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IMPROVEMENTS IN LIQUID CHROMATOGRAPHY COLUMN LIFE AND METHOD FLEXIBILITY BY SATURATING THE MOBILE PHASE WITH SILICA

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SUMMARY

We describe a technique which may expand the opportunities for liquid chromatography (LC) methods development. It has not been feasible to use high pH water-based mobile phases with silica packings, even those coated with octadecylsilane. A principal failure mode of such packings with both high pH and high temperature mobile phase is the dissolution of silica. We show that this can be controlled by equilibrating the mobile phase with silica using an appropriate column mounted in the oven ahead of the analytical column. We describe some early results of this technique for determining tricyclic antidepressant drugs at a mobile phase pH of 10.7 on uncoated 5-μm silica particles. Using an inorganic instead of an organic base also permits UV detection closer to 200 nm where most organic bases have become opaque. We measured the silicon dissolved in the column effluent by atomic absorption (AA). We used an injection procedure which permits the AA burner to take up solution at its optimum rate while the LC is used at any lower mobile phase flow-rate.

INTRODUCTION

It is well known in the literature that liquid chromatographic (LC) columns with silica substrates fail rapidly if a mobile phase of high pH is used. Horváth *et al.*[1] suggested that C_{18} bonded columns should not be used with eluents above pH 7. This failure may result from the increased solubility of silica as pH is increased.

Sosman[2] reports that the solubility of silica in water depends upon the physical characteristics of the silica and increases rapidly with temperature. For an amorphous form he reports a solubility of about 10 μg/ml at room temperature and 250 μg/ml at 200°.

In recent work Wehrli *et al.*[3] also used atomic absorption (AA) to measure the silicon (Si) content of strongly basic solutions, both organic and inorganic, in contact with C_{18} and C_8 bonded column packing materials. The rate of dissolution was linear with time. For NaOH more than 20% of the C_{18} packing had dissolved in less than 200 h while for some organic bases the rate was smaller. They also found the rate of dissolution to be even greater when using an alkylammonium hydroxide solution.

We have adapted an atomic absorption method to the detection of Si eluting from the column to measure quantitatively the loss of silica for different analytical conditions.

In gas chromatography, the eluting gas is sometimes pre-saturated with a compound that is known to bleed from the column during analysis. Such a procedure stabilizes volatile stationary phases and increases column life. LC column packings which have been coated with liquid substrate have also required mobile phase pre-saturated with the liquid phase in order to prevent loss due to solubilization. By analogy, we have presaturated the mobile phase with silica ahead of the analytical column. We add a silica column, referred to here as a guard column, before the sample injector to bring the Si content of the mobile phase to a level which greatly reduces the dissolution of silica from the analytical column. The guard column is mounted in the same oven as the analytical column, and comes to the same temperature. Thus, as the solubility of silica increases greatly with temperature, the concentration eluting from the guard column rises to the appropriate level for that temperature. The analytical column is thus automatically protected under varying operating conditions. We show that this arrangement provides useful life to an uncoated column packed with 5-μm silica particles even when a mobile phase pH of 10.7 at 65° is used.

In ref. 3, samples were taken for AA analysis from a mixture of test solutions and packing materials. We wanted to measure the dissolved Si in the mobile phase under variable chromatography conditions. We therefore attached the eluent from the column directly to the uptake capillary of the AA spectrophotometer. However the performance of the spectrophotometer is degraded if the flow-rate through the column is very different from the preferred rate for the AA spectrophotometer (about 7 ml/min).

We therefore adapted the AA injection method[4,5] which is the most efficient way to utilize very small samples to achieve the same detection limit for Si as is attained by conventional AA flame analysis. The eluent from the column is collected in drops of about 100 μl which fall into a conical PTFE block which is attached through capillary tubing to the uptake tube of the AA nebulizer (Fig. 1). One drop of sample is just large enough to permit the AA instrument and its recorder to reach a steady-state signal. Thus any mobile phase flow-rate may be used that is slower than the uptake rate of the nebulizer, about 7 ml/min. Changing flow-rate changes the frequency of drops, but has little effect on the sensitivity to Si.

EXPERIMENTAL

Materials and methods

We used a Perkin-Elmer Model 601 liquid chromatograph and a Perkin-Elmer Model Series 2/2 liquid chromatograph equipped with a Model LC-65T variable wavelength UV detector and a Rheodyne 7105 injection valve. Data for all experiments were recorded using Model 56 recorders. For some experiments a Model LC-420 Auto Sampler was also used. We used Perkin-Elmer Silica A/10, 10-μm uncoated silica packing, Silica B/5, 5-μm uncoated silica packing, and HC-ODS-Sil-X, 10-μm C_{18} bonded phase packing in 0.26 × 25 cm or 0.46 × 25 cm columns.

