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Preparative ion-exchange chromatography of proteins from dairy whey

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Abstract

A preparative-scale ion-exchange chromatographic process is described for the separation of the four major proteins and lactose from sweet dairy whey. Experiments using a commercial anion-exchange resin were carried out to determine the optimum conditions for initially separating the proteins α-lactalbumin, β-lactoglobulin, bovine serum albumin, immunoglobulin G and lactose from a sweet dairy whey mixture. The separation was accomplished with simultaneous step elution changes in salt concentration and pH. It was found that the anion-exchange step was most effective in separating β-lactoglobulin from the feed mixture. Following the anion-exchange separation, its breakthrough curve was processed using a commercial cation-exchange resin to further recover the valuable immunoglobulin G. The whey output from an east Tennessee cheese manufacturer was used as a feedstream for the preparative scale experiments and as a reference in scaling to an economically optimized production level operation.

Keywords: Preparative chromatography; Proteins; Whey

1. Introduction

Several different types of liquid chromatography are utilized industrially for separation and purification of multicomponent protein mixtures. These include size exclusion, ion-exchange, adsorption and affinity chromatographic processes. Within the last decade or so there has been increasing interest in these liquid chromatographic processes because of the growing biotechnology industry and needs from the pharmaceutical and specialty chemical industries for highly specific and efficient separation methods. This has driven much research to investigate the fundamentals of preparative chromatography and the important issue of scale up. This research needs to continue with specific research into industrially significant problems like multicomponent protein mixtures separation and purification.

An example of a multicomponent protein mixture containing proteins of commercial value is dairy whey. Dairy whey is characterized as the water-like liquid or serum that separates from the curds during cheese manufacturing [1]. There are two types of whey: sweet and acid. Acid whey is obtained from the manufacturing of cheese in which the caseins or principal milk proteins are removed from the milk by precipitation at a pH of 4.6. The proteins and other compounds remaining in the water-like liquid at this pH have a specific composition and the whey is

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characterized as acidic. Sweet whey is produced when the caseins are enzymatically coagulated using rennet type enzymes at a pH of 5.9 to 6.3 [2]. By far of the 1.18×10^{11} kg of whey produced as a cheese manufacturing by-product worldwide in 1997, nearly all of it was sweet whey. The amounts of whey produced worldwide increases each year by approximately 3% (w/w).

In general whey is a dilute liquid composed of lactose, a variety of proteins, minerals, vitamins and fat. Whey contains about 6% (w/w) solids of which 70% (w/w) or more is lactose and about 0.7% (w/w) is proteins [3]. Whey is a liquid mixture composed of a variety of these chemical compounds but unfortunately low in concentration. Because of this whey has long been considered a waste product of dairy operations rather than a by-product. This very fact has created the problem of utilizing whey for as long as the major milk proteins have been commercially used. This problem can be further grasped by considering that in the manufacture of cheeses or casein products only 10 to 20% (w/w) of the raw milk is utilized to obtain the final product; 80 to 90% (w/w) of the raw milk yields whey as a waste product [4]. Thus the problem of whey utilization is that a tremendous amount of whey is produced worldwide each year that is composed of valuable proteins and other biochemicals and yet is very dilute in concentration. There are three main types of whey utilization: animal feedstocks, lactose production and the production of whey powders and individual whey proteins [4]. The production of individual whey proteins and whey proteins concentrates was the focus of this project.

1.1. Available processes for whey proteins separation

The separation and purification of whey proteins on an industrial scale has for a long time presented a research problem to the dairy industry. Because of the high value placed on the individual proteins in a purified state, many different separation schemes have been utilized worldwide. Originally precipitation techniques were used with chemical complexes or other reagents used to form stable complexes. Today membrane technology along with chromatographic processes are utilized. Of course the nature of the desired product determines the separation method to be used. To produce whey protein concentrates containing 35–75% (w/w) whey proteins, a variety of techniques have been developed and utilized industrially.

Ultrafiltration is the most widely used commercial method of whey fractionation offering considerable diversity in manufacturing a wide range of whey protein concentrates. It would be employed after an initial chromatographic processing step to further separate the whey proteins. However, it suffers from the drawback of high capital and operating costs, membrane fouling, incomplete removal of low-molecular-mass solutes thereby requiring in-place cleaning and sanitation of the membrane to minimize microbial problems, and the generation of large permeate volumes – the low protein content stream [5]. The process is employed when the molecular mass cutoff point for solutes from proteins is 500 or greater. Below a molecular mass of 500 reverse osmosis is used [4]. Reverse osmosis is usually used in conjunction with ultrafiltration or gel filtration for the concentration and partial demineralization of whey up to 28% (w/w) dry matter and when small increases in the dry matter content of the whey are required [4]. The drawbacks associated with ultrafiltration apply as well to reverse osmosis. It was reported that in 1987 $100\cdot10^6$ lbs of whey protein concentrate were produced in the USA by ultrafiltration and this has certainly increased since then [2] (1 lb=0.454 kg). The primary reason for the success of ultrafiltration is that membranes have been developed that can tolerate the cleaning compounds and temperatures utilized for sanitation [2].

Microfiltration is a relatively new whey processing technique. In this process the pores of the membrane are slightly larger than those of ultrafiltration membranes. The pore size used, 1.4 or 0.8 μm, allows nearly complete exclusion of microorganisms, thereby enabling an in-place sterilization of the membrane without heat treatment [6]. This is important because it can change industry perception of whey, i.e., the fact that other membrane processes needed in-place cleaning methods thereby introducing foreign chemicals into the whey. Also, microfiltration permits whey from a variety of cheese sources to be pooled and it allows a uniform whey feedstock to be

generated in manufacturing whey protein concentrates. Microfiltration is also valuable in removal of residual lipid materials from whey. This is important because this material will concentrate with the main whey proteins thereby limiting their achievable content to about 75% (w/w) [6]. Finally, microfiltration must be used in combination with ultrafiltration and fat or cream removal methods to further enrich the proteins [4].

