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Preparative membrane adsorber chromatography for the isolation of cow milk components

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Abstract

Preparative membrane adsorber (MA) chromatography was used to process milk fractions such as the whey and the permeate commonly obtained during lactose production in modern dairies. In MA systems the fluid-dynamic and mass-transfer properties are superior to conventional HPLC or fast protein liquid chromatography (FPLC) columns. Since the flow resistance caused by the MA stacks is quite low, high throughputs can be realized without loss in resolution. Feed sizes were varied from the laboratory scale (several ml) up to batches of 10 l during the investigations. MAs based on modified cellulose filtration membranes (average single layer thickness 200 μm, average pore size 5.0 μm) were used for the small-scale experiments. The MAs are functionalized by covalent linkage of strong and weak ion exchanger groups to their surface. Three commercially available types were used [strong ion exchanger: MA Q15 (3 layers of 5 cm^2) and MA Q100 (5 layers of 20 cm^2); weak ion exchanger: MA D15 (3 layers of 5 cm^2); all Sartorius, Germany]. For the large-scale work a dead-end filtration unit containing up to 1300 cm^2 of MA-area was used. Here MAs based on a synthetic co-polymer, that were prepared from cut-out sheets, were inserted. Chromatographic conditions were transferable from the cellulose- to the polymer-based MA carrying the same functional groups. The influence of the flow-rate and the pH of the mobile phase on the separation was investigated. The flow-rate could be raised to the limit of the respective chromatographic systems and/or MA modules without loss in resolution. The use of the strong anion exchanger MA together with a mobile phase pH of 6.0 and a fine-tuned gradient allowed the separation of BSA, α-lactalbumin and the genetic variants of β-lactoglobulin, even though no baseline separation was possible in the latter case. The use of coupled modules rather than a single one is shown to improve the separation considerably.

Keywords: Preparative chromatography; Milk; Membrane adsorbers; Stationary phases, LC; Proteins; Lactalbumin; Lactoglobulines; Bovine serum albumine; Whey

1. Introduction

Bovine milk is a self-replenishing resource of high nutritional and pharmaceutical value [1]. Various milk components are also used in cosmetics and as washing powder additives [2,3]. Transgenic cows, on the other hand, may soon become interesting production units in biotechnology [4]. The conditioning of bovine milk, e.g. for adaption to human milk, the isolation of certain milk components, including the putative isolation of recombinant proteins from whole milk, are thus problems to be addressed at a large scale in the near future.

The composition of bovine milk is given in Fig. 1. Besides water, fat, carbohydrates (lactose) and minerals, up to 6% of the mass are made up by proteins

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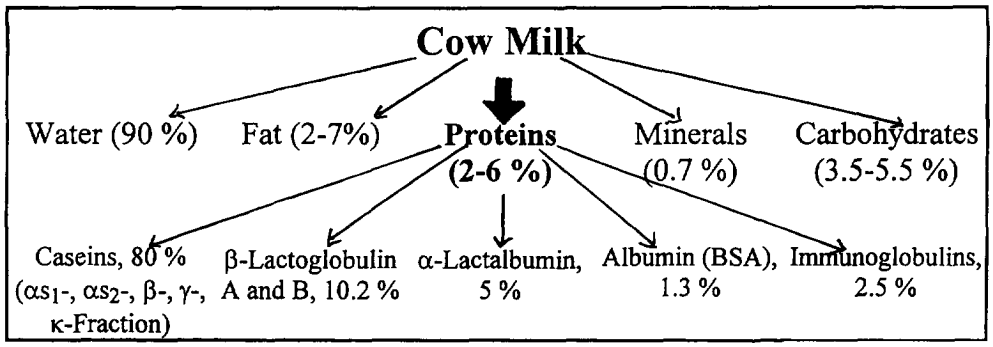

Fig. 1. Major components found in bovine milk. Mass percentages are given.

and peptides, among them a number of high value substances [5]. About 80% of the milk proteins are the caseins (αs_1-, αs_2-, β-, γ- and κ-casein), which are routinely separated from whole milk through acidic precipitation at their isoelectric point (p*I* ca. 4.6) and used for cheese making. Since milk is available in large amounts new areas of utilization have been sought and caseins have for some time now also been used in the paper, textile, dye, and polymer industry.

Through the removal of the caseins, the so-called whey fraction of the milk is obtained, which contains the remaining 20% of the milk proteins, called whey proteins. Besides traces of a number of other proteins, the main whey proteins are bovine serum albumin (BSA), some immunoglobulins, α-lactalbumin and the two naturally occurring genetic variants of β-lactoglobulin (Table 1) [6]. The latter differ by two amino acid residues.

