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THE SELECTIVE ADSORPTION OF POLYSACCHARIDES ON POLYAROMATIC SURFACES

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ABSTRACT

A new matrix, polymeric 1,3-diaminobenzene-coated Celite, which selectively adsorbs monomeric and polymeric carbohydrates, has been prepared. This matrix adsorbs glycogen and other branched polysaccharides, as opposed to neutral or charged monosaccharides. Other solid supports were coated with polymeric 1,3-diaminobenzene; some of these (coated Sephadex G10 and coated Bioglas 1,000) had better physical form for column packing. Coated Celite was considered to be the best support in view of its greater stability. The effects of ionic concentration, pH, temperature, and the concentration of carbohydrate solution on the adsorption of glycogen on to coated Celite were studied, and methods to prevent adsorption and remove adsorbed carbohydrate were investigated. A comparison is made with the adsorption of heterocyclic compounds by cross-linked dextran gels.

INTRODUCTION

Traditional methods for the fractionation of polysaccharides include gel filtration and ion-exchange chromatography but, recently, a number of novel methods for the separation and purification of polysaccharides have been reported. These include the use[1] of insoluble complexes formed by the specific interaction between branched-chain polysaccharides and concanavalin A. Concanavalin A attached to an insoluble matrix has been used[2] for affinity chromatography of monomeric and polymeric carbohydrates and extends the range of application achieved with the soluble form of the lectin. Concanavalin A is an important reagent for the isolation of biologically active and important materials, for fractionation of immunoglobulin and blood-group substances, and for cell typing, but not all situations demand the use of this particular lectin. Furthermore, the lectin is unstable (being easily biodegraded) and large-scale work would be costly. Therefore, we sought an alternative support, which would bind carbohydrates selectively according to their structure, and would be stable and relatively cheap to produce on a large scale.

It is known that insoluble polysaccharides (such as Sephadex, a cross-linked dextran) adsorb soluble heterocyclic, and, in particular, aromatic, compounds[16–20]. The main classes of adsorbed compounds are phenols, anilines, and benzoic acids. It

occurred to us that a similar effect might be obtained by using the reverse situation, namely, an insoluble aromatic amine and soluble carbohydrates. The insoluble aromatic amine used was polymeric 1,3-diaminobenzene, obtained by partial diazotisation of the monomer, coated on to insoluble supports, and we now report on the use of this system.

EXPERIMENTAL AND RESULTS

Materials. — The following inert solid-supports were used: Celite (30–80 mesh, British Drug Houses Ltd.), alumina (Woelm Neutral TLC, Koch–Light Laboratories Ltd.), Sephadex G10 (Pharmacia Fine Chemicals AB), Ballotini glass beads (No. 18 60-μm size, Jencons Scientific Ltd.), Bioglas 1,000 (Bio–Rad Laboratories), and titanium dioxide in the following forms: spheroids (300-μm large pore size, batch M_{27}, kindly provided by Dr. A. R. Thompson, A.E.R.E. Harwell), and Tioxides R-SM and A-DM (British Titan Products).

The carbohydrates used were: D-glucose and maltose (G.P.R., Hopkin and Williams Ltd.), amylose (from potato, blue value 1.20, degraded to 69% fall in specific viscosity with β-amylase), amylopectin (from potato, blue value 0.243), glycogen (from shellfish, type II, Sigma Chemical Co.), glycogen (from rat liver, Sigma), starch (soluble, AnalaR, Hopkin and Williams), linear dextran (T40, mol. wt. 44,400, Pharmacia), branched dextran (from *Betacoccus arabinosaceous*), D-glucopyranosyl phosphate (dipotassium salt dihydrate, puriss, Koch–Light), D-glucono-1,5-lactone (pure, Koch–Light), and 2-amino-2-deoxy-D-glucose hydrochloride (puriss, Koch–Light).

The following carbohydrates were used for the column elution; levan (from *Bacillus mesentericus*), gum tragacanth (viscosity 4), lactose (puriss, Koch–Light), and cyclohepta-amylose (prepared from *Bacillus macerans* and potato).

