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DISSOLUTION OF SILACEOUS CHROMATOGRAPHIC PACKINGS IN VARIOUS AQUEOUS ELUENTS

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SUMMARY

Two pairs of DuPont Zorbax PSM Bimodal gel permention chromatography columns, packed with porous silica particles have been used for the fractionation of dextran. High initial efficiencies decreased with use and shrinkage of the bed suggested silica was dissolving. The eluent required a certain ionic strength to prevent some of the dextran being excluded.

The dissolution of two types of porous silica packings, LiChroprep SI-60 and Zorbax BP-SIL, has been investigated in a batch system using several aqueous solutions of different ionic strengths at ambient temperature. It was found that where a low ionic strength was required to supress ionic exclusion of the dextran, the best eluents were 0.001 *M* potassium phthalate and 0.001 *M* potassium dihydrogen phosphate. However, silica dissolution with these eluents or even with high purity water will give columns packed with porous silica a finite life.

INTRODUCTION

We have recently reported our experiences with various chromatographic packings used in the aqueous gel permeation chromatography (GPC) of dextran[1]. We remarked in that paper on the doubtful long term stability of microparticular silaceous chromatographic packings in aqueous eluents.

Silica packings are used to a considerable extent with aqueous eleuents in reversed-phase chromatography and there has been little adverse comment on the long term stability of these packings. However, comparatively little high-performance GPC is carried out with aqueous eluents.

That silaceous chromatographic packings would dissolve in aqueous solvents with a pH greater than about 8 has long been recognised[2]. Only recently[3] has dissolution of silaceous reverse phase chromatographic packings been accepted as a problem at lower pH values and the rate of silica loss reported is extremely high.

It is not surpising that problems of silica dissolution in aqueous eluents should exist. The normally accepted[4] equilibrium solubility is about 100 ppm at pH values between 1 and 8 although this can be much greater for microparticles[5]. The complicated

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interaction between silica and water is summarised succintly by Unger[5]. It would appear that chromatography on silaceous packings in aqueous eluents is only possible because of the low rate of dissolution.

We have tested one of the new generation of high-performance silaceous packings and subjected some of this packing to stability tests in various aqueous eluents.

EXPERIMENTAL

Chromatography

Two pairs of DuPont Zorbax PSM Bimodal columns were tested. A simple chromatographic system was used, consisting of a pump (Series II; Metering Pumps Ltd., London, Great Britain), a sample injection valve (Type 30.501; Spectroscopic Accessory Co., Sideup, Great Britain) fitted with a 20-μl sample loop and a differential refractometer detector (Model 1107LJ; Laboratory Data Control, Stone, Great Britain).

Any deterioration in the chromatographic columns could be easily monitored by measurement of their efficiency. The efficiency was calculated, with glucose as the solute, by

$$N = 8\left(\frac{t_R}{W_{h/e}}\right)^2$$

where N is the number of theoretical plates, t_R is the peak retention time and $W_{h/e}$ is the peak width at the peak height, h, divided by e, the base of the natural logarithm.

The pairs of columns were calibrated using Dextran "T" fractions (Pharmacia, Uppsala, Sweden) and the elution of the solutes was compared in terms of the Wheaton and Bauman distribution coefficient, K_d[7]

$$K_d = (V_e - V_0)/V_i$$

where V_e is the elution volume of the solute and V_0 is the void volume, measured by the elution of a totally excluded solute, Dextran 2000 (Pharmacia). V_i is the internal pore volume which is assumed to be the difference between the elution volume of glucose, as a small solute, and the void volume.

A number of eluents were used in this work, all were made up from distilled water and analytical grade reagents.

For the second pair of Zorbax columns a pre-column (25 × 0.4 cm) filled with LiChroprep Si-60, 15 to 20 μm (E. Merck, Darmstadt, G.F.R.) was introduced. Such a pre-column acts as an eluent filter and should contribute towards saturating the eluent with silica.

Silica dissolution

Using static experiments, the dissolution of loose silaceous chromatographic packings in various possible eluents was measured. The silica dissolution was measured as the silicon content of the solutions as assayed by atomic absorbtion spectroscopy.