The milk sugar, lactose, is used as a taste- and odorless filler in pharmaceuticals and foodstuffs. Lactose at various purities is isolated by ultrafiltration from whey after a defattening step. The whey proteins are found in the retentate of that procedure, whereas the permeate contains the so-called non-protein-nitrogen (NPN)-fraction (Table 2) [7,8]. The permeate is expected to contain a certain number of biologically active peptides [9,10].

Table 2
Composition of the NPN-fraction (%) found in bovine milk [7]

Components	Composition (%)
Urea	48
Amino acids	15
Peptides	11
Kreatin	8.5
Kreatinin	5
Orotic acid	4
NH_4^+	3
Uric acid	2.5
Hippuric acid	1.5

Both the whey protein and the NPN fraction are produced at several hundred tons per day in modern dairies. Both fractions do contain valuable substances, whose marketability mainly depends on the difficulty and costs of their isolation. The preparative isolation of milk proteins has been shown to be

Table 1
Data on the major whey proteins [6]

Protein	Isoelectric point (p*I*)	Molecular mass (M_r)
β-Lactoglobulin A and B (Lg)	5.2–5.4	18 360 g/mol
α-Lactalbumin (La)	5.4	14 170 g/mol
Bovine serum albumin (BSA)	4.8	66 000 g/mol
Immunoglobulins (Ig)	5.8–7.8	IgG_1: 162 000 g/mol IgG_2: 152 000 g/mol IgA: ca. 400 000 g/mol IgM: ca. 900 000 g/mol

possible by conventional (low to medium pressure) column chromatography [11–14]. Often strong anion-exchange columns are used. In view of the considerable amounts of, e.g., whey that have to be processed per day, however, the commercial integration of such chromatographic steps into the overall milk processing scheme requires the utilization of chromatographic systems at extremely high throughputs. Due to their excellent fluid dynamic and mass transfer properties together with their easy scalability, membrane adsorbers may become the high-performance stationary phase of choice for this purpose.

Membrane adsorbers (MA) may be envisioned as filter membranes that have been functionalized by the linkage of interactive groups to their surface. Their use as stationary phase in protein chromatography has been discussed for some time now [15–21]. Modules containing short MA stacks (typically of no more than some mm of height with a diameter of several cm) have been substituted for columns in HPLC- and FPLC-systems [22–26]. The mass transport in MA phases occurs predominantly by convection rather than diffusion; thus, there is little deterioration of the separation efficiency at elevated flow-rates [20,23,27–29]. Since the flow resistance caused by the MA stacks is often quite low, high throughputs are possible. Scale-up might conceivably benefit from the experience gained in filtration, a unit operation which is often optimized towards processing large volumes at high throughputs. In this paper the applicability of anion-exchange MA chromatography for the isolation of milk components is investigated in general.

2. Experimental

2.1. Chemicals

All proteins were from Sigma. Bulk chemicals were from Fluka. The highest available purity was used. Permeate was supplied to us by Biolac (Harbansen, Germany). Permeate was used within 48 h after receipt and stored at 4°C for that time or kept at −80°C prior to use. Some lactose precipitation occurred under the latter storage conditions. Buffers were prepared with deionized water and passed through a 0.2-μm filter (Sartorius, Göttingen, Germany) before use. Samples were clarified by filtration (0.45-μm filter (Sartorius) or centrifugation (5 min, 14 000 rpm).

2.2. Instrumentation

The FPLC system (Pharmacia, Uppsala, Sweden) consisted of two P 500 pumps, the UV detector 2141, the fraction collector Superrac, and the injection valve MV7, all controlled by the controller LCC 500. The data were analyzed by FPLC software on a PS/2 computer. The automated HPLC system incorporated several preparative Knauer 64 pumps (Knauer, Berlin, Germany), a P1 pump (Pharmacia), injection valves (Valco, USA) and 7-port valves (Knauer), an ERMA 7251 UV detector (ERMA, Germany), an Uvicord UV detector (Pharmacia), and a conductivity detector (BioRad, USA). The gradients were formed by an Autochrom 300 system and an Autochrom valve box (both Autochrom, USA). The valves were controlled by the Autochrom 300 system and a Lab Timer (Knauer). Fractions and samples for analysis were collected by home-made devices. A CIM box (ERC, Germany) was used for data collection. Data analysis was carried out on a PC using APEX software (ERC).

2.3. Membrane adsorber (MA)

The MA had the functional groups (strong cation exchanger: sulfonic acid groups; weak anion exchanger: DEA-groups) attached to a support of a synthetic co-polymer or modified cellulose. According to the manufacturer, the average thickness was 180 μm and the average pore size 0.45 μm in case of the co-polymer-based MA and 200 μm and 5.0 μm, respectively, in case of the cellulose-based ones. The cellulose-based anion-exchange MAs are commercially available (strong anion exchanger: MA Q15 15 cm^2 total area, 3 layers of 5 cm^2; MA Q100 100 cm^2 total area, 5 layers of 20 cm^2; weak anion exchanger: MA D15 15 cm^2 total area, 3 layers of 5 cm^2; all from Sartorius). The MAs are stable within the pH range 2–12. A pressure limit of 7 bar is given by the manufacturer for the commercially available units.

