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Kinetic behaviour of a novel matrix ion exchanger, carboxymethyl-HVFM operated at high flow-rate

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ABSTRACT

The kinetic behaviour of a novel coherent ion-exchange matrix, CM-HVFM, a carboxymethyl derivative of cross-linked regenerated cellulose, was investigated using lysozyme as a model protein. The results obtained were compared with those for two other commercial carboxymethyl cellulosic ion exchangers, HC2 (Phoenix) and CM-52 (Whatman). The flow properties of the new matrix were by far superior to those for the other materials whilst the adsorption and desorption kinetics were also faster. The new resin was able to sustain a superficial velocity of up to 8.78 m h^{-1} in a 147-mm I.D. column. In a 26-mm I.D. column, an only slightly faster velocity of 10 m h^{-1} was obtained. This contrasted with a limiting flow of less than 1.5 m h^{-1} with the other two packings. It was found that the kinetic rates of adsorption and desorption depended on the flow-rate through the matrix improving as the flow-rate increased. It was concluded that the highest flow-rates external mass-transfer resistances had been virtually removed and any remaining transfer resistance was due to diffusion through the matrix. In adsorption it was possible to achieve 90% adsorption within 8 min and 90% desorption within 5 min. Whilst the Whatman material had similar desorption rates neither of the other materials were close on the adsorption rates taking almost three times as long at the fastest sustainable superficial velocity for the CM-52 resin. These fast kinetics were achieved with a high capacity ranging from over 800 mg per dry g at a relative high salt concentration to over 2 g lysozyme per dry g for the unsalted lysozyme solution. Finally, no obvious swelling and shrinking was observed when the CM-HVFM column of 110 × 10 mm I.D. was regenerated by 2 *M* NaCl or 0.7 *M* NaOH solution for 40 min.

INTRODUCTION

The high separation efficiency and low energy requirements of ion-exchange chromatography have led to its routine use in biochemical separation processes [1,2]. In 1986 Bonnerjea *et al.* [3] studied the published purification protocols and found that ion exchange was used in 75% of all purifications. Traditional synthetic ion exchangers have a high charge density, a high degree of cross-linking and hydrophobic character that lead to low protein capacities and denaturation of adsorbed biomolecules [4]. A series of ion exchangers based on naturally occurring organic macromolecules are better at separating macromolecules as they possess macropores and a hydrophilic surfaces with lower charge density which tends not to denature the proteins. The ion exchangers based on polysaccharides possess moderately good hydraulic properties but relatively poor kinetics. Those based on cellulose, fast kinetics but poorer flow properties and those based on inorganic supports, good flow

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properties but poor capacities. There are difficulties in using several of these materials on the large scale [5].

There are few publications referring to the use of high flow-rates. Microgranular materials usually use superficial velocities of around 0.3–0.4 m h^{-1}. Levison *et al.* [6], using the Whatman microgranular QA-52, or DE-52 in a 100-ml axial flow column (66 × 44 mm I.D.) achieved a flow-rate of up to 25 ml min^{-1}, 0.99 m h^{-1} superficial velocity, beyond which higher flow was impossible due to the compressibility of the resin. Levison *et al.* [7] also reported that they used a 160 × 450 mm I.D. column packed with DE-52, a microgranular cellulose anionexchanger, at a volumetric flow-rate of about 800 ml min^{-1}, *i.e.*, only 0.3 m h^{-1} superficial velocity to separate egg white proteins. Only in analytical-scale columns significantly higher velocities are used, sometimes reaching 6 m h^{-1}.

Flow-rates used with cross-linked dextran exchangers are even slower. An example of industrial ion-exchange chromatography to produce egg white lysozyme used CM-Sephadex C-25 to adsorb the enzyme which was then eluted with 1 *M* NaCl. The cycle time depended on whether the ionic strength was adjusted by dilution or by desalting on Sephadex G-25. In the former case, the cycle time was 42 h and in the second case 15 h [8]. Both cases indicated that one of the main reasons which caused such long) cycle time was the low flow-rate.

Flow-rates through ion exchangers are usually limited by the compressibility of the matrix. The granular materials with larger particle sizes can allow fast flows but the large particles create internal transfer resistances which control the overall process. The productivity of these processes for production purposes is thus limited by either the diffusion within the particles or the flow through the bed. It will be shown in this paper that the effect of low flow-rates through the bed is to reduce further the inherent velocity of the process leading to a potential reduction in throughput and hence efficiency of use of the system.

In addition, the ion exchangers based on the cross-linked cellulose and dextran in the physical form of fibre, microgranular and sphericity show swelling and shrinking when the ionic strength and pH of the carrier fluids are varied. This leads to the resin bed being deformed and cracked and causes channeling and subsequent loss of performance.

