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Comparison of anion-exchange and hydroxyapatite displacement chromatography for the isolation of whey proteins

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Abstract

In displacement chromatography, several substances may be isolated and concomitantly concentrated, which makes this separation procedure attractive for the processing of diluted product streams containing a number of high value substances. Here, the suitability of anion-exchange and hydroxyapatite displacement chromatography for the processing of technical dairy whey is investigated. The pH and flow-rate of the carrier, the displacer chemistry and, in case of the apatite, the particle diameter of the stationary phase are considered. As a consequence of the pH sensitivity of the β-lactoglobulin, one major whey component, apatite displacement chromatography is less then successful in whey separation. At a denaturing carrier pH (>8.5) the β-lactoglobulin zone is broad and stretches over the entire displacement train. At a lower carrier pH, previously successful polyanionic displacers do not bring about separation, while low-molecular-mass ones do, but they tend to overrun and thus contaminate the protein zones. In the case of anion-exchange displacement chromatography, polyanions, especially polyacrylic acid (PAA, M_r 6000), constitute suitable displacers. Here too, a carrier pH of 8.0 is most suited to the separation of the whey proteins. The low-molecular mass-displacer iminodiacetic acid (IDA, M_r 133.4), on the other hand, displaces only α-lactalbumin. The β-lactoglobulin remains on the column. PAA is used as the displacer to process a dairy whey sample.

Keywords: Whey; Hydroxyapatite; Displacement chromatography; Proteins; Lactalbumins; Lactoglobulins

1. Introduction

In addition to being an important foodstuff, bovine milk constitutes a source of a great number of putative and established high value substances [1]. Up to 6% of the total mass of bovine milk is made up of proteins. The majority of these proteins, the so-called caseins (>80% of the milk proteins), are removed by acid precipitation and used for cheese making. The so-called proteose peptone accounts for another 4% of the milk proteins. The remainder, called whey, contains the whey proteins (α-lactalbumin, bovine serum albumin, the genetic variants of β-lactoglobulin (A and B), and the immunoglobulins) together with a host of low-molecular-mass substances (LMWS).

Precipitation is an established, well understood, and easily scaleable operation in preparative protein chemistry. Similar reasoning can be applied to the recovery of the low-molecular-mass milk components such as lactose, which is routinely achieved by a sequence of filtration and spin drying steps. Recovery of the individual whey proteins, on the other hand, would require a more selective separation procedure. The separation may be achieved by conventional chromatography (e.g. Refs. [2–4]). The

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amount of whey that is produced daily in a modern dairy, however, makes some prerequisites concerning the minimum throughput of any separation process to be integrated in the overall scheme. Thus, the major part of the whey proteins are currently discharged at considerable costs, instead of being recovered for further use.

We have recently suggested the use of membrane adsorbers as the stationary phase for the chromatographic separation/isolation of the whey proteins in order to improve the throughput [5]. In this paper we would like to discuss the potential of the displacement rather than the elution mode of chromatography for the isolation of these substances. To afford separation, displacement chromatography depends on the competition of the feed components for a limited amount of binding sites on the stationary phase surface [6–8]. During loading of the feed mixture, the column's dynamic capacity is saturated to an experimentally determined optimum percentage, often between 50 and 80%. Some separation occurs during loading due to a "frontal" chromatographic effect. In the second phase, competition for the stationary phase's binding sites is enforced by the introduction (step-function) of the highly concentrated displacer solution, a displacer being a substance with even higher affinity for the stationary phase than any of the feed components. Due to its superior binding, the displacer will compete successfully with any of the feed components for the binding sites. Consequently, the competition between the various feed components is enforced as the displacer front traverses the column bed. In a system governed by Langmuir-type isotherms, the more strongly bound substances will push the less strongly bound ones ahead, until all sample components are focused into consecutive zones of pure substances. The so-called "displacement train" has been formed. Theoretically, the concentration in the individual protein zones depends solely on the displacer's concentration and the relative position of the respective substance's multicomponent isotherm to the displacer's isotherm, not on the concentration of feed.

Displacement chromatography has had its place in the separation of certain whey proteins, ever since Torres et al. [9] and Liao et al. [10] succeeded in demonstrating the resolving power of this particular chromatographic mode by separating the genetic variants of β-lactoglobulin by anion-exchange displacement chromatography. The two protein variants differ by less than 0.1 units in their isoelectric point (p*I*). The utilization of displacement chromatography for the separation of technical dairy whey has, to our knowledge, not yet been described, however. Here the potential of displacement chromatography for such a fractionation of dairy whey is discussed. Both anion-exchange and hydroxyapatite stationary phases are considered in the experiments.

2. Experimental

2.1. Materials

Proteins and fine chemicals were from Sigma (Deisenhofen, Germany) and bulk chemicals for carrier and regenerant preparation were from Fluka (Neu-Ulm, Germany). Technical whey, lacking only the casein fraction, was provided by Biolac (Harbansen, Germany). Polymeric displacers were from Polysciences (Warrington, PA, USA). The apatite stationary phases were from Asahi Optical (Tokyo, Japan; 2 μm particles) and from Bio-Rad (Munich, Germany), Asahi's European distributor (10 and 20 μm particles).

Strong anion-exchange columns (particles bearing quaternary ammonium groups at the surface) were also supplied by Bio-Rad (Bio-Scale Q2) and by TosoHaas Europe (Super Q, Stuttgart, Germany). Technical data on the stationary phases, as provided by the manufacturers, are summarized in Table 1.

