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High-performance liquid chromatography and ultrafiltration of whey proteins with inorganic porous materials coated with polyvinylimidazole derivatives

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ABSTRACT

A coated ultrafiltration membrane was developed which allows the highly selective extraction of lactalbumin from a whey protein mixture. A permeate containing only lactalbumin was obtained using inorganic membranes coated with polyvinylimidazole derivatives containing both ionic and hydrophobic groups. The phenomena involved were analysed with high-performance liquid chromatographic supports bearing similar coatings. With the hydrophobic layer, the selectivity enhancement can be explained through mixed interactions with whey proteins; increasing fouling is due to both lactoglobulin and lactalbumin contributions to the build-up of the boundary layer at the wall of the derivatized membrane.

INTRODUCTION

Ultrafiltration is a pressure-driven membrane process by which macromolecular solutes may be separated or concentrated. A decline in membrane permeability is usually observed; many phenomena are involved (for reviews, see refs. 1–3). In the first few seconds of the run, concentration polarization at the membrane interface is apparent, owing to solute accumulation. The chief result is fouling, which is almost or completely irreversible and which occurs in less than 1 h (sometimes in a few minutes). Owing to protein–membrane interactions, solute adsorption modifies the performance of all membranes with regard to both retention and permeation [4–10]. In fact, the retention of a given protein by an ultrafiltration membrane is dramatically increased if other components of the mixture are so retained, resulting in poor selectivity [4,11–14]. Contrary to common opinion, the selectivity or retention of an ultrafiltration membrane is not based chiefly on its pore size but on the physico-chemical environment of the solute and the chemical nature of the membrane.

The objective here was a highly selective extraction of the smallest component from a concentrated whey protein mixture. To improve the selective extraction of lactalbumin (LA, MW 15 500) *versus* the other main protein, lactoglobulin (LG, MW 35 600), inorganic ultrafiltration membranes of high molecular weight cut-off (100 000) were coated with polyvinylimidazole (PVI) derivatives in order to induce strong interactions between the membrane and the proteins to be concentrated.

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To elucidate the protein–membrane interactions involved, high-performance liquid chromatography (HPLC) of the mixture was performed using supports with the same coatings as the membranes. Both ionic and hydrophobic interactions were studied, using standard and whey proteins. The results were compared with those obtained with unmodified and modified membranes.

EXPERIMENTAL

Products

Standard proteins used in HPLC were α-lactalbumin (LA), β-lactoglobulin (LG) and lysozyme (LYS) and were purchased from Sigma (La Verpilliere, France). Bovine serum albumin (BSA, fraction V) was obtained from Fluka (Interchim, Montluçon, France). A concentrated whey protein mixture (90%, w/w; Eurial, Herbignac, France) was produced by ultrafiltration concentration and spray drying. This commercially available product contained 46% (w/w) LG and 14% (w/w) LA.

Reagents

PVI was synthesized by radical polymerization of N-vinylimidazole (Polysciences, Saint Goar, Germany) using azobisisobutyronitrile (AIBN) as initiator [15]. The molecular weight, determined by viscosimetry, was 13 300 [16]. Quaternization and cross-linking of the coated polymer were then performed with agents such as epichlorohydrin (ECl; Prolabo, Paris, France) or the diglycidyl ether of bisphenol A (DGEBA; Epikote 828) kindly provided by Shell (Rueil-Malmaison, France). All organic compounds were of reagent grade and used as received.

Potassium phosphate and chloride were of analytical-reagent grade. Acetonitrile (ACN) of spectroscopic grade was used for HPLC experiments.

Water was deionized and filtered with a 0.2-μm filter (Eau et Industrie, Le Perreux, France) for ultrafiltration runs. All HPLC eluents were filtered with a 0.45-μm filter (Sartorius, Palaiseau, France).

Inorganic porous materials

In order to compare HPLC and ultrafiltration experiments, we developed a similar reaction scheme for preparing coatings of PVI derivatives.

