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Improved separation of bovine serum albumin and lactoferrin mixtures using charged ultrafiltration membranes

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ABSTRACT

The aim of this work is to assess the isoelectric separation of a binary protein mixture containing bovine serum albumin (BSA) and lactoferrin (LF) using charged ultrafiltration membranes. First, a thorough characterization of the protein properties that could affect their separation, i.e., the zeta potential under different working conditions, the molecular size, and the tendency to form aggregates, was carried out. The influence of the following operational variables on the separation selectivity was then analyzed: (i) BSA/LF initial concentration ratio, (ii) protein isoelectric point (Ip), and (iii) membrane charge using three composite regenerated cellulose (CRC) membranes: negatively charged, positively charged, and unmodified. The results were used to identify conditions that yielded the optimum separation of proteins. Under those conditions, LF was completely retained in the feed mixture, BSA was characterized by an Ip of pH 5.0, and the use of an unmodified membrane yielded a maximum BSA permeation flux of 30.31 g m^{-2} h^{-1}. By contrast, BSA was completely retained by the negatively charged membrane at an LF Ip of pH 9.0, and LF yielded a maximum permeation flux of 1.07 g m^{-2} h^{-1}. This work provides theoretical and experimental insights into the phenomena that influence the membrane separation of mixtures of similarly sized milk proteins. This work also offers a phenomenological explanation of the main features of the separation process.

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1. Introduction

Amino acids, which are supplied by proteins in the diet, are required for the development and maintenance of cells and tissues in an organism. Some proteins play an important role as regulators of physiological processes. Due to the large number of potential applications of protein isolates, quite a few chromatographic processes have been developed for the isolation of high-purity protein fractions [1]. Affinity chromatography is one of the most powerful techniques available for purifying biomolecules in large-scale production and is commonly applied in downstream processes [2]. Compared to resin-based chromatography, membrane separations are simple, energy efficient and readily scalable from the laboratory to industrial settings [3]. Despite the significant effort that has been applied toward developing new materials, conformations, and configurations, the industrial application of this technology has not yet been achieved. The low selectivity and/or flux, especially during the separation of similarly sized proteins, are issues that need further improvement before an industrial application will be viable.

Previous studies of the separation of similarly sized protein mixtures have examined the separation of (i) β-lactoglobulin (β-lg) and α-lactalbumin (α-la) mixtures [4–11], where Chang and Zydney reported a flux of 10.08 L m^{-2} h^{-1} and a selectivity exceeding 55 for β-lg [6]; (ii) lysozyme (LYS)/myoglobin (MYO) [12,13], where Yunos and Field reported a flux of 26.5 L m^{-2} h^{-1} and a selectivity of 12.14 for MYO [14]; and (iii) bovine serum albumin (BSA)/hemoglobin (Hb) mixtures, where Cheng et al. reported a flux of 31.08 L m^{-2} h^{-1} and a total selectivity for BSA [15].

In addition to these well-studied mixtures, several examples of similarly sized protein mixtures have been analyzed by other authors. These systems include (iv) BSA/ovalbumin by Rao and Zydney [16]; (v) BSA/Fab DNA by van Reis et al. [17]; or (vi) cytochrome C/MYO/α-la by Rohani et al. [18]. The last example mixture displayed the best separation performance upon application of the modified CRC UF membranes with the addition of a zwitterionic ligand. On the other hand, the separation of high molecular weight proteins found in natural whey has deserved less attention [19,20]. The best results were obtained by Nyström et al. [19], who reported a flux of 240 L m^{-2} h^{-1} and a selectivity of 2.5 for BSA in a synthetic BSA/LF mixture in which each protein was present in an initial concentration of 0.1 g L^{-1}.

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Nomenclature

A	Hamaker constant (J)
BSA	bovine serum albumin
c_o	concentration (mol m^{-3})
C_p	concentration in the permeate (mol m^{-3})
C_t	concentration in the tank (mol m^{-3})
CRC	composite regenerated cellulose
F	Faraday's constant (96500 C mol^{-1})
Fab DNA	Antigen-binding fragment of DNA
I	ionic strength (mol L^{-1})
Ip	isoelectric point
Hb	hemoglobin
HPLC	high-performance liquid chromatography
LF	bovine lactoferrin
LYS	lysozyme
MWs	molecular weight
MYO	myoglobin
P	pressure (Pa)
Q	quantity of protein in permeate (g)
Q_o	initial quantity of protein (g)
R	gas constant (8.314 J mol^{-1} K^{-1})
R_v	recovery (%)
S_o	observed sieving coefficient (g L^{-1} g^{-1} L)
T	absolute temperature (K)
t	time (h)
T_M	melting point of proteins (°C)
Tr	transmittance (g L^{-1} g^{-1} L)
V	permeate volume (L)
Vd	diavolume volume permeate vs. initial volume (L L^{-1})
V_o	initial volume (L)
WPI	whey protein isolate
z	surface charge of the protein