2.2. Displacement chromatography

The preparative system was assembled from a Gynkotech 300 CS pump (Gynkotech, Germering, Germany), a Shodex pulse damper (Showa Denko, Tokyo, Japan) and a Valco 10-port valve (Valco, Houston, TX, USA). A 1-ml loop was used for sample injection, the displacer was introduced from preparative sample loops (Knauer, Berlin, Germany) that could hold 5 and 11 ml, respectively. For the apatite stationary phases, the columns were packed at

Table 1
Technical data of the stationary phases/columns used in the investigation

Material	Particle diameter[b] (μm)	Pore size[b] (nm)	Column dimensions (mm)
Hydroxyapatite	2	100	250×4.0
Hydroxyapatite	10	100	250×4.0
Hydroxyapatite	20	100	250×4.0
Strong anion-exchanger (Bio-Scale Q2)	10	100	52×7.0
Strong anion-exchanger (Super Q)[a]	35	100	85×7.5

All materials/columns: Bio-Rad, except [a]: TosoHaas, the anion-exchange columns were obtained pre-packed and ready for use, the hydroxyapatite particles were packed in the laboratory.
[b] Average values given by the respective manufacturers.

170 bar using a self-assembled packing apparatus. Self-packed columns were 25 cm×4 mm I.D. The anion-exchange columns were used as received from the respective manufacturers. Column dimensions were 85×7.5 mm for the Super Q column and 52×7 mm in the case of the Bio-Scale Q2.

A flow-rate of 0.1 ml/min was used in all displacement experiments unless indicated otherwise. The effluent from the displacement column was analyzed twice per minute by the analytical RPC. For apatite chromatography, a 0.4 *M* phosphate buffer (pH 6.8) was used to regenerate the column after the displacement run. Prior to each displacement, apatite columns were conditioned by a wash with 2 ml of a 0.2 *M* $CaCl_2$ solution, prepared in the carrier to be used during the respective displacement experiment. For anion-exchange columns, 1 *M* NaCl was added to the carrier, to afford regeneration. No column conditioning was required in this case.

2.3. Analytical high-performance liquid chromatography (HPLC) by RPC

The high pressure gradient system for the analytical HPLC was assembled from two IRICA S 871 pumps (ERC, Alteglofsheim, Germany) controlled by an Autochrom gradient controller (ERC, Alteglofsheim, Germany) and a dynamic mixing chamber (0.25 l, Knauer, Berlin, Germany). An SF 757 UV detector (ABI, Applied Biosystems, Foster City, CA, USA), equipped with a 0.5 ml/1 mm micro flow cell (0.1 s filter time) and with the detection wavelength set to 214 nm, was used. For sample injection (5 μl) a 6-port motor valve (E6UWT, Valco) was used. A 30×4.6 mm column, packed with non-porous 2 μm Micropell C_{18} beads (Glycotech, Hamden, CT, USA), was used. Eluent A consisted of deionized water with 0.1% (v/v) trifluoroacetic acid (TFA). Eluent B consisted of acetonitrile with 0.08% (v/v) TFA. The gradient was run from 20 to 60% B. By using a flow-rate of 1 ml/min and an operating temperature of 60°C, each analysis was completed within 120 s. Data collection and interpretation was carried out on a personal computer using APEX Andromeda chromatography software (Techlab, Braunschweig, Germany).

3. Results and discussion

A prerequisite for the use of displacement chromatography in the processing of dairy whey is the separation of α-lactalbumin and β-lactoglobulin, the two major proteins found in technical whey (for data on the major whey proteins, see Table 2). Concentration of the protein fraction is also desired to facilitate putative follow-up purification steps.

3.1. Hydroxyapatite displacement chromatography of whey proteins

Hydroxyapatite is a ceramic, autoclavable material, which has been used in biopolymer separation since the 1950s [11]. Only recently has a material suited to HPLC application, i.e. beads able to withstand at least 200 bar, become available [12].

Table 2
Data on the major whey proteins

Protein	Isoelectric point	Relative molecular mass	Percentage of the whey protein fraction[a]
β-Lactoglobulins A and B	5.2–5.4	18 400	56.3
α-Lactalbumin	5.4	14 200	25.0
Bovine serum albumin (BSA)	4.8	66 000	6.3
Immunoglobulins	5.8–7.8	IgG: ca. 150 000 IgA: ca. 400 000 IgM: ca. 900 000	12.5

[a] According to [22].

Protein interaction with apatite surfaces is a rather complex process, aspects of which are still under discussion [13–15]. Normally, a phosphate buffer of near neutral pH is used as the mobile phase in apatite (elution) chromatography. Under these conditions, the apatite surface can be assumed to carry a negative net charge. The positively charged residues of basic proteins presumably interact with this surface. In contrast, acidic proteins have a net negative charge at neutral pH and are therefore repelled by the apatite surface under standard conditions. The fact that they bind at all is ascribed to a chelating interaction between the carboxylate groups of their aspartate and glutamate residues and the calcium sites on the apatite surface. Due to this complex behavior, protein separations on apatite columns are difficult to predict. The selection of a suitable protein displacer, which tends to be a problem in displacement chromatography at any time, becomes a special challenge in apatite displacement chromatography.

The rational design and synthesis of the displacer substance has only been addressed fairly recently [16–20]. A typical protein displacer is often still a medium-to-high-molecular-mass substance, which has been found by trial-and-error to suit a specific separation problem. Difficulties associated with the displacer, such as heterogeneity, solubility, detectability, column regeneration, etc., tend to impede the use of displacement chromatography. In the past, we have used apatite phases several times, with success, in the isolation of biotechnical high-value products such as recombinant h-AT III [16]. A number of putative displacers of acidic proteins, both of the high- and the low-molecular-mass type, have been identified in the course of this work [16,21]. The active principle of these substances, e.g. EGTA [ethylene glycol bis(β-aminoethylether)-N,N,N′,N′-tetraacetic acid] and PGA (polyglutamic acid), is presumably the competitive chelating of the calcium sites on the apatite surface. A carrier pH of at least 9.0 was required for efficient displacement separation in these cases [16].

