Lait (1998) 78, 391-400

Original article

Rapid separation of bovine whey proteins by membrane convective liquid chromatography, perfusion chromatography, continuous bed chromatography, and capillary electrophoresis

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(Received 19 September 1997; accepted 26 November 1997)

Abstract – Membrane convective liquid chromatography is a technique based on porous cellulose membranes designed for the separation of biomolecules in few minutes at high flow-rates and low back-pressures. Bovine whey proteins are separated in less than 10 min, at pH 8.5, with a flow-rate of 5.6 mL·min^{-1} and with a 0–0.2 mol·L^{-1} NaCl linear gradient. Three other rapid methods are also proposed. With the ion-exchange perfusion liquid chromatography based on beads with large pores and with the continuous bed chromatography based on a polymer matrix, separations are achieved in only 10 min. Capillary zone electrophoresis using an untreated fused-silica capillary allows the separation of whey proteins in a single run of 8 min without the presence of polymeric additives. These rapid methods are suitable in the quality control of wheys and could be applied in dairy industry or in research. © Inra/Elsevier, Paris

membrane liquid chromatography / MCLC / perfusion chromatography / continuous bed chromatography / capillary electrophoresis / whey protein

Résumé – Séparation rapide des protéines du lactosérum bovin par chromatographie liquide sur membranes à flux convectif, par chromatographie par perfusion, par chromatographie en lit continu et par électrophorèse capillaire. La chromatographie liquide sur membranes à flux convectif est une technologie utilisant des membranes de cellulose poreuses destinées à la séparation des biomolécules en quelques minutes à des débits élevés et des contre-pressions faibles. Les protéines du lactosérum bovin sont bien séparées en moins de 10 min à pH 8,5, avec un débit de 5,6 mL·min^{-1} et un gradient linéaire de 0 à 0,2 mol·L^{-1} de NaCl. Trois autres méthodes rapides sont également

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proposées. La chromatographie d'échange d'ions par perfusion, utilisant des billes à larges pores, et la chromatographie en lit continu utilisant une matrice polymérisée donnent des séparations bien résolues en seulement 10 minutes. L'électrophorèse capillaire utilisant un capillaire en silice fondue non traité permet de séparer les protéines lactosériques principales en une seule étape et en 8 minutes, sans adjonction de polymères. Ces méthodes rapides sont idéales pour le contrôle de la qualité des lactosérums et pour l'étude de l'hydrolyse ou de l'agrégation des protéines lactosériques.

chromatographie liquide sur membranes / MCLC / chromatographie par perfusion / chromatographie en lit continu / électrophorèse capillaire / protéine de lactosérum

1. INTRODUCTION

Membrane convective liquid chromatography (MCLC) is a biomolecule separation method designed to overcome the limitations of conventional liquid chromatography or fast protein liquid chromatography (FPLC). Unlike in traditional bead based soft gel and HPLC or FPLC columns, mass transport of the molecules being separated is achieved with cellulose membranes of 1.2 μm pore size through convective rather than diffusive flow [4]. This allows the flow to be increased and separations to be achieved in a few minutes.

Perfusion chromatography was also developed for the rapid separation and purification of proteins. This technology utilizes packed columns, in which particules have a bidisperse porous structure composed of 600–800 nm through-pores transecting the particules and 50–150 nm diffusive-pores that line the through-pores [1].

The newly introduced continuous bed technology was developed from the previous work of Hjerten et al. [6] and is based on the polymerization of advanced monomers and ionomers directly in the chromatographic column. The polymer chains aggregate into a dense network of nodules consisting of micro-particles with an average diameter of 200 nm. The channels between the nodules are large enough (7 to 15 μm diameter) to permit a high hydrodynamic flow. The first successful results in the development of a new continuous matrix were obtained with an amphipathic macroporous gel plug, consisting of a copolymer of acrylic acid and N,N'-methylenebisacrylamide [6].

On the other hand, capillary zone electrophoresis (CZE) is a technique that takes much less time and labour, and requires smaller sample and buffer volumes than gel electrophoresis and HPLC or FPLC. As MCLC or perfusion chromatography, CZE is applicable for rapid separation and measurement of proteins [7, 16].