An ideal ion exchanger is sought with the characteristics of fast kinetics, high flow-rate and stable physical form when varying the ionic strength and pH value. The novel ion-exchanger, carboxymethyl-HVFM, (CM-HVFM) supplied by Biotechnology Process Services (Durham, U.K.) is based on cross-linked regenerated cellulose and is in a coherent rather than particulate form. It is shown in this paper to exhibit high flow-rates at low pressures, and a high capacity. Because of its coherent form it is not possible to form cracks in the bed when it is subjected to changing ionic strengths.

Here we report some of these characteristics of the new matrix and leave a theoretical development for a future paper. Firstly, we examined the adsorption and desorption kinetic behaviour of CM-HVFM under a variable column flow-rate. The differential bed technique was used which allows us to assume the whole column (4 mm long) was at the same condition.

MATERIALS AND METHODS

Lysozyme (Sigma, Dorset, U.K.) was dissolved in 0.01 *M* acetic acid–sodium acetate buffer of pH 5.0.

A novel cross-linked cellulosic ion-exchanger (batch W1) (Biotechnology Process Services) was obtained in the carboxymethyl form (CM-HVFM). It was found to have a maximum lysozyme capacity of 2100 mg per dry at pH 5.0. The 3-mm high discs were supplied in diameter 2% larger than the I.D. of the columns, which were used to pack the columns. Two other carboxymethyl cellulosic ion-exchange resins were obtained commercially. CM-52, a microgranular CM ion-exchanger, was purchased from Whatman BioSystems (Kent, U.K.) and Indion HC2 (CM resin, size between 350–400 μm, batch 1246) was obtained from Phoenic Chemical, Waitaki NZ Refrigerating (Nelson, New Zealand).

A Sartorius (Surrey, U.K.) D-3400 filter holder with distributor was packed with either 2.7 g (0.3 g dry weight) CM-HVFM or 5 g of 1:1 slurry of CM-52 or 4.3 g of 1:0.8 slurry of HC2. This produced a short 5.8-ml column (4 mm × 43 mm I.D.

A 160 × 10 mm I.D. adjustable glass column (Amicon, Stonehouse, U.K.) packed with 0.5 g (dry weight) CM-HVFM was used for the 110 × 10 mm I.D. column of 8.64 ml volume. A gear micropump (Micropump, Concord, CA, U.S.A.) was used for the higher flow-rates otherwise a Millipore (Bedford, MA, U.S.A.) peristaltic pump or Autoclude peristalic pump (F.T. Scientific Instruments, Tewkesbury, U.K.) were used. A LKB (Bromma, Sweden) Uvicord II monitor was used on-line to detect the lysozyme concentration at 280 nm.

The experiments were carried out using the systems shown in Fig. 1. The sample

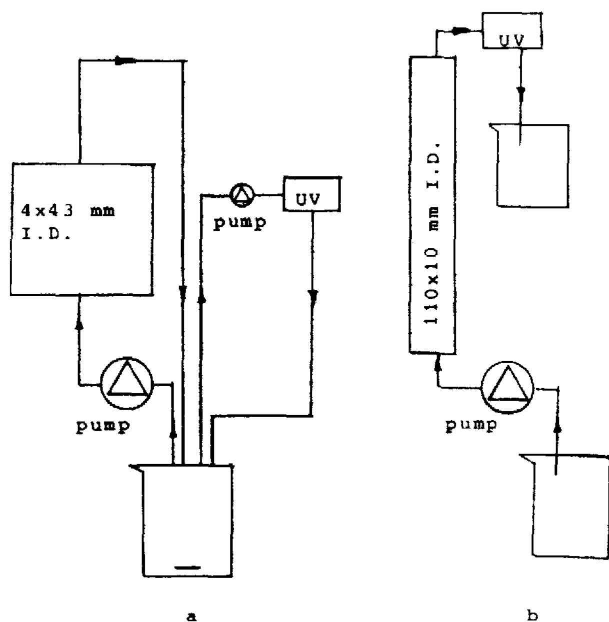

Fig. 1. Experimental devices. (a) Short column; (b) long column.

to be loaded was placed in a stirred beaker from which it was pumped upwards through the column. With the short column (Fig. 1a) a separate stream was pumped from the beaker to the UV monitor and back to the beaker at a velocity of 1800 m h^{-1} whilst for the longer column (Fig. 1b) the stream passed from the top of the column through the UV detector. The short column was operated at a fast recycle rate and acted as a differential bed in order to study the kinetics of adsorption and desorption on the ion exchangers. Before each adsorption run, the ion exchanger was equilibrated with pH 5.0 of 0.01 *M* sodium acetate buffer then the whole system (Fig. 1a) was drained before starting the run. Washing with pH 5.0 of 0.01 *M* sodium acetate buffer was followed by the adsorption and then desorption with 0.7 *M* NaCl solution. The CM-HVFM was regenerated by 2 *M* NaCl or 0.7 *M* NaOH solution. A load–wash–elution process was also performed on the longer column.

