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Journal of Chromatography A, 925 (2001) 69–78

JOURNAL OF CHROMATOGRAPHY A

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Continuous superporous agarose beds in radial flow columns ☆

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Received 13 March 2001; received in revised form 6 June 2001; accepted 8 June 2001

Abstract

Continuous superporous agarose beds constitute a new support material for chromatography, biocatalysis and electrophoresis. The bed consists of a single piece of agarose gel, homogeneously transected by flow-carrying pores, which easily can be varied in the range of 10–100 μm. In this work, large diameter beds (60 mm) were prepared and used in specially designed radial flow columns. The basic chromatographic properties of the beds were investigated by size-exclusion chromatography experiments. In an affinity chromatography application one bed was derivatized with Cibacron Blue 3GA and used for the purification of lactate dehydrogenase from a crude bovine heart extract. In a biotransformation application one bed was provided with immobilized β-galactosidase and used in the production of lactose-free milk.

Keywords: Continuous superporous agarose beds; Radial flow columns; Monoliths; Lactate dehydrogenase

1. Introduction

Continuous beds (rods, monoliths) have become, since their introduction as a separation material for biomolecules in the late 1980s, an interesting alternative to columns packed with beads. These beds consist of a single piece of material, a monolith, transected by channels large enough to permit a chromatographic flow through the column. Due to their configuration they may be easier and cheaper to manufacture than beaded support and they have a more favourable flow-rate/pressure drop relationship [1–4]. For example, Bidlingmaier et al. [1] compared the performance of a 5-μm particle column with a continuous bed of the same base material. The two formats gave approximately the same chromatographic efficiency for small molecules but, more importantly, the continuous rod gave three to five times lower pressure drop. In the light of such potential advantages it is not surprising that an arsenal of continuous beds have been developed from a wide range of standard chromatography base materials. For instance, continuous beds made of acrylates [3,4], polystyrene and methacrylates [5–7], silica [2,8] and agarose [9–11] have been prepared and used for the separation of biomolecules. A mathematical model has also been presented for monolithic structures to aid in their design and to predict their behaviour [12]. Some of these continuous beds have also reached the market [1,7,13].

In previous reports [9–11] we described continu-

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0021-9673/01/$ – see front matter
PII: S0021-9673(01)01027-5

ous beds made of agarose. The agarose bed was transected with large superpores (besides the diffusion pores normally found in agarose gels), which carried the chromatographic flow through the column. These continuous superporous agarose beds were used for protein isolation [10], as supports for enzymes in rapid on-line ELISA applications [11] and as an electrophoretic medium [10]. The superpores in these beds were produced by an emulsification procedure and could be varied in the range of 10–100 μm.

Although the continuous superporous agarose beds were used successfully in small columns (0.5–10-ml columns), a problem was discovered when large-diameter beds were prepared and used for chromatography. The problem was due to the difficulty of achieving a uniform cooling of the bicontinuous agarose–solvent phase mixture during the solidification step. In large diameter columns the central parts of the bicontinuous phase system solidified considerably later than the outer part, giving the phase system time to mature into coarser structures. Thus, the resulting gel showed an uneven distribution of flow pore size; the central parts had larger pores than the peripheral parts. As expected such an uneven pore size distribution led to an uneven flow profile and the reduction of column efficiency. A related phenomena described by Peters et al. [14] is the inhomogeneities in the porous structure of continuous beds made of polystyrene or methacrylates, caused by the heat of polymerization. The proposed remedies, e.g., gradual addition of the polymerization mixture to the reaction vessel and decreasing the rate of polymerization are obviously not applicable in our case. However, another promising method was tested by Podgornik et al. [7], where annular monoliths of different diameters were polymerized and merged into a single large monolithic unit, exhibiting good chromatographic performance. An early remedy to our problem was to prepare superporous agarose membranes of about 5 mm thickness and stacking these membranes in a column to the desired height [10]. By this method large-diameter beds could be prepared, showing good chromatographic properties. However, the method was rather cumbersome and only suited for rather shallow beds. On the other hand, the superpore size in cast columns would only be expected to vary in the radial direction and should only cause problems when an axial flow direction is used. Consequently, to circumvent the problem we investigated the combination of radial flow column technology and continuous superporous beds.