Rapid and easy analyses for whey proteins, β-lactoglobulin variant A and variant B (β-LG A and B), α-lactalbumin (α-LA), bovine serum albumin (BSA), and immunoglobulins mainly class G (IgG) are of major interest for the dairy industry for the quality control of wheys and the production of whey protein concentrates and for research on hydrolysis and aggregation of whey proteins. Classical chromatographic and electrophoretic techniques have been widely used for the isolation of milk proteins (for review, see [13]). Recently, rapid separation and determination of the major whey proteins have been performed by reverse-phase perfusion HPLC [14] and CZE [3, 9, 10].

In this paper, a rapid separation of the major whey proteins was optimized using an ion-exchange membrane connected to an FPLC system and compared to separations achieved by ion-exchange perfusion chromatography, continuous bed chromatography or CZE. In the case of MCLC, effects of

pH, flow-rate, mass loading and concentration of NaCl used for protein elution on the retention times of the major whey proteins were studied.

2. MATERIALS AND METHODS

2.1. Whey protein samples

For MCLC, perfusion chromatography and continuous bed chromatography, whey proteins were prepared from bovine raw skim milk after isoelectric precipitation of the whole casein and, then, dialysed and freeze-dried. For CZE, whey was diluted twice with pure water before direct injection onto the capillary. For the four separation techniques used (MCLC, perfusion chromatography, continuous bed chromatography and CZE), proteins were identified with β-LG A, β-LG B, α-LA, and BSA purified by anion-exchange FPLC with a MonoQ HR5/5 column (Pharmacia) according to Andrews et al. [2].

2.2. MCLC

The diethylaminoethyl (DEAE) MemSep 1000 (0.5 × 1.9 cm ID; 1.4 mL bed volume) ion-exchange cartridge (Millipore Co., Bedford, MA, USA) was made of cellulose membrane disc filters placed on top of each other in a polypropylene housing. Weak anion exchangers (DEAE groups) were linked on the surface of each membrane. The MemSep unit was connected to an FPLC system (Pharmacia-LKB Biotechnology, Uppsala, Sweden) which included a Model GP-250 gradient controller, two Model P-500 pumps, and a V-7 injection valve. The MemSep effluent was monitored at 280 nm by a Model UV-1 detector and an Omniscribe B-5000 recorder (Houston Instrument, Austin, TE, USA). The elution buffer was 0.02 mol·L^{-1} Tris, adjusted to different pH ranging from 7.5 to 9.5 with HCl and retention of the major whey proteins was studied according to the pH. Flow-rate varied from 1.4 (one bed volume per min) to 7.0 mL·min^{-1} (five bed volumes per min) in order to evaluate the efficiency of the DEAE MemSep 1000 in linear gradient chromatography. Volumes of 100 µL containing 1, 2, 3, or 4 mg of whey proteins and a volume of 200 µL containing 8 mg of proteins were loaded onto the MemSep cartridge in order to study the effect of the mass loading on the performance of the MemSep. Four linear gradients (10, 20, 40, and 80 mmol·L^{-1} NaCl·min^{-1}) were tested to examine the effect of salt on protein retention time with a constant gradient volume of 56 mL.

The chromatography conditions varied according to the parameters studied as follows:

– effect of pH: Flow-rate 5.6 mL·min^{-1}, mass loading 3 mg, linear elution gradient 20 mmol·L^{-1} NaCl·min^{-1};

– effect of flow-rate: Elution buffer at pH 8.5, mass loading 3 mg, linear elutient gradient 20 mmol·L^{-1} NaCl·min^{-1};

– effect of mass loading: Elution buffer at pH 8.5, flow-rate 5.6 mL·min^{-1}, linear elution gradient 20 mmol·L^{-1} NaCl·min^{-1} (in these conditions, the gradient time was kept constant but not the gradient volume);

– effect of linear elution gradient: Elution buffer at pH 8.5, flow-rate 5.6 mL·min^{-1}, mass loading 3 mg.

2.3. Perfusion chromatography and continuous bed chromatography

Perfusion chromatography of the major whey proteins was achieved with a Poros 20HQ (10 × 1 cm ID; 7.9 mL bed volume) column (PerSeptive Biosystems, Framingham, MA, USA) connected to the FPLC system. A volume of 100 µL containing 3 mg of whey proteins was adjusted to pH 7 with HCl. The flow-rate was 5 mL·min^{-1} and a linear gradient of NaCl ranging from 0 to 0.35 mol·L^{-1} was applied with a gradient volume of 30 mL.