Preparation of HPLC materials

Vydac 101TP and Nucleosil 300–10 silica (10 μm; 300 Å pore diameter) were used as stationary phases. Silica was coated with a PVI solution in methanol (4%, w/v) [17,18]. Cross-linking of vinylimidazole was achieved through a tertiary amine group or adjacent carbon [19,20]. Quaternization and cross-linking of the coated polymer were then performed with agents such as ECl or DGEBA. With ECl, a second quaternization step was effected by the use of methyl iodide [18]. The route is similar to that in previous work described by Régnier and co-workers [21,22] for polyethyleneimine-coated silica.

All characteristics of the columns are listed in Table I.

Preparation of modified inorganic membranes

Ultrafiltration membranes (Carbosep, Tech-Sep, Miribel, France) of molecular

TABLE I

ADSORPTION OF PVI AND IONIC CAPACITY ON POROUS SILICA

For abbreviations see text and List of Symbols.

Silica	A_s (m^2/g)[a]	PVI (mmol/g)[b]	Cross-linker	Capacity (mmol/g)[c]	Ref.	Column	
						I.D. (mm)	Length (cm)
Vydac 101TP	90	0.56	ECl	0.45[d]	QPVI	4.6	7.5
Nucleosil 300–10	100	0.66	DGEBA	0.16	KPVI	4.6	15

[a] Manufacturer's data.
[b] From nitrogen analysis.
[c] Ionic capacity (see text).
[d] From ref. 18.

weight cut-off 100 000 (M_1 type) were used. The separation layer was mainly composed of zirconium oxide, deposited on the internal wall of a tube of porous carbon (O.D. and I.D. 1.0 and 0.6 cm, respectively, length 60 cm). Membranes were modified with PVI by the same process as described above. One-step quaternization and cross-linking of the polymer coating were achieved with the difunctional reagents ECl or DGEBA. The ammonium content was determined by argentimetric back-titration; a potassium iodide solution ($2 \cdot 10^{-2}$ *M*) was recirculated against the membrane without any pressure so that mainly membrane surface charges were involved. The ionic capacity was found to be in the range 10–20 mmol m^{-2} membrane area.

HPLC apparatus

All HPLC runs performed with PVI silica derivatives (see Table I) included a pump (Waters Model 6000 A), a valve (Rheodyne Model 7125), a UV detector (Varian Model VUV 10, 280 nm) and a potentiometric recorder (Séfram, PE type). The flow-rate was adjusted to 1 ml/min and 50-μl samples were injected.

Size-exclusion chromatography with a TSK 3000 SW column (30 cm × 0.72 cm I.D.) was used for analysis of UF samples. An automatic sample injector (Gilson Model 231-401, 50 μl) and an integrator (Shimadzu Model CR3A) were added to the HPLC line. The flow-rate was 0.7 ml/min.

Ultrafiltration module

The ultrafiltration module was developed at the Centre de l'Énergie Atomique (CEA-Cadarache, St. Paul-Lez-Durance, France). The principles and operating conditions have been described elsewhere [23,24]. Accurate measurements of the pressure drop over the membrane length allowed the determination of τ_w, the wall shear stress.

Experimental procedures

Standard proteins (1 g l^{-1}) or whey proteins (5 g l^{-1}) were dissolved in phosphate buffer or triethanolamine (TEA) buffer (0.05 *M*). The pH was adjusted to the appropriate value with either dilute KOH or HCl. For the sake of clarity, ionic strength is expressed as KCl content, the relevant variable here.

HPLC procedure. Between two buffers (isocratic mode), each column was equilibrated with at least five column volumes. Sample injections were repeated until a 2% reproducibility of the capacity factors (k') was obtained. Each reported value is the average of at least three runs. A protein was considered to be not eluted (N.E.) if elution required over 30 column volumes.

Ultrafiltration procedure. Prior to ultrafiltration runs, water and buffer permeation (J_o and J_b, respectively) were determined as reference fluxes.