2. Experimental

2.1. Materials

Agarose powder (Sepharose quality) was a gift from Amersham Pharmacia Biotech (Uppsala, Sweden). Polyoxyethylenesorbitanmonooleate (Tween 80) and sodium borohydride were obtained from Merck-Schuchardt (Munich, Germany). Cyclohexane (puriss.) and sodium *meta*-periodate were obtained from Merck (Darmstadt, Germany). Cibacron Blue 3GA, *o*-nitrophenyl-β-D-galactopyranoside (ONPG), sodium cyanoborohydride and glucose (HK) assay kit were obtained from Sigma (St. Louis, MO, USA). Sodium azide was obtained from Fluka (Buchs, Switzerland). Blue Dextran (M_w=2 000 000) was obtained from Amersham Pharmacia Biotech (Uppsala, Sweden). A protein assay kit (Bradford-type) was obtained from Bio-Rad Labs. (Hercules, CA, USA). A purified β-galactosidase preparation (Lactozym 3000 L, type HP-G) which had a specific activity of 85 lactose units/mg or 65 ONPG units/mg (at 30% substrate conversion) was obtained from Novo Nordisk (Bagsvaerd, Denmark). Glycidol was obtained from Acros Organics (Geel, Belgium).

2.2. Construction of radial flow columns

A radial flow column was constructed (type 1, Fig. 1a) consisting of a plexiglass cylinder (61 mm I.D., 80 mm O.D. and 100 mm long), a plexiglass bottom and top plate and a central flow distributor made of stainless steel (8 mm dia. and 150 mm long). The flow distributor, besides distributing the flow into or out of the column, also made it possible to adjust the position of the top plate. Nylon nets positioned between the bed and the cylinder aided in the task of centering the bed in the column and improving the flow distribution. The column was constructed from transparent plexiglass to allow inspection of the bed during operation.

An alternative, simplified construction, mainly

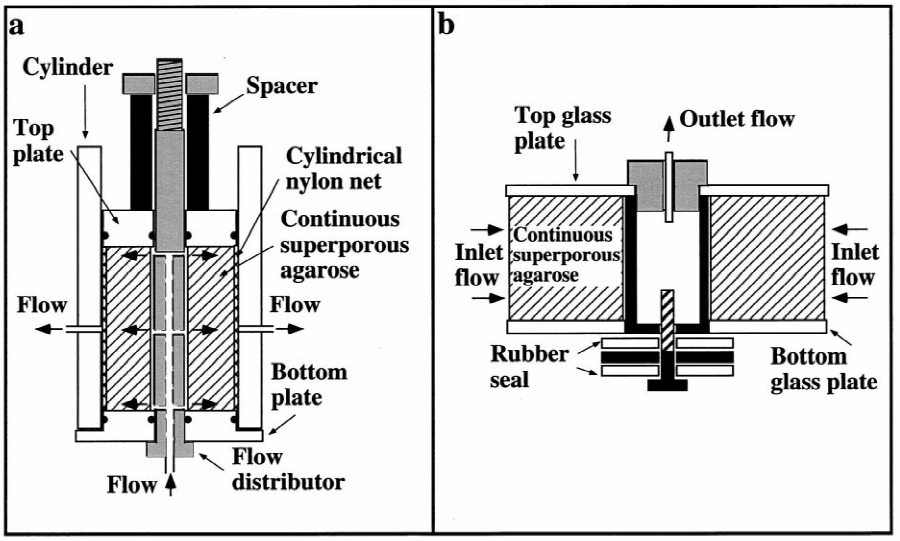

Fig. 1. Drawings of radial flow columns used with continuous superporous beds. (a) Type 1 column constructed to work in both flow directions (centrifugal and centripetal flow). (b) Type 2 column with simplified design used with a centripetal (inward) flow. The figures are not drawn to scale and dimensions are given in the text.

used for washing of freshly prepared gel beds (type 2, Fig. 1b) was prepared from two glass plates and a standard chromatography solvent filter. The chromatography solvent filter (sintered stainless steel, 10-μm pore size; Upchurch Scientific, Oak Harbor, WA, USA) acted as an outlet flow distributor. Two octagonal glass plates (80 mm) each with a central hole functioned as end pieces. The simplified construction was used submerged and the liquid was drawn through the column by attaching a pump (or a vacuum) to the centre outlet.