To fractionate the whey proteins by continuous bed chromatography, the anion-exchange UNO Q-1 (3.5 × 0.7 cm ID; 1.3 mL bed volume) column (Bio-Rad Laboratories, Hercules, CA, USA) was connected to the FPLC system. The conditions were slightly different from those used with the Poros 20HQ column. Flow-rate was 3 mL·min^{-1} and the linear gradient was ranged from 0 to 0.30 mol·L^{-1} with the same gradient volume.

2.4. CZE

Capillary zone electrophoresis was carried out with an untreated fused-silica capillary (72 cm × 50 µm ID; 49 cm from injector to detector) connected to a Model 270A-HT system (Applied Biosystems, Foster City, CA, USA). Diluted whey was introduced for 1 s by hydro-

static injection onto the capillary filled with electro-elution solution of 0.18 mol·L^{-1} phosphoric acid at pH 3.6. Proteins were separated at 37 °C at a constant potential difference of 22 kV and detection was monitored at 214 nm. Data were processed with the Model 600 Data Analysis System version 1.0.2 software (Applied Biosystems).

3. RESULTS AND DISCUSSION

Bovine acid whey contains β-LG A and B (3.2 g·L^{-1}), α-LA (1.2 g·L^{-1}), IgG (0.75 g·L^{-1}), and BSA (0.4 g·L^{-1}) as major proteins and small amounts of more than 30 other proteins and peptides also called proteose peptones [9, 11].

In the present work, rapid separation of whey proteins was performed by three chromatography methods (MCLC, perfusion chromatography and continuous bed chromatography) and by capillary zone electrophoresis (CZE). With the three chromatography techniques, the flow-rate could easily increase without loss of resolution caused by small pore diffusion typical of conventional porous beads. In a classical bead based column, the molecules (whey proteins) travel only by diffusion from the interstitial fluid, i.e., the volume of buffer between beads, to the adsorption sites located mainly inside the porous beads. Thus, flow-rates are slow (1 mL·min^{-1} with the MonoQ column; [2]). Resistance to diffusive transport has been minimized with chromatographic methods based on large porosity materials, where the mass transfer is mainly achieved through convective flow.

The competition adsorption of α-LA and BSA to a sulfopropyl ion-exchange membrane (SP MemSep 1010) was studied [17]. Indeed, these ion-exchange membranes qualitatively display local-equilibrium behaviour and are an useful tool for examining competitive protein sorption behaviour.

In our study, the major whey proteins were eluted with the DEAE MemSep 1000 cartridge using smaller salt concentrations than those required with the MonoQ column. With classical bead-based columns, modifications of salt concentration performed in the interstitial fluid need some time to be effective inside the porous beads where 90 % of the adsorbed proteins are localized. With membrane-based cartridges, however, the salt concentration remains the same in the bulk fluid and inside the pores due to minimization of the diffusion phenomenon. Under convective flow conditions, elution of proteins does not require mobile phases as concentrated in sodium chloride as those used in bead-based chromatography techniques.

On the MCLC profiles of whey proteins, IgG were not or weakly retained onto the MemSep cartridge at pH 8.5. The elution order remained the same as that observed with bead-based columns for α-LA, β-LG B and A. BSA, however, was eluted last and corresponded to a broad minor peak. In classical anion-exchange FPLC, IgG are weakly adsorbed onto the MonoQ column [2] and their retention capability increases with pH [5]. BSA is co-eluted with α-LA at pH 6.3 and co-eluted with β-LG B at pH 8.0. At neutral pH, BSA is identified by an individual broad peak between the two peaks of α-LA and β-LG B [5]. The other genetic variant of β-LG is eluted at the end of the linear gradient.