Two other larger diameter columns were used to measure the relationship between flow-rate and pressure drop through the columns. These were both about 100 mm long × 26 mm or 147 mm I.D. Pressure was increased by increasing flow.

RESULTS AND DISCUSSION

The CM-HVFM isothermal adsorption curves shown in Fig. 2 were obtained for lysozyme solutions adjusted to different ionic strengths with salt. The curve for 0.767 mS cm^{-1} ionic concentration was fitted to a Langmuir [9] isotherm with a dissociation constant, K_d, of 0.08 mg/ml and a lysozyme capacity (q_m) of 2010 mg/g. Such high protein capacity might result from the large surface area to volume ratio of the open irregualor network structure of the CM-HVFM. At a very low lysozyme concentration the CM-HVFM showed a high equilibrium protein capacity, *i.e.* 1300 mg g^{-1} in equilinium with 0.2 mg/ml lysozyme. When the ionic concentration was increased 21

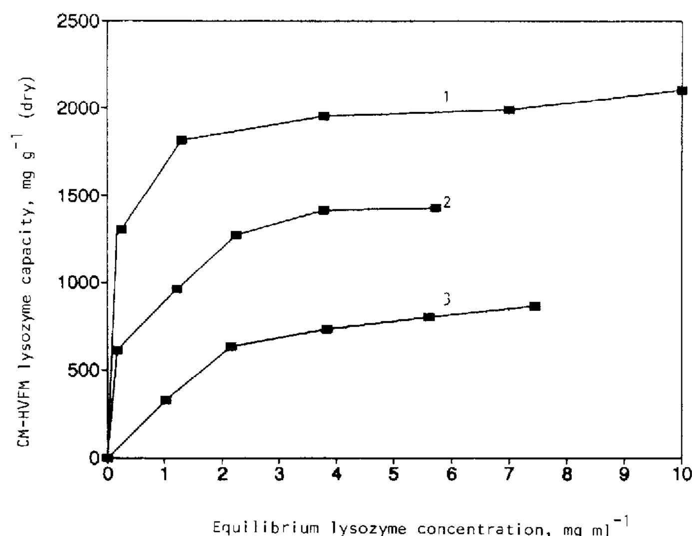

Fig. 2. CM-HVFM adsorption isotherms. Effect of ionic concentration. Curves: 1 = 0.767 mS cm^{-1}; 2 = 5.66 mS cm^{-1}; 3 = 16.3 mS cm^{-1}.

times to 16.3 mS cm^{-1}, the q_m value of CM-HVFM only dropped to 863 mg g^{-1}. Thus it is suggested from Fig. 2 that CM-HVFM could be used to recover protein from relatively low concentration streams at moderate ionic concentrations between 0.7 and 8 mS cm^{-1}.

When applying the CM-HVFM to low protein concentration streams of large volume, there are two other performance factors, kinetic process and flow-rate, which have to be considered and those will be discussed below.

The CM-52 and HC2 are also based on cross-linked cellulose thus they were chosen as the controls. The first is in the physical form of irregular microgranules (diameter 60–150 μm) and the second as almost spherical particles (diameter 350–450 μm). Fig. 3 shows the effect of pressure on superficial velocity for all three materials. The superficial velocity through the CM-HVFM or CM-52 or HC2 in the short column (4 mm × 43 mm I.D.), could reach 92.76 m h^{-1} for CM-HVFM, 32.00 m h^{-1} for Indion HC2 and only 23.64 m h^{-1} for Whatman CM-52 at an inlet pressure of 1 bar where all three kinds of ion exchangers tended to plateau and when the inlet pressure was increased to 1.7 bar no further increase in flow was observed. These materials are not suited to high-pressure chromatography.

Although very high flow-rates are obtainable for very short beds, this is not possible for long beds with the conventional materials. When the CM-HVFM or 60 g of 1:1 slurry of CM-52 or the HC2 was packed in a 100-mm deep polycarbonate column of 26 mm I.D. (Fig. 4), it can be seen that at an inlet pressure of 0.4 bar the superficial velocity of CM-HVFM could still reach 9.50 m h^{-1} in the upflow mode. However, the CM-52 and HC2 could not maintain their previous performance. The maximum superficial velocity of both of them was only about 1.00 m h^{-1} and the CM-52 inside the column was compressed to about 18% of its original length at the inlet pressure of 0.7 bar due to pressure forces within the system. This accords with the findings of Levison *et al.* [6]. The superficial velocity through the HC2 was slightly