A whey protein solution (5 g l^{-1}) was then ultrafiltered for 3 h under pressure ($\Delta P = 2 \cdot 10^5$ Pa) and with a tangential flow-rate ($V_L = 4.4$ m s^{-1}) and a wall shear stress ($\tau_w = 75$ Pa) resulting in a hydrodynamically turbulent regime to minimize fouling [23,24]. For all experiments, the concentration of the feed solution was kept constant by permeate remixing in the feed tank. The experimental temperature of the solution was controlled (20°C). Permeate was sampled during the run.

At the end of each run, the membrane was rinsed with water and the water permeation flux was then measured (J_a). The following membrane-cleaning procedure was applied: NaOH (0.1 *M*, 40°C, 40 min) with a final addition of NaOCl (300 ppm of active chlorine) for 3 min; rinsing with tap water (20 min); HNO_3 (0.05 *M*, 30°C, 10 min); rinsing with tap water until the permeate pH was neutral; and water permeation flux measurement (J_f); the cleaning procedure was repeated if the ratio J_f/J_o was lower than 0.95.

RESULTS AND DISCUSSION

HPLC results

Capacity factor determination. The capacity factor (k') was obtained from the equation

$$k' = (V_e - V_m)/V_m \quad (1)$$

where V_e and V_m are, respectively, the elution volume of the solute and the mobile phase volume, which was determined by injection of water. In fact, water injection resulted in a positive UV peak followed by a negative peak. For V_m determination, we selected the positive peak which results from phosphate displacement by water, as this peak was little affected by the eluent conditions (pH, ionic strength).

The k' value of a protein should be negative from eqn. 1 if the protein elution is based on a size-exclusion mode; the more negative k' is, the more excluded from the pores the protein is. Moreover, this definition allows the comparison of the elution volumes of proteins which are of different size without using the theoretical SEC volume of each protein.

Strong anion-exchange chromatography with QPVI silica. Supports based on silica coated with epichlorohydrin PVI derivative (QPVI) have been evaluated as protein sorbents by HPLC [18]. The results obtained with a QPVI silica in TEA buffer (50 m*M*, pH 7) are shown in Table II. The k' of LYS is negative owing to its positive net charge (pI = 11) at pH 7; at low ionic strength, negatively charged LG (pI = 5.1–5.3) and BSA (pI = 4.9) were not eluted. At high ionic strength, all negatively charged proteins display k' values close to zero but not reaching the theoretical negative SEC k' values. Hence, the elution of BSA and LG with QPVI support in

TABLE II

INFLUENCE OF IONIC STRENGTH ON CAPACITY FACTORS (k') OF PROTEINS WITH QPVI SILICA UNDER ISOCRATIC CONDITIONS: TEA BUFFER (0.05 M), [KCl] AS I

pH	I	k'			
		LA	LG	BSA	LYS
7	0.1	5.92	N.E.[a]	N.E.	−0.11
	1	0.03	0.08	−0.03	0.02

[a] N.E. = Not eluted.

TEA buffer is governed by ionic interactions. On the other hand, increasing ionic strength induced a higher retention of LYS owing to enhanced hydrophobic interactions.

As phosphate occurs naturally in milk and whey, the influence of phosphate ions on the mechanism of fouling in whey ultrafiltration has been explored [25–27]. Therefore, in chromatographic runs we used phosphate buffer instead of TEA buffer as the eluent. The influence of the pH, at fixed ionic strength (0.1 M), on the elution of whey proteins (LA, LG) is shown in Fig. 1. The elution volumes decrease as the pH is close to the protein isoelectric point. The retention and the band width of the LA (pI = 4.8) peak decrease with increase in pH, as is predictable from the protein charge. On the other hand, a higher retention of LG is observed in this pH range (7–5); no elution is observed from pH 7 until a separation in two subunits appears at pH 5.