Study of the effect of pH showed a weaker adsorption of proteins onto the MemSep cartridge than onto the MonoQ column since α-LA was not retained at pH values lower than 8.0–8.5 (*figure 1*). To fractionate the major whey proteins, more basic conditions (pH ≥ 8.5 instead of pH = 7.0 for the MonoQ column) were needed with the MemSep cartridge. We have observed a similar phenomenon with a classical bead based column called Bio-Scale Q (Bio-Rad Laboratories), where α-LA was not retained at a pH lower than 8.0 (result not shown). According to the manufacturer, the matrix of this strong anion-exchanger is more hydrophilic than the Monobeads™

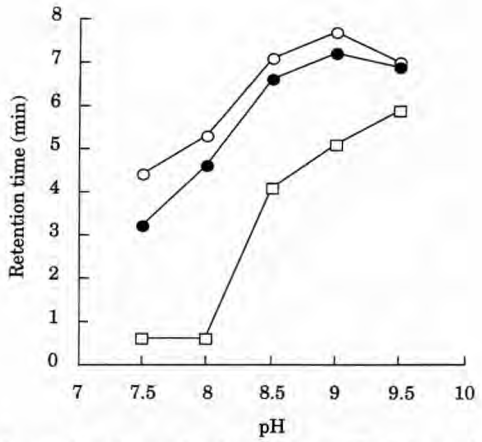

Figure 1. Variation of retention times of α-lactalbumin (□), β-lactoglobulin A (○), and β-lactoglobulin B (●) as a function of pH. A volume of 100 µL containing 3 mg of whey proteins was injected onto a diethylaminoethyl MemSep 1000 cartridge and separation was performed using a linear elution gradient of 20 mmol·L^{-1} NaCl·min^{-1} in 20 mmol·L^{-1} Tris buffer at various pH values for 10 min with a flow-rate of 5.6 mL·min^{-1}.

Figure 1. Évolution des temps de rétention de l'α-lactalbumine (□), de la β-lactoglobuline A (○) et de la β-lactoglobuline B (●) en fonction du pH. Un volume de 100 µL contenant 3 mg de protéines lactosériques a été injecté dans une cartouche MemSep 1 000 porteuse de groupements diéthylaminoéthyles. La séparation a été réalisée avec un gradient linéaire de 20 mmol·L^{-1} NaCl·min^{-1} en tampon Tris 20 mmol·L^{-1} à différents pH pendant 10 minutes, avec un débit de 5,6 mL·min^{-1}.

constituting the MonoQ stationary phase. For a given pH value, the adsorption of α-LA was dependent of the hydrophilic or hydrophobic nature of the matrix of the column. In the case of the MemSep 1000, the cellulose membranes seemed to be more hydrophilic than the Monobeads™ and was not able to retain α-LA at a pH lower than 8.5.

An increasing linear gradient slope corresponded to decreasing retention times for the three principal proteins (*figure 2*). Curves of the retention times of the different proteins versus the linear gradient slope approximated to hyperbolic functions, except in the case of α-LA which separated with a linear gradient slope lower than 20 mmol·L^{-1} NaCl·min^{-1}. The behaviour of α-LA toward the MemSep was complex. In fact, when the linear gradient slope was increased from 10 to 20 mmol·L^{-1} NaCl·min^{-1}, this protein was adsorbed onto the membrane more strongly. With a slope of 10 mmol·L^{-1} NaCl·min^{-1}, only a few of the protein's interaction sites interacted effectively with the DEAE ligand due to a specific folding of the protein in the presence of a small salt concentrations. Conformational changes were possible when salt was added to the solution and adsorption of α-LA improved. Indeed, it was reported that addition of ions induces the important conformational changes of α-LA observed by fluorescence studies [8]. Thus, due to the particular behaviour of α-LA on the MemSep membranes, i.e., a weak affinity for the cellulose matrix and an ionic strength-sensible spatial conformation, good separations between α-LA and β-LG were obtained with linear gradients of 10 or 20 mmol·L^{-1} NaCl·min^{-1}.

After pH and linear gradient optimization, influence of the flow-rate was tested (*figure 3*). The difference in retention times of α-LA and β-LG was improved by increasing the flow-rate from 1.4 mL·min^{-1} (one bead volume) to 7.0 mL·min^{-1} (five bed

