Note

Stability of silica packing materials towards a mixed aqueous–organic eluent at alkaline pH

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The use of silica-based packing materials with alkaline eluents is frowned upon by most practising chromatographers. It is a commonly held belief that columns used with eluents having a pH > 7.5 will undergo rapid loss of performance due to dissolution of the silica matrix. This view, which can be traced back to a single report[1], has been perpetuated by a number of leading workers in the field, as well as by most manufacturers of high-performance liquid chromatography (HPLC) packing materials despite more recent reports to the contrary. Wehrli *et al.*[2] studied the dissolution of reversed-phase materials in different eluent types using atomic absorption spectrophotometry. They concluded that when using acetonitrile as the organic modifier, the rate of dissolution was very dependent on the aqueous content and the type of base in the eluent, *i.e.*, ammonia, alkylamine, sodium hydroxide or quaternary ammonium salt. Similar findings were obtained by Atwood *et al.*[3] who showed that for non-bonded silica, dissolution was reduced when the eluent had a high methanol or acetonitrile content. They also noted a sharp increase in dissolution with temperature, and they showed how the inclusion of a "sacrificial" column (often misnamed as a guard column) between pump and injector could effectively minimise silica dissolution.

In the analysis of basic compounds, the use of silica with mixed aqueous–organic eluents of high pH (typically 9 to 10) offers many advantages over reversed-phase methods. In presenting data on such systems, one of us (B.L.) has often faced scepticism from other workers on the viability of such systems due to the above mentioned belief. Apart from a report by Wheals[4], who stated that silica dissolution using such a system was not a problem providing the organic content was high and ammonia was the source of hydroxyl ions, no hard chromatographic data existed to support the stability of such systems.

The aim of the present work therefore was to produce quantitative data to demonstrate the stability of silica in the presence of an organic-rich alkaline eluent.

EXPERIMENTAL

Materials

Chromatography was carried out using a Waters 6000A HPLC pump, a Rheodyne 7125 injection valve and an LDC UV-III fixed-wavelength (254 nm) UV detec-

tor. The column was 200 × 4.6 mm I.D. packed with Spherisorb S5W silica (batch No. 5068) with characteristics typical of this type of material.

The eluent was methanol–ammonium acetate buffer (9:1), pH 9.2 measured using a Whatman PHA270 pH meter calibrated with aqueous standards. The buffer (pH 10.0) was prepared from ammonia (65 ml; 25%, w/v), acetic acid (11 ml) both AnalaR grade and water 848 ml.

Two test mixtures were used; the first to study chromatographic efficiency contained diphenylamine (t_0 marker), caffeine, propranolol, chlorcycloguanil and benzethonium chloride. The second, which was used to study changes in selectivity contained the antimalarial drugs proguanil and chlorproguanil and their metabolites, cycloguanil, 4-chlorophenylbiguanide, chlorcylcoguanil and 3,4-dichlorophenylbiguanide. These solutions were prepared in methanol and injections of approximately 10 μl were made.

Method

The column was packed using a conventional slurry technique and after conditioning with 45 ml of eluent (*ca.* 20 column volumes) tested using the two mixtures with the eluent delivered at 1 ml/min.

To simulate long and continued use, the following procedure involving recycling of the eluent was adopted.

After the initial testing 500 ml of fresh eluent was placed in the reservoir and the eluent recycled at a flow-rate of 2 ml/min, the waste from the column being fed back directly to the eluent reservoir. This system was run continuously for over three weeks, the eluent being replaced by a fresh 500-ml batch every morning, with the exception of weekends when the volume of eluent was 1 l. At appropriate intervals, using fresh eluent and a flow-rate of 1 ml/min the column was retested.

After 63 l of eluent had passed through the column, the packing material at the inlet was discoloured and showed a slight depression. The top 2–3 mm were removed and replaced with fresh packing material and the recycling process continued. The top mesh was also replaced after 3 l of eluent had passed through the column.

All measurements were made in duplicate or triplicate, efficiency (N) reduced plate height (h) and capacity factor (k') were calculated using the following standard relationships:

$$N = 5.54 \ (t_R/W_{1/2})^2; \ k' = (t_R - t_0)/t_0; \ h = L/(Nd_p)$$

where t_R and t_0 are the retention times of the compound under investigation and the unretained marker, respectively. $W_{1/2}$ is the width of peak at half height, L the column length and d_p the particle diameter.

RESULTS AND DISCUSSION

It is common practice where alkaline eluents are used to employ a sacrificial or saturator column between the pump and injection valve. However, to present a worst case, this precaution was omitted in the present work. The recycling of the eluent —a common practice in some laboratories— will obviously have had some protecting effect on the column. Given the length of the experiment (over 3.5 weeks) and the fact
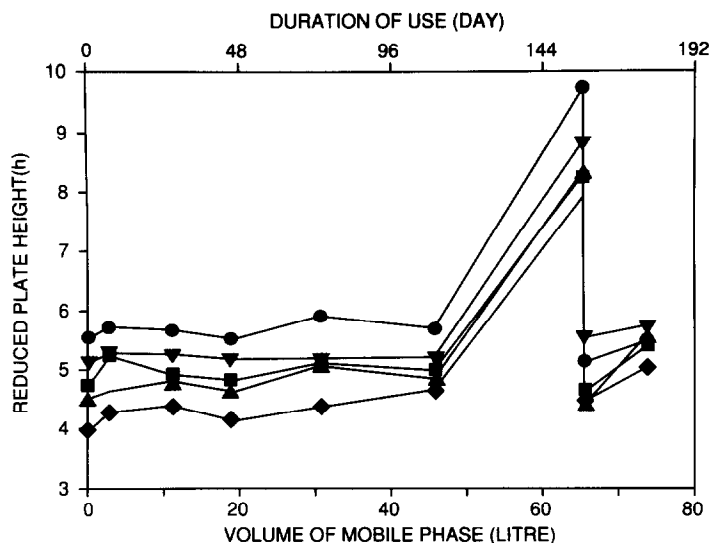
Fig. 1. The variation in reduced plate height (h) for five compounds as a function of the volume of eluent passed through the column and duration of use. ● = Diphenylamine; ▲ = caffeine; ■ = propranolol; ◆ = chlorcycloguanil; ▼ = benzethonium chloride.

that the eluent was renewed every day (except weekends) the benefit of this practice to the present case was considered to be minimal.