MAs based on the synthetic co-polymer were available as cut-outs from membrane sheets. For the

Table 3
Filter modules used in the experiments

Module type	Number/size MA	Maximum flow-rate investigated (ml/min)
Upchurch A-340 (module 1)	up to 10×0.5 cm^2	10
Upchurch A-333 (module 2)	up to 15×2.5 cm^2	50
Millipore XX4504700 (module 3)	up to 20×9.6 cm^2	100
Sartorius SM 16517 (module 4)	up to 10×3.4 cm^2	50
Sartorius SM 16275 (module 5)	up to 10×130 cm^2	500

integration of the cut-out MAs into the chromatographic system the re-usable filter holders listed in Table 3 were used according to the manufacturer's instructions.

Prior to the first use, MAs are stored dry at room temperature in the dark. Between use, the MAs are kept in an appropriate buffer with a bacteriostatic agent added. When blocking or a significant decrease in capacity is observed, the ion-exchange MAs are regenerated by washing with 1 *M* NaOH followed by 1 *M* HCl.

2.4. Methods

Protein concentrations in solution were established by the BCA assay and the micro-Lowry assay (Pierce, USA) according to the manufacturer's instructions. Total nitrogen was determined according to the Kjehdahl method and correlated via the product (i.e. bovine milk) specific factor of 6.38 to the protein concentration [8]. The sample is treated with H_2SO_4, K_2SO_4 and $CuSO_4$ for 1 h to transform all nitrogen present in the sample components into ammonia. The latter in turn is changed into NH_3, transferred via destillation into a boronic acid solution and quantitated by titration. Orotic acid was quantitated photometrically according to Adachi et al. [30]. Urea and ammonia were quantitated with a commercially available assay kit (Boehringer Mannheim, Germany) according to the manufacturer's instructions. Amino acids were analyzed by reversed-phase chromatography following pre-column derivatization with OPA (*o*-phthalic acid) [31]. Reproducible signals observed in these chromatograms that could not be correlated to milk amino acids were ascribed to peptides. Peak identification in chromatograms in general was done by spiking.

The static capacity of the MAs was established by loading the MAs until saturation at a flow-rate of 1 ml/min, followed by elution and quantitation of the retained protein. The dynamic capacity was established by loading the MAs until the first protein broke through and calculating the amount of protein retained until that moment from the respective concentration and volume of the feed. A separation capacity was usually determined for β-lactoglobulin and α-lactalbumin. It corresponds to the maximum total amount of these proteins that could still be resolved with a resolution (R_s) of 1, using: $R_s=(t_{R2}-t_{R1})/0.5(w_2+w_1)$, with t_R being the retention time (peak maximum) for components 1 and 2, respectively, and w being the peak width at half height.

3. Results and discussion

3.1. Isolation and separation of permeate components on strong anion-exchange MAs

The permeate supplied to us contains a remainder of 300 μg/ml of whey proteins as well as several other UV-active substances, mainly amino acids and peptides. The total nitrogen content as determined according to the Kjehdahl method amounts to 0.16% of the total mass.

When the permeate is passed through a strong anion-exchange MA under conditions that favour retention, i.e. a high pH and a low salt concentration in the mobile phase, approximately 17% of the UV-active substances break through, while the others are retained and may be eluted by an NaCl step gradient (1 *M* NaCl). That ratio is not influenced by the flow-rate in the investigated range of 1 to 10 ml/min. The same behaviour is observed when the

loading is halved (dilution or smaller sample size). As expected, urea and ammonia are found mainly in the breakthrough, while the majority of the amino acids, at least two peptides, the remaining whey proteins, orotic acid and riboflavin are retained on the MA.

Several of the modules compiled in Table 3 were used for the enrichment of the permeate components (see Table 4). Both the capacity, i.e. the amount retained per area of the MA, and the final concentration, i.e. the amount found per unit of the eluted volume, need to be considered. The latter is largely determined by the dead volume of the MA module and thus an MA with good retention power may yet yield an inferior product concentration due to poor outlet design, a common problem in MA chromatography [20].