For the sake of comparison, Fig. 2 shows the chromatograms of LYS and BSA eluted with QPVI silica under isocratic conditions in phosphate buffer at pH 7 and at low ionic strength (0.1 M). A broad peak of BSA (pI = 4.9) is observed owing to its negative net charge at pH 7. On the other hand, LYS is not retained (negative k').

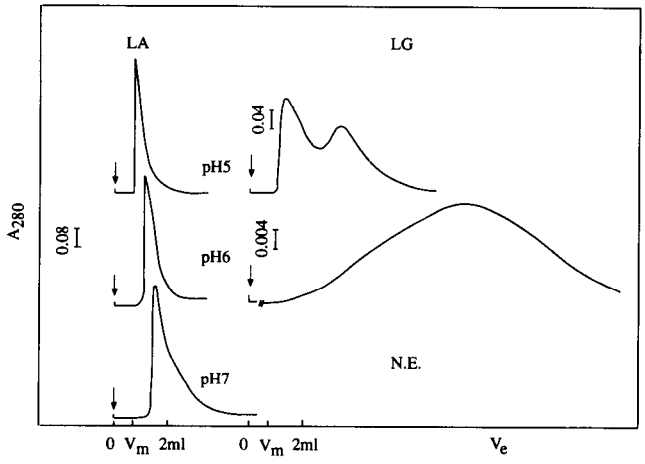

Fig. 1. Influence of pH on chromatograms of LA and LG with QPVI silica under isocratic conditions. Phosphate buffer (0.05 M), KCl (0.1 M); flow-rate, 1 ml/min; V_{inj}, 50 μl.

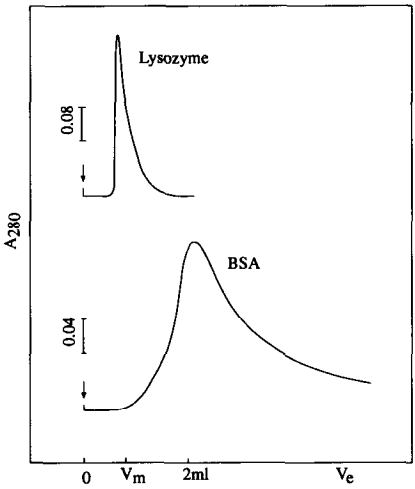

Fig. 2. Chromatograms of LYS and BSA with QPVI silica under isocratic conditions. Phosphate buffer (0.05 *M*, pH 7), KCl (0.1 *M*); flow-rate, 1 ml/min; V_{inj}, 50 μl.

The k' values of all proteins *versus* pH at low and high ionic strength are given in Table III. At high ionic strength, all k' values became negatieve or close to zero. Phosphate buffer is a better displacer than TEA (Table II), as a pure size-exclusion mode is never reached with this latter buffer at pH 7. Particular attention must be paid to the k' values at pH 5, where enhanced hydrophobic interactions arise at high salt content. It has been reported previously that ion-exchange packings with PEI coatings exhibit significant hydrophobic interactions, resulting in mixed-mode chromatography for protein separations [28–31]. On the other hand, the increase in k' for lysozyme in the SEC mode at high salt content is in agreement with previous studies [32–35].

TABLE III

INFLUENCE OF pH AND IONIC STRENGTH (I) ON CAPACITY FACTORS (k') OF PROTEINS WITH QPVI SILICA

Phosphate buffer (0.05 *M*), [KCl] as I.

pH	I	k'			
		LA	LG	BSA	LYS
7	0.1	1.26	N.E.[a]	1.78	−0.08
6	0.1	0.72	18.8	1.29	−0.10
5	0.1	0.18	0.88[b]	0.51	−0.15
			3.42[b]		
7	1	−0.01	−0.01	−0.04	−0.08
6	1	−0.08	−0.10	−0.10	−0.10
5	1	0.03	−0.03	0.00	−0.01

[a] N.E. = Not eluted.
[b] Two subunits.

