AN INVESTIGATION OF CELLULOSE ION EXCHANGERS

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ABSTRACT

The aim of this thesis was to prepare a range of polysaccharide ion exchangers and to explore their potential for use in chromatographic methods and their ability to remove protein from solution.

The ion exchangers were prepared from regenerated cellulose cross linked with epichlorohydrin and hydroxyalkylated with propylene oxide. The preparations of the DEAE -, CM- and SP- derivatives were investigated and the products shown to be chemically stable, to allow high flow rates and to have excellent capacities for adsorbing protein.

Practical applications of these ion exchangers were demonstrated. The DEAE-derivative was used for the chromatographic fractionation of serum proteins and an enzyme purification. The CM- and SP- derivatives were found to be useful for removing protein from whey. The conditions, such as pretreatment of whey and pH, were investigated to find the conditions necessary for good protein uptake and from the results a new process was developed for efficient recovery of protein from whey by ion exchange.

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INTRODUCTION

1.1 Background

An ion exchanger is an insoluble material containing chemically bound charged groups and mobile counter ions. If the matrix carries positive groups the counter ions will be negative. Such an ion exchanger will exchange negative ions and is therefore termed an anion exchanger. In the same way, if the matrix carries negative groups the counter ions will be positive. Since the positive ions are exchangeable the term cation exchanger is used.

Ion exchangers have been around for a long time. As early as 1850, Thompson (1) and Way (2) reported the ability of soils to exchange ions, such as ammonium for calcium. Other early developments involved the demonstration that a number of natural minerals, particularly the zeolites are capable of ion exchange (3-4). While research continued on these points it was not until 1905 that Gans (5-6), synthesized examples of inorganic ion exchangers. In 1935, one of the most important events in the history of ion exchangers was the recognition by Adams and Homes (7-8) that synthetic resins have ion exchange properties. Since 1936 patents in this field have proliferated.

The ion exchangers which were first obtained by polycondensation came to be replaced increasingly by polymerization products after 1945, when d'Alelio (9) succeeded in incorporating sulfonic acid groups into a cross-linked polystyrene resin. Further developments dealt with improvements and the production of special resins with specific ion exchange properties.

The phenomenon of separating specific compounds from a mixture with the aid of ion exchangers was first called base exchange and was interpreted as a chemical process in 1856 (10). The mechanism by which separation is obtained on an ion exchanger is one of reversible adsorption. In two stages there is the binding of substances to the ion exchanger followed by the removal of these one at a time, separated from eachother. Separation is possible since substances normally have different electrical

properties and are released from the ion exchangers by change in ionic strength or a shift in pH. Such a separation is today referred to as ion exchange chromatography.

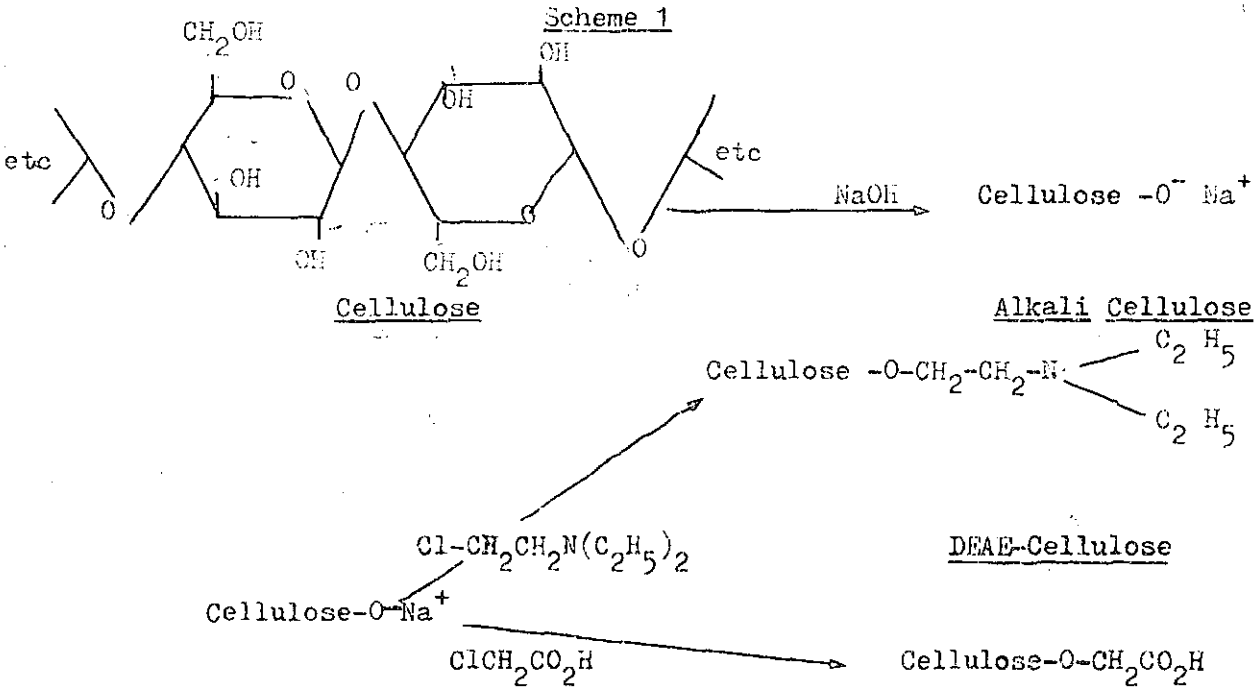
The problem with synthetic ion exchangers with a few exceptions was their failure to be established as useful media for polyelectrolyte fractionation. Being specifically designed for application to problems involving inorganic ions, their molecular structure is inaccessible to polyelectrolytes of higher molecular weight. To have sufficient ion exchange groups accessible to polyelectrolytes on a macrosurface, would necessitate reducing the material to an impracticably small size. Also, synthetic resins show irreversible adsorption to proteins by forming too many electrostatic bonds with the protein, preventing the disruption of these bonds under elution conditions consistent with maintaining the configuration of the protein.