Si measurements were made with a Perkin-Elmer Model 373 atomic absorption spectrophotometer using a nitrous oxide–acetylene flame. It is the silica dissolved

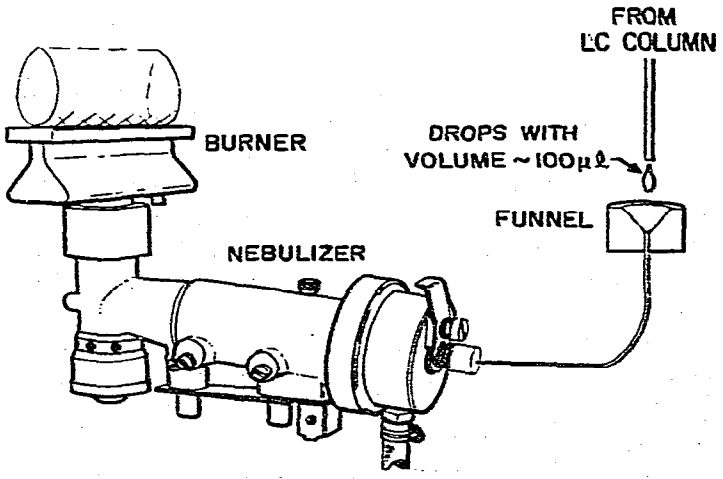

Fig. 1. The AA injection method applied to the eluent from an LC column. The tube mounted above the funnel is connected directly to the column. Its diameter at the lower end is such that a drop falls when it has grown to about 100 μl. The 100-μl drop is drawn into the nebulizer of the AA burner where its metal content produces a spike signal. The chromatogram formed from the envelope of these spikes is shown in Fig. 3.

from the column packing substrate which we are measuring, but in this paper we report this as concentration of Si in parts per million (ppm), weight per volume. This is what atomic absorption measures. The equivalent silica is 2.14 times the Si reported.

The guard column was mounted in the same oven as the analytical column to assure that the Si content of the mobile phase would be at equilibrium at the temperature of the analytical column. A layout of the system is shown as Fig. 2.

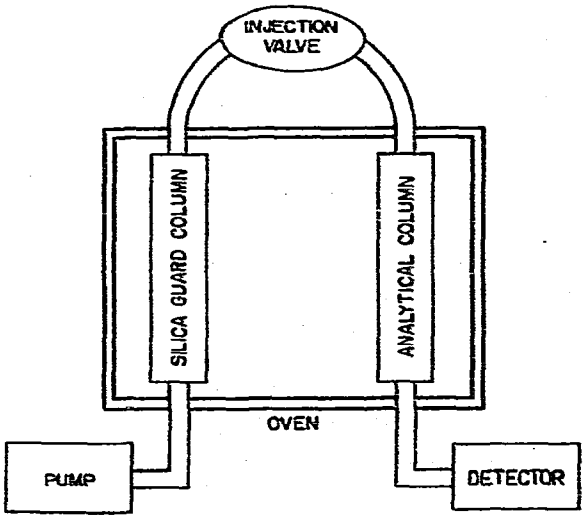

Fig. 2. Schematic layout of the guard column in the LC system.

RESULTS

Our experience supports the systematic experience of Wehrli *et al.*[3] in that the concentration of Si increases slowly with the length of time the mobile phase is in contact with the packing. Thus, the Si concentration in the eluate decreases with increasing flow-rate. We also find that the organic C_{18} coating on reversed-phase packing materials protects the silica to a great extent so that the Si content is very much less in a particular eluted mobile phase at a particular flow-rate using the C_{18} packing as compared to the uncoated silica A materials. There is a significant increase in the Si content of solutions with increasing temperature.

When a new column filled with 10-μm uncoated silica was filled with water at room temperature and left overnight, the concentration of Si in the first column volume of water eluted was 42 μg/ml. A column of the same packing material with water as the mobile phase at 1 ml/min flow-rate contained 38 μg/ml Si. Thus water flowing through the column at room temperature approaches the equilibrium concentration.

The Si content rises very rapidly with increasing temperature. With the same 10-μm uncoated silica column and deionized water as the mobile phase at a flow-rate of 1 ml/min, approximately 100 μg/ml Si were measured in the eluate when the column oven was maintained at 60°.

