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## STATIC COATING OF CAPILLARY COLUMNS: SOME PRACTICAL CONSIDERATIONS

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### SUMMARY

Some of the practical problems involved in the preparation of wall-coated open tubular (WCOT) glass columns have been examined, and precautions are described to overcome or minimize these problems when non-polar silicone gums are used as the stationary phase. Modifications have been made that simplify the preparation of WCOT columns. The labour involved in cleaning an old column and recoating it amounts to about 2 h work, plus monitoring those steps that proceed automatically. About half this time is spent in cleaning the old column.

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### INTRODUCTION

Many people are deterred from using capillary columns for gas-liquid chromatography (GLC) not only because they are both expensive to buy and relatively difficult to prepare but also because the useful life of these columns is relatively short. It is normal practice to purchase these columns and, whether they are coated by the supplier or by the user, to discard them once the column characteristics deteriorate. Since the cost of pre-coated columns is high, and even uncoated ones are moderately expensive, finance is often the factor that limits the use of these columns. For these reasons the simple procedures described in this paper have been developed for cleaning used columns and coating them. The equipment required for this is to be found in most laboratories. Due to practical problems, only wall-coated open tubular (WCOT) columns have been prepared; SCOT columns require specialised equipment. The technique used is essentially a development of that of Bouche and Verzele<sup>1</sup>. Although more difficult to employ than the dynamic coating technique<sup>2,4</sup>, unlike this procedure it can be used with viscous phases to yield a column with a loading that is adequate for most practical applications.

In principle, it appears simple to allow a solution of GLC phase to evaporate in a capillary column *in vacuo*, so that as it evaporates, the phase is left as a thin film on the surface of the glass; however, in practice there are numerous problems, some of which are easily overcome, but others are more difficult to resolve. Although it is possible to obtain an even coat on the column, many solvents and phases do not

yield an even coat without taking special precautions, *e.g.* etching the surface to form sodium chloride crystals<sup>3</sup>, or pre-coating with barium carbonate<sup>2</sup>. The technique described here will, in fact, yield an even coating without taking such precautions, but only with the solvent and phases described.

#### PREPARATION OF COLUMN

For this work, commercially available Pyrex glass capillaries, (50 m × 0.5 mm I.D.) in coils of 13-cm diameter, have been used. Previously used columns can be recovered and re-used if they are cleaned as follows: two solvents are chosen, one in which the coating phase is soluble (*e.g.* diethyl ether for SE-30) and the other in which it is insoluble (*e.g.* acetone for the above phase). The latter phase is introduced into the column, 2 ml at a time. This can be facilitated by using a coating reservoir purchased from SGE (London, Great Britain), at 50 p.s.i. Portions of a mixture of the solvents are then introduced, slowly increasing the concentration of the solvent in which the phase dissolves, but not so fast that the phase is stripped off the column so suddenly that the column becomes plugged. This is continued until the phase has been removed. Two ml of acetone are introduced, then 2 ml water, followed by 5 ml 2 *N* hydrofluoric acid, 2 ml water and a final wash with acetone. The hydrofluoric acid removes brown material from the column without significantly affecting the surface of the glass; this treatment would be more hazardous with soda glass, which is more readily attacked by hydrofluoric acid. Not only does it attack soda glass; it produces a precipitate that can clog the column irreversibly. The column is now ready for pre-treatment.

For use with SE-30 Ultraphase, pre-treatment with *N,O*-bis(trimethylsilyl)-acetamide is adequate. A 2% solution in an inert solvent is passed through the column, left for about 30 min and the excess is washed out with more solvent. The pre-treatment is inadequate for other phases if they are to be used under conditions of maximal resolution, since they are often deposited as droplets on the inside of the column instead of a film. For use with these phases (*e.g.* OV-101), more rigorous pre-treatment such as barium carbonate coating<sup>2</sup> is required. Even for these phases the above pre-treatment is adequate if only about 50% of the maximal resolution is required, *i.e.* about 1500 plates/m for compounds of 18 methylene units under conditions of maximal resolution. The cleaning and pre-treatment steps take several hours but only about 1 h of a technician's time is involved.

#### CHOICE OF SOLVENT

The solvent used must, of course, dissolve the phase used. In addition, it must be volatile. In practice, this means that the boiling point of the solvent should be less than 50° above the temperature at which the column is to be coated. As an example, the volatility of benzene is scarcely great enough to be used if the evaporation is to be carried out at room temperature.