Photographs of these two types of radial flow columns are shown in Fig. 2.

2.3. Casting of continuous agarose beds for radial flow columns

A suspension of agarose in water (150 ml, 6%, w/v) was heated to 95–100°C in a microwave oven (with occasional shaking to keep the agarose powder well suspended), and kept at that temperature for 1 min. The agarose solution was cooled to 60°C in a stirred glass reactor and a mixture of 100 ml cyclohexane and 7.5 ml Tween 80 (60°C) was added. The mixture was emulsified by stirring at 1250 rpm for 5 min. The emulsion was poured into a glass column (60 mm I.D. and 80 mm long) fitted with a rubber plug at the bottom end and pre-warmed to 60°C in a water bath. The emulsion was briefly stirred at 400 rpm to remove any disturbances in the emulsion caused by the pouring procedure. The emulsion was cooled by transferring the column to a water bath (5°C). The solidified continuous agarose bed thus obtained was carefully trimmed to a length of 25 mm. A central hole (13 mm dia.) was punched out from the bed with the aid of a sharp and thin-walled cylindrical stainless steel puncher. The organic phase in the superpores was removed by placing the bed in a radial flow column (type 2, Fig. 1b) and subsequently pumping water, ethanol–water (50:50, v/v) and finally degassed water through the continuous agarose bed.

2.4. Investigation of basic chromatographic properties

The homogeneity and efficiency of the continuous beds were investigated in breakthrough and pulse injections experiments using sodium azide (penetrates the whole gel) and blue dextran (confined to the superpores) as tracer-molecules. The mobile phase was 0.1 *M* sodium phosphate buffer, pH 7.0,

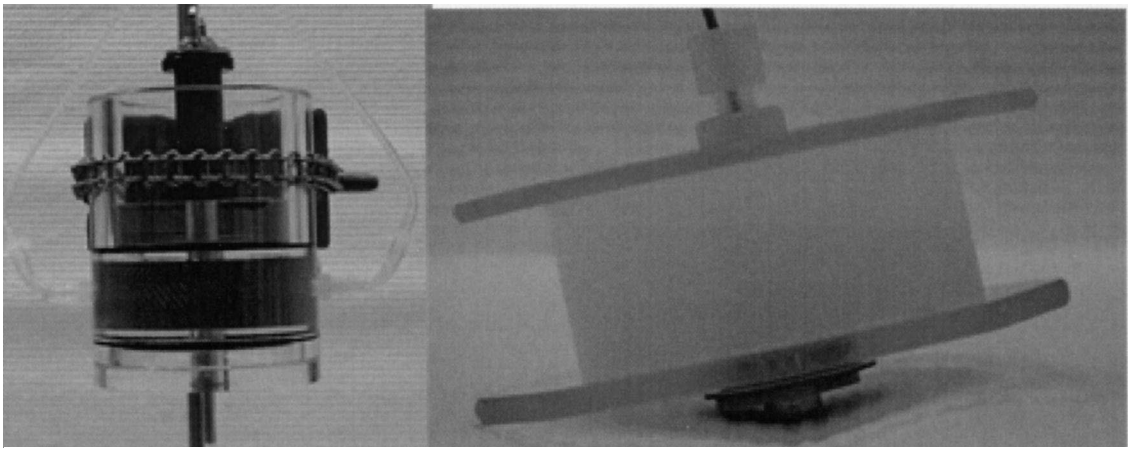

Fig. 2. Photographs of the two types of radial flow columns constructed. (Left) Type 1 column containing the continuous bed derivatized with Cibacron Blue; (Right) Type 2 column containing an underivatized bed.

which was pumped into the radial column (type 1, Fig. 1a) and through a UV-detector by a peristaltic pump. The HETP data were calculated using the formula:

$$\text{HETP} = L/5.54\,(t_R/w_h)^2 \quad (1)$$

where t_R is the retention time and w_h is the width of the band at half-height (fairly symmetric peaks). L in this case represents the length of the flow path in the continuous bed (23 mm). Extra-column band broadening was usually around 15% of the total band broadening and was corrected for. Since most of the dead volume of the system was due to the volume of the tubing, appropriate data for corrections could be obtained by injecting samples when the column had been disconnected from the system.