Separation of α-lactalbumin and β-lactoglobulin under similar conditions using the low-molecular-mass substance EGTA (M_r 380.4) as the displacer, is not possible, however (Fig. 1). β-Lactoglobulin is quite sensitive to the pH [22,23]. Between pH values of 3.5 and 7.5, the monomer exists only in equilibrium with its oligomers, up to the octamer. Above a pH value of 8.5, β-lactoglobulin denatures irreversibly. In accordance with the theory of protein retention on apatite, any denaturation should reduce the binding strength [14,15]. Consequently, we observed a broad β-lactoglobulin zone in the displacements that stretched over the entire displacement train and deeply into the displacer zone as well, when the displacement was carried out at the denaturing pH value of 9.0. Moreover, only 41% of the α-lactalbumin was displaced in these experiments, compared to over 99% of the β-lactoglobulin. The rest of the α-lactalbumin was recovered in pure form during the column regeneration procedure. The separation quality should improve when the carrier pH is lowered, e.g. to a value of 8.0. At the lower pH, however, certain of the apatite protein displacers, including the PGA (polyglutamic acid, M_r 13 400) used successfully before, no longer worked (Fig. 2). The displacer front is diffuse and except for fractions 8 and 9 (which contain low concentrations of α-

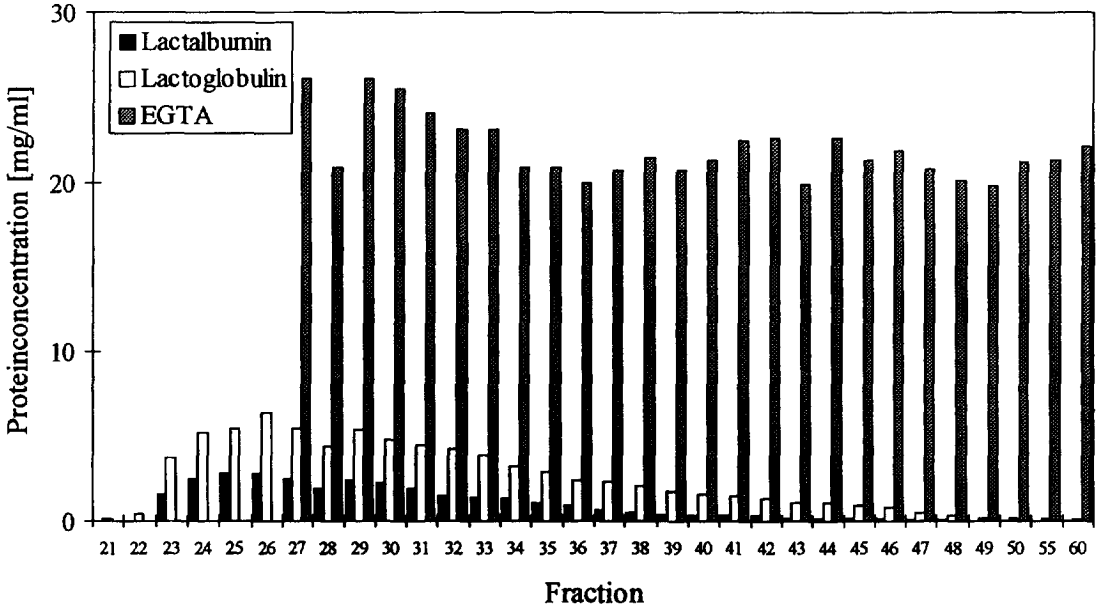

Fig. 1. Displacement of whey proteins on hydroxyapatite using EGTA as the displacer. Stationary phase, 2 μm porous hydroxyapatite; carrier, 0.02 *M* Tris–HCl, pH 9.0; feed, 3.7 mg/ml α-lactalbumin, 3.8 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 41% α-lactalbumin, 99% β-lactoglobulin.

lactalbumin) both whey proteins are found in the protein fractions. Both proteins are, however, recovered to a reasonable extent (84% α-lactalbumin, 76% β-lactoglobulin).

The particle diameter of the stationary phase and the flow-rate of the mobile phase may [24] or may not [25,26] influence the quality of a displacement separation. To investigate this, the displacement of the whey proteins by EGTA was repeated at a carrier pH of 8.0 on columns packed from with 2, 10 and the 20 μm apatite beads (Fig. 3). Compared to the displacement depicted in Fig. 1, the separation of the whey protein is improved by the switch to pH 8.0. Especially in the case of the column packed with the

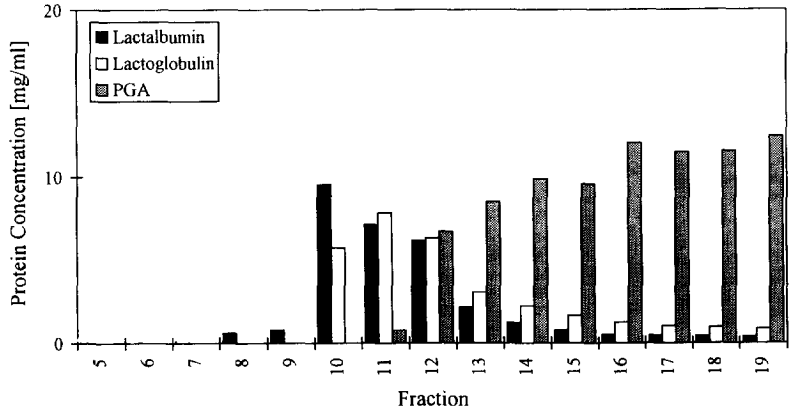

Fig. 2. Displacement of whey proteins on hydroxyapatite using PGA as the displacer. Stationary phase, 2 μm porous hydroxyapatite; carrier, 0.02 *M* Tris–HCl, pH 8.0; feed, 3.2 mg/ml α-lactalbumin, 3.3 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 84% α-lactalbumin, 76% β-lactoglobulin.