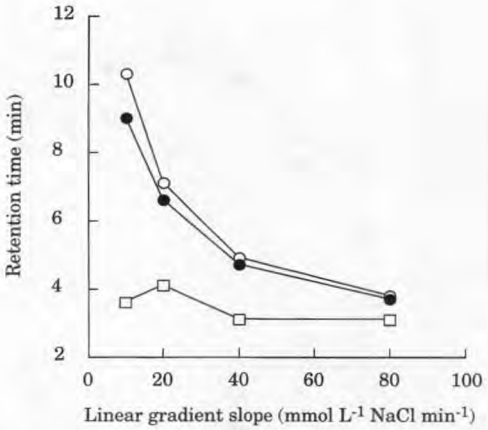

Figure 2. Variation of retention times of α-lactalbumin (□), β-lactoglobulin A (○), and β-lactoglobulin B (●) as a function of the linear elution gradient. A volume of 100 µL containing 3 mg of whey proteins was injected onto a diethylaminoethyl MemSep 1000 cartridge and separation was performed using various linear elution gradients of NaCl in 20 mmol·L^{-1} Tris buffer at pH 8.5 for 10 min with a flow-rate of 5.6 mL·min^{-1}.

Figure 2. Évolution des temps de rétention de l'α-lactalbumine (□), de la β-lactoglobuline A (○), et de la β-lactoglobuline B (●) en fonction du gradient linéaire d'élution. Un volume de 100 µL contenant 3 mg de protéines lactosériques a été injecté dans une cartouche MemSep 1000 porteuse de groupements diéthylaminoéthyles. La séparation a été réalisée avec différents gradients linéaires de NaCl en tampon Tris 20 mmol·L^{-1} à pH 8,5 pendant 10 minutes, avec un débit de 5,6 mL·min^{-1}.

volumes). In our conditions, resolution could also be slightly improved by changing the gradient time but not the gradient volume. Efficiency of the MemSep 1000 cartridge was determined by reporting the plot of the theoretical plate height (h) versus the flow-rate (*figure 3*; [15]). The factor h is defined by the relation h = L/n, where L is the height of the stack of the cross-linked cellulose membranes and n the theoretical plate number calculated from the different peaks of α-LA obtained at each flow-rate tested. The plate height was on the order of 8 µm and was relatively insensitive to flow-rate. Thus, dispersive contributions from fluid flow irregularities were minimal in the MemSep 1000 cartridge under our conditions of flow-rate of 1.4–7.0 mL·min^{-1}. Similarly, the plate heights determined for human transferrin and cytochrome *c* were constant under operating conditions of 1–10 mL·min^{-1} [4]. A flow-rate of 5.6 mL·min^{-1} offered, however, a good compromise between a short run time and a high level of back pressure generated on the MemSep cartridge. The back pressure does not exceed 7 bar. In the case of the DEAE MemSep 1000 cartridge, a flow-rate range of 2 to 6 mL·min^{-1} is recommended by the manufacturer.

The mass loading could be increased from 1 to 8 mg without significantly changing the resolution. The retention times were, however, increased by about 0.5–1 min (*figure 4*). A slight increase of the back pressure was observed during runs where the loaded protein mass was increased from 2 to 4 mg. This was probably the result of a local increase in the mobile phase viscosity inside the MemSep cartridge that extended the retention times of each whey protein.

Finally, a satisfactory separation of each major proteins could be achieved in a short time (< 10 min) at pH 8.5, with a flow-rate of 5.6 mL·min^{-1} and with a 20 mmol·L^{-1} NaCl·min^{-1} linear gradient for 10 min (*figure 5a*). The recent work of Splitt et al.

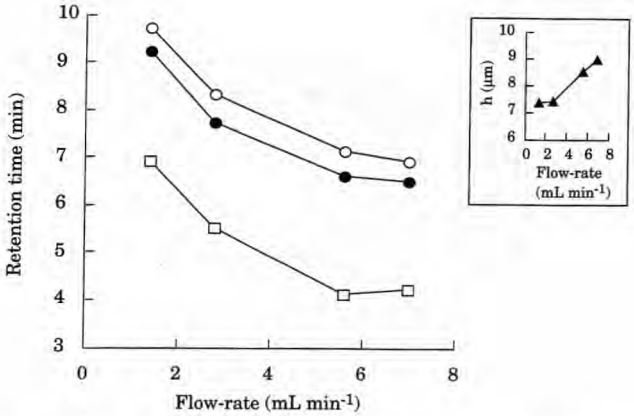

Figure 3. Variation of retention times of α-lactalbumin (□), β-lactoglobulin A (○), and β-lactoglobulin B (●) as a function of the flow-rate. The inset shows the Van Deemter plot [15] representing the theoretical plate height (h) calculated for α-lactalbumin as a function of the flow-rate. A volume of 100 µL containing 3 mg of whey proteins was injected onto a diethylaminoethyl MemSep 1000 cartridge and separation was performed using a linear elution gradient of 20 mmol·L^{-1} NaCl·min^{-1} in 20 mmol·L^{-1} Tris buffer at pH 8.5 during 10 min with various flow-rates.

