steps and the second part gives the corresponding working instructions.

The optimization of our procedure does not, of course, exclude the possibility of there being a better procedure based on a different approach. It would not be useful to discuss recommendations by other workers, however. Such recommendations may be valuable in a different approach, but hardly fit into our optimization scheme. Thus, this report does not review actual research into column technology.

DISCUSSION OF SINGLE STEPS

Glass variety

In addition to mechanical differences (friability, behaviour under drawing), different glasses vary primarily in their amenability to the production of an ion-free silica surface with minimum deviation from the original compact and smooth glass surface. Top-quality columns can be made from all common laboratory glasses. However, producing the ideal surface is less critical with borosilicate than alkali glasses.

The problem with alkali glasses seems to be the relatively easy migration of calcium and magnesium ions from lower levels through the hydrated surface layer during leaching. Consequently, deep leaching is required, which may lead to an excessively open silica structure. Combining sufficient deionization with minimum surface porosity requires a critical optimization that has to be carried out individually for the different alkali glasses.

With borosilicate glasses, ion diffusion from lower, intact levels is less intense. Consequently, less deep leaching is required in order to produce an ion-free surface, and optimization of the leaching process is less critical. Our preferred and almost universally used glass is Duran 50, which combines the typical behaviour of a borosilicate glass with some advantages of soft glasses (less friable and easier to draw than Pyrex).

We have no results on the effect of rinsing the glass tubing prior to drawing. Our only precaution is to remove adhering dust with compressed air.

Leaching

We introduced intense acidic leaching in order to produce an ion-free support surface for barium carbonate crystals[3]. The use of the leaching procedure as a basis of persilylation did not cause us to adopt any substantial modifications, in spite of the considerable criticism of Lee and Wright[4], who claimed their dynamic leaching to be superior to our static procedure. In fact, we have been unable to obtain constant column quality by dynamic leaching (see also under *Rinsing*). Similarly, despite all our efforts we have so far failed to replace the non-specific action of the hydrochloric acid solution by a specific deionization based on complexing agents.

We have long been aware[5] that intense, or excessive, leaching results in an ion-free silica layer, the structure of which may be termed "open" or microporous. We have reported adverse effects of microporosity[6]. Recently, the experimental evaluation and interpretation of a potentially microporous silica surface has been greatly facilitated by the advent of fused silica, which may serve as an ideal model exhibiting an ion-free surface without any microporosity. A comparison of glass leached according to our optimized procedure with fused silica showed very little difference, which is negligible in most applications.

In contrast to the procedures of rinsing and dehydration, the optimization of leaching is easy and straightforward. Leaching borosilicate glass at 160°C or lower temperatures results in a residual column activity that we attribute to insufficient deionization. Leaching at 180°C produces the first clear signs of a microporous surface. Thus, 170°C is the optimum temperature.

During the leaching process, the column should be completely filled with liquid. This is ensured when the column is sealed, at room temperature, with an empty space amounting to 7% of the total volume of the column. As the empty space is regularly expressed as 7% of the column length, problems may arise from varying diameters of single turns of a coiled column, or from a fluctuating internal width of a glass capillary.

Rinsing

The purpose of rinsing is simply to eliminate the dissolved metal ions from the leached column. We know, however, that the process is complex, and may be carried out in several ways, leading to unsatisfactory columns. Some experimental observations exemplifying this complexity are as follows:

(i) We have never been able to rinse 50–100-m columns in the same ideal way as is feasible with 20-m columns.

(ii) Prolonged rinsing (in terms of time and rinsing volume) with the aim of ensuring complete elimination of dissolved material may result in more active columns than are obtained by briefer rinsing.

(iii) The entire rinsing process should occur under strongly acidic conditions (no distilled water should be applied).

(iv) Completing the rinsing process by flushing the bulk of water with acetone or methanol may have an adverse effect on quality.

We conclude from these observations that the theoretical understanding of the process is still insufficient to serve as a basis for experimental work. We hope that the gap will be filled in the near future. The hope is supported by the work by Venema and Beltman[7,8], who recently discussed the latter two observations, and by work of the Eindhoven group[9]. The currently unknown details of the process that may be responsible for the negative effect of prolonged flow are the probable reason for the unsatisfactory results obtained by dynamic leaching.

Dehydration

There is now wide, although not general, agreement that dehydration should eliminate adsorbed water while preserving surface hydroxylation (as a basis for silylation). However, the recommended temperatures for dehydration (150–400°C) obviously show that different workers achieve different results by using different working conditions. At first, it seems contradictory that all of these workers are able to achieve comparable column qualities. We believe free ammonia to be the agent that solves this apparent contradiction (see under *Persilylation*).