The use of macroreticular ion exchange resins in adsorbing biological substances has been subjected to a number of restrictions. Using the macroreticular ion exchange resin, Amberlite IRC-50, Pollio and Kunin (11) found the exchanger to be limited to substances of low molecular weight (m.w. 10,000 - 70,000). In comparing both Cytochrome C, (m.w. 11,000 - 13,000) and Lysozyme (m.w. 14,000 - 19,000) it was found that such factors as the size of the organic molecule and resin particle size had a great effect on the rate and capacity of these types of ion exchangers. For larger molecules, for example Haemoglobin (m.w. 68,000) adsorption was considerably more difficult (11, 12). Overall macroreticular synthetic ion exchangers have a limited capacity for proteins.

1.2 Celluloses with Ion Exchange Properties.

Early cellulose based ion exchangers included, Oxycellulose (a weakly acidic carboxyl type ion exchanger) (13, 14), cellulose succinic half esters (15) and a variety of treated cottons (16, 17). The diethylaminoethyl ether of cellulose was prepared in 1930 (18) and subsequently by Hoffpauir and Guthrie (16) by heating a mixture of β-chloroethyldiethylamine with alkali cellulose.

Scheme 1



However, the chromatographic separation of polyelectrolytes such as proteins did not really become possible until Peterson and Sober (19-21) prepared the cation exchanger, carboxymethyl-cellulose (CM) by treating strongly alkali cellulose with chloroacetic acid and the anion exchanger, diethylaminoethyl-cellulose (DEAE) by treating strongly alkali cellulose with 2-chlorotriethylamine hydrochloride. Both the phospho-cellulose (P) and epichlorotriethanolamine-cellulose (ECTEOLA) were prepared as well. Using their preparations, made with cellulose powder, they used the column situation in the separation and concentration of proteins.

Since this initial work others have studied cellulose based ion exchangers. Guthrie and Black (22), found ion exchange celluloses able to combine a relatively low total binding strength with an adequate capacity for polyelectrolytes. The products being finer, presented a larger surface area than ordinary resins and also their open and porous structure allowing larger molecules to enter. Fractionation of serum proteins using DEAE-Cellulose as investigated by James and Stanworth (23), who observed an increase in specific adsorption capacities with degree of substitution. A relatively high capacity was found. They did not elaborate whether increased capacity was due to an increased number of adsorption sites on the ion exchanger or to an increased selectivity. Peterson and Sober performed similar experiments but with a lower capacity being obtained.