Fig. 3 represents a similar experiment with a 5-μm uncoated silica column that had been stored for 5 days filled with deionized water. As fresh deionized water flowed through the column the Si content gradually decreased towards a steady state. The column oven was turned on at about 5 ml and the Si content gradually rose to 60 μg/ml when the column reached 70°. When the oven was turned off the Si content decreased to the room temperature equilibrium level of 13 μg/ml.

Using methanol as a flowing mobile phase with the uncoated packings, Si cannot be detected. Even after methanol was left in a 10-μm uncoated silica column for 3 months there was less than 1 μg/ml Si, the least concentration we could detect in this experiment. Using a mobile phase of 40% acetonitrile in water (pH 10.7) at 65° at a flow-rate of 1.5 ml/min, the mobile phase contained approximately 123 μg/ml Si.

Some experiments were run with columns packed with C_{18} bonded phase materials. At room temperature with water as a mobile phase the Si content was less than the minimum 0.3 μg/ml that we could detect with the experimental procedure we used. At 60° with water at a flow-rate of 1 ml/min, the Si content was approximately 0.7 μg/ml. When water at 60° was left in the column for 32 min, the Si content increased to about 18 μg/ml.

In contrast with the experience with the uncoated silica, it appears that the C_{18} coating greatly reduces the amount of Si dissolved by the flowing mobile phase. However, after large volumes of mobile phase, especially at higher temperatures and high pH the loss of silica from the C_{18} packings is significant.

Some experiments with the C_{18} packed columns were run with 40% acetonitrile in water as the mobile phase. The dissolution of silica was very small and even when the mobile phase was left in the column overnight at room temperature, the Si content rose only to about 4 μg/ml. When 45% acetonitrile in water was left in the column for 14 days, the Si content rose to 40 μg/ml.

An experiment was run using a 10-μm uncoated silica column as a guard col-

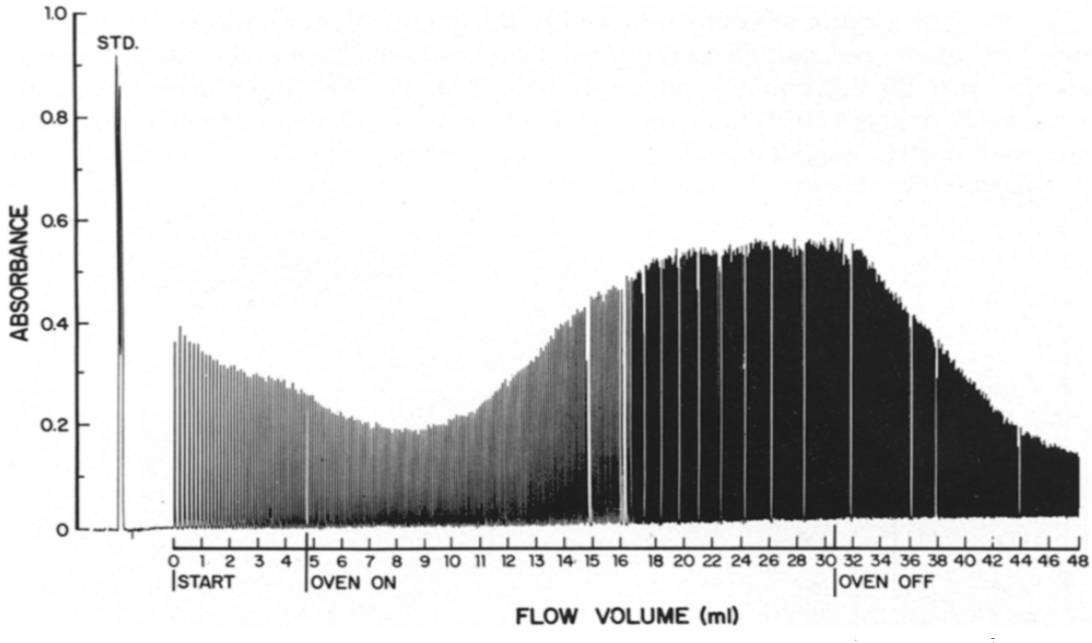

Fig. 3. The AA injection method used to measure Si in the column eluent. An 0.46 × 25 cm column packed with 5-μm uncoated silica was stored for 5 days with water at room temperature. The experimental arrangement of Fig. 1 was used to record continuously the Si in the column eluent, flowing at 1 ml/min. The standard shown contained 100 μg/ml Si. As the mobile phase that stood in the column was replaced by fresh mobile phase, the Si dropped towards a lower steady state concentration. At about 5 ml the oven was turned up to 70°. As the column gradually heated, the Si content rose to a steady state concentration of about 60 μg/ml Si. When the oven was turned off the Si content gradually returned to a concentration of about 13 μg/ml.