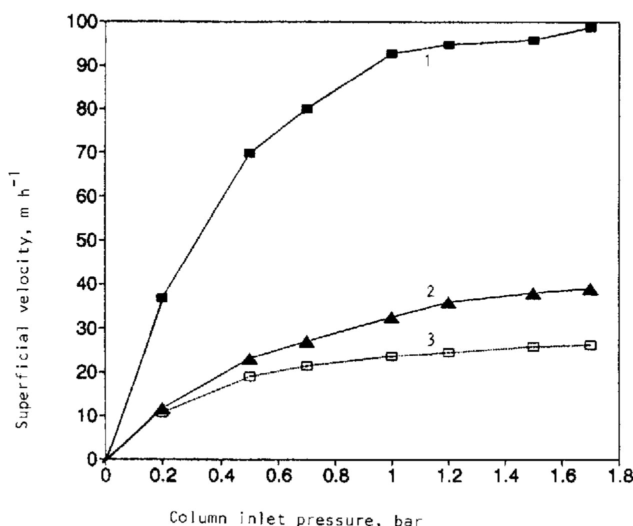

Fig. 3. The effect of pressure on superficial velocity for CM-HVFM (1), HC2 (2) and CM-52 (3) in a 4 mm × 43 mm I.D. column.

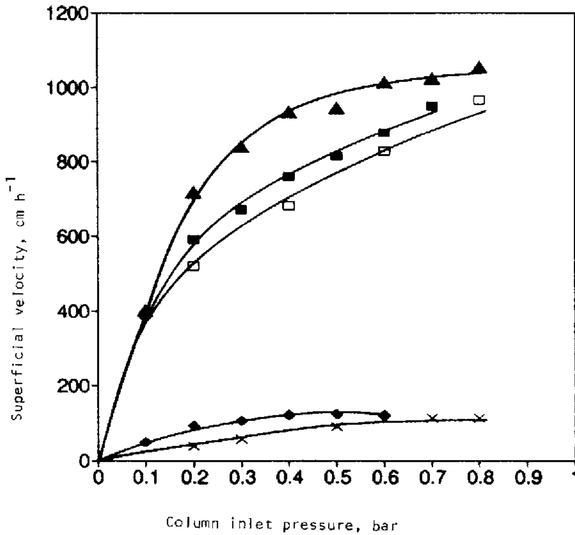

Fig. 4. The effect of column diameter and pressure on superficial velocity for CM-HVFM and comparison with 26 mm diameter columns containing CM-52 or HC. □ = 100 × 147 mm I.D. CM-HVFM, up-flow mode; □ = 100 × 147 mm I.D. CM-HVFM, down-flow mode; ▲ = 100 × 26 mm I.D. CM-HVFM, up-flow mode; × = 100 × 26 mm I.D. CM-52, up-flow mode; ◆ = 100 × 26 mm I.D. HC2, up-flow mode.

higher than that of CM-52 since the particle size of HC2 was 3–4 times larger than that of CM-52. It did not seem practical to examine the effect of increased column diameters as this would have decreased performance of these exhangers still further. It was also decided to examine the adsorption and desorption kinetics only up to a flow-rate that could be sustained in the 110-mm long column for the latter exchangers.

Scaling the column diameter up 5.7-fold to 147 mm I.D. would be expected to create a serious flow problem as the effect of the wall support is lost. Data from Levison *et al.* [7] shows that with DEAE-cellulose DE-52 the most practicable flow-rate for diameters above 100 mm is 300 mm h^{-1}. With CM-HVFM (Fig. 4) the increase in diameter only caused a further loss of flow of about 15% depending slightly on whether the flow was up or down through the column. The superficial velocity was not affected at 0.1 bar, and at 0.4 bar reached 7 m h^{-1} which suggests that a superficial velocity of 5 7 m h^{-1} might be applied on the process scale.

Figs. 5 and 6 show the relationship between the kinetic rates of adsorption and desorption and flow-rate for 5.8 ml of CM-HVFM in the differential bed (4 × 43 mm I.D. column) when first fed with 180 ml of 0.5 mg ml^{-1} of lysozyme in 0.01 *M* sodium acetate buffer at pH 5.0 to adsorb the lysozyme and then eluted with 180 ml of 0.7 *M* NaCl to desorb the adsorbed lysozyme at various superficial velocities. The same elution volume as adsorption was used so that an indication of the overall mass balance and the percentage adsorption can be gauged readily from the UV chart trace.

By using the differential bed column the column wall effect, axial dispersion and uneven distribution could be neglected due to the recycle mode of the differential bed. It is also possible to show directly how flow can affect the kinetics by altering the