According to the manufacturer, the MA Q15 modules have been optimized for MA chromatography. The fact, that these modules have the highest capacity in terms of $\mu g/cm^2$ of all systems tested supports this claim. With 18 ml the elution (dead) volume is also the smallest of all units investigated. However, module 4 equipped with five membrane layers yields a higher final concentration due to the larger retention area. It should be noted, that the average capacity per area decreases when a larger number of membranes is integrated into filter module 4. This is an effect generally observed for non-optimized filter modules adapted to MA chromatography. It is ascribed to the fact that little lateral mass transfer takes place within the membranes and insufficient flow distribution quickly leads to a deterioration in average membrane capacity. This phenomenon is not observed for the commercially available units, where, e.g., the average capacity per area of a MA Q15 module (three layers) will be quite similar to that of a MA Q100 module (five layers) (see below).

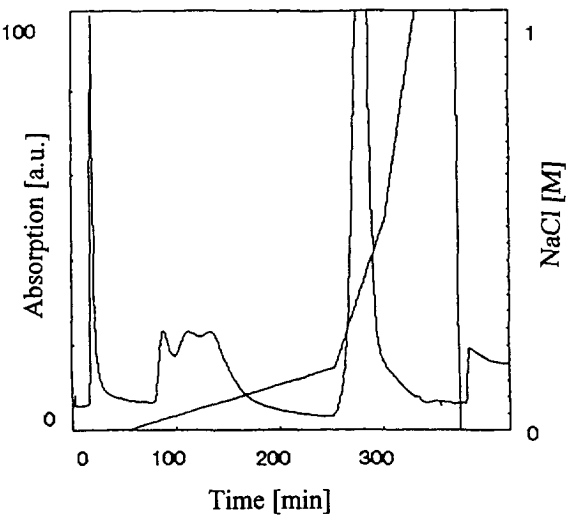

Fig. 2. Chromatogram of the permeate fraction of bovine milk obtained on a strong anion-exchange MA. Buffer A, 20 m*M* borate, pH 9.5; buffer B, 20 m*M* borate, pH 9.5, +1 *M* NaCl; flow-rate, 0.1 ml/min; sample, 50 μl permeate; MA, 5 cm^2 total area. Module 1, ten membrane layers, strong anion-exchange groups.

Whenever possible the protein/peptide concentration in the MA eluate was also determined by standard protein assay. We find a higher protein concentration than that extrapolated from the Kjehdahl factor [8], i.e. a selective adsorption of proteins and peptides. This is to be expected if mainly urea and ammonia break through the MA.

When a linear gradient is used instead of the NaCl step (Fig. 2), the permeate may be separated in three fractions (module 1). Fraction 3 contains mainly orotic acid, fraction 1 is the breakthrough. A scale-up

Table 4
Retention of UV-active components from the permeate on strong anion-exchange MAs

Module	Number of MA layers	Total area (cm^2)	Capacity[a] ($\mu g/cm^2$)	Final concentration[a] ($\mu g/ml$)
Module 3	16	211	4.1	27.91
Module 4	1	78	27.5	39.00
Module 4	5	390	12.2	86.51
MA Q15	3	15	58.9	49.08

[a] Both are given in terms of total nitrogen (according to Kjehdahl) in $\mu g/cm^2$ and $\mu g/ml$, respectively.

was attempted using modules 2 and 3. However, the larger modules only allowed a separation into two fractions, i.e. the breakthrough and a fraction of retained substances.

3.2. Separation of whey proteins using MA chromatography

The isolectric points of the whey proteins are mostly below 7. However, note that some immunoglobulins have an p*I* as high as 7.8. The separation of whey proteins under non-denaturing conditions (especially β-lactoglobulin is becoming unstable above a pH of 8.5) is usually done on a strong anion-exchange column, such as the Mono Q column from Pharmacia [11–13]. A separation of α-lactalbumin and β-lactoglobulin on a MA Q15 module under conditions optimized for the Mono Q column resulted in a decidedly worse separation (Fig. 3). Broader peaks coincided in the MA chromatogram with an inability of the MA to resolve the genetic variants of β-lactoglobulin. When BSA is added to the sample under similar conditions, this substance is separated on neither stationary phase. It co-elutes with the β-lactoglobulin in case of the MA and with the β-lactoglobulin B variant in case of the Mono Q column.

If the MA Q15 and the MA Q100 modules are compared, we find a similarity in separation efficiency, dynamic capacity (0.87 μg/cm^2 of β-lactoglobulin), static capacity (1.3 μg/cm^2 of β-lactoglobulin) and 'separation capacity' (0.4 μg/cm^2 of β-lactoglobulin/α-lactalbumin). The influence of the loading on the resolution is shown in Fig. 4.