Figure 3. Évolution des temps de rétention de l'α-lactalbumine (□), de la β-lactoglobuline A (○), et de la β-lactoglobuline B (●) en fonction du débit. L'encart montre la courbe de Van Deemter [15] représentant la hauteur de plateau théorique (h) calculée pour l'α-lactalbumine en fonction du débit. Un volume de 100 µL contenant 3 mg de protéines lactosériques a été injecté dans une cartouche MemSep 1000 porteuse de groupements diéthylaminoéthyles. La séparation a été réalisée avec un gradient linéaire de 20 mmol·L^{-1} NaCl·min^{-1} en tampon Tris 20 mmol·L^{-1} à pH 8,5 pendant 10 min, avec des débits variables.

[12] reported the preparation of major whey proteins from whey and permeate obtained during lactose production in modern dairies using Sartorius MA Q15 ion-exchange membranes. The authors obtained a partial resolution of the two variants A and B of β-LG. The resolution can be, however, improved by coupling two MA Q15 units in series. Moreover, separation of BSA is achieved using these two consecutive modules with local isocratic elution conditions. Separation of whey proteins is thus achieved by judicious module coupling together with a fine tuned gradient.

MCLC was compared with ion-exchange perfusion chromatography, continuous bed chromatography, and CZE. With the Poros 20HQ and UNO Q-1 columns, mass transport was achieved through convective flow. The elution order and resolution of the different whey proteins were similar to those obtained with a classical MonoQ column (*figure 5b, c*). Unlike the MemSep 1000 cartridge, the two matrixes constituting Poros 20HQ and UNO Q-1, have a behaviour similar to that of the MonoQ anion-exchanger, and whey proteins, especially α-LA, were retained onto the columns even at a pH lower than 8.0. Separations were achieved in 8 to 10 min (at high flow-rates) instead of 20–25 min in the case of conventional FPLC. A perfusion reversed-phase HPLC method was recently developed by Torre et al. [14] for the rapid separation of the major whey proteins in less than 3 min with a Poros1 10R (5 × 0.21 cm ID) column, with a bed volume of 0.17 mL instead of the 7.9 mL of our Poros 20HQ column. However, perfusion ion-exchange chromatography is more suitable for preparative fractionations than the reverse-phase method due to its lower protein denaturation effect.

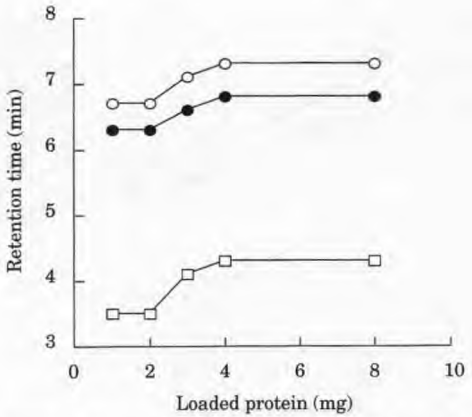

Figure 4. Variation of retention times of α-lactalbumin (□), β-lactoglobulin A (○), and β-lactoglobulin B (●) as a function of the mass of proteins loaded onto the MemSep cartridge. A volume of 100 µL containing various masses of whey proteins was injected onto a diethylaminoethyl MemSep 1000 cartridge and separation was performed using a linear elution gradient of 20 mmol·L^{-1} NaCl·min^{-1} in 20 mmol·L^{-1} Tris buffer at pH 8.5 during 10 min with a flow-rate of 5.6 mL·min^{-1}. To load 8 mg of proteins, an injection volume of 200 mL was required.