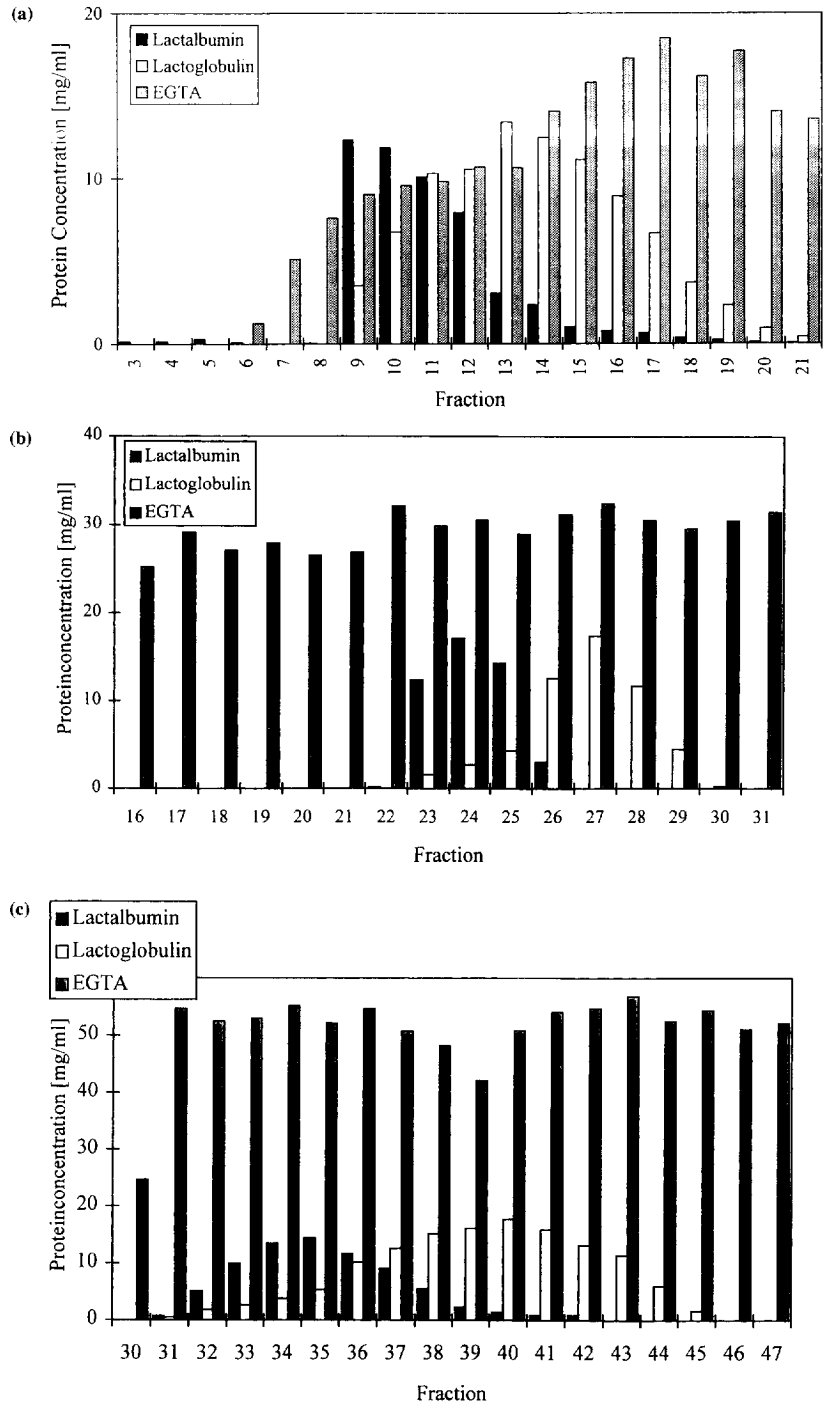

Fig. 3. Displacement of whey proteins on hydroxyapatite as a function of the particle diameter. Carrier, 0.02 *M* Tris–HCl, pH 8.0; displacer, EGTA. (a) Particles (2 μm); feed, 4.3 mg/ml α-lactalbumin, 4.1 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 79% α-lactalbumin, 99% β-lactoglobulin. (b) Particles (10 μm); feed, 5.1 mg/ml α-lactalbumin, 5.1 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 46% α-lactalbumin, 54% β-lactoglobulin. (c) Particles (20 μm); feed, 5.1 mg/ml α-lactalbumin, 5.1 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 36% α-lactalbumin, 64% β-lactoglobulin.

2 μm material, we now find good yields for both proteins (ca. 80% α-lactalbumin, 99% β-lactoglobulin). However, a pronounced tendency of the displacer to "overrun" the protein zones is obvious in all cases. The yield decreases as the diameter of the particles used to pack the columns increases (10 μm hydroxyapatite, 46% α-lactoglobulin, 54% β-lactoglobulin; 20 μm hydroxyapatite, 36% α-lactalbumin, 64% β-lactoglobulin). Previously such a behavior had only been observed for the 20 μm material, whereas the 10 μm material had yielded a separation quality and yield similar to that of the 2 μm material while the concentrating factor was superior [21]. Also the attempts to accelerate the separation by increasing the flow-rate to 0.3 and 0.5 ml/min, respectively, had to be abandoned, due to rapid deterioration of the separation quality under these conditions.

The separation of the displacer EGTA from the target proteins in the fractions can be achieved based on the size difference of the involved molecular species. After all, filtration steps already play an important part in milk fractionation. This would, however, add to the complexity and the costs of the isolation procedure. Considering this only as a last resort, the application of anion-exchange displacement chromatography to the separation of whey was studied instead. The interaction of proteins with ion-exchange materials should be more straightforward than the protein–apatite interaction.

3.2. Anion-exchange displacement chromatography of whey proteins

Strong anion-exchangers have long since been used in the chromatographic separation of whey proteins. Their use in the displacement mode requires a strongly interacting anionic or even polyanionic molecule as a protein displacer. In the case of the Super Q column investigated first (TosoHaas, 85×7.5× mm, 35 μm particles), polyanions such as PGA and PAA (polyacrylic acid) failed to act as protein displacers for the whey proteins, regardless of the pH of the carrier and adjustmust of the flow-rate (data not shown). Similarly, EGTA yielded only slightly "concentrated" fractions and exceptionally low recoveries (<10%) (Fig. 4).