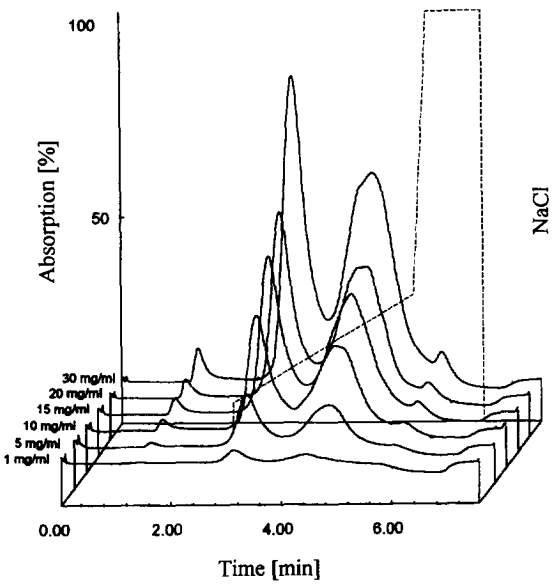

Fig. 4. Influence of the sample concentration on the separation of α-lactalbumin and β-lactoglobulin by strong anion-exchange MA. Buffer A, 20 m*M* Tris, pH 7.4; buffer B, 20 m*M* Tris, pH 7.4, +1 *M* NaCl; flow-rate, 4 ml/min; sample, 50 μl of the indicated concentration. Module, MA Q15.

The pH strongly influences the protein retention on ion-exchange MAs. While the pH range accessible to us is restricted to a maximum of 8.5 by the pH sensitivity of the β-lactoglobulin, a lowering of

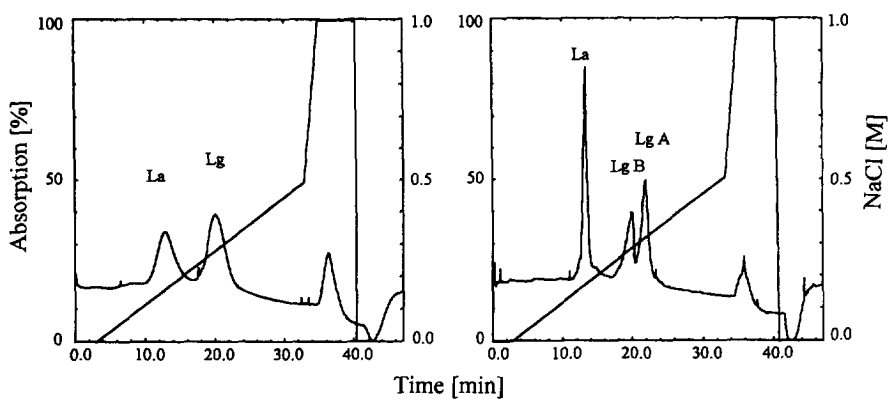

Fig. 3. Comparison of the separation of whey proteins on a MA Q15 module (left) and a Mono Q column (right). Buffer A, 20 m*M* Tris, pH 8.0; buffer B, 20 m*M* Tris, pH 8.0, +1 *M* NaCl; flow-rate, 1 ml/min; sample, 50 μl [1.25 mg/ml α-lactalbumin (La), β-lactoglobulin A and B (Lg)].

the pH is expected to result in a decrease in protein-binding strength due to a reduction of interactive sites. We find an improvement of resolution on the MA when the pH of the mobile phase is lowered (Fig. 5, left). At pH 7.5 and below, there is some separation of the genetic variants of β-lactoglobulin. At the same time the retention times of all substances decrease, therefore the formal resolution calculated from the retention times (peak maxima) and peak width of the α-lactalbumin and the β-lactoglobulin signal is not necessarily improved.

Weak anion exchangers differ in protein resolution from strong ones. A comparison of the MA D15 module (DEA groups) with the MA Q15 one, however, yielded worse results from the weak anion exchanger over the entire pH range investigated (Fig. 5, right). This particular MA type was therefore not considered in the following experiments.

The efficiency of MA chromatographic separations in general tends to show little dependency on the flow-rate. Deviations of this rule have so far mainly been observed for slow bioaffinity-based interactions, not for ion-exchange MAs [25,28]. In the case of the whey protein separation an increase of the volumetric flow-rate from 1 ml/min to 9 ml/min results in a considerable reduction of the separation time, while the resolution and the retention volume remain constant (Fig. 6). The flow-rate is only limited by the pressure limit of the modules (7 bar).

3.3. Improvement of the separation by module coupling

The influence of the loading, i.e. the amount of protein adsorbed onto the MA prior to elution, on the separation efficiency is demonstrated in Fig. 4. Resolution decreases with increased loading, wether by increasing the volume or by increasing the concentration of the feed, until $R_s = 1$ is observed when an average load corresponding to the separation capacity has been reached. In preparative chromatography it is obviously desirable to work close to that separation capacity in order to optimize the throughput. In analytical MA chromatography we usually find an increase in total capacity with increasing MA size and number, but little concomitant influence on the resolution [20]. If anything, the influence is slightly negative, since the dead volume largely responsible for protein zone mixing and dilution increases when several modules are connected via their Luer Lock connectors. However, in analytical MA chromatography one usually aims for working in the linear range of the adsorption isotherm, i.e. far from approaching the separation capacity during sample introduction.