Theoretical HETP curves for homogeneous agarose beads were generated using the van Deemter equation:

$$\text{HETP} = 4R_p + 2D_m/u + (2R_p^2(1-\varepsilon)\varepsilon_p)u/(15D_p(\varepsilon + (1-\varepsilon)\varepsilon_p)^2) \quad (2)$$

where R_p is the particle radius, D_m is the free diffusion coefficient, D_p is the pore diffusivity, ε is the column voidage, ε_p is the total particle porosity and u is the linear flow velocity.

2.5. Cibacron Blue coupling to continuous agarose

Cibacron Blue 3GA was coupled to a continuous agarose bed essentially as described by Angal and Dean for beaded agarose [15]. However, the method was slightly modified to suit the continuous bed format. Accordingly, the radial flow column (type 2, Fig. 1b) was submerged in a beaker containing 240 ml water, 1.0 g of dissolved Cibacron Blue and 6.0 g of dissolved NaCl. This solution was recirculated through the column at a flow-rate of 10 ml/min for 30 min by a peristaltic pump, positioned after the column. Then 60 ml water containing 3.0 g of dissolved sodium carbonate were added and the recirculation was continued for 40 h at a temperature of 45°C. The coupling was ended by pumping warm water (45°C) through the column. The immobilized dye content was determined by acid hydrolysis of thin slices of the bed and subsequent measuring the absorbance of the solution at 620 nm [16]. A molar absorption coefficient for Cibacron Blue of 13 600 l mol^{-1} cm^{-1} was used [17].

2.6. Purification of bovine lactate dehydrogenase

A crude extract of lactate dehydrogenase was prepared from bovine heart, using disintegration, centrifugation and ammonium sulphate fractionation (30–60% saturation), essentially as described earlier [18]. The precipitated enzyme was dissolved in 0.02

M sodium phosphate buffer, pH 7.0. The extract had a protein content of 6.0 mg/ml, determined according to the method of Bradford [19] and an activity of 80 U/ml (see Section 2.9).

One hundred ml of this extract were mixed with 100 ml of 0.02 *M* sodium phosphate buffer, pH 7.0, containing 1 m*M* EDTA and 2 m*M* β-mercaptoethanol and the solution was pumped through the continuous bed column (type 1, Fig. 1a) by a peristaltic pump (10 ml/min). The effluent from the column was monitored at 280 nm. After the adsorption step, the column was washed with 180 ml of buffer to remove unbound proteins. Elution was carried out by pumping 160 ml of buffer containing 1 m*M* NADH through the column (5 ml/min). The eluate was collected at 1-min intervals by a fraction collector and the fractions were analysed for lactate dehydrogenase activity and protein content (Bradford method using a commercial kit).

2.7. Immobilization of β-galactosidase

β-Galactosidase was immobilized on a continuous agarose bed by the reductive amination method [20], slightly modified to suit the continuous bed format. This method includes pre-treatment of the agarose matrix with glycidol (to produce a periodate-oxidizable support) and subsequent treatments with periodate to produce aldehyde groups. The aldehyde groups were then used to couple β-galactosidase by a reductive amination step.

A continuous bed was positioned in the radial column (type 1, Fig. 1a) and 300 ml of degassed water was pumped through the column, followed by 300 ml of 1.0 *M* NaOH. A solution consisting of 6 ml glycidol, 60 ml 1.0 *M* NaOH and 60 mg $NaBH_4$ was then recirculated through the column overnight (16 ml/mm) at room temperature to create vicinal hydroxyl groups on the agarose. The reaction was stopped by pumping 500 ml water, 500 ml 1.0 *M* NaCl and finally 500 ml water through the column.

Periodate activation of the bed was performed by adding 2.6 g sodium *meta*-periodate to 60 ml water and then recirculate this solution through the column for 90 min at room temperature. The reaction was terminated by pumping 500 ml water and 500 ml 0.1 *M* phosphate buffer, pH 7.0, containing 1 m*M* $MgCl_2$ through the column.