IDA (iminodiactetic acid, free acid, M_r 133.4), on the other hand, performed more favourably (Fig. 5). A reduction of the flow-rate from 1.25 to 1.0 ml/min resulted in an increase in the concentration of the fraction. A further decrease to 0.5 ml/min was of no further benefit. An increase in the pH of the carrier from 7.7 to 8.5 resulted in a slight decrease in the fraction's protein concentration (Fig. 6a). For the anion-exchanger, however, there was no pronounced

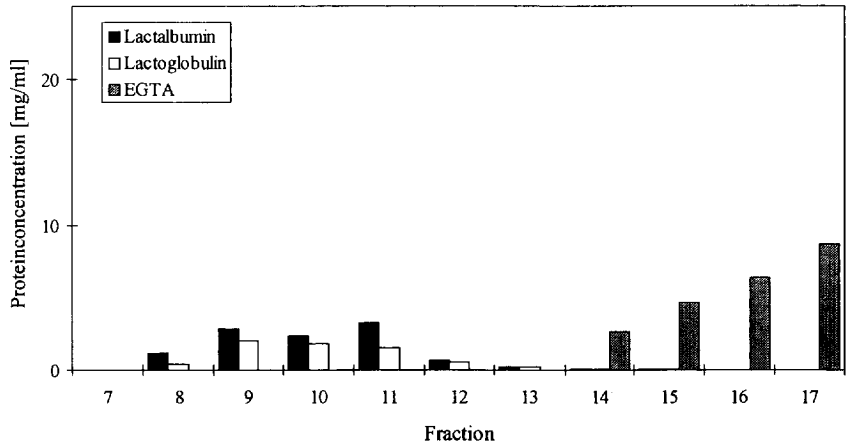

Fig. 4. Displacement of whey proteins on a "Super Q" anion-exchange column using EGTA as the displacer. Carrier, 0.02 *M* Tris–HCl, pH 7.7; flow-rate, 1.0 ml/min; feed, 5.4 mg/ml α-lactalbumin, 5.5 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 10% α-lactalbumin, 6% β-lactoglobulin.

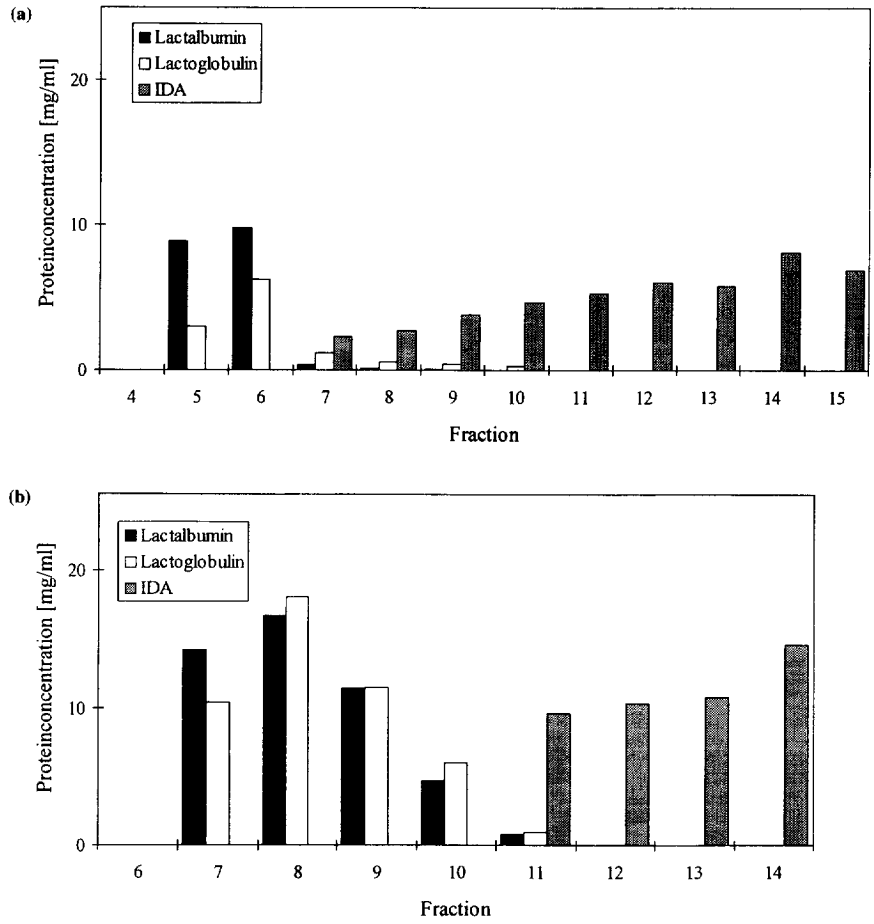

Fig. 5. Displacement of whey proteins on a "Super Q" anion-exchange column using IDA as the displacer. Carrier, 0.02 *M* Tris–HCl, pH 7.7. (a) Flow-rate, 1.25 ml/min; feed, 6.4 mg/ml α-lactalbumin, 6.4 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 15% α-lactalbumin, 8% β-lactoglobulin. (b) Flow-rate, 1.0 ml/min; feed, 7.0 mg/ml α-lactalbumin, 7.5 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 17% α-lactalbumin, 16% β-lactoglobulin.

change in the separation quality when the pH-value of the carrier was increased further to 9.0 (Fig. 6b). Apparently, denaturation of β-lactoglobulin is less fatal in anion-exchange chromatography than in apatite chromatography.

Even under optimized conditions, however, the two whey proteins were by no means separated into individual zones and the "yields" remained below 20%. Extrapolating from the results gained using the apatite material, one may assume that the particle diameter (35 μm) of the material used to pack the Super Q-column is at least partially responsible for the encountered problems, especially at the flow-rates required because of the minimum backpressure limit of our HPLC system. Better results in terms of actual protein separation should therefore ensue using the Bio-Scale Q2 column (Bio-Rad, 52×7 mm, 10 μm particles).