General preparation of polyaromatic surfaces. — Solid support (10 g) was mixed with a solution of 1,3-diaminobenzene (puriss, Koch–Light; 5 g unless otherwise stated) in 1.0M hydrochloric acid (200 ml). A 4.5% aqueous solution of sodium nitrite (200 ml), precooled to 0°, was added to the mixture at 0° and the resulting suspension was stirred for 30 min at 0°. The solid was then centrifuged off at 0° and washed with 0.2M sodium acetate buffer (pH 5.0, precooled to 0°, 3 × 50 ml), using a Griffin Christ refrigerated centrifuge. The solid was then stirred for 2 h at 4° with the minimum of 0.2M sodium acetate buffer (pH 5.0), a solution of 2-naphthol (saturated in saturated sodium acetate, filtered through a No. 4 sinter, 200 ml precooled to 4°) was added, and the suspension was stirred for 2.5 h at 4°. The solid was then washed with 0.1M sodium acetate buffer (pH 6.0, precooled to 4°, 5 × 50 ml) and stored as a suspension in the same buffer (50 ml) at 4°. When a different buffer was required in the subsequent adsorption experiments, an aliquot of the suspension was centrifuged, and the supernatant was removed and replaced by an equal volume of the required buffer. In order to determine the amount of solid phase, an aliquot (500 μl) of the suspension was filtered, washed with distilled water, and dried *in vacuo* over phosphorus pentaoxide at 20° for 24 h. Polyaromatic surfaces on Celite were also

prepared in this manner by using decreased amounts of 1,3-diaminobenzene (2.5, 1.0, and 0.5 g), all other parameters being unaltered.

Methods of assay for carbohydrates. — With the exception of 2-amino-2-deoxy-D-glucose and D-glucono-1,5-lactone, carbohydrates were assayed by the L-cysteine–sulphuric acid assay[3]. 2-Amino-2-deoxy-D-glucose and D-glucono-1,5-lactone were assayed by determination of formaldehyde released on periodate oxidation[4].

General method of adsorption of carbohydrates by the polyaromatic surfaces. — An aliquot (500 μl) of the prepared suspension was diluted with buffer (500 μl), and a solution of the carbohydrate in the same buffer (1 mg/ml; 400 μl) was added. The suspension was stirred magnetically for 18 h at 20° (unless otherwise stated) and centrifuged. Aliquots of the supernatant were assayed for carbohydrate. Control carbohydrate solutions, which had been similarly stirred in the absence of solid phase, were similarly assayed. Each adsorption and assay was carried out in duplicate.

Effect of various parameters on the adsorption of carbohydrates by the polyaromatic surfaces. — A range of carbohydrates was subjected to adsorption on to Celite coated with four amounts of 1,3-diaminobenzene, using sodium phosphate buffer (0.1M, pH 6.0) at 20° (Table I). The effect of the various parameters on the adsorption of carbohydrates was studied by using a suspension containing 48.6 mg of solid phase (5 g of 1,3-diaminobenzene/10 g of Celite)/500-μl aliquot. With glycogen, the effect of ionic concentration of the buffer was studied by using sodium acetate buffers (pH 6.0) at 25° (Table II). The effect of pH on the adsorption was studied by using citric acid–sodium phosphate, sodium acetate, and sodium phosphate

TABLE I

ADSORPTION OF VARIOUS CARBOHYDRATES BY POLYAROMATIC-COATED CELITE

	Carbohydrate adsorbed (%) by Celite having various coatings of 1,3-diaminobenzene			
	1,3-Diaminobenzene (g/10 g of Celite)			
Carbohydrate	*5.0*	*2.5*	*1.0*	*0.5*
D-Glucose	0	0	0	0
Maltose	3.3	6.4	0	3.3
Amylose	65.6	40.6	8.0	6.3
Amylopectin	93.5	59.1	1.3	0.3
Glycogen	89.0	71.1	0	0
Starch	42.9	35.6	11.0	3.5
Dextran (linear)	11.7	5.1	10.2	2.5
Dextran (branched)	78.6	49.3	9.2	3.8
D-Glucopyranosyl phosphate	0	7.4	4.1	2.5
D-Glucono-1,5-lactone	0	0	0	0
2-Amino-2-deoxy-D-glucose	0	4.1	0	0
Weight of solid phase (mg/500-μl aliquot)	67.2	75.5	58.8	85.2