Figure 4. Évolution des temps de rétention de l'α-lactalbumine (□), de la β-lactoglobuline A (○), et de la β-lactoglobuline B (●) en fonction de la quantité de protéines chargée dans la cartouche MemSep. Un volume de 100 µL contenant des quantités variables de protéines lactosériques a été injecté dans une cartouche MemSep 1000 porteuse de groupements diéthylaminoéthyles. La séparation a été réalisée avec un gradient linéaire de 20 mmol·L^{-1} NaCl min^{-1} en tampon Tris 20 mmol·L^{-1} à pH 8,5 pendant 10 minutes, avec un débit de 5,6 mL·min^{-1}. Pour déposer 8 mg de protéines, un volume d'injection de 200 µL a été nécessaire.

The most satisfactory resolution was obtained with continuous bed chromatography. According to Hjerten et al. [6], the reasons for this are that a gel plug has more homogeneous structure than a packed bed of beads, e.g., Porous 20HQ, and that the gel plug was compressed, which has a favourable effect on the resolution. It is also likely that the continuous bed is non-porous, i.e., the 'walls' of the channels in the gel are impermeable to proteins, which in combination with compression of the bed gives good resolutions independently of flow-rate [6]. Thus, continuous bed chromatography combines the advantages of cellulose membrane based chromatography, i.e., the ease of use and the efficient resolutions independent of flow-rate, and the advantages of bead based perfusion chromatography, i.e., the high adsorption capability of the polymer matrix.

CZE was utilized differently for the separation of whey proteins with an untreated fused-silica capillary. Otte et al. [9] show that, in phosphate buffers at pH 7.0 or more and with a potential difference of 22 kV, these conditions are not suitable for the separation of BSA and β-LG, although separation of the two genetic variants of β-LG is achieved. According to these authors, BSA and β-LG are well-separated at low pH values (pH = 2.5) but not the A and B variants which co-elute. Injection of standard proteins shows that IgG are electro-eluted last in a broad peak at an injector to detector migration time of 7 min. Under the conditions used by Recio et al. [10], α-LA, BSA, and β-LG B and A are well-separated in borate buffer at pH 8.2 with a run time of 12 min and a potential difference of 7 kV. In our conditions, at pH 3.6 and with a potential difference of 22 kV, the electro-elution order we determined was BSA, β-LG A, β-LG B, α-LA and IgG (*figure 6*). IgG was not easily detectable when whey was directly