The atomic absorption spectrophotometer was a Model 151 from Instrumentation Laboratories Inc. (Lexington, MA, U.S.A.), fitted with a silicon hollow cathode lamp from S & J Juiper and Co. (Harlow, Great Britain). The instrument set-

tings were as recommended by the manufacturer: lamp current 12 mA; slit width 80 μm; wavelength 251.6 nm; a rich acetylene–nitrous oxide flame with a burner height of 7 mm.

Two silaceous chromatographic packings were used in this work: (i) Li-Chroprep Si-60, a 15–25 μm packing normally used for preparative chromatography (E. Merck); (ii) Zorbax BP-SIL, a microparticular packing very closely resembling the Zorbax PSM 60 used in chromatography.

The various solutions were made up from analytical grade reagents in either deionised water or "Water for Liquid Chromatography" (BDH, Poole, Great Birtain).

The experimental procedure involved preparing 50 cm^3 of the solutions, monitoring the pH and then placing 0.2 g of the packing in the solution. The solutions were vigorously agitated occasionally, though they were allowed to settle for at least 1 h before the dissolved silicon concentration was determined directly by aspirating solutions from above the solid packing. Measurements were taken as the average of five 4-sec integrated signals. It was assumed that all the silicon was dissolved silica, the concentrations being expressed as parts per million (ppm) SiO_2.

All the work was carried out using polythene containers although the "Water for Liquid Chromatography" was supplied in glass bottles.

RESULTS

Chromatography

The initial characteristics of the first pair of Zorbax PSM Bimodal columns were very impressive (Table I). A flow-rate of 1 cm^3/min was maintained throughout the life of this pair of columns.

TABLE I

INITIAL CHARACTERISTICS OF THE FIRST PAIR OF ZORBAX COLUMNS

Column	*Efficiency (plates)*	*Pressure drop (bar)*
PSM 60	7000	55
PSM 1000	10,000	55
Combined	18,400	120

A slow deterioriation in efficiency was observed over the first 80 h use, but then there was a dramatic drop in efficiency which coincided with the application of a number of samples of Dextran 2000, used to determine the void volume. After this sudden drop in efficiency the columns maintained a steady performance for a further 250 h use. During this latter period of operation a variety of dextran samples were successfully analysed. It was not the reduced efficiency which caused problems but the non-Gaussian peak shape. When the inlet ends of the columns were opened up it was found that a definite void of about 2 mm existed on the PSM 60 column and a slight settlement of the PSM 1000 also appeared to have taken place. The final characteristics of this first pair of columns are shown in Table II.

A second pair of Zorbax PSM Bimodal columns were brought into use. The initial characteristics of this pair of columns were even more impressive (Table III).

TABLE II

FINAL CHARACTERISTICS OF THE FIRST PAIR OF ZORBAX COLUMNS

Column	*Efficiency (plates)*	*Pressure drop (bar)*
PSM 60	3600*	60
PSM 1000	8000	60
Combined	5000*	100

* Poor peak shape.

TABLE III

INITIAL CHARACTERISTICS OF THE SECOND PAIR OF ZORBAX COLUMNS

Column	*Efficiency (plates)*	*Pressure drop (bar)*
PSM 60	9900	88
PSM 1000	17,200	72
Combined	21,200	145

The fractionating range of both pairs of columns was ideal for the analysis of clinical dextran fractions with no noticeable lower limit and an exclusion limit of about 10^6 daltons. However, the second pair of columns did show a consistent excluded peak with dextran samples. This was presumed to be due to ionic exclusion and previous experience had shown us that this could usually be suppressed by increasing the ionic strength of the eluent. Our experiences with a number of eluents is summarised in Table IV.

TABLE IV

EXPERIENCES WITH VARIOUS ELUENTS

Eluent	*Hours used*	*Samples analysed*	*Comments*
Distilled water	60	160	Small excluded peaks
0.5% NaCl	1	3	*
0.02% NaN_3	3	3	*,**
0.02% Sodium pentachlorophenol	2	3	**
0.02% KH_2PO_4	80	140	***
0.02% Potassium hydrogen phthalate	9	4	***, also a dramatic drop in efficiency

* Suppressed excluded peaks, but it produced large negative peaks due to the absence of salt in the sample.