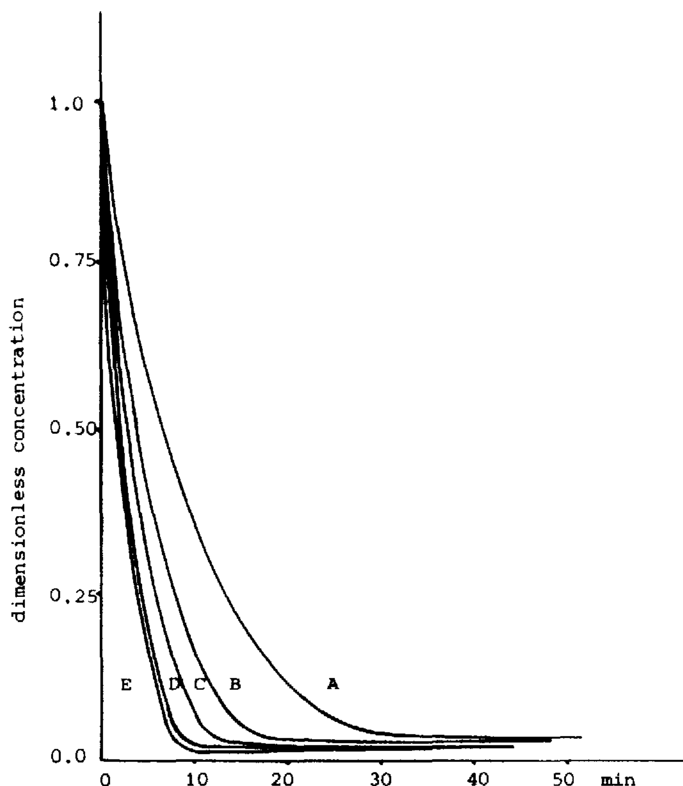

Fig. 5. The adsorption of 180 ml of 0.5 mg ml^{-1} lysozyme in 0.01 *M* sodium acetate buffer pH 5 in a 4 mm × 43 mm I.D. column A = 297 cm h^{-1}; B = 644 cm h^{-1}; C = 1388 cm h^{-1}; D = 4443 cm h^{-1}; E = 9276 cm h^{-1}.

external mass transfer coefficient. This demonstrates the significant effect which mass transfer external to the particles has on chromatographic kinetics. It is also clear that to achieve these effects on a large scale it is important to have a matrix which will sustain high velocities on a wide diameter column.

From Figs. 5 (curves A–E) and 6 (curves A–E) with the superficial velocity varying from 2.97 m h^{-1} to 92.76 m h^{-1}, it can be seen that the rates of adsorption and desorption increased with increasing superficial velocity. 80% of the total lysozyme was adsorbed in 14 min and 90% of adsorbed lysozyme was eluted in 10 min at a superficial velocity of 2.97 m h^{-1} (Figs. 5 and 6, curves A), while at a superficial velocity of 13.88 m h^{-1}. 90% of lysozyme was adsorbed in 8 min and 90% of adsorbed lysozyme was eluted in 6 min (Figs. 5 and 6, curves C), further increasing the superficial velocity to 44.43 m h^{-1} at the inlet pressure 0.21 bar required only 3.5 min for 90% of lysozyme to be adsorbed and only 5 min for 90% of the adsorbed lysozyme to be desorbed (Figs. 5 and 6, curves D). These times of adsorption and desorption were only slightly improved when the flow velocity was increased to 92.76 m h^{-1} at an inlet pressure of 1 bar (Figs. 5 and 6, curves E). It appears that the flow-rate is no longer the main limiting factor above about 50 m h^{-1}, and that at this flow-rate external film mass

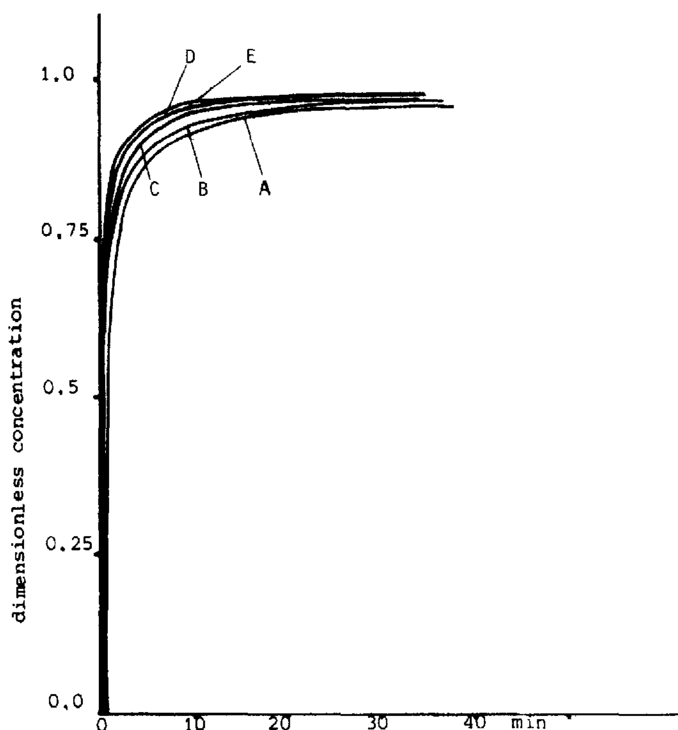

Fig. 6. The desorption of the adsorbed lysozyme with 180 ml of 0.7 *M* NaCl in a 4 mm × 43 mm I.D. column at various superficial velocities. A = 297 cm h^{-1}; B = 644 cm h^{-1}; C = 1388 cm h^{-1}; D = 4443 cm h^{-1}; E = 9276 cm h^{-1}.

transfer is no longer very significant. Conversely, at lower flow-rates the fastest kinetics are not attainable due to increased mass transfer in the fluid phase. The adsorption time for 90% lysozyme adsorption and desorption of 90% adsorbed lysozyme was reduced by factors of 5.7 and 2 respectively by increasing the superficial velocity 15-fold, from 2.97 to 44.43 m h^{-1}.