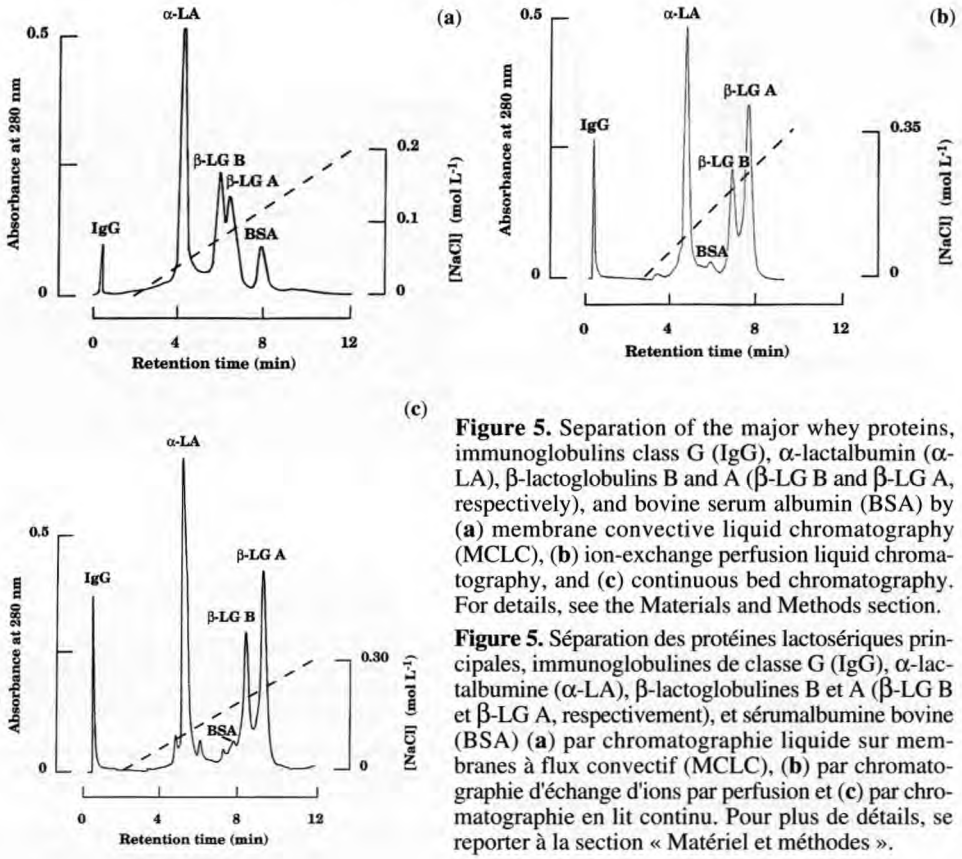

Figure 5. Separation of the major whey proteins, immunoglobulins class G (IgG), α-lactalbumin (α-LA), β-lactoglobulins B and A (β-LG B and β-LG A, respectively), and bovine serum albumin (BSA) by (**a**) membrane convective liquid chromatography (MCLC), (**b**) ion-exchange perfusion liquid chromatography, and (**c**) continuous bed chromatography. For details, see the Materials and Methods section.

Figure 5. Séparation des protéines lactosériques principales, immunoglobulines de classe G (IgG), α-lactalbumine (α-LA), β-lactoglobulines B et A (β-LG B et β-LG A, respectivement), et sérumalbumine bovine (BSA) (**a**) par chromatographie liquide sur membranes à flux convectif (MCLC), (**b**) par chromatographie d'échange d'ions par perfusion et (**c**) par chromatographie en lit continu. Pour plus de détails, se reporter à la section « Matériel et méthodes ».

injected onto the capillary. Separation of BSA, β-LG A and β-LG B was achieved in a single run of 8 min. In another work, Cifuentes et al. [3] separated β-LG A+B, α-LA and BSA by protein size in 10 min using a capillary of a 20-cm effective length. The separation by size is carried out at 16 kV with a Tris-borate buffer at pH 8.6 containing polymeric additives (sodium dodecyl sulfate and polyethylene glycol 8 000). The separation of the two genetic variants of β-LG is, however, not achieved.

4. CONCLUSION

MCLC, perfusion chromatography, continuous bed chromatography, and CZE are four rapid suitable methods applicable to the separation and determination of the major whey proteins and could be applied in research on hydrolysis or aggregation of whey proteins. Continuous bed chromatography based on a polymer matrix has not yet been used for the separation of milk proteins. The use of the UNO Q-1 column is suitable for the separation of the major whey proteins and also of caseins (results not shown). The MemSep cartridge offered a good separation efficiency of whey proteins only at a pH greater than 8.0. This was due to the weak adsorption capability of the cellulose matrix, compared to the Poros 20HQ, UNO Q-1 and MonoQ matrix. Due to the particular technology based on stacked membranes, MCLC seemed to us easier to handle than perfusion chromatography or continuous bed chromatography, especially for scale-up in preparative fractionations.

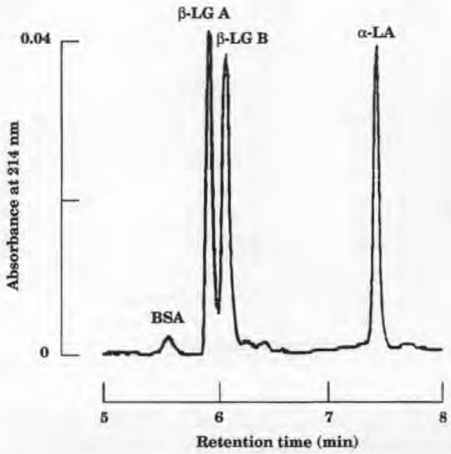

Figure 6. Separation of the major whey proteins, immunoglobulins class G (IgG), α-lactalbumin (α-LA), β-lactoglobulins B and A (β-LG B and β-LG A, respectively), and bovine serum albumin (BSA) by capillary zone electrophoresis (CZE). For details, see the Materials and Methods section.

Figure 6. Séparation des protéines lactosériques principales, immunoglobulines de classe G (IgG), α-lactalbumine (α-LA), β-lactoglobulines B et A (β-LG B et β-LG A, respectivement), et sérumalbumine bovine (BSA), par électrophorèse capillaire. Pour plus de détails, se reporter à la section « Matériels et méthodes ».

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