In the non-linear preparative MA chromatography investigated here, however, we found that the resolution improved whenever we used two MA modules in series, even though the sample volume had been raised correspondingly, i.e. the loading per cm^2 has been kept constant (Fig. 7). In other words, we find a higher value for the separation capacity in the case of the two-module approach than for only a single one. This effect is observed for the smaller MA Q15 as well as for the larger MA Q100 ones (Fig. 8). No further improvement is observed when more than two modules are stacked (Fig. 7), which on the other hand will increase the flow resistance of the 'column' and is thus better avoided [32].

The improvement in separation power is not due to the increase in membrane layers or simply to an increase in total area, since two consecutive MA Q15 modules (a total of six membrane layers) will give a better resolution of a given loading than a single MA Q100 one (a total of five membrane layers). At the same time will four consecutive MA Q15 modules (60 cm^2) give a better resolution for a given sample (16 ml, 1.3 mg/ml total protein concentration) than a single MA Q100 one (100 cm^2). By judicious module coupling together with a fine tuned gradient, it is thus possible to considerably improve the resolution of a whey protein mixture by MA chromatography (Fig. 9).

3.4. Scale-up

As long as the separation capacity of a given system is not surpassed, MA are excellent systems for the concentration of diluted product streams. According to the manufacturer, fluids may, e.g., be passed through module 5 at flow-rates of up to 500 ml/min. For a first investigation, a MA Q15 module was used (Table 5). A total of 2 mg of α-lactalbumin, β-lactoglobulin and BSA, albeit at different dilutions, was loaded onto the MA using different flow-rates. The flow-rate maximum was restricted to