For the Bio-Scale column, IDA, for example, served as the displacer only for the α-lactalbumin, while the β-lactoglobulin was retained on the column (Fig. 7). The protein was concomitantly concentrated

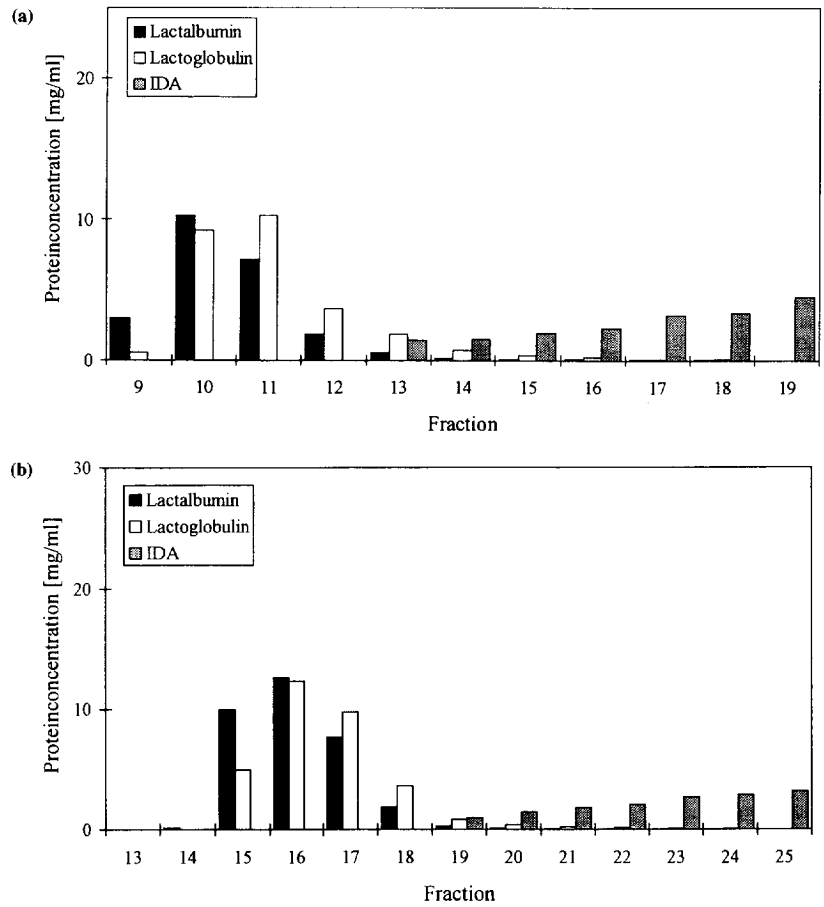

Fig. 6. Displacement of whey proteins on a "Super Q" anion-exchange column using IDA as the displacer. (a) Carrier, 0.02 *M* Tris–HCl, pH 8.5; flow-rate, 1.0 ml/min; feed, 6.4 mg/ml α-lactalbumin, 6.4 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 9% α-lactalbumin, 10% β-lactoglobulin. (b) Carrier, 0.02 *M* Tris–HCl pH 9.0; flow-rate, 0.5 ml/min; feed, 5.8 mg/ml α-lactalbumin, 5.4 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 19% α-lactalbumin, 20% β-lactoglobulin.

by a factor of two over the feed concentration (5.7 mg/ml). Unlike the situation with the Super Q column, both PAA and PGA acted as protein displacers on the Bio-Scale Q2 column. At a carrier pH of 9.0 and a flow-rate of 0.25 ml/min, broad substance zones, with a very diffuse PGA front, were found (Fig. 8a). The displacer was detected from fraction 24 onward, i.e. from the very first substance-containing fraction. As observed before in the case of displacement by EGTA on the hydroxyapatite columns, yields below 50% (32% α-lactalbumin, 44% β-lactoglobulin) were observed for both proteins, which were concomitant with this overrunning of the protein zones by the displacer.

The shape of the displacer front, if not the broadness of the protein zones (and the concomitant low concentration of the fractions) and the low yields (29% α-lactalbumin, 42% β-lactoglobulin), could be improved by lowering the pH of the carrier to 8.0 (Fig. 8b). With a carrier pH of 8.0, fractions 32 to 35 contained only α-lactalbumin, while the first discernible amount of the displacer was found in fraction 38. A considerable improvement in the separation, not only in terms of the protein concentration of the

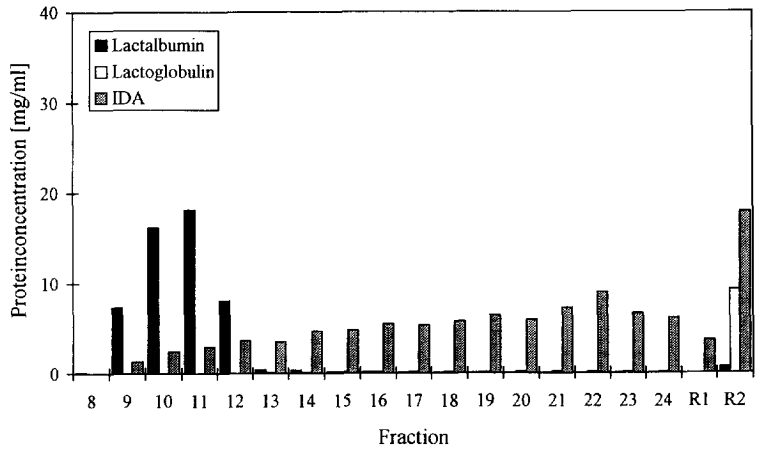

Fig. 7. Displacement of whey proteins on a "Bio-Scale Q2" anion-exchange column using IDA as the displacer. Carrier, 0.02 *M* Tris–HCl, pH 8.0; flow-rate, 0.3 ml/min; feed, 5.7 mg/ml α-lactalbumin, 5.4 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 30% α-lactalbumin, 0% β-lactoglobulin.

individual fractions but, more importantly, in the yield (77% α-lactalbumin, 95% β-lactoglobulin), could be effected by lowering the flow-rate to 0.1 ml/min (Fig. 8c). Once again, maintaining conditions that should limit the influence of putative kinetic and mass transfer limitations on the separation helped to improve the quality of the separation.