Most of the volatile solvents tested in which the phase is soluble are unsuitable (*e.g.* dichloromethane), since they deposit the phase as droplets of about 0.01–0.05 mm diameter. Diethyl ether, however, has an unusual property which makes it suitable for this purpose. When a dilute solution of SE-30 Ultraphase in diethyl ether evapo-

rates, a thin highly viscous coat of the phase forms on the surface of the solution which readily adheres to the glass, instead of being concentrated in the body of the solution as happens with some other phases, sometimes to the extent that the concentrated solution forms a plug that blocks the column. This property is observed, to a lesser extent with the silicone gums E-30, E-301 and E-350, whereas E-351 does not show any distinctive evidence of this property. Thus, when a solution of SE-30 Ultraphase evaporates in the column, the phase is deposited as a film on the surface of the glass, instead of being drawn back with the body of the solvent to form a highly concentrated solution at the air-surface interface that breaks up on the glass surface as droplets.

#### FILLING THE COLUMN

The most critical factor in the preparation of a column is maintenance of an unbroken solvent thread in the column, both during filling and during the evaporation stage. Special precautions must be taken during the process of filling to prevent the thread breaking subsequently. This involves degassing the solution and keeping it degassed throughout the procedure. A solution (about 0.4%) of SE-30 Ultraphase in diethyl ether is prepared, and doped with OV-25 to alter the GLC characteristics. For separation of phenolic acids (as their ethyl ester-TMS ethers) SE-30 Ultraphase and OV-25 in the ratio of 4:1 will give the desired characteristics. A flask containing the solution is attached to a vacuum line and shaken by hand without warming until the volume has been reduced by about 30%. The flask is then warmed with the vacuum line closed off, and the flask is detached only when its temperature is about 30°. It is then warmed to 37° and kept at this temperature during the filling process. At 37°, if the flask is kept free from draughts, there is little loss of solvent and little sign of boiling.

The column is filled by placing one end of it in the solvent and applying a gentle vacuum to the other end. A high vacuum can cause the thread of solvent to break and evaporate, which may cause a plug of phase to form in the column. The column fills, rapidly at first, and then more slowly, taking about 1 h to fill a 50 m column.

When filled, one end of the column has to be plugged off. In our hands this has proved to be the most difficult part of the procedure. We have had problems with the silicate procedure of Bouche and Verzele<sup>1</sup> so we have developed a quite different technique. The essential factor is the necessity to eliminate air bubbles and dissolved gases from the region of the plug and sharp discontinuities in composition of the material in the column, otherwise the liquid thread is liable to break at, or near the plug. A suitable material for use as a plug is paraffin melle (Vaseline) hardened slightly by addition of 1/6 vol. of histological wax (m.p. 56-58°). This is sufficiently fluid to prevent it breaking away from the glass of the capillary when it is cooled to 20°; if it breaks away, gas collects in the gaps and this may act as a source of bubbles. In practice, if the wax shows a tendency to break away from the glass, the proportion of Vaseline should be increased. This cannot be used in direct contact with the ethereal solution, since a junction between this wax and ether is a potential source of gas bubbles, as it is not practicable to degas the wax adequately. Therefore, a 1:1 mixture of hexane and diethyl ether (degassed) is drawn about 30 cm into the column, prior

to insertion of the plug. The hexane allows some degree of mixing the plug, thus preventing sudden changes of composition, and the diethyl ether will dissolve traces of gas released from the plug.

To make the plug, the wax mixture is melted at 50–60° and the end of the column is dipped into the wax whilst avoiding the introduction of air bubbles. The wax is drawn quickly into the column for a total distance of about 15 cm by squirting a jet of acetone onto the coiled column, cooling the column rapidly by evaporation. Other methods would not draw the wax in sufficiently fast to allow it to enter significantly before becoming solidified. If the plug of wax is then melted (by using a hair dryer), the risk of bubble formation is somewhat reduced, and it is our impression that this risk is further reduced by allowing the column to stand for several days prior to evaporation. Plugging the column takes about 5 min, compared with overnight for the silicate procedure.