Greek letters

α-la	alpha lactalbumin
$\alpha_{i/j}$	selectivity, defined as the ratio between the sieving coefficients
β-lg	beta macroglobulin
ε_r	dielectric constant of the fluid (78.5)
ε_o	vacuum electrical permittivity (8.854×10^{-12} C V^{-1} -m^{-1})
κ^{-1}	thickness of the electrical double layer, Debye layer (m)
σ_P	effective surface density (C m^{-2})
μ	viscosity (Pa s)
ζ	zeta potential (V, mV)
z_i	ion valence
Λ	solution conductivity (Ω^{-1} m^{-1})

Milk whey is a mixture of a variety of secreted proteins. The mixture displays a wide range of chemical, physical, and functional properties [21]. Whey proteins have been satisfactorily separated into different fractions (casein and other major and minor proteins) [22–24] as well as the isolation of the major proteins, β-lg and α-la [4–9]; however, the separation of high-value minor proteins of similar size (BSA, LF, and immunoglobulin) remains a scientific challenge. BSA and LF have a high nutritional value because they contain essential amino acids and are easily digested. BSA is a 66.5 kDa protein with an isoelectric point (Ip) approaching 5.0 and has foaming and gelling properties. Its concentration in milk is 0.4 g L^{-1}, on average. LF is a 78 kDa iron-binding protein with an Ip around 9.0. LF has important nutraceutical, anti-inflammatory, and antimicrobial properties, it plays an important role in iron metabolism [25], and it is present in a concentration of 0.1 g L^{-1} in milk. LF and BSA have comparable molecular weights (MWs). Both proteins tend to prevent foam formation at their isoelectric points by reducing the surface tensions [26], which makes their separation even more difficult.

In this work, the separation of a BSA/LF target mixture was examined by diafiltration using 100 kDa composite regenerated cellulose (CRC) ultrafiltration membranes. The protein zeta potentials, sizes, and charges were determined, and an experimental study was conducted to examine the influence of the operation variables (protein initial concentration ratio, pH, and membrane charge, i.e., negatively charged, positively charged, or uncharged membrane) on the permeation flux and selectivity during the separation of model LF-BSA mixtures. A comparison of the results to the literature data revealed an improvement in the separation process.

2. Materials and methods

2.1. Protein solutions

Experiments were performed using mixtures of native BSA (Catalog A-6003 Sigma Chemical, Spain) and native LF (NutriScience Innovations, USA). BSA is readily soluble in water and requires high concentrations of neutral salts, such as ammonium sulfate, to induce precipitation. Bovine LF is highly soluble in water (2%, 20 °C) and has an Fe^{3+} content of 3.0 mg/100 g protein. The isoelectric point of BSA is close to 4.9, its molecular weight is 66.5 kDa, and the protein shape is a prolate ellipsoid with dimensions of 14 × 3.8 × 3.8 nm [27]. LF has an isoelectric point around 9.0, a molecular weight of 78 kDa, and a globular shape with dimensions of 4.0 × 5.1 × 7.1 nm, as determined using the lattice cell parameter data [28,29].

Protein solutions were prepared by adding the protein powder to the desired buffer solution, pH 5.0 (Acetate) or pH 9.0 (borax/hydrochloric acid (Fluka, Spain)). The solutions were stored in the refrigerator until complete dissolution had been achieved. Shaking was avoided to prevent foam formation, which can seriously interfere with analytical procedure. The solutions were analyzed only after any foam had naturally dissipated.

Protein solutions were used within the subsequent 24 h to minimize the likelihood of protein aggregation. All experiments were performed at ambient temperature, and the samples were stored in the refrigerator (the melting points, T_M, of both proteins were around 65 °C [30–32]).

2.2. Protein analysis and characterization

Both proteins in the binary mixture were quantitatively analyzed using high performance liquid chromatography (HPLC, according to the method of Adam et al. [33]) using an HPLC Waters 2690 Separation Module (Waters) fitted with a CIMac™-SO_3 analytical column (BiaSeparations) and a Waters 996 diode array spectroscopic detector with a wavelength range of 210–400 nm (UV, Waters). The method was sensitive to protein concentrations below 0.03 g/L, and the detection limit was still lower.

The separation process was analyzed by characterizing the protein physicochemical properties using a Zetasizer Nano ZS (Malvern Instruments) [34,35]. The charges on the proteins in the two buffer solutions were first determined by phase analysis

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