When PAA was used as the displacer of the whey proteins under identical conditions, similar or even superior separations were achieved (Fig. 9). For example, the displacer front was sharper for the somewhat smaller PAA (M_r 6000 vs. 13 400 for PGA). Somewhat higher β-lactoglobulin concentrations were recorded, while the overlap between the two protein zones was less pronounced. Close to 80% of the α-lactalbumin and well over 90% of the β-lactoglobulin were recovered. PAA was thus chosen as the displacer in an attempt to separate dairy whey by anion-exchange displacement chromatography.

3.3. Displacement chromatography of a technical dairy whey

According to an analysis by analytical RPC (Fig. 10) the technical whey supplied to us contained 3.45 g/l α-lactalbumin and 12.65 g/l β-lactoglobulin, together with some other UV-active components (dubbed LMWS) that were not further identified by us. Spiking experiments were carried out to verify that neither bovine serum albumin nor bovine immunoglobulins were present in detectable amounts in our sample. The conductivity of the material was 548 μS. The whey (1 ml) was applied directly to the Bio-Scale anion-exchange column, without further preparation.

Compared to the feed, both whey proteins were concentrated by a factor of three during the displacement separation (Fig. 11). This was brought about by a judicious increase in the displacer concentration. Yields were similar to those obtained previously, i.e. 78% for the α-lactalbumin and 92% for the β-lactoglobulin. The displacer front was sharp and little contamination of the preceding protein zones took place. Some LMWS were found throughout the fractions until deep into the displacer zone. The exact chemistry of these substances needs to be elucidated before a prognosis concerning their possible removal can be made. However, the whey considered here had only had the casein fraction removed. A different "whey fraction" can be selected at some other point in the milk fractionation process, e.g. after the majority of the low-molecular-

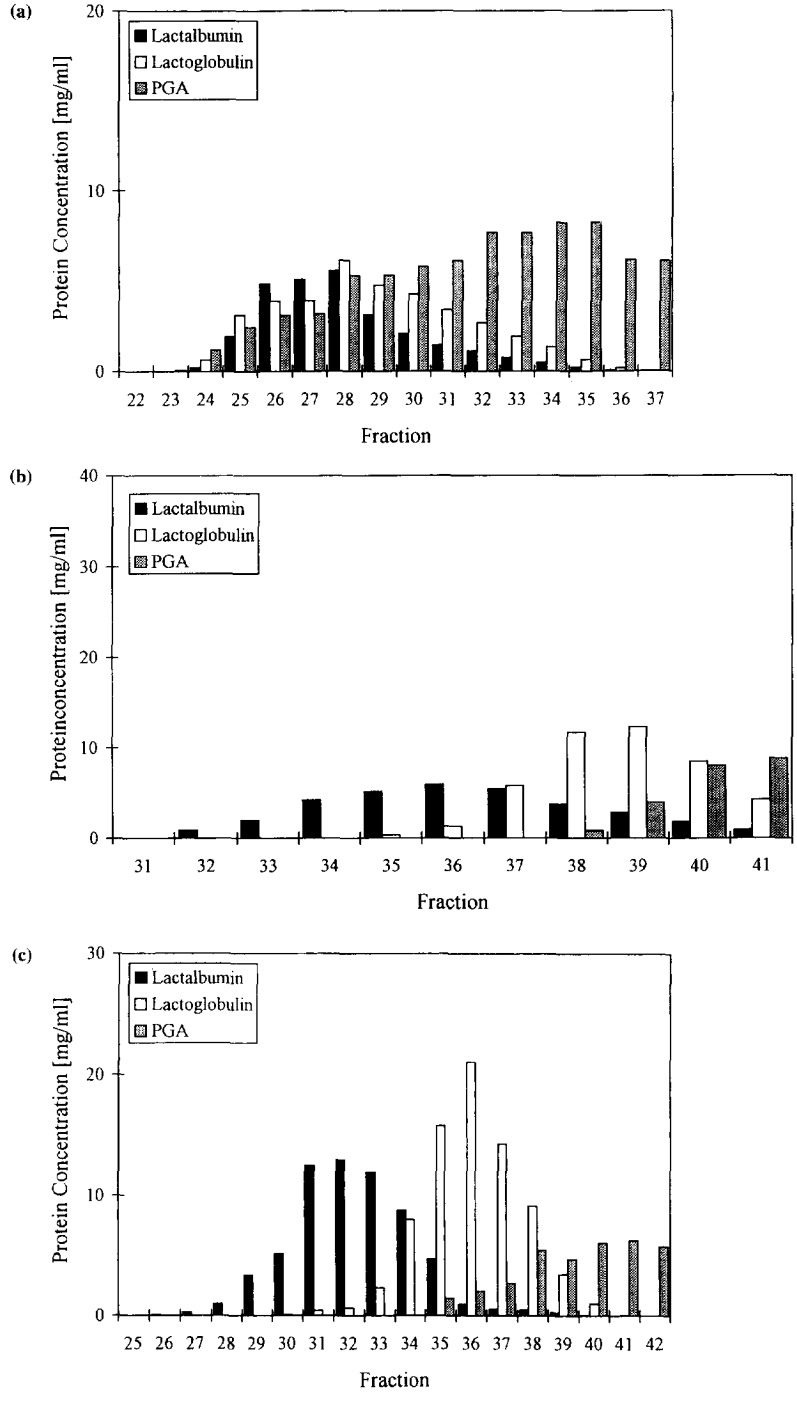

Fig. 8. Displacement of whey proteins on a "Bio-Scale Q2" anion-exchange column using PGA as the displacer. (a) Carrier, 0.02 *M* Tris–HCl, pH 9.0; flow-rate, 0.25 ml/min; feed, 4.3 mg/ml α-lactalbumin, 4.3 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 32% α-lactalbumin, 44% β-lactoglobulin. (b) Carrier, 0.02 *M* Tris–HCl, pH 8.0; flow-rate, 0.25 ml/min; feed, 5.7 mg/ml α-lactalbumin, 5.3 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 29% α-lactalbumin, 42% β-lactoglobulin. (c) Carrier, 0.02 *M* Tris–HCl, pH 8.0; flow rate, 0.10 ml/min; feed, 4.1 mg/ml α-lactalbumin, 4.0 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 77% α-lactalbumin, 95% β-lactoglobulin.