** With pH values of less than 7 these eluents were expected to enhance silica dissolution.

*** Suppressed excluded peaks with only a small negative peak introduced.

The efficiency dropped slowly during the period of time that phosphate was used in the eluent. After 50 h use of the phosphate eluent the combined column efficiency was 16,200 plates. After the sudden drop in efficiency it was no longer possible to maintain the 1 cm^3/min flow-rate. The column efficiencies were measured after being used with the phthalate eluent (Table V).

TABLE V

CHARACTERISTICS OF THE SECOND PAIR OF ZORBAX COLUMNS AFTER 150 h USE

Column	*Efficiency (plates)*	*Pressure drop (bar)*	*Flow-rate (cm^3/min)*
PSM 60	1190	115	1
PSM 1000	8040	115	1
Combined	6650	130	0.7

* Poor peak shape.

When the inlet ends of the second pair of columns were opened up it was found that there had been a considerable amount of settling with 2-mm and 1-mm voids on the PSM 60 and PSM 1000 columns respectively. The columns were "topped-up" using the Zorbax BP-SIL material and a combined efficiency of 15,000 plates was obtained. A further 250 h use was obtained from these columns with occasional "topping-up". Although the combined efficiency was always kept well above 5000 plates, there was a steady increase in pressure drop and decrease in flow-rate. The phthalate eluent was used for this later work.

The elution volumes of Dextran 2000 and glucose were recorded regularly to allow the use of the Wheaton and Bauman distribution coefficient. No change in the calibration of these columns was observed.

Silica dissolution

The initial silica dissolution work was carried out with the LiChroprep Si-60 packing. The silica dissolution with time is recorded in Table VI.

TABLE VI

DISSOLUTION OF LICHROPREP Si-60

Solution	*Approx. molarity*	*Silica concentration (ppm)*				*Initial pH*
		16 h	*98 h*	*122 h*	*27 days*	
Deionised Water	—	41	73	92	118	5.5
0.5% NaCl	0.1	51	81	105	113	5.6
0.05% NaCl	0.01	41	75	101	113	5.2
0.05% KH_2PO_4	0.004	21	43	60	88	5.0
0.05% $KO_2C(C_6H_4)CO_2H$	0.003	9	15	21	49	4.2
0.05% KSCN	0.001	32	65	94	88	5.7
0.05% NaN_3	0.01	51	79	103	113	7.3

Zorbax BP-SIL was used in two separate dissolution experiments. In the first experiment the solutions were made up in deionised water and the results are summarised in Table VII. In the second dissolution experiment with Zorbax, the solutions were made up in "Water for Liquid Chromatography" (Table VIII).

TABLE VII

DISSOLUTION OF ZORBAX IN SOLUTIONS FROM DEIONISED WATER

Solution	*Silica concentration (ppm)*			*Initial pH*
	1 h	*24 h*	*48 h*	
Deionised water	2.1	2.4	9.0	5.7
0.1 *M* NaCl	3.4	24.2	54.6	5.7
0.05% NaN_3	5.7	≫40	≫70	7.3
0.1 *M* KH_2PO_4	4.3	25.3	65.4	4.5
0.01 *M* KH_2PO_4	3.2	18.9	44.6	4.5
0.001 *M* KH_2PO_4	2.1	9.0	19.1	5.2
0.1 *M* $KO_2C(C_6H_4)CO_2H$	7.1	24.4	50.1	3.8
0.01 *M* $KO_2C(C_6H_4)CO_2H$	1.5	16.7	31.9	4.0
0.001 *M* $KO_2C(C_6H_4)CO_2H$	2.4	7.9	15.0	4.2
0.1 *M* KSCN	2.1	20.6	48.4	5.5
0.1 *M* HCl	1.7	7.9	12.0	0.6

TABLE VIII

DISSOLUTION OF ZORBAX IN SOLUTIONS FROM "WATER FOR LIQUID CHROMATOGRAPHY"

Solution	*Silica concentration (ppm)*		*Initial pH*
	48 h	*114 h*	
Deionised water	2.1	8.8	5.7
"Water for Liquid Chromatography"	3.2	9.2	4.8
0.001 *M* $KO_2C(C_6H_4)CO_2H$	8.9	19.3	4.2
0.1 *M* Na_2SO_4	34.4	50.6	6.2
0.01 *M* Na_2SO_4	22.5	35.4	6.8
0.001 *M* Na_2SO_4	11.5	23.4	6.8