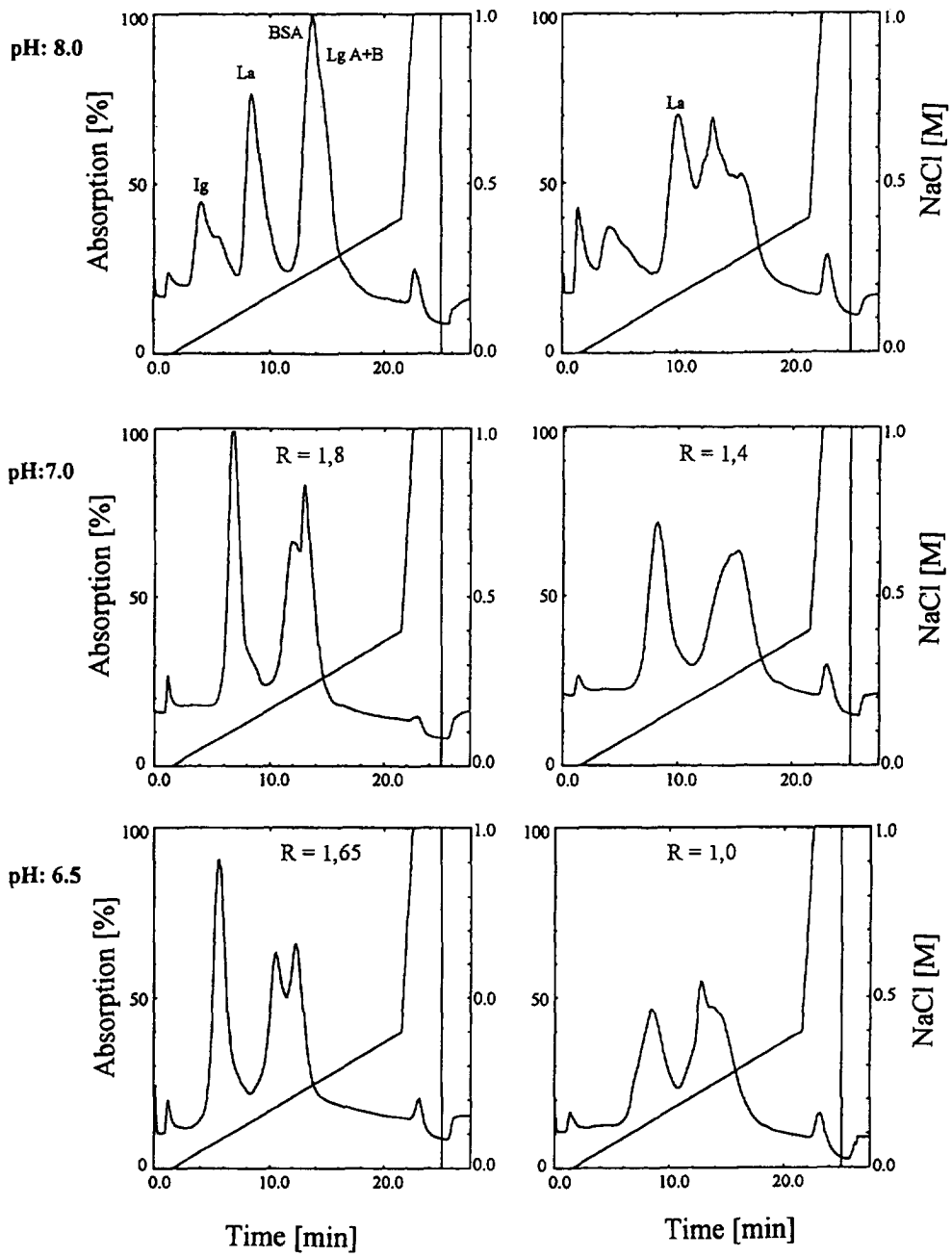

Fig. 5. Comparison of weak (MA D15) and strong anion-exchange module (MA Q15) for the separation of whey proteins at varying mobile phase pH. Right hand side: weak anion exchanger (MA D15). Left hand side: strong anion exchanger (MA Q15). Buffer A, 20 m*M* Tris, pH 8.0 and 7.0 (20 m*M* piperazin in case of pH 6.5); buffer B, 20 m*M* Tris, pH 8.0 and 7.0, +1 *M* NaCl (20 m*M* piperazin in case of pH 6.5); flow-rate, 2 ml/min; sample, 100 μl [1 mg/ml immunoglobulins (Ig) and La, 2 mg/ml Lg and BSA].

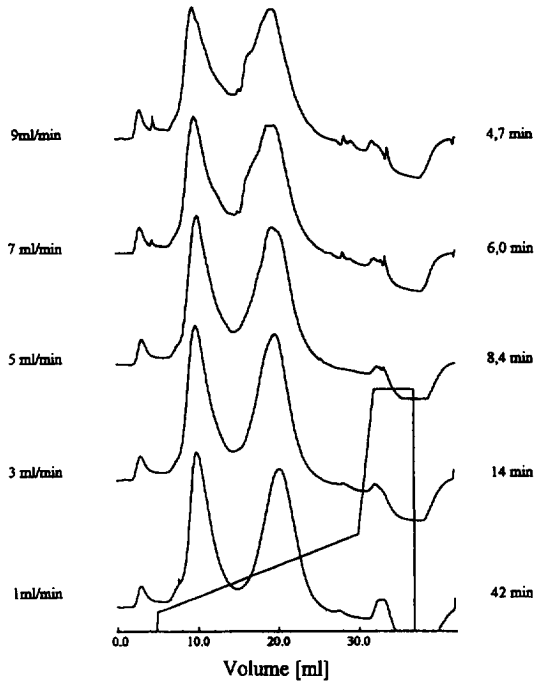

Fig. 6. Influence of flow-rate on the separation of whey proteins by MA chromatography. Buffer A, 20 m*M* Tris, pH 7.4; buffer B, 20 m*M* Tris, pH 7.4, +1 *M* NaCl; sample, 500 μl (1.0 mg/ml total protein). Module, MA Q15.

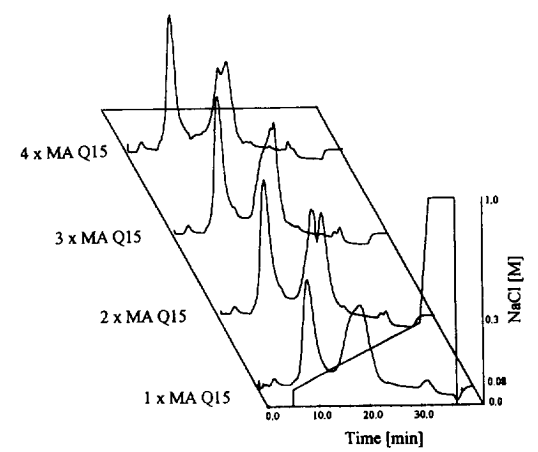

Fig. 7. Influence of module coupling on the separation of whey proteins. Buffer A, 20 m*M* piperazine, pH 6.5; buffer B, 20 m*M* piperazine, pH 6.5, +1 *M* NaCl; flow-rate, 1–4 ml/min (constant retention time); sample, 0.5 mg/ml total protein. Module, MA Q15.