Mixed-mode chromatography with KPVI silica. In order to obtain a mixed-mode material, another coated silica was developed by using PVI and a hydrophobic cross-linking agent (DGEBA) (hereafter called KPVI silica).

Experimental results *versus* ionic strength, at fixed pH (pH 7), are given in Table IV. These results are different from the QPVI results. At low ionic strength, any protein is adsorbed on the KPVI coating except LYS, which is strongly retained. The hydrophobic character of the polymer coating is indicated by the high k' value (about 26) of LYS, for which no ionic interactions can be invoked, as both stationary phase and LYS are positively charged. Similar results were obtained with TEA buffer (not shown).

A weakly polar organic solvent (acetonitrile) was added to the mobile phase buffer to suppress hydrophobic interactions. The acetonitrile content lies in the range 0–30% (v/v) because of the poor solubility of proteins in this buffered eluent at high salt concentration.

Fig. 3 shows LA and LG peak profiles in a mobile phase including 30% acetonitrile, phosphate buffer and a high ionic strength. LG appears as a double peak of 2 subunits, not well resolved; LA, which is a more hydrophobic protein than LG, is not entirely displaced from the KPVI phase by this eluent. Table IV gives the capacity factors of all proteins, depending on acetonitrile content, ionic strength and pH. For clarity, Table IV does not include k' values corresponding to 10 and 20% acetonitrile contents; elution, if any, is indicated in the text. The ionic part of the retention mechanism of these proteins is clearly shown as these proteins are not eluted at low ionic strength in acetonitrile-containing eluents. Nevertheless, mixed (ionic and hydrophobic) interactions between negatively charged proteins and the KPVI support still remained ($k' > 2$) at high ionic strength in acetonitrile media. On the other hand, BSA is eluted according to a size-exclusion mode with the KPVI column, at high ionic strength only, revealing that ionic BSA–support interactions are involved in the adsorption of this protein in a low-salt medium. From Table IV, it appears that the

TABLE IV

INFLUENCE OF ACN CONTENT AND IONIC STRENGTH ON PROTEIN CAPACITY FACTORS (k') WITH KPVI SILICA

Phosphate buffer (0.05 *M*, pH 7), [KCl] as *I*.

ACN (%)	*I*	Phase	k'			
			LA	LG	BSA	LYS
0	0.1	KPVI	N.E.[a]	N.E.	N.E.	26.4
	1		N.E.	N.E.	N.E.	N.E.
	1[b]		N.E.	N.E.	–	–
30	0.1	KPVI	N.E.	N.E.	N.E.	−0.13
	1		2.54	2.02[c] 2.96[c]	−0.26	−0.12
	1[b]		2.95	2.33	−0.26	−0.14

[a] N.E. = Not eluted.
[b] pH 6.
[c] Two subunits.

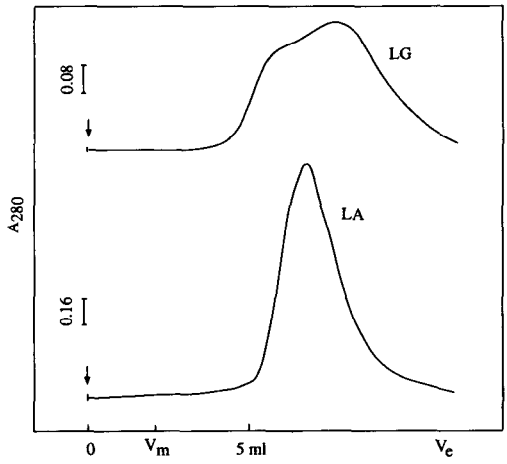

Fig. 3. Chromatograms of LA and LG with KPVI silica under isocratic conditions. Phosphate buffer (0.05 *M*, pH 7), KCl (1 *M*), ACN 30% (v/v); flow-rate, 1 ml/min; V_{inj}, 50 μl.

behaviour of BSA is less hydrophobic than that of whey protein towards the KPVI phase. Finally, the LYS capacity factor becomes negative whatever the ionic strength if the eluting buffer includes acetonitrile.