It is interesting to note that the desorption of CM-HVFM is less influenced by external mass transfer (Fig. 6). Since the commercially useful flow-rates of CM-52 and HC2 are constrained (Fig. 4), the kinetic behaviour of adsorption and desorption of CM-52 and HC2 is only described at flow velocity less than 1 m h^{-1}. Superficial velocities of 0.99 and 0.51 m h^{-1} were used but otherwise the loading and process conditions for the kinetics study of both CM-52 and HC2 were exactly the same as for CM-HVFM.

Figs. 7a and b illustrate that the kinetic processes of CM-52 are faster than those of HC2 in both cases due to the large surface area to volume ratio of microgranular material. With the same loading conditions as for the CM-HVFM, it needed 17 and 31 min to adsorb 80% of loaded lysozyme for CM-52 at the superficial velocities 0.99 and 0.51 m h^{-1}, respectively, whilst for HC2 it needed 27 min and 37 min under the same conditions (Fig. 7a). Although the CM-52 exhibits faster desorption than HC2, it still needed 10 and 14 min to elute 90% of the adsorbed lysozyme at 0.99 and 0.51 m h^{-1},

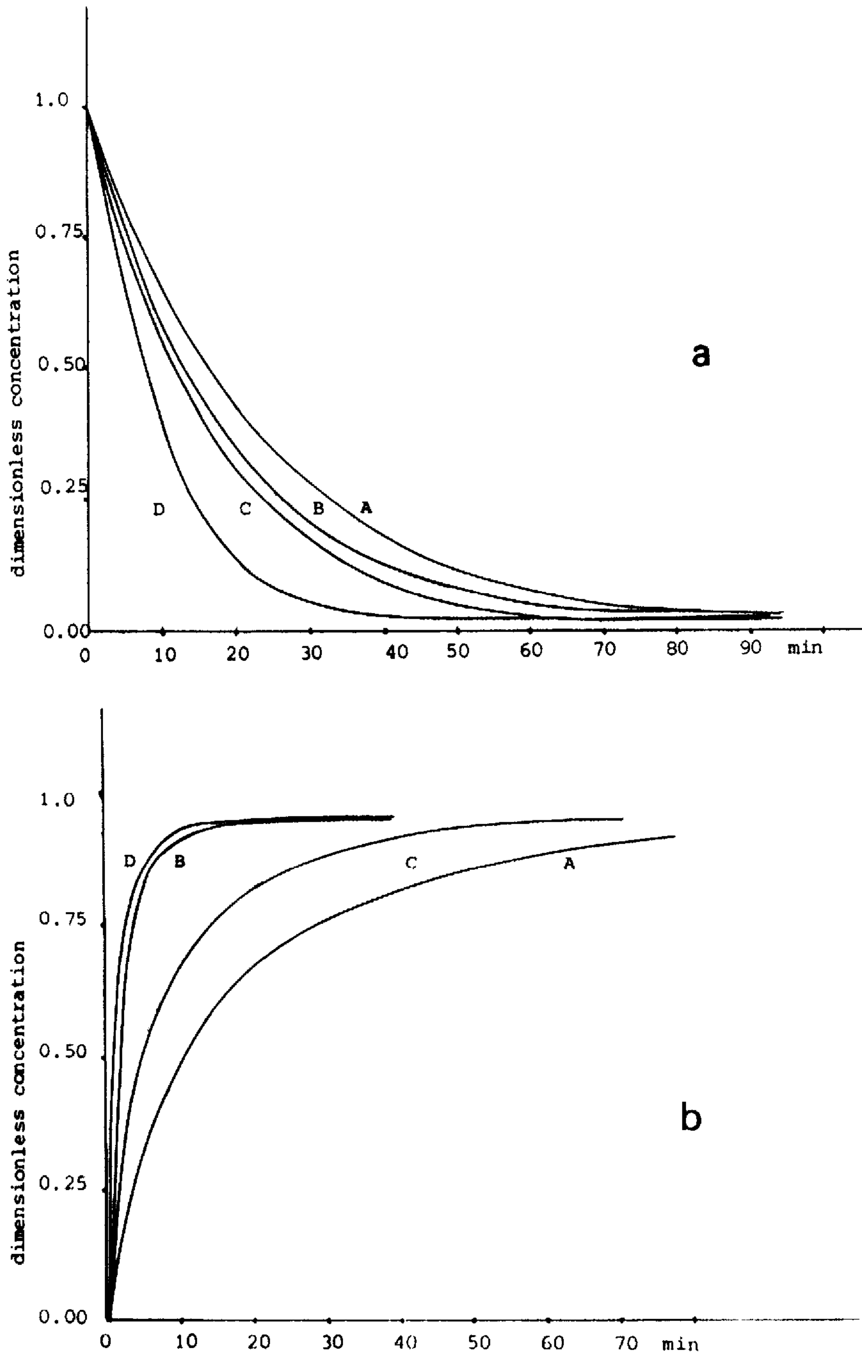

Fig. 7. (a) The adsorption of 180 ml of 0.5 mg ml^{-1} of lysozyme in 0.01 *M* sodium acetate duffer at pH 5 in a 4 mm × 43 mm I.D. column at various superficial velocities. (b) The desorption of adsorbed lysozyme with 180 ml of 0.7 *M* NaCl in a 4 mm × 43 mm I.D. column at various superficial velocities. Curves: A = HC2, 51 cm h^{-1}; B = CM-52, 51 cm h^{-1}; C = HC2, 99 cm h^{-1}; D = CM-52, 99 cm h^{-1}.

respectively. The HC2 needed more than 40 min to elute the same amount of protein. This is attributed to the larger particle sizes. The experiment described earlier (Fig. 4) indicated that it was difficult to operate the 100 × 26 mm I.D. column packed with CM-52 at a superficial velocity of more than 0.90 m h^{-1} because the microgranular material was getting