Dehydration involves transport of water out of the column. This transport can be achieved by a flow of carrier gas, by applying a vacuum or by means of a water-miscible solvent. Our experience with the three methods is as follows.

A flow of carrier gas will inevitably cause a gradient along the column, the importance of which increases with increasing column length. A carrier gas is relative-

ly inefficient in removing the large amounts of water that remain after rinsing with dilute hydrochloric acid. Consequently, drying under a flow of carrier gas is suitable for short columns. It may be applied to longer columns from which the bulk of the water has previously been removed by a solvent.

A vacuum is an efficient means of drying a column that is fully wetted with dilute hydrochloric acid. Symmetrical dehydration is obtained by connecting both column ends to a vacuum via a T-piece, or by alternating the connection between the two ends.

Solvent flushing, particularly with soft glass, may affect the surface structure of the hydrated silica layer. With a long column it may nevertheless be a suitable first step of dehydration, as the removal of large amounts of water by evaporation may be difficult anyway.

Persilylation

We believe that the term "per-" correctly denotes the essential purpose of the process to produce an organic surface coverage of maximum density. The reason why all silylation procedures as applied before 1977 yielded columns of unsatisfactory inertness and thermal stability was their inability to cover the surface densely. The breakthrough started with Welsch *et al.*'s[10] high-temperature reaction (300°C, untreated glass), which had to be completed by increasing the temperature to 400°C and by using leached glass[11].

We suppose that, in addition to prolonged reaction at high temperature, ammonia is an essential agent in producing dense surface coverage. At first, steric problems may cause residual unreacted areas. In the presence of ammonia, siloxane bridges may be opened and restored, causing the steric situation to be continuously modified. At the moment of a temporary, favourable situation, additional silylation may occur, thus approaching maximum density.

Our interpretation is in agreement with the fact that excessive dehydration (*i.e.*, partial dehydroxylation) does not exclude *per*silylation as long as ammonia is present. It is also in agreement with our previous experience that just repeating a silylation does not complete an insufficient first silylation that has completely consumed all available silanol groups. The failure of a second reaction is then caused by the absence of ammonia, which is normally produced by the regular silylation reaction. We have recently confirmed that a second silylation does occur, provided that silylating agent plus ammonia is introduced before sealing the column. The interpretation is also in agreement with the fact that more bulky silylating agents require higher reaction temperatures.

We have no experimental evidence to lead us to replace disilazanes by one of the other recommended silylating agents. As long as other agents are not proved to be superior, we prefer disilazanes for convenience (viscosity, volatility, price).

We still find that diphenyltetramethyldisilazane (DPTMDS) produces slightly higher wettability (higher separation efficiency) and higher thermal stability of the columns than hexamethyldisilazane (HMDS). The advent of immobilization at first brought an argument against introducing phenyl groups into the support surface, as phenyl groups are supposed to hinder surface bonding. However, broad experimental comparisons have greatly reduced the weight of this argument. As long as apolar phases are used, the difference in the degree of immobilization as observed on surfaces

with and without phenyl groups is surprisingly small, whereas the advantages of phenyl groups are fully effective.

For the preparation of non-polar columns, we do not regularly introduce vinyl groups into the support surface. As reported previously[12], persilylation with a vinyl-containing agent results in weak, but clearly observable, column activity. The columns show predominantly basic behaviour including, however, slight acidic characteristics simultaneously. Thus, whenever surface bonding as supported by vinyl groups is not of essential importance, we prefer vinyl-free silylating agents in the interests of achieving maximum inertness.

Originally, we carried out persilylation under vacuum. In the interests of technical simplicity, we have now optimized the procedure for persilylation at atmospheric pressure as reported by the Ghent group[13].

We recommend checking the oven (muffel oven, oven of a gas chromatograph) used for persilylation. Built-in thermometers may indicate greatly deviating temperatures, and the heat distribution in the oven may be very inhomogeneous.

Rinsing after persilylation

As a regular side-product, persilylation produces trace amounts of a white powder, which is easily dissolved in water and is weakly soluble in methanol. We assume the powder to be a mixture of ammonium salts. Trace amounts of this solid material remaining in the column will act as active points causing bubble formation, and breakthrough, in the course of static coating. Rinsing with methanol before coating solves this problem[14].

Coating

For reasons of easy immobilization (see below), and of maximum thermal stability, our standard stationary phase is SE-54. Alternative non-polar phases are used only when the slightly different selectivity is of importance. SE-54 shows all positive aspects of a gum phase. Its additional advantages are relatively rapid dissolution in pentane and a relatively low viscosity in solution, permitting coating with concentrations up to 4%, corresponding to 3–3.5 μm thick films in 0.3 mm I.D. columns.

