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## SILICA GEL AS AN INSOLUBLE CARRIER FOR THE PREPARATION OF SELECTIVE CHROMATOGRAPHIC ADSORBENTS

### THE PREPARATION OF 8-HYDROXYQUINOLINE SUBSTITUTED SILICA GEL FOR THE CHELATION CHROMATOGRAPHY OF SOME TRACE METALS

J. M. HILL

*Biochemistry Department, Rothamsted Experimental Station, Harpenden, Herts. (Great Britain)*

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#### SUMMARY

Aminopropyl silica gel made by treating chromatographic grades of silica gel with 3-aminopropyl triethoxy silane, is reacted with *p*-nitrobenzoyl chloride and the  $-\text{NO}_2$  group reduced to  $-\text{NH}_2$  with sodium dithionite. Diazotisation and reaction with 8-hydroxyquinoline produces a dark red silica gel derivative which can be used for the removal, concentration and separation of trace amounts of copper and other biologically important trace-metal cations from solutions of high ionic strength. The preparation of other selective adsorbents with different reactive groups is discussed.

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#### INTRODUCTION

Chromatographic adsorbents are often needed with greater selectivity than that given by the commercially available materials. While preparing silica-gel-based adsorbents for the affinity chromatography of metallo-enzymes, some were found which removed and concentrated copper, zinc and other biologically important trace-metal cations from salt solutions of high ionic strength. Such a modified silica gel, with a terminal functional 8-hydroxyquinoline group, can be used to remove and separate some transition elements from solutions of salts of the alkali and alkaline earth metals.

#### EXPERIMENTAL

##### *Preparation of the 8-hydroxyquinoline derivative of silica gel*

Silica gel (BDH chromatographic grade, 60-120 mesh) was dried at 450° for 12 h and cooled in a desiccator. Acetone was dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Amino-propyl silica gel (APSG) was prepared by a method similar to that described for the silation of porous glass<sup>1</sup>. Dried silica gel (100 g) was mixed with 4% 3-amino-propyl triethoxy silane (100 ml) in anhydrous acetone and incubated in a sealed flask

at 45°. The reaction was complete within 4 h. The APSG was filtered, washed with dry acetone and dried under vacuum. Calculating from the amount of trinitrobenzene sulphonic acid absorbed from an 0.1% solution in saturated sodium tetraborate, APSG contained 197  $\mu$ moles of bound  $\text{NH}_2$  groups per gram.

Dried APSG was benzoylated in a sealed flask at 50° with 200 ml of a solution in chloroform containing *p*-nitrobenzoyl chloride (10 g) and triethylamine (10 ml). The reaction was complete within 48 h when the silica gel was no longer stained by trinitrobenzene sulphonic acid. Filtering and washing with chloroform removed excess reagents and the silica gel was then dried under vacuum. Incubating the silica gel with 200 ml 5% sodium dithionite in a sealed flask at 45° overnight, reduced the  $-\text{NO}_2$  group to  $-\text{NH}_2$ ; washing with distilled water removed excess reagents. This aminophenyl derivative was then diazotised for 30 min at 0° with 200 ml of 2% (w/v)  $\text{NaNO}_2$  in 1% acetic acid. The colour of the silica gel changed from yellow to orange. After filtering and washing with cold distilled water the silica gel was mixed with 100 ml of 2% 8-hydroxyquinoline in ethyl alcohol. The colour changed immediately to deep red and excess 8-hydroxyquinoline was removed with ethyl alcohol.

This 8-hydroxyquinoline derivative of silica gel (8-OHSG) was ready for use after washing with  $3 \times 100$  ml of 1 N HCl and then with distilled water to remove HCl.