TABLE 1

Sephadex derivatives available

TYPE	USEFUL pH RANGE	FUNCTIONAL GROUPS	IONIC FORM	DESCRIPTION
DEAE	2-9	Diethylaminoethyl	$-C_2H_4N^+(C_2H_5)_2H$	Weak base:anion exchanger
QAE	2-10	Diethyl-(2-hydroxy-propyl)amino-ethyl.	$-C_2H_4N^+(C_2H_5)_2$ $CH_2CH(OH)CH_3$	Strong base:anion exchanger
CM	4-10	Carboxymethyl	$-CH_2COO-$	weak acid:cation exchanger
SP	2-10	Sulphopropyl	$-C_3H_6SO_3$	strong acid:cation exchanger

Staelhelim et al (24) and Tenser et al (25) studied the strength of interaction between ion exchanger molecules and the adsorbed polyelectrolytes and found this to be primarily dependent on the cumulative electrostatic binding between oppositely charged sites on the two.

There are a number of cellulose derivatives available today as ion exchangers. The most important of these are the DEAE and CM Celluloses. The diethylaminoethyl cellulose is prepared by heating alkali cellulose with B-chloroethyldiethylamine hydrochloride (CED). The carboxyl cellulose is prepared by reacting alkali cellulose with chloroacetic acid as shown in scheme 1.

There are a number of DEAE celluloses commercially available, with small ion capacities in the range of 0.1 - 1.1 meq/g. Other available celluloses include the triethylaminoethyl (TEAE), $-O-CH_2-CH_2-N^+(C_2H_5)_3$; quaternary aminoethyl (QAE), $-C_2H_4N^+(C_2H_5)_2CH_2CH(OH)CH_3$; epichlorotriethanolamine (ECTEOLA); and phospho (P), $-O-\overset{\overset{O}{\|}}{\underset{\underset{OH}{|}}{P}}-OH$, with various small ion capacities.

The other main ion exchangers used for protein fractionation are those based on spherical beads of cross-linked dextran and marketed under the name of Sephadex. The derivatives available are shown in Table 1.

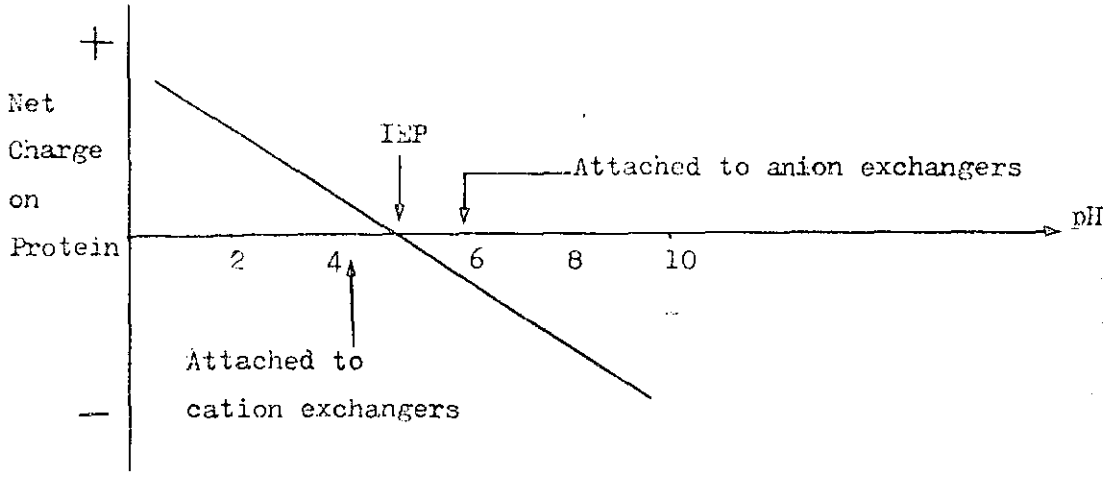
Other polysaccharides such as Starch and Agarose have also been used as the matrix for ion exchangers after being stabilized by crosslinking.

1.3 Choice of Ion Exchanger and Conditions of Use

The choice of ion exchanger depends on the net charge of the substance to be chromatographed. Substances which carry both negatively and positively charged groups are amphoteric and their net charge is thus dependent on pH. At low pH the net charge is positive, at high pH it is negative. At the point of zero net charge, the isoelectric point (IEP), the substances are not bound to any type of ion exchanger. Proteins are amphoteric polyelectrolytes and can normally be bound to both anion and cation exchangers by a suitable choice of conditions. The net charge on a typical protein as a function of pH is shown in Figure 1.

Figure 1

The net charge of a protein as a function of pH



It can be seen that below the IEP the protein has a net positive charge and is therefore adsorbed by cation exchangers. Above the IEP the protein can be adsorbed by anion exchangers since it carries a net negative charge. The choice of ion exchanger maybe determined by the range of stability of the protein. The ion exchange derivative used will be one that has the correct charge to bind the protein within the pH range of stability of the protein used.