Static coating is carried out as usual. We have recently described a practical procedure[14] that we have carefully optimized for maximum simplicity and safety.

Immobilization

The advantages of immobilized coatings are so obvious that for almost 2 years we have no longer prepared non-polar columns with extractable coatings. Immobilization requires very little additional work, consisting only in adding the peroxide to the coating solution and keeping the sealed column at the reaction temperature before regular conditioning. The earlier evaluation of peroxides[15] has not been modified. Dicumyl peroxide (DCUP) remains the standard reagent. Additional experience has shown that more peroxide can be added without causing significant activity.

Originally we carried out the radical reaction in a very low flow of carrier gas to flush undesirable by-products (possibly water). In the interest of simplicity we have further studied the reaction in the sealed capillary. We have found that relatively intense flushing before the reaction can replace the permanent flushing during the reaction. This permits immobilization in the sealed column without any decrease in column quality.

Certain details concerning the work with immobilized columns have still to be studied. Additional information is required about the selection of solvents for column washing, as well as about the ideal procedure (frequency, intensity, detection of desirability) for column washing.

Column end sections

Straightening coated and immobilized column ends without affecting column quality is not always easy. We recommend one of two techniques.

Keeping 5–10% of column length at both ends free of coating is of general desirability. In particular, an uncoated end should be available to serve as a retention gap[16]. Low-temperature, electrical straightening of uncoated ends is not critical. Producing an uncoated end during static coating (at the open column end) causes no problems[14]. The same result may be achieved at the opposite end by washing out the coating immediately after coating, and before immobilization or by having the end filled with pure solvent during static coating.

An alternative technique is simply to cut the coiled column, and to connect the cut end to a short piece of empty fused-silica tubing, using a recently described technique[17]. Fused-silica capillaries with suitable dimensions are available from SGE (Victoria, Australia).

Column renewal

It would be very desirable to have a standard procedure capable of restoring the original column quality after routine application involving heavy stress to the column. Unfortunately, there is no general treatment available. If contamination is the source of degradation, column washing may be fully effective. If there is damage to the phase, *e.g.*, by strong acids or bases, additional silylation may be helpful. In other instances, additional coating (sandwich coating) may solve the problem.

By far the most frequent damage to inert, immobilized columns is contamination by polar, high-molecular-weight sample by-products, possibly introduced in trace amounts. Depending on their volatility, they may remain in the inlet section of the column, or may slowly penetrate into the column. If they are soluble, they may be eliminated by back-washing, the standard solvent sequence being methylene chloride, methanol, methylene chloride. If they are insoluble, the inlet section has to be replaced. In both instances early reaction is recommended to prevent excessive accumulation or deep penetration into the column.

Especially with samples of natural, *e.g.*, biomedical, origin, it is purely a matter of experience to know their influence on a column. They may be heavily contaminated (no clean-up applied) without doing any harm to the column. In contrast, even careful clean-up may not remove sufficiently a specifically "poisonous" by-product. In too many instances we meet users worried by column degradation which they erroneously attribute to poor column quality or insufficient thermal stability. They should realize that it is an intrinsic characteristic of inert columns to be sensitive to certain contaminants which, by their simple presence, destroy the inertness.

SUMMARIZED INSTRUCTIONS

The raw material

Duran 50 (Schott-Ruhrglas, P.O. Box 2924, D-8580 Bayreuth, G.F.R.) is particularly suitable for drawing glass capillary columns. Before drawing, mechanical cleaning without washing or chemical purification is recommended.

Leaching

By means of compressed gas, force 20% hydrochloric acid into the column. When the column is filled, continue to introduce hydrochloric acid until 20–30% of the filling volume has been collected at column outlet. Accurately mark the first 7% (4% in the case of soft glass) of the column length. When the rear meniscus of the hydrochloric acid plug reaches the mark, disconnect the inlet end from the pressure source and connect it to a vacuum. When the liquid has reoccupied at least 10% of the empty section, seal the vacuum-connected end in a flame. Evacuate and seal the opposite end. Keep the column overnight (12–16 h) at 170°C (140°C in the case of soft glass). The column must not touch heated parts of the oven.

The quality of the leaching treatment is independent of column length and the longest available pieces may therefore be leached.

Rinsing

From the leached and hydrochloric acid-filled column (which can be stored without damage), break away the shortest useful piece. Displace the filling by 1–2 filling volumes of 1% hydrochloric acid at a rate of not more than 2 cm/sec. If the alternative dehydration method is to be applied, 1% hydrochloric acid may be followed by half the column volume of acetone (not recommended for soft glass).