Ultrafiltration results

Ultrafiltration performances. The permeation flux of pure solvent passing through the membrane can be related to the applied pressure (ΔP) by Darcy's law:

$$J = \Delta P/\mu R_M \tag{2}$$

where μ is the solvent viscosity (Pa s) and R_M is the hydraulic resistance (m^{-1}) of the membrane before use.

Fouling could be introduced as an apparent serial of hydraulic resistances opposite to the mass transfer, so that Darcy's law becomes

$$J = \Delta P/\mu_t(R_M + R_{BL}) \tag{3}$$

where μ_t is the feed solution viscosity (Pa s) and R_{BL} the hydraulic resistance of the boundary layer (m^{-1}).

During ultrafiltration runs, the permeation flux decline (J_t) can be analysed through hydraulic resistance ratios with the following relationship:

$$R_{BL}/R_M = (\mu_b/\mu_t)(J_b/J_t) - 1 \tag{4}$$

where J_b and J_t are the buffer flux and the permeation flux during ultrafiltration at time t, respectively ($l\ h^{-1}\ m^{-2}$). Eqn. 4 is a convenient way to define an overall fouling index without any assumption as to cause.

Retention ratios are defined by the following relationship:

$$R(\%) = 100(1 - c_p/c_o) \quad (5)$$

where c is the concentration and subscripts p and o refer to permeate and feed solution, respectively.

Permeate concentrations were determined by SEC (1% accuracy; see Experimental).

Ultrafiltration with M_1-QPVI and unmodified membranes. Three inorganic Carbosep membranes were tested: an unmodified M_1-type membrane and two functionalized membranes, hereafter called M_1-QPVI and M_1-KPVI, where the PVI coating was cross-linked with ECl or DGEBA, respectively.

In a first step, we compared the ultrafiltration performances of unmodified M_1 and M_1-QPVI membranes. Ultrafiltration of LYS and BSA solutions by these membranes has been reported previously [13,36]. The physico-chemical conditions (pH 7; 0.2 *M* KCl) were derived from Table III in order to suppress ionic interactions between LA and QPVI and to achieve a selective extraction of LA from the whey protein mixture. Retention ratios and fouling indexes (R_{BL}/R_M) *versus* time are shown in Fig. 4. The LG retention ratio is higher with the M_1-QPVI than with the unmodified M_1 membrane, but complete retention of LG was expected in 0.2 *M* medium based on HPLC data. Moreover, ionic interactions between LG and the M_1-QPVI membrane also induce an increase in the LA retention ratio (from 52.5 to 66.2%). On the other hand, the fouling index, R_{BL}/R_M, is reduced with the M_1-QPVI membrane.

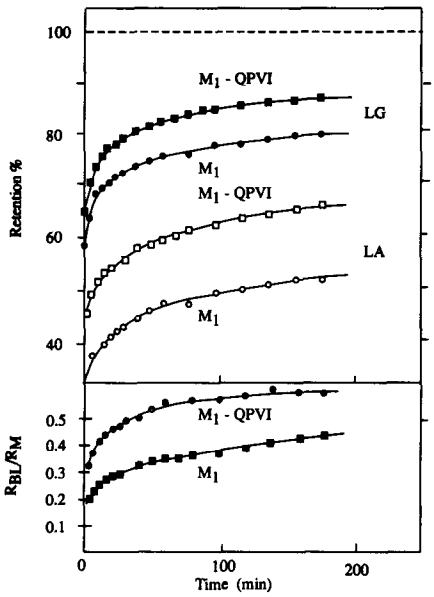

Fig. 4. LA and LG retention ratios (top) and fouling indexes (bottom) with an unmodified M_1 membrane and with an M_1-QPVI membrane. Phosphate buffer (0.05 *M*, pH 7); KCl (0.2 *M*). $\Delta P = 2 \cdot 10^5$ Pa; $V_L = 4.4$ m s^{-1}; $\tau_w = 75$ Pa; $T = 20$°C.