umn for an 0.26 × 25 cm C_{18} reversed-phase column. A pure water mobile phase at 60° was left in both columns for 17 h by stopping the flow of the mobile phase in the column. The effluent was then analyzed for Si. The steady state Si concentration using this guard column was measured at 98 μg/ml. The eluent that had been standing in the C_{18} column showed no additional Si indicating that the Si concentration provided by the guard column prevented the dissolution of silica.

Tricyclic drug separation

To confirm that the guard column protects an analytical column, we separated tricyclic drugs on an 0.46 × 25 cm, 5-μm uncoated silica column using a mobile phase with pH of 10.7. In our previous experience with this separation[6] we found that column performance was degraded below usefulness after 50 runs at a pH of only 7.2.

Preliminary experiments showed that we obtained very good separation with 40% acetonitrile in water adjusted to pH 10.74 with NH_4OH. We used a mobile phase flow-rate of 1.5 ml/min and column temperature of 65°.

Two new 0.46 × 25 cm, 5-μm uncoated silica B/5 columns were used for the experiment. The tricyclic drug mixture was separated on one column without any precolumn. After several runs and 80 min of mobile phase flow, the column performance became noticeably degraded. The measured Si content in the mobile phase had gradually increased over that period from 103 to 117 μg/ml.

This silica column was then moved to the guard column position and the second silica column was mounted in the analytical position. The initial separations were very similar to the first column and are shown in Fig. 4a. The Autosampler was set up to repeat the analysis 102 times overnight. There was no noticable degradation of the performance of the analytical column over this period of time (Fig. 4b). The measured Si content of the eluent was about 98 μg/ml.

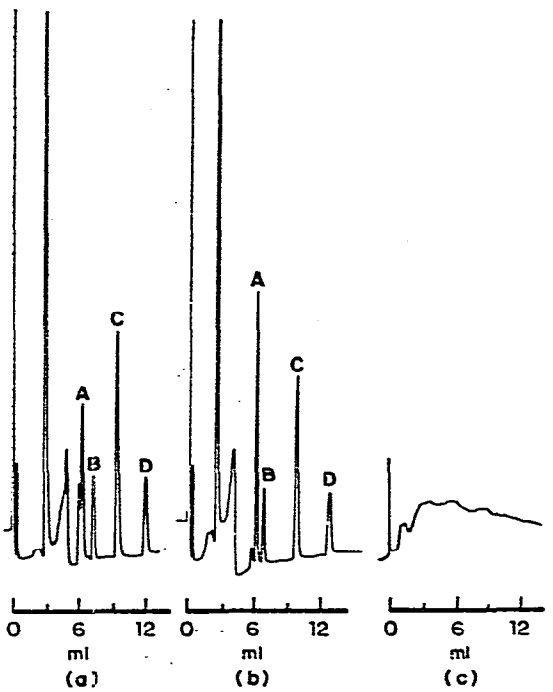

Fig. 4. Chromatograms of a tricyclic drug separation on an 0.46 × 25 cm, 5-μm uncoated column using a mobile phase with a pH of 10.7. The mobile phase flow-rate was 1.5 ml/min and the column temperature was 65°. The experimental protocol is described in the text. (a) Separation on a new analytical column; (b) results from an analytical column after 102 runs; (c) result when the guard column was used as an analytical column after 102 runs. The tricyclic drugs are: A = amitriptyline; B = imipramine; C = nortriptyline; D = desipramine.

The guard column was then retested in the analytical position. The performance of the column had become degraded to the point where no peaks were seen, Fig. 4c. It is not surprising when it is calculated that about 0.36 g of packing were dissolved while 1700 ml of mobile phase removed silica at the rate of 100 μg/ml Si. This is about a 15% loss of the original packing. When the column was opened the packing level had fallen about 3 mm and channelling had developed to one side. The pressure drop across both columns had remained at about 1000 p.s.i. during the experiment.

The same analytical column was used for approximately 400 determinations with these conditions, replacing the guard column three times. The first two replacements of the guard column were necessitated by its developing a very high pressure drop. Finally, both the third guard column and the analytical column developed excessive pressure drops. We speculate that this final failure of the analytical column was caused by transfer of fine silica particles from the guard columns.

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