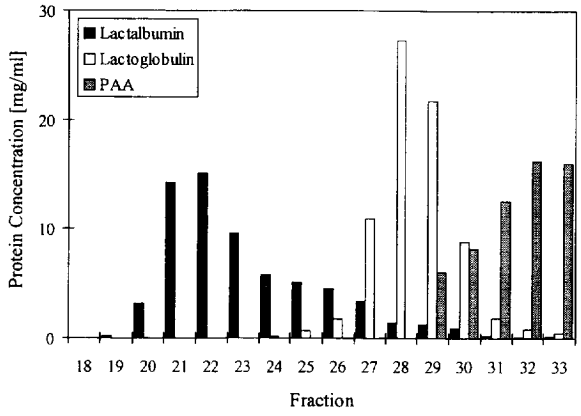

Fig. 9. Displacement of whey proteins on a "Bio-Scale Q2" anion-exchange column using PAA as the displacer. Carrier, 0.02 *M* Tris–HCl, pH 8.0; flow-rate, 0.1 ml/min; feed, 4.1 mg/ml α-lactalbumin, 4.0 mg/ml β-lactoglobulin; feed size, 1 ml; yield, 79% α-lactalbumin, 93% β-lactoglobulin.

mass fractions have been removed by ultrafiltration and only the separation of the residual proteins is required. Further investigations to optimize the displacement step in the processing of milk are currently under way in our laboratory.

4. Conclusion

HPLC has reached a stage where the efficient separation even of substances that are very similar and difficult to differentiate is possible. Scale and throughput limitations may, however, prevent the use of the technique in large scale plants and for diluted substance streams, especially when the recovery of a substance of only intermediate value is considered. Displacement chromatography may provide a solution in this case, as it allows the isolation and concentration of the substance stream while making good use of the dynamic stationary phase capacity.

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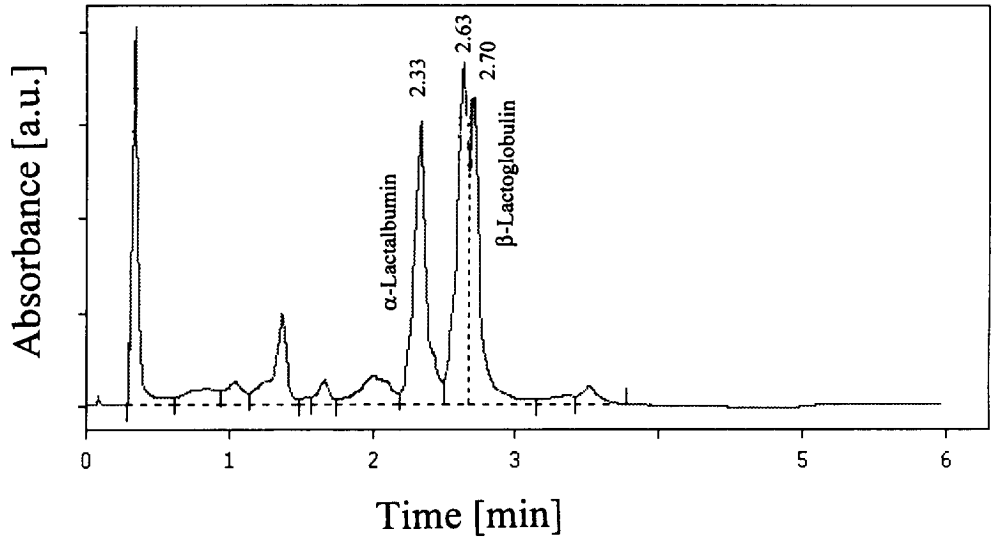

Fig. 10. Analysis by RPC of a sample of the technical dairy whey.

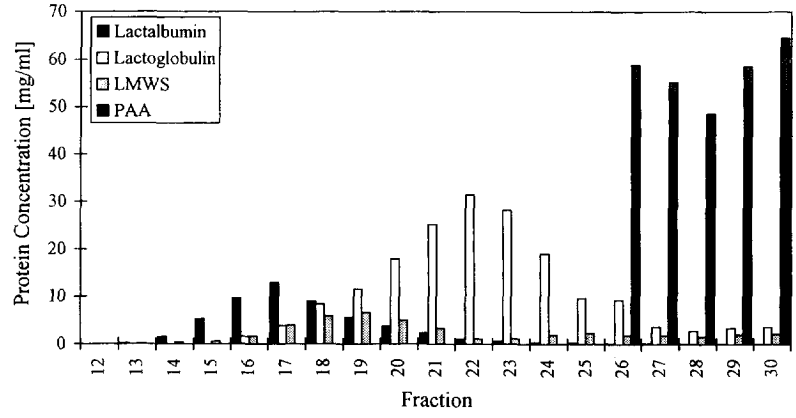

Fig. 11. Displacement of dairy whey on a "Bio-Scale Q2" anion-exchange column using PAA as the displacer. Carrier, 0.02 *M* Tris–HCl, pH 8.0; flow-rate, 0.1 ml/min; feed, dairy whey containing ca. 3.5 mg/ml α-lactalbumin and 12.7 mg/ml β-lactoglobulin; Feed size, 1 ml; Yield, 78% α-lactalbumin, 92% β-lactoglobulin.

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