Coupling of β-galactosidase was performed by adding 10 ml of Lactozym preparation (330 mg protein, 85 lactose units/mg or 65 ONPG units/mg) to 50 ml of coupling buffer (0.1 *M* phosphate buffer, pH 7.0, containing 1 m*M* $MgCl_2$ and 360 mg sodium cyanoborohydride) and adjusting the pH to 7.0. The coupling solution was then recirculated through the column for 24 h at 4°C. The coupling was terminated by pumping 300 ml 0.1 *M* phosphate buffer, pH 7.0, containing 1 m*M* $MgCl_2$, 300 ml 1.0 *M* NaCl, 300 ml water and finally 300 ml 0.1 *M* phosphate buffer, pH 7.0, containing 1 m*M* $MgCl_2$ through the column.

2.8. Production of lactose-free milk

A radial column (type 1, Fig. 1a) containing β-galactosidase gel was connected to a peristaltic pump. One liter of room temperature milk (fat content $<0.1\%$, pH 6.8) was supplied with 1 m*M* $MgCl_2$ and recirculated through the column at a flow-rate of 86 ml/min. The breakdown of lactose to glucose and galactose was followed by taking 100-μl samples every 10 min and analysing the glucose content by a commercial glucose (HK) assay kit [21].

2.9. Protein and activity analysis

The lactate dehydrogenase activity was assayed by following spectrophotometrically (340 nm) the oxidation of NADH by pyruvate at room temperature. Ten μl of suitably diluted samples were added to 1.00 ml of an assay solution (0.22 m*M* NADH, 1.0 m*M* pyruvate, 1 m*M* EDTA, 1 m*M* mercaptoethanol, 0.02 *M* sodium phosphate buffer, pH 7.0). The protein content was analysed according to the method of Bradford [19].

3. Results and discussion

3.1. General properties of continuous agarose beds in radial flow columns

Radial flow columns, compared to conventional axial flow columns, use a radial flow direction to maximize the cross-sectional area available to flow

and to minimize the bed height, thereby reducing the pressure drop over the column [22]. The technique has found use when scaling-up chromatographic separations to production level in all types of adsorption chromatographic modes. Due to the shallow bed height, the technique is unsuited for non-interactive modes such as size-exclusion chromatography. A characteristic feature of radial flow columns is the varying cross-sectional area of the bed in the direction of flow. As a consequence, the linear flow velocity in the column varies, giving a decelerating velocity in the case of centrifugal flow (outward flow) or accelerating velocity in the case of centripetal flow (inward flow). The consequences of this concerning band broadening was studied by, e.g., Tharakan and Belizaire [23]. Since the radial dispersion and mass transfer coefficients in a radial flow column cannot be assumed constant, they concluded their study by pointing out that trial experiments are necessary when implementing a radial flow column at production scale. A theoretical model for radial flow chromatography has been developed by Gu et al. [24].

Our continuous beds had an inner cross-sectional area of 10 cm^2 and an outer cross-sectional area of 47 cm^2. At a volumetric flow-rate of 100 ml/min this corresponds to a linear flow velocity range of 2–10 cm/min.

Apart from the main objective of solving the uneven flow profile problem in large diameter beds (as explained in Section 1), the combination of continuous agarose beds and radial flow column technology showed some additional benefits.

Firstly, any wall effects, due to flow channeling between the bed and the column wall was easily controlled by the present set-up. The two constructions allowed the end pieces to execute a homogeneous pressure along the whole flow path (Fig. 1). This cannot be attained by axial flow columns except in the case of radial compression technology [25].

Secondly, since the superporous continuous beds are self-supported structures, their combination with radial flow technology simplify the packing procedure. The superporous continuous beds were inserted and removed in the radial columns over and over again, both easily and reproducibly. Moreover, an easy exchange could be made from one radial column type to the other with maintained chromatographic efficiency (similar HETP values). In addition, the self-supported structure of the continuous bed allowed the use of a simplified low-cost column (Fig. 1b), which cannot be used in the case of particle support formats.

Thirdly, the flow properties of the radial beds were excellent. Flow rates of up to 100 ml/min were frequently used with a pressure drop over the column of less than or equal to 1 bar. The upper flow-rate range was dictated by the peristaltic pump rather than on the mechanical stability of the bed. Flow rates in excess of 100 ml/min were not tried, since this upper limit was considered adequate for the present applications.