TABLE II

EFFECT OF IONIC CONCENTRATION AND pH OF ENVIRONMENT UPON ADSORPTION OF GLYCOGEN BY 1,3-DIAMINOBENZENE-COATED CELITE

Buffer	*Molarity*	*pH*	*Glycogen adsorbed (%)*
Acetate	0.01	6.0	98.7
Acetate	0.05	6.0	97.8
Acetate	0.10	6.0	95.7
Acetate	0.30	6.0	96.2
Acetate	0.60	6.0	98.5
Acetate	1.00	6.0	88.2
Acetate	0.05	3.6	96.3
Acetate	0.05	4.7	96.7
Acetate	0.05	5.8	96.0
Phosphate	0.05	5.8	98.5
Phosphate	0.05	6.9	98.2
Phosphate	0.05	8.0	98.2
Citrate–phosphate	0.05	2.3	96.8
Citrate–phosphate	0.05	4.7	97.8
Citrate–phosphate	0.05	5.8	97.6
Citrate–phosphate	0.05	6.9	97.2
Hydrochloric acid	0.10	1.0	95.6

buffers (all 0.05M), and 0.1M hydrochloric acid at 20° (Table II). The effect of temperature on the adsorption was investigated by using 0.05M sodium acetate buffer (pH 6.0) at 3.0, 25.0, 37.5, and 50.0°, the percentages of glycogen adsorbed being 97.5, 97.8, 97.0, and 97.2%, respectively. The effect of concentration of the glycogen solution was investigated by using 0.05M sodium acetate buffer (pH 6.0) at 20° (Table III).

The effect of reusing the solid phase to readsorb glycogen was studied by using 0.05M sodium acetate buffer (pH 5.0) at 20°, the initial supernatant being replaced with an equal volume of buffer containing 400 μl of glycogen solution (1 mg/ml). In three, successive adsorptions, the amounts of glycogen taken up were 417, 417, and 32 μg, respectively.

TABLE III

EFFECT OF GLYCOGEN CONCENTRATION UPON ADSORPTION ON 1,3-DIAMINOBENZENE-COATED CELITE

Weight of glycogen (μg/1.4 ml)			
Initial	*Adsorbed*	*Initial*	*Adsorbed*
0	0	1,185	996
126	114	2,240	900
280	267	5,850	945
434	423	11,000	800
560	547	48,600	1,000
882	871		

The effect of varying the nature of the solid support was studied by using D-glucose and glycogen in 0.1M sodium phosphate buffer (pH 6.0) at 20° (Table IV). The variation in the weight of the solid phase used in the adsorption experiments arises from the different bulk densities of the solid supports and the different weights of 1,3-diaminobenzene coated on to the different solid supports. The suspensions were made up by adding 0.1M sodium phosphate buffer (pH 6.0, 50 ml) to the coated, solid support (prepared from 10 g of solid support).

Prevention of adsorption of glycogen by Celite coated with 1,3-diaminobenzene. — Adsorption experiments were performed with 48.6 mg/500-μl aliquot of solid support and sodium borate buffers (mixtures of 0.05M boric acid and 0.05M sodium tetraborate) and phenolic sodium citrate–sodium phosphate buffers (mixtures of 0.05M citric acid and 0.05M disodium hydrogen phosphate, each containing 0.286 g of phenol/litre) over a range of pH values at 20° for 18 h (Table V).

TABLE IV

EFFECT OF CHANGING THE SOLID SUPPORT COATED WITH 1,3-DIAMINOBENZENE ON THE ADSORPTION OF D-GLUCOSE AND GLYCOGEN

Solid support	*Carbohydrate adsorbed*		*Weight of solid phase (mg/500 μl of suspension)*
	D-*Glucose* (%)	*Glycogen* (%)	
Alumina	1.4	87.5	31
Sephadex G10	8.0	85.8	63
Ballotini beads	7.5	5.4	27
Bioglas 1000	0	92.0	127
Titania spheroids	5.4	28.6	41
Tioxide R-SM	0	85.0	72
Tioxide A-DM	0	85.8	75

TABLE V

EFFECT OF BUFFERS ON THE ADSORPTION OF GLYCOGEN BY 1,3-DIAMINOBENZENE-COATED CELITE

Buffer[a]	*pH*	*Glycogen adsorbed* (%)
0.05M Boric acid	5.25	94.5
0.05M Sodium borate	6.30	94.2
0.05M Sodium borate	7.30	93.7
0.05M Sodium borate	8.20	92.3
0.05M Sodium tetraborate	8.80	93.9
Phenolic 0.05M citrate–phosphate	2.20	96.9
Phenolic 0.05M citrate–phosphate	3.50	96.8
Phenolic 0.05M citrate–phosphate	4.70	93.3
Phenolic 0.05M citrate–phosphate	5.80	96.1
Phenolic 0.05M citrate–phosphate	6.80	97.0
Phenolic 0.05M citrate–phosphate	8.30	97.0

[a]Concentration of phenol, 0.286 g/litre.