## RESULTS

### *Adsorption of $\text{Cu}^{2+}$ ions from water and salt solutions*

A column (1  $\times$  14 cm) containing 10 g of 8-OHSG was equilibrated with water and then slowly washed with 0.01 M  $\text{CuSO}_4$  until  $\text{Cu}^{2+}$  appeared in the eluate. After washing the 8-OHSG with distilled water until the eluate did not react with sodium diethyldithiocarbamate (DIECA), the remaining adsorbed copper was removed by washing with 10 ml of 1 N HCl followed by 20 ml of deionised water. This eluate contained 44.5 mg of  $\text{Cu}^{2+}$ , determined with DIECA<sup>3</sup>, which is equivalent to 70  $\mu$ mole adsorbed per gram 8-OHSG.

Traces of copper and other transition elements were removed from solutions of 2 M  $\text{KNO}_3$ , 2 M  $\text{Ca}(\text{NO}_3)_2$ , 1.33 M  $\text{Na}_2\text{HPO}_4$ , and 0.75 M  $\text{MgSO}_4$  by extraction with dithizone in carbon tetrachloride<sup>3</sup>.  $\text{CuSO}_4$  was then added to give 1  $\mu$ M  $\text{Cu}^{2+}$  (63.5  $\mu$ g  $\text{Cu}^{2+}$ /l) and 1 l of each solution was passed through a column (2  $\times$  15 cm) containing 45 g of 8-OHSG. After washing with water to remove the salt solution, the adsorbed copper was removed by washing with 10 ml of 1 N HCl followed by deionised water. In all cases between 60 and 65  $\mu$ g of  $\text{Cu}^{2+}$  were recovered, suggesting that all the copper added to the salt solutions was adsorbed by 8-OHSG and released by acid. The 8-OHSG was regenerated by washing with distilled or deionised water until the pH was  $>5$ ; alkaline treatments were neither necessary nor desirable.

$\text{CuSO}_4$  was also added to 1 l of 2 M  $\text{KNO}_3$  to give  $5 \times 10^{-8}$  M (3.18  $\mu$ g)  $\text{Cu}^{2+}$ . This solution was passed through the column and eluted as in the earlier experiments; 3.4  $\mu$ g of  $\text{Cu}^{2+}$  was recovered in the eluate.

### *Separation of manganese, copper and zinc*

A mixture containing 50  $\mu$ moles each of  $\text{MnSO}_4$ ,  $\text{CuSO}_4$  and  $\text{ZnSO}_4$  was adsorbed

onto a column ( $2 \times 15$  cm) of 8-OHSG and then eluted successively with 50 ml of water and 20 ml each of 0.1 *M*  $\text{KH}_2\text{PO}_4$  (pH 6), 0.1 *M* sodium acetate (pH 5), 0.1 *M* sodium formate (pHs 4 and 3), 0.1 *M* KCl (pH 2) and 0.1 *M* HCl (pH 1). Manganese was completely eluted at pH 3, zinc at pH 2, and copper at pH 1.

#### *Stability of 8-OHSG*

Samples (100 mg) of 8-OHSG were mixed with 5 ml each of 10 *M* HCl, 0.1 *M* HCl, 0.1 *M* KCl (pH 2), 0.1 *M* sodium formate (pHs 3 and 4), 0.1 *M* sodium acetate (pH 5), 0.1 *M* potassium phosphate (pHs 6, 7, 8 and 9), and 0.1 *M* sodium carbonate (pHs 9, 10, 11 and 12) and the absorption spectra of the supernatant liquids were plotted periodically. Within 24 h of mixing the solutions at pHs 10, 11 and 12 were distinctly pink and absorbed light strongly at 500 nm. At the end of one month the solution at pH 9 and in 10 *M* HCl were still almost colourless but absorbed slightly at 500 nm; even two months after mixing the other solutions were still colourless and did not absorb at 500 nm. The 8-OHSG was also stable to chloroform, benzene, acetic acid, and acetone for at least two months.

The diazo bond is cleaved by reducing agents; a 1% solution of dithionite in water rapidly reduced the diazo bond of 8-OHSG. The aminophenyl silica gel derivative formed could be rediazotised and coupled with more 8-hydroxyquinoline or other suitable compounds.