3.2. Characterization by size-exclusion chromatography experiments

Size-exclusion chromatography experiments were carried out to characterize the chromatographic efficiency of the radial beds and specifically to detect the presence of uneven flow through the radial beds. Uneven flow was earlier shown to be a problem in large diameter continuous beds operated in the standard, axial direction.

A number of radial beds were prepared and tested for chromatographic efficiency. The breakthrough curves were used to detect inhomogeneities in the bed, to determine the total bed volume (small-molecular-mass marker, sodium azide, $M_w = 65$) and the superpore volume (large-molecular-mass marker, blue dextran, $M_w = 2\,000\,000$) from the 50% breakthrough level. The values obtained were consistent with each other and corresponded to a bed volume of 65 ml and a superpore volume of 28 ml. The progress of the Blue dextran sample through the column could be followed visually through the transparent plexiglass. In the case of a poorly performing bed, this visual inspection made it easy to distinguish between the two most likely problems, wall effects or a set of fast flowing superpores within the bed.

The pulse injections of sodium azide were used to generate HETP data. Table 1 lists generated size-exclusion data of four continuous beds, which shows their consistent performance. Bed number 4 was further analysed by pulse injections of sodium azide at various flow-rates between 1 and 45 ml/min. Fig.

Table 1
HETP data for superporous continuous beds, obtained in size-exclusion chromatographic experiments

Bed	Flow rate (ml/min)	HETP (mm)
1	2.2	0.51
2	2.0	0.52
3	1.8	0.50
4	2.8	0.47

Column: 65 ml radial flow column type 1. Sample: 2.0 ml sodium azide, 2.0 mg/ml.

3 shows the result of this experiment, together with theoretical HETP curves for 220- and 160-μm agarose beads as a comparison. The theoretical HETP curves were generated using the spreadsheet program MS Excel and Eq. (2) [26]. This comparison was considered appropriate since the continuous bed showed a HETP-minimum of 440 μm at a flow-rate of 3.6 ml/min. This flow-rate corresponds to a reduced velocity of 4.5 using the middle cross-sectional area of the continuous bed (23.6 cm^2) and a diffusion coefficient for sodium azide of 10^{-5} cm^2/s. The HETP-minimum of 440 μm indicates a reasonable chromatographic performance, considering that the diffusion distances between the superpores in the continuous beds were around 150 μm (as measured by microscopy on thin slices of the continuous agarose material). Thus, the observed HETP-minimum is reasonably close to twice the thickness of the agarose structures.

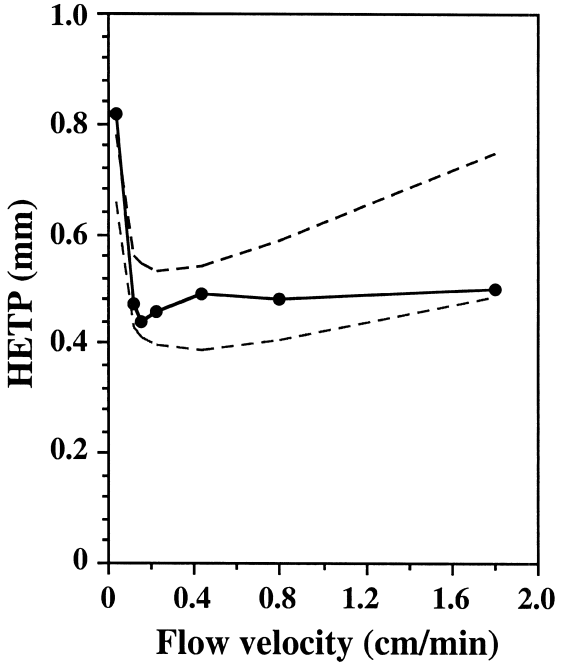

Fig. 3. HETP as a function of the linear flow velocity. Column: 65 ml radial flow column type 1. Sample: 2.0 ml sodium azide, 2.0 mg/ml. The dashed curves in the figure represent theoretical HETP curves for 220 μm (upper curve) and 160 μm (lower curve) homogeneous agarose beads calculated from the van Deemter equation (Eq. (2)) using a particle porosity of ε_p of 0.88, a column voidage ε of 0.4 and a diffusion coefficient for sodium azide of 10^{-5} cm^2/s (both D_m and D_p).