Methods of removal of glycogen adsorbed on Celite coated with 1,3-diaminobenzene. — Glycogen was adsorbed on to coated Celite (48.6 mg), as described in the general method, and the supernatant was removed by centrifugation and replaced by a solution (1.2 ml) of α-amylase (1 mg/ml, in 0.05M sodium acetate buffer, pH 5.0). The resulting suspension was incubated, with magnetic stirring, at 35° for 24 h. After centrifugation and removal of the supernatant for carbohydrate assay, the solid was washed with 0.05M sodium acetate buffer (pH 5.0, 2×2 ml), the first wash solution being boiled for 10 min with the solid to denature the enzyme. Sodium acetate buffer (0.05M, pH 5.0) and glycogen solution (1 mg/ml in 0.05M sodium acetate buffer, pH 5.0; 400 μl) were added to give a total volume of suspension of 1.4 ml, and this mixture was kept for 18 h at 20° (Table VI).

TABLE VI

EFFECT OF α-AMYLASE ON THE ADSORPTION OF GLYCOGEN BY CELITE COATED WITH 1,3-DIAMINOBENZENE

	Weight of glycogen (μg) in 1.4 ml		
	Initial	*Not adsorbed*	*Total adsorbed*
Adsorption	378	3	375
Degradation	0	187	188
Readsorption	378	332	234

A solution containing glycogen (1 mg/ml) in a solution of α-amylase (1 mg/ml) in 0.05M sodium acetate buffer (pH 5.0) was incubated at 35° for 24 h, and aliquots (400 μl) of the resulting dextrin solution were used in adsorption experiments with coated Celite, 10% of the dextrins being adsorbed.

Investigation of application of coated solids as column packings. — Coated Sephadex was packed under gravity into a column (1×10 cm), using 0.1M sodium phosphate buffer (pH 6.0). The eluate from the column was monitored by an automated version of the L-cysteine–sulphuric acid assay[5,6]. Aliquots of carbohydrate solutions (1 mg/ml, 50 μl) were introduced at the top of the column, and the flow rate was controlled by a peristaltic pump. When the carbohydrate was not eluted by the 0.1M sodium phosphate buffer (pH 6.0), 0.1M sodium borate buffer (pH 6.0) was used (Table VII).

Elution of a mixture of solutions (1 mg/ml) of D-glucose and glycogen (25 μl of each) with 0.1M sodium phosphate buffer (pH 6.0) gave a peak which had a very pronounced shoulder. Both the D-glucose and glycogen were eluted; the amount of carbohydrate eluted was equivalent to a 25% excess. When a column to which dextran (linear, 50 μl, 1 mg/ml) had been adsorbed was treated with D-glucose (50 μl, 1 mg/ml) followed by elution with phosphate buffer, there was no desorption of the dextran; the amount of carbohydrate eluted corresponded to 100% elution of D-glucose.

Coated Bioglas was packed into a column and the elution effected with 0.1M

TABLE VII

USE OF 1,3-DIAMINOBENZENE-COATED SEPHADEX AS A COLUMN PACKING

Carbohydrate	*Amount eluted* (%)	
	Phosphate buffer	*Borate buffer*[a]
D-Glucose	100	—
Glycogen	2	100
Dextran (linear)	3	100
Levan	0	100
Maltose	100	—
Gum tragacanth	100	—
Lactose	100	—
Cyclohepta-amylose	100	—

[a]See discussion.

sodium acetate buffer (pH 6.0) as described above, except that, as poor elution of linear dextran was obtained with 0.1M sodium borate buffer (pH 6.0), the pH of the sodium borate buffer was raised to 9.0 in an attempt to obtain sharper peaks (Table VIII).