Dehydration

Standard method. Place the wet column in an oven with both ends accessible outside the oven. Keep the column at 300°C for 2 h (15–20 m) or 3 h (30–50 m). Connect one end to vacuum and alternate the vacuum connection for the two ends every 10–20 min.

Alternative method (slightly less suitable, but simpler). Mount the column in a column oven without connecting the outlet end. Adjust the carrier gas flow to correspond to roughly 20% of the flow that one would give the particular column under regular chromatographic conditions.

Whereas the leached (hydrochloric acid-filled) and dehydrated columns can be stored, the rinsed columns should be dehydrated immediately.

Persilylation

The standard reagent is diphenyltetramethyldisilazane (DPTMDS) from Fluka (CH-9470 Buchs, Switzerland; No. 43340). For simplicity, the pure reagent may be used. For economy of time, a 1:1 (v/v) solution in pentane is recommended.

Suck into the column about 10% of the column length of reagent. By means of a dry inert gas, move the liquid at a rate of 0.5 cm/sec for the pure reagent or 2 cm/sec for the pentane solution. When the liquid has left the column, immediately seal both ends in a flame when using the pure reagent or, when using the pentane solution,

increase the carrier pressure 2–4-fold and apply a vacuum to the exit end. After 2 min for a 20-m or 10 min for a 50-m length, disconnect the vacuum in order to fill the column with inert gas, and seal both ends.

Place the column in the oven of the gas chromatograph, or into an equivalent high-temperature oven. After heating at full power to 150°C, continue to heat at a rate of 2°C/min. Keep the column overnight (12–15 h) at 400°C. Turn off the heating and allow the column to cool slowly in the closed oven. Cut both ends and rinse the column with toluene, methanol and diethyl ether, each solvent amounting to 30–50% of the column volume.

Coating

Prepare a fresh solution of SE-54 (or another non-polar silicone gum stationary phase) in pentane with a concentration within the range 0.02–4.0%. Select the concentration to yield a desired film thickness according to the equation

$$\text{Film thickness } (\mu\text{m}) = \text{I.D. (mm)} \cdot \text{stationary phase concentration } (\%)/0.4$$

When the stationary phase has dissolved, add dicumyl peroxide (DCUP; Elfa, Beethovenstrasse 48, CH-8002 Zurich, Switzerland). Standard amounts of DCUP, expressed as a percentage of the dissolved phase, are SE-54 0.4%, SE-30 and OV-1 0.6% and SE-52 and OV-73 1.0%.

To avoid misinterpretation, a practical example is given: adding 0.4% DCUP to 10 ml of a 0.5% solution of SE-54 in pentane means adding 10 µl of a 2% solution of DCUP in toluene.

For continuation, use the recent, detailed description of static coating[14].

Immobilization

Mount the freshly coated column into a gas chromatograph without connecting the outlet end. Set a flow of carrier gas corresponding to double the flow one would set for chromatographic purposes. Keep the column at 40–50°C for about 3 min. Remove the column, and immediately seal the ends in a flame. Keep the column for 1 h at 160°C and 1 h at 180°C.

The column may now be washed (with two column volumes of methylene chloride for low and medium film thicknesses, up to five column volumes for thick films) to flush the by-products of immobilization. If one wishes to determine the degree of immobilization, condition the column for 1 h at 200°C, run a test[18], wash the column and repeat the test to check the decrease in film thickness. Washing may well be postponed after a first period of routine application.

Conditioning

The "fresh test" run after brief conditioning at 200–220°C serves a basis for column evaluation. For a careful evaluation, run a second test after heating overnight to 250°C. A properly prepared column should not show any degradation due to this treatment, except for some change in acidity. If the column is not intended to be used above 250°C, further conditioning is useless. For high-temperature application, heat the column at 300°C for another night. Slight alcohol adsorption in the next test run does not necessarily indicate degradation. Inert, immobilized columns tend to recover

from heat stress. A further test run the next day may show the original characteristics.

With immobilized columns, conditioning with the aim of cleaning the column is preferentially replaced by washing.

CONCLUSION

Our recommendations may create an impression of pronounced, possibly excessive, sophistication or refinement. In fact, for moderately demanding analytical applications, columns prepared in a simpler way may be satisfactory. On the other hand, we wish to emphasize that increasing column quality is not just a matter of striving for perfection or "play". It is our experience that every quality improvement further broadens the range of applications feasible on a given column. Accordingly, increasing column quality may be an essential factor in the laboratory preparation of columns, as the columns obtained may render services that cannot be expected from average commercial columns.

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