The protein adsorbed on the ion exchanger can be eluted from the ion exchanger by shifting the pH so that the charge on the protein is changed or by raising the ionic strength which increases the competition for charged groups on the ion exchanger and thus reduces the interaction between the ion exchanger and the protein thereby causing their elution.

1.4 Regenerated Cellulose Ion Exchangers

The use of cellulose based ion exchangers is restricted due to their fibrous structure. These ion exchangers usually suffer from the disadvantage of poor hydraulic properties. They generally have low flow rates and tendto become easily clogged by particles of suspended matter. These difficulties were overcome by Grant (26) who used regenerated cellulose containing chemical cross-links as a matrix to which ionizable groups were attached.

Regenerated cellulose is prepared from natural cellulose by anyone of several processes. The Xanthate process, discovered in 1893 (27), involves rendering cellulose soluble by reaction with NaOH and carbon disulphide to form sodium xanthate. The sodium cellulose xanthate is soluble in caustic soda solution and gives a solution known as "viscose".

Xanthation $\quad R\ Cell\ ONa + CS_2 \longrightarrow R\ Cell\ OCSSNa$

Regeneration $\quad 2R\ Cell\ OCSSNa + H_2SO_4 \longrightarrow 2R\ CellOH + Na_2SO_4 + 2CS_2$

Regeneration may be affected by heat or acid and it is possible to considerably modify the microstructure and physical properties of the regenerated cellulose by varying the "viscose" composition and regeneration conditions.

Regenerated cellulose was proposed as an ion exchange material in 1959 (28). It was of no practical value since no cross-linking was proposed. The use of cross-linking agents to inhibit solubilization of regenerated cellulose ion exchangers was investigated by Selegny *et al* in 1966 (29). Murphy (30) investigated the reaction with isocyanate and amines to produce cross-linked ion exchange material from natural cellulose and regenerated cellulose.

The major work on use of regenerated cellulose for ion exchange preparation was that introduced in 1968 by Grant (26). Grant proposed the making of an ion exchanger comprising the introduction of cross-linking residues into regenerated cellulose together with or followed by the introduction of groups capable of anion or cation exchange. Groups capable of anion exchange suggested by Grant were: amino, alkylamino and quaternary ammonium groups. Groups capable of cation exchange were sulphonic acids, phosphate and carboxyl groups.

According to Grant, for both the cross-linking reaction and the introduction of exchange groups, the water content of the reaction

mixture should be carefully controlled to give optimum results. In general the water content preferably should be in the range, 50 to 100 percent of the weight of regenerated cellulose. It should be introduced with the exchange groups and depends somewhat on the grain size of the cellulose used.

Since Grant's patent other patents have appeared for ion exchangers based on cross-linked regenerated cellulose. These involve a modification of the Xanthate process. One of these (31), involved the introduction of ionizable groups at the soluble Xanthate stage before regeneration of the cellulose. This allows the regenerated cellulose exchangers to be produced in a variety of forms such as sponges, fibres, rods, filaments and yarn as well as particles as used by Grant. Other patents (32, 33) describe the production of "cellulose pearls" by regenerating the cellulose after an emulsion of the xanthate solution had been formed in an organic solvent. This gave highly swollen beads with excellent protein capacities (1000 - 2000 mg/g) but the flow rate through the ion exchangers when packed in a column are not as high as those obtainable with Grant's product.

Since 1969 the commercial development of the Grant ion exchangers has been hampered by their low protein capacity, i.e. 200 - 500 mg/g., but the potential is there for large scale application because of their robust nature, low attrition properties and high flow rates of ground regenerated cellulose particles.

Sheerin (34) first noted the ability of propylene oxide to swell regenerated cellulose. Instead of using the difficult regeneration procedure of Pharmacia (32, 33) with organic solvents to produce highly swollen regenerated cellulose the effect of propylene oxide on particles of ground regenerated cellulose has been investigated further in this thesis to see if improved protein capacities could be obtained for the ion exchangers. Although many commercial ion exchangers are available for work in extracting protein from solution, ones with hydroxypropyl groups attached to the cellulose back bone have not been reported before. The anion exchangers, DEAE-hydroxypropylated regenerated cellulose, and cation exchangers, CM- and Sulphopropyl hydroxypropylated regenerated cellulose have been investigated to find preparations with good protein capacity, reasonable water volume and stability to repeated use. The preparation of the cation exchangers were investigated for use in extracting protein from whey by use of a large column of ion exchanger .