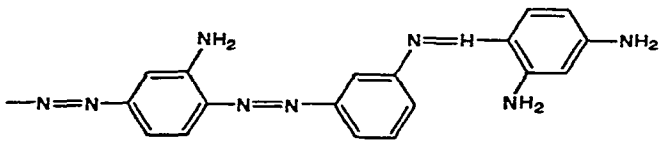
TABLE VIII

USE OF 1,3-DIAMINOBENZENE-COATED BIOGLAS AS A COLUMN PACKING

Carbohydrate	*Column load* (μg)	*Amount eluted* (%)	
		Acetate buffer	*Borate buffer*
D-Glucose	50	100	—
Dextran (linear)	50	0	100
Maltose	50	100	—
Glycogen (shellfish)	50	26	54
Glycogen (shellfish)	25	42	46
Glycogen (rat liver)	50	54	34

DISCUSSION

Treatment of 1,3-diaminobenzene in acid solution with sodium nitrite at low temperatures yields a brown, polymeric material. This material is commercially manufactured under the name "Bismarck Brown"[7,8] and is produced by the coupling of the diazotised or tetrazotised[9] molecules with unreacted or diazotised molecules to give a compound which may be represented[10] as **1**. Other, similar structures have been proposed for the dye[11–14], which probably consists of a complex mixture of polymers having different chain lengths and degrees of branching. Thus, it is possible only to postulate average structures, although certain features, such as the presence of diazo- and diazoamino-linkages, can be identified.



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When the diazotisation process takes place in the presence of an insoluble matrix such as Celite, the resulting dye forms a coating on the solid. That the nature of the solid support is important can be seen from the results for the adsorption of glycogen by the different coated solids (Tables I and IV). The non-porous surface (Ballotini beads) appeared to be insignificantly coated, judging by the lack of colour on the beads and their inability to adsorb glycogen. The solids (Celite, Sephadex, Bioglas, and the titanium dioxides) that adsorbed glycogen were coloured, showing that a coating had been obtained. These solids are highly porous and have structures which, to varying degrees, are either polar or can be induced to give polar interactions. Therefore, it was originally considered that the oligomers and polymers derived from 1,3-diaminobenzene form a coating on the particles of solid support with the aid of physical adsorption. However, this view must be modified to take into account the low surface area–volume ratios of the solid particles and the possibility of charge repulsion.

The various colours of the coated solids indicated that the coatings were not all identical. The polymeric material itself was a very dark brown, whereas the coatings on Sephadex and Tioxides were red–brown, those on titanium dioxide spheroids and Bioglas were purple–brown, and on Celite a range of colours was obtained according to the initial ratio of 1,3-diaminobenzene to Celite (ratios of 5:10, 2.5:10, 1:10, and 0.5:10 gave red–brown, red–brown, peach, and pale pink, respectively). Similar effects have been observed for coated celluloses, as was also a sensitivity of the reaction to small variations in temperature at the diazotisation stage; much darker products are obtained at lower temperatures[15].

The final product may contain several functional groups, including unreacted amino groups and hydroxyl groups formed by reaction of diazonium groups with the 2-naphthol added for this purpose to stabilise the polymer. The coating also contains a number of phenyl and naphthyl rings and diazo and diazoamino bonds which give rise to areas of delocalised electrons and polar regions.

A range of carbohydrates was used to study the effect of molecular weight, degree of branching, and charge on the adsorption by coated Celite. The carbohydrates can be considered in four groups. Thus, maltose, amylose, amylopectin, glycogen, and starch contain the same α-(1→4) links and vary in molecular weight and degree of branching. The linear and branched dextrans contain α-(1→6)-linked D-glucose residues, and have different degrees of branching. The effect of non-neutral carbohydrates was studied by using D-glucopyranosyl phosphate, D-glucono-1,5-lactone, and 2-amino-2-deoxy-D-glucose hydrochloride. Studies with the coated Celite showed that when a large proportion of 1,3-diaminobenzene coating is present,

compounds of low molecular weight (D-glucose and maltose) and the charged monomers are not adsorbed, linear polysaccharides are partially adsorbed, and the highly branched polysaccharides of high molecular weight are completely adsorbed (Table I).

The starch used was a soluble starch, which is a mixture of amylopectin and amylose in partially degraded form having a molecular weight and degree of branching lower than for amylopectin and amylose as separate entities, giving a lower absorption than amylopectin or amylose.

Adsorption was independent of ionic concentration, buffer system, and pH (Table II), and temperature. A given weight of coated solid support could adsorb the same maximal amount of glycogen when the polysaccharide was added in one or in several portions (Table III). The coated titanium dioxide spheroids adsorbed less glycogen (0.27% of dry, coated, solid support) than did the coated Celite (1.86%), possibly because of the lower surface area to volume ratio of the spheroids.