3.3. Purification of lactate dehydrogenase

Cibacron Blue is the most used of the many synthetic dyes available for affinity chromatography purifications and has affinity for many dehydrogenases and kinases that require adenylic cofactors (NAD^+, $NADP^+$ and ATP) for their activity. Due to their low cost and stability under operating conditions, these dyes have replaced nucleotides as general-purpose ligands for these enzymes.

Fig. 4 shows the affinity chromatography purification of lactate dehydrogenase by a radial flow column containing a continuous agarose bed derivatized with Cibacron Blue. The flow-rate was 10 ml/min for the adsorption and wash step, which corresponds to approximately 25.4 cm/h using the middle cross-sectional area (23.6 cm^2) of the column. The flow-rate was decreased to 5 ml/min (12.7 cm/h) for the elution step to minimize dilution of the target enzyme. The continuous bed used had a dye content of 1.2 μmol/ml bed, which corresponds to approximately 78 μmol Cibacron Blue in the whole bed. The applied extract had a total activity of 8000 U and a total protein content of 600 mg. The purification was completed in 68 min with a purification fold of 23 (from 13.3 to 308 U/mg) and an activity yield of 73% (5830 U). The slight LDH activity breakthrough seen during sample application corresponded to 8% of the inlet activity level of 40 U/ml. The tailing protein curve was probably the result of a not fully optimized adsorption and wash step. In this case the purification should probably have benefited from a longer wash step containing an optimized salt concentration. Non-specific adsorption

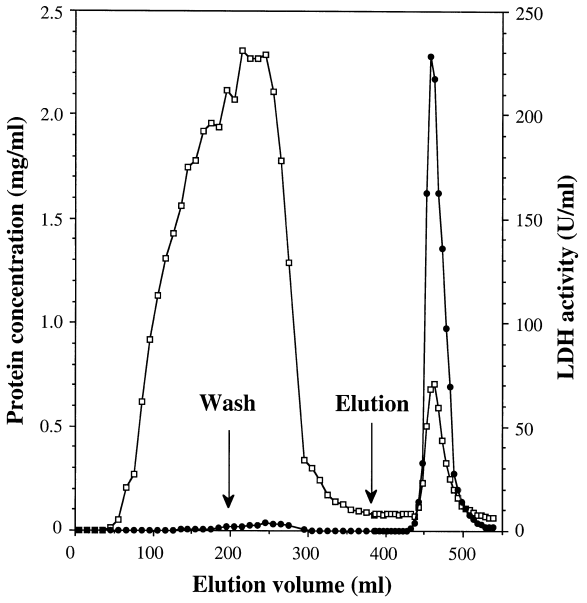

Fig. 4. Affinity chromatography purification of lactate dehydrogenase on a superporous continuous agarose bed derivatized with Cibacron Blue 3GA. Column: 65 ml radial flow column type 1. Sample: 200 ml of a crude bovine lactate dehydrogenase extract. Adsorption — wash buffer: 0.02 *M* sodium phosphate buffer, pH 7.0, containing 1 m*M* EDTA and 2 m*M* β-mercaptoethanol. Elution buffer: adsorption buffer containing 1.0 m*M* NADH. The adsorption — wash step was carried out at a flow-rate of 10 ml/min and the elution step was carried out at a flow-rate of 5 ml/min. Further details in Section 2.

is often a problem in dye affinity chromatography. The recently introduced polymer-shielding concept might be a way to reduce these non-specific interactions [27], where a preadsorbed polymer masks the non-specific adsorption sites on the adsorbent.

3.4. Production of lactose-free milk

Enzymatic conversion of lactose to glucose and galactose by β-galactosidase (lactase) is of interest to promote an extended use of lactose-containing products such as milk and cheese whey. Lactose in food products is a problem for lactose-intolerant persons and lactose in whey is an obstacle for the efficient use of whey in the food industry and as a fermentation substrate (e.g., solubility problem) [28,29]. β-Galactosidase from various sources (bacteria, yeasts and fungi) has been immobilized to various supports by entrapment, covalent linkages and by whole cell immobilization [28–31]. An obstacle for immobilized β-galactosidase utilization has been the high cost for the purification of this intracellular enzyme. However, economic evaluations seem to indicate that immobilization of the enzyme is economically feasible compared to the use of the free enzyme [32].