Efficiency

The change in chromatographic efficiency as a function of the volume of mobile phase passed through the column was measured using five compounds covering the k' range 0 (diphenylamine) to 8.6 (benzethonium). The data, reported as reduced plate height (h) are shown in Fig. 1. Although there were small variations in efficiency up to 46 l, most of these changes were not significantly different from the starting values ($p < 0.05$). Between a mobile phase volume of 46 and 65 l, however, there was a dramatic loss in efficiency as shown by increased h values. Efficiency was easily restored however, by repacking the top of the column, and with the exception of chlorcycloguanil, the restored reduced plate height values were not significantly different ($p < 0.05$) to the starting values.

The data show that there is little significant change in chromatographic efficiency when 45 l of eluent has passed through the column. Assuming a standard flow-rate of 1.0 ml/min and a 7-h working day, then this represents 107 actual days or the equivalent of 5 months continued use without significant loss in efficiency. By simply repacking the top of the column, use can be further extended to at least 74 l, 176 days, or the equivalent of nearly 9 months of routine use.

Selectivity

The biguanide mixture was chosen for this part of the study because previous experience[5] had shown these compounds to be good markers for selectivity changes using this type of HPLC system.
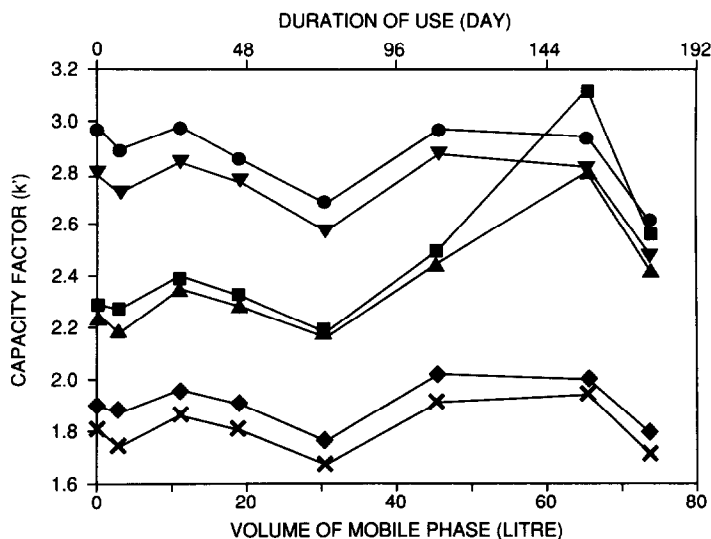
Fig. 2. The variation in capacity factor (k') for six antimalarial drugs and metabolites as a function of the volume of eluent passed through the column and duration of use. × = Proguanil, ◆ = chlorproguanil; ▲ = 3,4-dichlorophenylbiguanide; ■ = chlorophenylbiguanide; ▼ = chlorcycloguanil; ● = cycloguanil.

The variation in k' for the biguanide compounds as a function of mobile phase volume is shown in Fig. 2. Between 0.045 and 45 l (107 days) the variation in k' was relatively small ($< 10\%$ of the mean). The profiles for most of the compounds over this range were roughly parallel indicating that selectivity did not vary a great deal. Between 45 and 65 l (107 to 155 days), however, there was a significant change in k', coincidental with the loss in efficiency. At this point the retention of the two desalkyl metabolites increased relative to those of the other compounds in the mixture, resulting in a change in the order of elution. Repacking the top of the column resulted in a further change in selectivity; although the excellent separation between the four metabolites observed intially could not be regained.

Column backpressure

After 3 l of the mobile phase had passed through the column (approximately 7 days), the backpressure during the analytical phase of the work (flow-rate 1 ml/min) increased to approximately 3000 p.s.i. Replacing the top mesh re-established the pressure at 900 p.s.i. where it remained throughout the remainder of the work. This problem could be avoided in general work through the use of a sacrificial column or an in-line filter to remove particulate matter from the eluent. However, the similar backpressure at the start and end of the experiment, indicated that silica dissolution leading to settling of the bed or blocking of the bottom mesh with fines was not occurring.

CONCLUSIONS

These data indicate that contrary to popular belief silica-based packing materials can show excellent stability when used with certain types of alkaline eluent. Using

bare silica —which is more prone to dissolution than bonded materials[2,3]— with an eluent of 90% methanol, ammonia as the source of hydroxyl ions and a pH of 9.2, chromatographic efficiency was stable and reproducible for 107 days equivalent to 5 months of regular use. This could be extended to 176 days (*ca.* 9 months) by repacking the top of the column. A significant change in selectivity was observed after 107 days use which could not be restored to the original condition. It is possible that this change was due to irreversible adsorption onto the column which could be avoided by the use of a sacrificial and/or guard column in the system. These precautions were omitted in this experiment to present an aggrevated situation, although they are to be recommended for routine use.

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