The stability of the coating was affected by dilute acid (0.1M hydrochloric acid), by buffers of high pH (above pH 9.0), and by more concentrated solutions of glycogen. This instability was observed as a coloration of the supernatant, having a maximal absorbance at 380 nm. Thus, the coating was not irreversibly bound to the solid support but could be removed by physical means.

It is known[16] that Sephadex (a cross-linked dextran) adsorbs aromatic and heterocyclic compounds, possibly by hydrogen bonding of the hydroxyl, amino, and carboxyl groups of phenols, anilines, and benzoic acids[17] to the ether groups of the cross-linking agent in Sephadex[18]. Hydrogen bonding between hydroxyl groups in the Sephadex and π-electrons or unshared electron pairs (of nitrogen atoms in the case of purines), dipole–dipole and dipole–induced dipole interactions, and dispersion forces could also be involved[19,20]. These phenomena have been utilised in the fractionation on Sephadex (highly branched polysaccharide) of aromatic acids and neutral compounds derived from catechol and indole amines[21], and the separation of maleic and fumaric acids[22].

The system described herein, which involves a solid-phase, aromatic heterocyclic compound that interacts with soluble, branched polysaccharides, is the reverse situation, but similar mechanisms of adsorption apply.

Adsorption of the glycogen on the coated Celite was not prevented by using borate buffers (Table V), presumably because the formation of borate complexes (which should oppose adsorption[23]) was less favourable than the formation of the glycogen-coating complex. Phenolic buffers were also ineffective, although they prevent the adsorption of peptides containing aromatic amino acids on to Sephadex[24].

With the aim of allowing the surface to be reused to adsorb fresh solutions of glycogen an attempt was made to remove the adsorbed glycogen by using α-amylase to break randomly the α-(1→4) linkages and so decrease the molecular weight. An amount of carbohydrate was thus removed, but the solid phase was unable to adsorb more glycogen (Table VI). The products obtained by digestion of glycogen with α-amylase were not strongly adsorbed by the solid phase, so it can be assumed that

only part of the glycogen had been degraded enzymically and removed, and that the residual, adsorbed carbohydrate prevented further adsorption. It appeared that α-amylase also affected the readsorption of glycogen, since in control experiments fresh samples of coated Celite digested with the enzyme would not adsorb glycogen even after heat deactivation of the enzyme.

The analogous adsorption of aromatic, heterocyclic compounds on Sephadex led to the use of Sephadex columns as a means of separation and purification. Attempts were therefore made to use the coated solids as column packings to fractionate and purify carbohydrates. It was not possible to use coated Celite, as the flow rates were not reproducible and there was a strong tendency for the columns to block. The physically similar, coated alumina and Tioxides could not be used for the same reason. However, columns of coated Bioglas and Sephadex could be run reproducibly for long periods of time, Bioglas being preferable because, with Sephadex columns, erroneous peaks were recorded by the automated analysis system. Whenever the buffer system was changed to sodium borate, a peak was recorded which corresponded to the solvent front and was attributed to washing of carbohydrate, coated carbohydrate, or polymeric 1,3-diaminobenzene from the column as a result of disruption of the Sephadex structure by the diazotization process. The coated Bioglas adsorbed certain polysaccharides when eluted with sodium acetate buffer, but these could be eluted by use of borate buffers (Table VIII). This desorption of carbohydrates by borate buffers is due to the different equilibria obtained in column elution compared to the test-tube experiments, but the high pH of the buffers required (pH 9.0) had an adverse effect on the stability of the polyaromatic coating. The adsorption of glycogen was partially prevented by the presence of D-glucose, the glycogen being eluted by phosphate buffer but not with the same elution volume as D-glucose. In most cases, the adsorbed carbohydrates were eluted by borate buffers with an elution volume which corresponded to the solvent front. Use of solid supports of better physical form, the adoption of solvent gradients, and elution with D-glucose solutions could lead to the use of these coated-solid supports as column packings for adsorption chromatography.

In the field of pollution control, there is a need for substances which can remove polysaccharides (starch and cellulose in particular) in low concentrations from solution. Coated Celite has a number of properties which make it useful in this field. Celite is a cheap, inert, solid support which is easily obtained and non-toxic. In coated form, it can be used over a wide range of pH values and temperatures with no adverse effects, and may be readily removed from solution by filtration or centrifugation.

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