DISCUSSION

It can be seen from the results reported here on chromatography with the DuPont Zorbax PSM Bimodal columns that a useful practical life can be obtained from microparticular silaceous GPC packings when used with aqueous eluents. The first pair of columns exhibited an early dramatic drop in efficiency which was then followed by a long period of stability. With the second pair of columns a significant period of high efficiency was achieved before a sudden drop in efficiency again followed by a steady lower efficiency. The later silica dissolution work indicates that there is probably no connection between the sudden drop in efficiency of the second pair of columns and the change to the phthalate eluent solution.

The difference in the initial pressure drops across the two pairs of columns is to some extent explained by the introduction of the pre-column when using the second pair. However, the higher efficiency and the higher presure drop of the second pair of columns probably reflect an improvement in the column packing technique of the manufacturer.

The very high efficiency found with the silaceous microparticular columns is not generally required in GPC, though the short analysis time is a great asset. When a

void is created at the top of the column, a shoulder will usually be seen on the glucose peak. It should be stressed that it was this lack of quality in the peak shape, rather than the drop in efficiency, that caused problems. However these voids at the top of the column can be "topped-up", even with a different packing, to improve the peak shape and increase the effective life of the column.

The silica dissolution work with LiChroprep Si-60 was originally carried out to test the experimental method on a less valuable chromatographic packing. Since this packing is likely to have different surface properties to the Zorbax, the results have been included to allow comparisons to be made.

The two experiments with the Zorbax BP-SIL packing used two test solutions common to each experiment [deionised water and 0.001 *M* $KO_2C(C_6H_4)CO_2H$]. That the measured silica dissolution is so different in each case indicates that there must be other important parameters (*e.g.*, the degree and frequency of agitation) and only the relative solubilities should be considered.

From the first experiment with Zorbax BP-SIL it can be clearly seen that the concentration of salts in solution have a dramatic effect on the amount of silica dissolution. The concentration of salts is obviously more important than small changes in pH around this mildly acidic area. The more extreme pH values have a large effect on silica dissolution. The effect of sodium azide solution is of particular interest; its pH of 7.3 would normally be considered acceptable and in fact sodium azide is frequently used to inhibit bacterial growth either in aqueous samples or aqueous eluents. However, these results suggest that sodium azide enhances dissolution of silica quite significantly.

The second experiment with Zorbax BP-SIL was principally intended to compare the silica dissolution properties of deionised water and "Water for Liquid Chromatography", though an additional salt, recommended to suppress "ghost" peaks[8], sodium sulphate was also tested. There appeared to be no significant difference between the silica dissolution properties of deionised water and "Water for Liquid Chromatography". It was also surprising that this "Water for Liquid Chromatography" had no recordable silica concentration as it was supplied in glass bottles; this may well be because the ultra pure water does not contain the salts necessary to promote silica dissolution.

Comparison of the phthalate and sodium sulphate solutions suggests that sodium sulphate will cause similar silica dissolution to the other salts used at the same concentration.

Subsequent to the work reported here it was found[9] that if the columns are flushed with and stored in acetone after use, their lifetime is considerably increased. It is also reported[8] that aqueous methanol (10% methanol–90%water, to which 2% glycerol is added) has been successfully used as eluent elsewhere.

CONCLUSIONS

There would appear to be always a significant dissolution of microparticular silaceous chromatographic packings in aqueous eluents. In the slightly acidic region of pH (3–6), frequently used for chromatography, the concentration of salt in solution is the important parameter. Usually only a low concentration of salts is required to suppress any adverse ionic effects of a chromatographic column. We have found that

0.001 *M* potassium phthalate or potassium dihydrogen phosphate suppresses the small excluded "ghost" peaks, without introducing large negative peaks and these solutions do not significantly enhance silica dissolution.

The life of the new generation of microparticular silaceous chromatographic columns must be limited when used in aqueous eluents because of silica dissolution. Whether or not the life of microparticular silaceous chromatography columns will justify the cost of manufacturers pre-packed columns will depend on the application. Where bulk chromatography packings are available this is probably a more sensible approach as the columns can be readily re-packed.

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