#### DISCUSSION

The preparation of resins with selective adsorbent properties have been described<sup>4,5</sup>. Most are substituted polystyrene resins or are condensation polymers; iminodiacetate-polystyrene resins for metal chelation are available commercially but slowly release iminodiacetate groups on standing<sup>6</sup>. The methods of formation of most of these resins are specific and do not permit much modification of the properties of the resultant resin. Recently specific adsorption or affinity chromatography has been developed for both separation and insolubilisation of enzymes and other biochemically important compounds, usually using derivatives of cellulose, Sepharose, and agarose, or porous glass, as adsorbents<sup>7-9</sup>. Dithizone has been coupled to carboxymethyl cellulose and used for the extraction of trace metals<sup>10</sup> but little else has been done to extend the techniques used in affinity chromatography to routine analytical and preparative chemistry.

Silanes containing hydrolysable groups condense with silanol groups of silicas, clays, and glasses, a reaction which is industrially important for the covalent coupling of organic groups to these materials<sup>11</sup>. Aminoalkoxy silanes are used to modify the porous glasses used as carriers in affinity chromatography<sup>1</sup> but these glasses are too expensive for general use in routine analytical chemistry. Silica gels are cheap, easily prepared and available commercially in grades suitable for both thin-layer and column chromatography. Unlike cellulose, agarose, and Sepharose, they do not readily support microbial growth and like other silica derivatives they have silanol groups which can condense with alkoxy silanes. WOLF *et al.*<sup>12</sup> showed that after reaction with aminoalkoxy silanes, silica gels contained from 300 to 800  $\mu\text{equiv.}$  of bound  $\text{NH}_2$  per gram in contrast with less than 100  $\mu\text{equiv./g}$  found for porous glass<sup>1</sup>.

In this paper aminopropyl silica gel after benzylation, reduction and diazotisation, is coupled with 8-hydroxyquinoline. Diazonium salts couple with 8-hydroxy-

quinoline in the 5-position and the phenolic group and heterocyclic nitrogen atom used in chelation are not involved<sup>13</sup>. The stability constant of 8-hydroxyquinoline is greater than that of iminodiacetate<sup>14</sup> and the silica gel derivative now described should be even more effective at removing transition elements from solutions than the commercially available chelating resins. Because this 8-hydroxyquinoline silica gel derivative removes traces of copper from strong solutions of other salts it is probable that chelation, and not ion exchange is involved.

The terminal adsorbing group of this silica gel derivative can be easily changed. After reducing the diazo bond with sodium dithionite, the aminophenyl derivative formed can be re-diazotised and coupled with other functional groups. Both salicylal-doxime and salicylic acid have been coupled to the diazonium salt; the diazonium salt has been heated to form the 4-hydroxyphenyl compound and has also been reduced with dithionite to form the phenylhydrazine derivative. This phenylhydrazine derivative successfully removed pyridoxal from a mixture of pyridoxal and pyridoxine. Many other alkoxy silanes could be used to couple different organic groups initially to the silica gel<sup>11</sup>. The adsorbent properties of the modified silica gel would depend largely on the initial silating agent and the subsequent coupling reactions.

Anhydrous conditions were used for the silation reaction to prevent hydrolytic condensation of the aminopropyl siloxane and to allow maximum substitution of the silanol groups. Completely anhydrous conditions may not be necessary provided the silating agent is in dilute solution; in some industrial applications water is used as a solvent for siloxanes<sup>11</sup>.

Silica gels, like porous glasses, are dissolved by alkalis and are unsuitable for use above pH 9. The results obtained suggest that these silica gel derivatives are suitable for long-term use in many organic solvents and, except for concentrated mineral acids, in aqueous solutions below pH 9. The silica gel derivative now described was designed both for use in analysis and for removing traces of transition elements from solutions used for the study of nutrient deficiencies in plants. This or other derivatives could also be useful in preparative work particularly where an insoluble reactant, as in the introduction of an amino group by diazonium coupling, would facilitate the subsequent separation and purification of the products.

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