increasingly compressible. This resulted in higher and higher pressures with the same flow-rates. It is apparent from the above data that this would result in significant mass transfer limitation in the external film. It was thus not possible to use the microgranular CM-52 or HC2 at their optimum kinetic rate owing to the flow restrictions.

The higher flow velocities attainable through the new matrix, CM-HVFM, allow much faster kinetics to be exhibited. Even this material has reduced flows on the larger-scale columns and whilst 40 m h^{-1} would be kinetically optimum only 7 m h^{-1} can be sustained on the 100 × 147 mm I.D. column. This is about 20 times faster than attainable on the Whatman and Indion materials.

By loading 50 mg lysozyme to the 110 × 10 mm I.D. column and then eluting with 1 *M* NaCl at various superficial velocity from 0.910 to 13.750 m h^{-1} (Fig. 8) it is found that desorption times decrease monotonically with increasing velocity. There is a point, around 5.0 m h^{-1}, in both curves beyond which the time for eluting 80% and 90% of total lysozyme is reduced only slowly.

The advantages of very high velocities and fast kinetics can be realised in a practical application. In Fig. 9, a complete adsorption desorption cycle that has not been optimised is show for 0.5 g dry weight CM-HVFM packed into the longer cylindrical column (110 × 10 mm I.D.). The feed was 340 ml of 0.588 mg ml^{-1} of lysozyme in 0.01 *M* sodium acetate buffer at pH 5.0 with a superficial velocity of 8.87 m h^{-1}, 1.7 bed volumes/min. After loading the column was washed for 2 min with 2.7 bed volumes of buffer at the same flow-rate and then eluted with 1 *M* NaCl fed at 8.41 m h^{-1}. A sharp peak and short tail for the elution are observed with complete absence

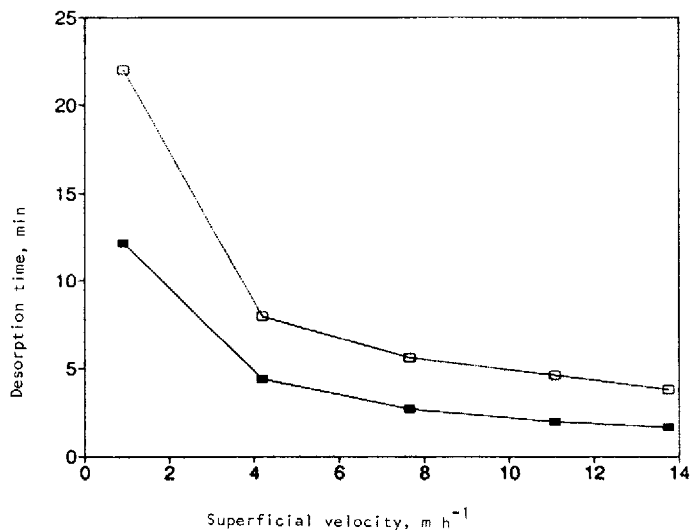

Fig. 8. Desorption time of 80% (■) and 90% (□) of total elution lysozyme at various superficial velocities.