### EVAPORATION STEP

The column is placed in a large vacuum desiccator, preferably with a sheet of black polythene at the bottom. The coils near the open end are spread out so that the progress of the solvent front can be observed in the first 20 coils. After the temperature in the desiccator has been allowed to stabilize with the lid on, air is pumped out very slowly so that the pressure is reduced at about 10 mm Hg/min. The desiccator should be kept at a constant temperature although there is no need to place it in a thermostat. The slow rate of evacuation allows the gas in the desiccator to remain at this temperature; adiabatic expansion of the gas brought about by sudden application of a vacuum cools the gas and the column, thus causing the liquid in it to contract by perhaps 10 cm or more. If this contraction is rapid, it can cause the thread of liquid to break, probably near the plug, and when the pressure drops below the vapour pressure of diethyl ether, the column will discharge its contents. If the thread has remained intact the liquid will expand when the column begins to warm up. Then, if the pressure has dropped below the vapour pressure of diethyl ether and the solvent begins to evaporate from the surface before the column has fully warmed again, there will be a stage when the rate of evaporation will equal the rate of expansion of the liquid; the surface of the liquid will remain stationary relative to the column and the phase will build up into a plug, thus making the column unusable. This is a hazard that can also occur at any time during the evaporation if the ambient temperature rises fast enough to cause a relatively rapid expansion of the solvent; this problem could be rather more acute with solvents less volatile than diethyl ether. In practice, it is usually adequate to keep the desiccator free from draughts or sudden temperature fluctuations.

At first, the pressure is reduced from atmospheric pressure until the diethyl ether evaporates slowly, and the liquid front retreats at about 1 cm/min at 20°. This temperature is close to the optimum; at a higher temperature the risk of breaking the thread is increased and at a lower temperature the rate of evaporation diminishes. It is inadvisable to reduce the pressure further until about 20 coils have been coated, otherwise there is a risk that the liquid thread may break. From time to time diethyl ether vapour must be slowly pumped out of the desiccator and a lower final pressure can be attained after about 20 coils have been coated, without undue risk of the

thread breaking, although the pressure should not be reduced below about 100 mm Hg. The pressure must not be allowed to rise to the vapour pressure of diethyl ether, since this causes condensation in the coils that have already been coated.

In principle, this method of coating is applicable to solvents with a higher boiling point by keeping the desiccator at a higher temperature, although we have not examined its potential.

#### SCHEDULE FOR PREPARING A COLUMN

Since the preparation of a column takes about a week overall it helps to have a time schedule to prevent crucial steps occurring at awkward times, e.g. during the weekend. The following scheme has been found to be satisfactory:

The column is cleaned and filled on a Friday. Since it takes several hours for the phase to dissolve, this is carried out with continuous stirring while the column is being cleaned and pre-treated. After filling, the column is placed in a desiccator. On Monday, the pressure is reduced until evaporation has been established. On each subsequent day, diethyl ether vapour is pumped out of the desiccator. The progress of evaporation can be followed by observing the appearance of the column. When filled with solution the coils are quite clear, but after coating they are distinctly translucent. A 50-m column should be completely coated by Friday, and a 90-m column has been prepared successfully by coating it during the week, evacuating the desiccator finally on Friday and leaving it unattended over the weekend. This, of course, requires a desiccator that is not only entirely air tight, but also has an adequate volume to hold the vapour. A total capacity of 10–15 l is adequate.

#### INSTALLATION OF THE COLUMN

After completing the coating procedure the plug must be removed by warming the plugged end and applying pressure or a vacuum to drive the plug out of the column. Most of the residual plug adhering to the glass can be removed by gently heating the section that contained the plug with a Bunsen flame, while a stream of nitrogen is passed through the column. Residual traces are removed during purging, the plug end being open to the atmosphere.

We have experienced problems in connecting these capillaries to a GLC instrument. Commercially available non-metallic ferrules of 1/16-in. bore are usually too hard to use with the column, since the glass collapses when the metal fittings are tightened onto the ferrule sufficiently firmly to make a gas-tight seal. This problem has been overcome by binding PTFE tape round the end of the column. The tape deforms sufficiently as the fittings are tightened to form a gas-tight seal, without crushing the glass.

It is advisable to store the columns in the dark after preparation, as there are some indications that columns which have been exposed to light from fluorescent lamps possess poorer GLC properties than those that have not been exposed.

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