Table V shows the influence of the ionic strength and pH on the properties of UF membranes. Retention ratios of whey proteins with an unmodified membrane are not sensitive to ionic strength. It should be noted that ZrO_2 has a pH of zero electric charge of about 5.7 [37], so that the membrane interface is negative at pH 7. In contrast to the chromatographic results, the LA and LG retention ratios with a functionalized M_1-QPVI membrane were only slightly dependent on ionic strength. This emphasizes the minor effect of ionic strength on the retention properties of this type of inorganic membrane; nevertheless, the fouling index was significantly decreased when the ionic strength was raised to 1 *M*.

At lower pH and ionic strength (0.1 *M*), the retention ratios of LA and LG increase by over 80% and 90%, respectively. A marked rise in the fouling index (R_{BL}/R_M = 1.0) of the M_1-QPVI membrane is noted at pH 5, very close to the p*I* values of whey proteins. This result agrees with other work [6,10,38,39] which correlates an increase in fouling with the amount of adsorbed protein, which is maximum at a pH close to the p*I*. This long-term fouling can be related to hydrophobic interactions, which are predominant under these pH conditions.

The whey protein retention ratios are enhanced at pH close to protein p*I* whereas HPLC *k'* data (Table III) suggest only weak ionic interactions. Ultrafiltration, with various hydrodynamic conditions not discussed here, is based on a more complicated retention mechanism than HPLC zonal elution.

Ultrafiltration with M_1-KPVI membrane. A functionalized M_1 membrane with enhanced hydrophobic character (Bisphenol A moiety) was then developed in order to obtain complete retention of LG and to improve the selectivity. Fig. 5 shows that the LG retention ratio is 100% during the entire run at low ionic strength (0.2 *M*) and pH 7. There is a time lag of nearly 30 min before LA appears in the permeate, owing to an adsorption step. The LA retention ratio at the steady state (87%) depends on the complete retention of LG. The hydrophobic moieties of KPVI induce complete retention of LG, an increasing fouling index (1.19) and an increase in LA retention. Table V reports M_1-KPVI membrane performances *versus* pH and ionic strength. A

TABLE V

INFLUENCE OF pH AND IONIC STRENGTH ON THE ULTRAFILTRATION OF WHEY PROTEINS WITH AN UNMODIFIED M_1 AND TWO FUNCTIONALIZED M_1 MEMBRANES

Phosphate buffer (0.05 *M*), [KCl] as *I*.

pH	*I*	Membrane	R_{LA}	R_{LG}	R_{BL}/R_M
7	0.2	M_1	52.5	79.5	0.61
	1		52.8	78.9	0.42
7	0.2	M_1-QPVI	66.2	86.7	0.45
	0.4		63.4	86.7	0.37
	1		59.0	84.5	0.33
6	0.1		80.6	92.4	0.67
5	0.1		83.7	93.2	1.0
7	0.2	M_1-KPVI	87.0	100.0	1.19
	1		83.4	97.2	0.84
5	0.2		93.6	>98	1.24

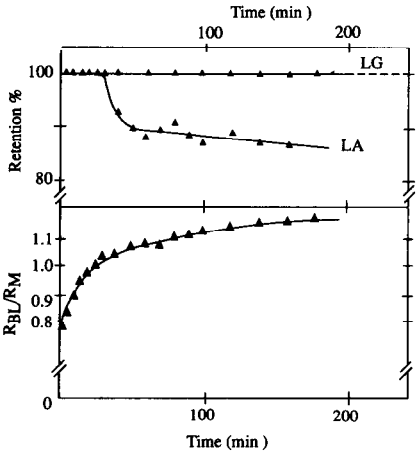

Fig. 5. LA and LG retention ratios (top) and fouling index with an M_1-KPVI membrane (bottom). Phosphate buffer (0.05 *M*); KCl (0.2 *M*); pH 7. $\Delta P = 2 \cdot 10^5$ Pa; $V_L = 4.4$ m s^{-1}; $\tau_w = 75$ Pa; $T = 20$°C.

high ionic strength decreases the fouling index again but is not a basic parameter for retention properties. When the pH is close to the p*I*, the LA retention ratio is increased owing to its increased hydrophobic character and consequently the fouling index increases; the contribution of LA to the build-up of the boundary layer is thus demonstrated.