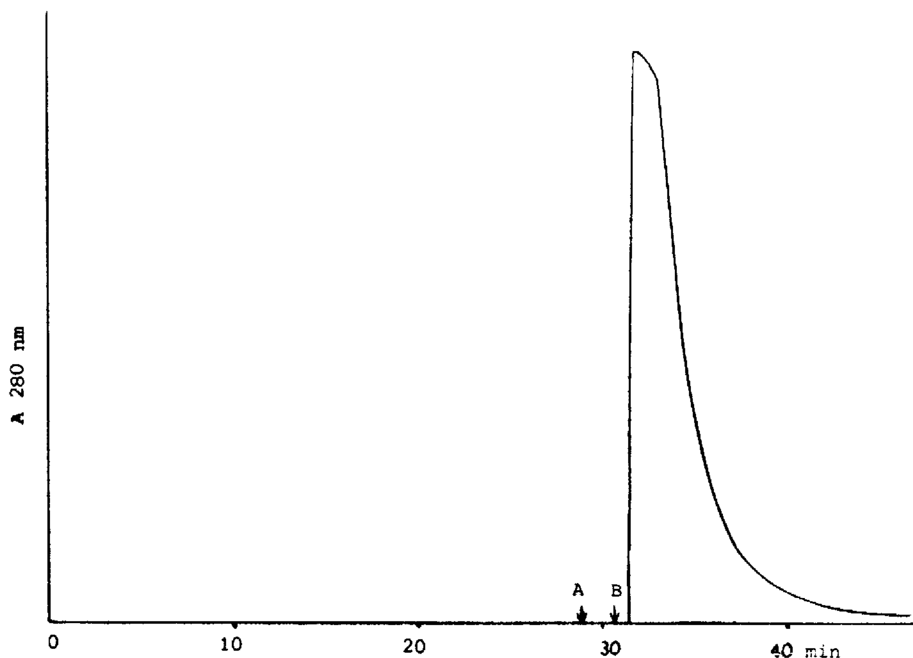

Fig. 9. Protein concentration in the column eluent. Adsorption started time 0, washing started at A, elution at B. CM-HVFM in a 110 mm × 10 mm I.D. column, loaded with 340 ml of 0.588 mg/ml lysozyme. Superficial velocity 8.87 ml h^{-1}.

of any measurable breakthrough even though the column was loaded to 50% of its total capacity with 200 mg of protein on 8.6 ml or 0.5 g of matrix. The whole process took less than 40 min. In a later experiment breakthrough was observed only when the 110 × 10 mm I.D. column had adsorbed 43 bed volumes or 365 ml of 1 mg ml^{-1} lysozyme at a superficial velocity 6.34 m h^{-1}. The column capacity at the breakthrough point was thus 365 mg of protein or 42 mg protein ml^{-1} of column. At this flow-rate that took only 45 min for the adsorption. It was thus possible to load a solution containing 1 mg ml^{-1} of protein at nearly 1 bed volume/min with total adsorption on a bed only 110 mm long.

During the above operation, the 110 × 10 mm I.D. column was regenerated by 2 *M* NaCl or 0.7 *M* NaOH for 40 min and there was no obvious swelling and shrinking observed which indicates a potential further advantage of the CM-HVFM matrix.

CONCLUSION

The new CM-HVFM matrix has several advantages over the microgranular and spherical cellulosic ion exchangers:

(1) The kinetics of adsorption of proteins by carboxymethyl cellulosic ion exchangers are controlled by external mass transfer at normal operational flow-rates. Higher flow velocities allow faster adsorption and desorption kinetics.

(2) The bed of the CM-HVFM matrix maintains an open structure at high

flow-rates, whilst the beds of microgranular and spherical matrix tend to be compressed and block. The new matrix can thus sustain a superficial velocity of over 7 m h^{-1} in a 100 mm × 147 mm I.D. column which is over five times the velocity that can be sustained by the other materials. Flow-rates are normally restricted by compression which tends to become worse in larger diameter columns leading to slower kinetics in large-scale columns than small-scale columns. The faster flows mean that longer beds are needed and thus it is important to have fast flow even in long beds. A bed of 110 mm is long enough to avoid breakthrough when over 30 column volumes have been loaded at 1 bed volume/min. The new material can thus be used to treat large volumes of solution.

(3) It is suggested that the new matrix possesses an open structure for flow which is not easily compressed but is clearly not rigid yet there are sufficiently short diffusion pathways within the material that kinetics of desorption are not slowed down. The fine structure of the new matrix is such that there are short diffusion pathways within the cellulose and a very high percentage of the surface is accessible to direct convective flow which leads to rapid kinetics that also are commensurate with the flow rate allowing very fast large scale separations. The desorption rates are intrinsically faster than adsorption and less influenced by mass transfer for the HVFM and HC2 although the large particles of HC2 are influenced by mass transfer in the external phase.

(4) CM-HVFM can withstand changes of ionic strength and pH without obvious swelling and shrinking, which can avoid the column bed being deformed and cracked.

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