In this investigation a commercial β-galactosidase preparation from the yeast *Kluyveromyces* was used. During coupling, both the protein content and the enzymatic activity of the coupling solution were measured. The values obtained were consistent with each other and showed that about one-third of the added enzyme had been coupled to the gel, which would indicate that the β-galactosidase content on the gel should be about 1.8 mg/ml gel bed. This value can be compared with normal values reported in the literature with this coupling method on agarose of around 1 mg β-galactosidase/ml gel [33], which indicates that a higher coupling yield would be obtained with less added amount of enzyme.

Fig. 5 shows the production of 1 l lactose-free milk by the radial bed containing immobilized β-galactosidase. This was done by recirculating the milk through the radial flow column at a flow-rate of 86 ml/min. The figure shows that all of the lactose was converted to glucose and galactose in 90 min which corresponds to an immobilized activity of 1400 U, giving an expressed immobilized activity yield of 24% of the coupled activity (at 100% lactose conversion). The low activity yield of 24% is probably caused by inactivation of the enzyme during the immobilization procedure. This conclusion is supported by previous work showing similar figures in the range of 34–50% activity yields [28,33]. However, a better approach to increase the throughput for this production (0.5 g converted lactose per ml gel and hour or 10 ml lactose-free milk per ml gel and hour) would be the use of a larger radial bed. For example, a simple calculation show that to double the volume of the continuous bed, an increase in diameter of only 2 cm is necessary. An alternative approach to double the

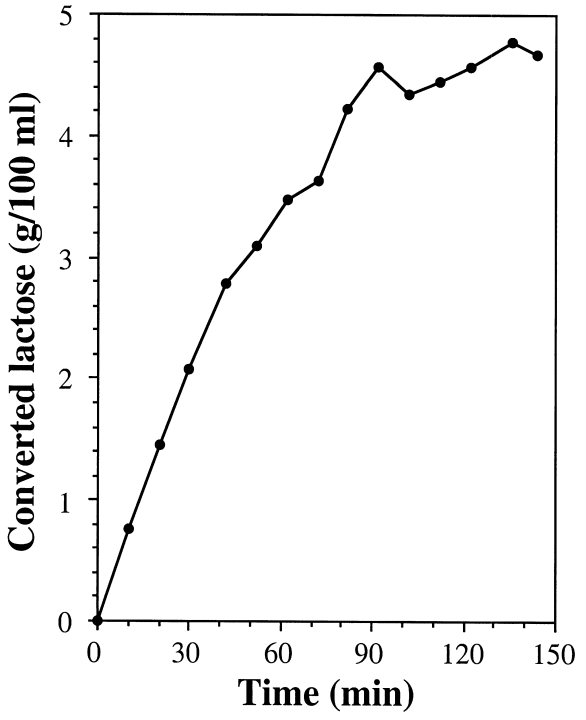

Fig. 5. Production of lactose-free milk using a superporous continuous agarose bed derivatized with β-galactosidase. Column: 65 ml radial flow column type 1. Sample: 1 l milk (lactose content 4.8 g/100 ml, fat content <0.1%). Flow rate: 86 ml/min (circulating). The breakdown of lactose to glucose and galactose was followed by taking samples every 10 min and analysing the glucose content.

throughput would be to use two radial columns coupled in series or parallel.

4. Conclusion

We have prepared and evaluated superporous continuous agarose beds in radial flow columns for chromatographic and biocatalytic applications. By employing a radial flow, problems previously experienced with large diameter continuous beds in conventional axial columns were removed.

The characteristic features of radial flow column technology were retained when combined with the continuous beds, making it possible to use high flow-rates through the columns with low pressure drops. The time-consuming procedure of packing the columns was avoided by the use of the continuous beds.

Acknowledgements

Economic support from The Swedish Center for Bioseparation is gratefully acknowledged.

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