This new type of membrane involving mixed interactions appears to be promising for the separation of protein mixtures in the most difficult case, *i.e.*, to separate proteins of the same charge.

CONCLUSIONS

The chromatographic experiments with a quaternized hydrophilic polymer coating (QPVI) show that lactalbumin is not retained at pH 7 and at low salt content (0.1 *M*) whereas lactoglobulin is not eluted. Ultrafiltration of these proteins from concentrated whey proteins with this functionalized membrane demonstrates enhanced retention compared with an unmodified membrane. However, the separation selectivity is not improved. The main difference between these two types of experiments is due to the large excess of protein with respect to the membrane sites compared with the low protein/ionic sites ratio in zonal chromatographic elution [40]. Further, in UF, the LA retention is dependent on the LG retention, which agrees with previous conclusions regarding the selectivity of protein separations [4,11–14]. The separation properties of an ultrafiltration membrane are dependent on both protein–membrane and protein–protein interactions. An M_1-QPVI membrane had previously achieved a highly selective extraction of lysozyme with a complete retention of ovalbumin [13,41]. A highly selective extraction can be achieved with strong ionic interactions between the membrane and the proteins to be concentrated, but in the case of the whey protein mixture the difference in interactions is too weak to obtain a highly selective separation.

With a more hydrophobic coating (KPVI), a mixed mode governs the chroma-

tography of LA and LG, as high acetonitrile and salt contents are necessary for eluting these proteins. Ultrafiltration results with this membrane, using aqueous media, show the complete retention of LG and a permeate containing only lactalbumin. Hence, no correlation can be established between UF retention and HPLC capacity factors. Nevertheless, these UF results are more comprehensive judging from the HPLC data. Strong ionic and hydrophobic interactions between proteins (LA and LG) and a modified KPVI membrane are involved in such a way that LA contributes both to complete LG retention and to fouling; the build-up of the boundary layer, composed of protein multilayers, depends on interactions between membrane sites and proteins and/or on the salt used. The boundary layer at the wall of the functionalized membrane is composed of completely retained LG and partially retained LA. The reason for the difference in protein retention is not clear, but perhaps is due to differences in mixed interactions with the KPVI membrane. It should be remembered that the hydrodynamic conditions (not developed here) of the UF process are optimized for this highly selective extraction [23–24]. More detailed HPLC studies with overloaded conditions must be made in order to obtain a better correlation between HPLC and UF runs.

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ABBREVIATIONS AND SYMBOLS

ACN	Acetonitrile
BSA	Bovine serum albumin
c	Concentration
DGEBA	Diglycidyl ether of bisphenol A
ECl	Epichlorohydrin
I	Ionic strength
J	Flux ($l\ h^{-1}\ m^{-2}$)
J_0	water flux before use
k'	Capacity factor
KPVI	Quaternized PVI with DGEBA
LA	Lactalbumin
LG	Lactoglobulin
LYS	Lysozyme
QPVI	Quaternized PVI with ECl
pI	Isoelectric point
PVI	Polyvinylimidazole
R	Membrane hydraulic resistance (m^{-1})
R (%)	Retention ratio
TEA	Triethanolamine
V_L	Tangential velocity of the feed solution ($m\ s^{-1}$)

Greek letters

ΔP	Applied pressure (Pa)
μ	Viscosity (Pa s)
τ	Shear stress (Pa)

Subscripts

a	After ultrafiltration
b	Buffer
BL	Boundary layer
f	After the cleaning procedure
M	Membrane
o	Feed solution
p	Permeate
t	Time
w	Wall

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