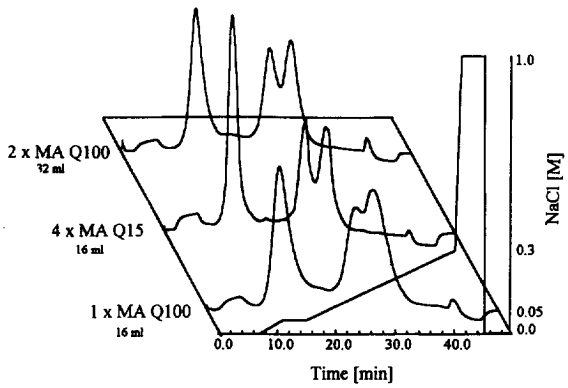

Fig. 8. Influence of module coupling on the separation of whey proteins. Buffer A, 20 m*M* piperazine, pH 6.5; buffer B, 20 m*M* piperazine, pH 6.5, +1 *M* NaCl; flow-rate, 4 and 8 ml/min (constant retention time); sample, 0.5 mg/ml total protein. Module, MA Q100.

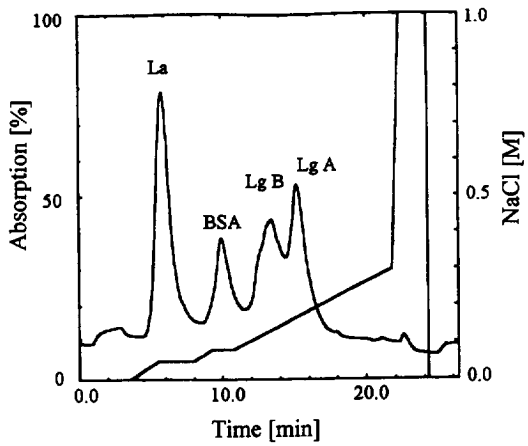

Fig. 9. Optimized separation of whey proteins by MA-chromatography. Buffer A, 20 m*M* piperazine, pH 6.5; buffer B, 20 m*M* piperazine, pH 6.5, +1 *M* NaCl; flow-rate, 4 ml/min; sample, 8 ml (La and Lg 0.5 mg/ml, BSA 0.125 mg/ml). Module, two MA Q15 in series.

Table 5
Enrichment of whey proteins from diluted solutions by the MA Q15 module

Feed	Flow-rate (ml/min)	Recovery (%)	Enrichment factor
1 ml (2.0 mg/ml)	1	91	0.046
10 ml (0.2 mg/ml)	2	87	0.435
100 ml (0.02 mg/ml)	10	82	4.100
1000 ml (0.002 mg/ml	20	79	39.500

20 ml/min by the limited mechanical stability of the MA module in question. Especially the recovery of the least retained protein, α-lactalbumin, deteriorates at higher dilution (Table 5), while the flow-rate per se has no influence for a given dilution (data not shown). Nearly 100% of the β-lactoglobulin and BSA are recovered.

In order to simulate a large-scale separation, the preparative module 5 was fitted with ten membrane layers (based on a synthetic co-polymer) each with an area of 130 cm^2. A feed solution (5 l) containing 0.065 mg/ml α-lactalbumin and β-lactoglobulin as well as 1 μg/ml BSA were passed through the system at a flow-rate of 50 ml/min. Using a two-step NaCl-gradient (up to 1 *M* NaCl) 248 mg of protein were eluted within 14 min: 116 mg in step 1 (10% B, α-lactalbumin), 132 mg in step 2 (50% B, mixture of BSA and β-lactoglobulin). The recovery was 72.5%; an enrichment factor of 60.4 was calculated. If an even higher dilution is used, an enrichment factor of up to 114 could be obtained.

4. Conclusion

Due to their superior mass transfer and fluid-dynamic properties together with the putative ease of scale-up, membrane adsorbers appear to be the optimal high-performance stationary phase for the chromatographic processing of large volumes of raw solutions. An example for such a separation problem is cow milk, where fractions such as the whey and the NPN are produced at large amounts during standard milk processing, which still contain a number of high-value substances. Their chromatographic isolation per se should be straightforward, the integration of such chromatographic steps into the overall milk processing scheme, however, requires an efficient chromatographic system, which is compatible to extremely high throughputs, e.g. one using membrane adsorbers as stationary phase.

By optimizing the chromatographic conditions (mobile phase pH, gradient shape, interactive groups) the separation efficiency of the MA is improved to a point where the major whey proteins as well as several fractions of the permeate (NPN) can be separated. The separation does not depend on the flow-rate and the highest flow-rates compatible with the instrument and the MA module may therefore be used. Scale-up is at present still limited by the restricted number of MA modules available; most of the commercially available, re-usable filter holders cause problems in MA chromatography due to their large outlet volume. Module coupling is shown to improve the separation capacity. First attempts to isolate and enrich whey proteins from diluted solutions (up to 10 l) using 1300 cm^2 of MA yielded promising results.

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