Chromatographic methods, particularly ion-exchange adsorption processes, for whey proteins separation and whey protein concentrates production, have been developed and utilized successfully on a commercial scale. The Vistec process uses a cellulose-based exchanger in a stirred tank reactor. The process, utilized by the Davisco Co. of LeSuer, MN, USA, involves pH adjustments of the whey and sequential elution of lactose and proteins. Subsequent ultrafiltration, evaporation and spray drying yield a whey protein concentrate containing 95% (w/w) protein content [7]. Because of the proprietary nature of the process, the exchanger resin and other process details are not available.

Another chromatographic process, the Spherosil process, uses Spherosil S and QMA resins. The process is capable of handling acidic and sweet whey. It was reported that this process yields a whey protein concentrate with only 60–65% (w/w) protein content and a high ash content, up to 20% (w/w). It was also reported that the concentrates displayed poor protein functionality and solubility, making it unattractive for further industrial use [5]. Problems with ion-exchange processes in general are the production of large volumes of rinse, chemical solutions and deproteinized whey that must be treated and disposed of. These solutions can become up to two and a half times the volume of the original whey feed. Also, the dilute eluate protein fractions require concentration by ultrafiltration and spray drying.

One promising new technology that attempts to overcome the inherent problems of ion-exchange chromatography is ion-exchange membranes. In this process the whey is passed through a membrane unit containing micron-sized pores with ion-exchange groups appended to the membrane surface. Operated in a cyclic manner, this process incorporates the desirable features of membranes and ion-exchange resins. The diffusional limitations of ion-exchange processes are negligible because the whey is passing through the membrane by convective means [8]. Possible drawbacks of the process are fouling of the membrane, high capital costs and difficulty in scaling up.

With this background of processes available, a preparative chromatography study was conducted to investigate the performance of Pharmacia Biotech's process-grade Q- and SP-Sepharose Big Beads resins for the initial separation of the four major whey proteins from sweet whey: α-lactalbumin, β-lactoglobulin, bovine serum albumin (BSA), immunoglobulin G (IgG) and lactose. The goal of the project was to separate these components into individual peak fractions and then scale up the process in an economically optimized manner. The resulting peak fractions would then have to be further processed by ultrafiltration and spray drying to produce individual proteins or whey protein concentrates, depending on the economics of the scaled up process. It was not an objective of this study to investigate the necessary ultrafiltration and spray drying operations of the overall process. These issues are left to future studies.

2. Experimental

2.1. Whey proteins system, reagents

To conduct these studies an actual dairy whey protein system was utilized. A dairy whey stream produced at the Dairyman, Inc. plant in Greeneville, TN, USA was used for this project. This plant produces cheddar cheese products and consequently approximately $127 \cdot 10^6$ lbs per year of sweet whey.

After the cheese making process produces the sweet whey, it is further processed by reverse osmosis to increase the solids content from approximately 5.5% (w/w) to 14.6% (w/w). This is carried out in order to facilitate economical transportation of the whey to traditional end users. These include land spreading of the whey for feed at poultry farms or selling it to pet food manufacturers, depending on market demand for the whey. Samples of the sweet whey used in the project were taken after the reverse

osmosis step in order to take advantage of the increase in solids content.

Samples of the whey stream as well as chromatographic peak fractions were collected and analyzed for lactose, proteins and minerals content. An analysis of the whey feed is shown in Table 1.

All chemical reagents used in this study for the preparation of buffers and cleaning agents were food and/or pharmaceutical grade in quality, depending on availability. The reason for this was to minimize the introduction of impurities into the preparative system and subsequently into the resolved chromatogram peaks. On the proposed larger scale of operation, food and/or pharmaceutical grade quality reagents would also be specified.

Sodium acetate, used in the preparation of the salt step elution buffers for both ion-exchange processes, was purchased from Fisher Scientific with a purity rating of U.S.P./N.F. Glacial acetic acid, used in the pH adjustment of the various buffer solutions and in the cleaning portion of the ion-exchange process cycles to lower the column pH, was purchased from J.T. Baker with a purity rating of U.S.P./F.C.C. Sodium hydroxide, used in the cleaning portion of the ion-exchange process cycles, was purchased from J.T. Baker with a purity rating of N.F./F.C.C.

Before a set of column experiments was conducted, 20-l batches of each buffer and cleaning cycle component were prepared and degassed for at least one half hour with high-purity helium prior to use. The water used to prepare the buffers was from a laboratory deionized water supply unit.

Table 1
Composition of Greenville, TN dairy plant sweet whey after reverse osmosis processing in % (w/w)

Parameter, component	% (w/w) value
α-Lactalbumin	0.30
β-Lactoglobulin	1.31
BSA	0.038
IgG	0.11
Lactose	11.03
Other protein	0.15
Total protein	1.91
Ash	1.37
Fat	0.21
Sodium	0.088
pH	5.8
Total solids	14.61
Density (g/ml)	1.057

The analysis of the raw sweet whey and chromatogram peak fractions, upon determination of best resolution visually, was conducted by Kendrick Labs. (Madison, WI, USA). The samples were analyzed by two-dimensional electrophoresis to quantitate the individual and total proteins content. Silliker Labs (Stone Mountain, GA, USA) analyzed the samples for lactose, mineral and total solids content and density. These laboratories were selected based on recommendations from the dairy products industry.

2.2. Equipment

The centerpiece of this research project was a preparative chromatography apparatus constructed at the Chemical Technology Division at Oak Ridge National Laboratory, Oak Ridge, TN, USA. The apparatus consists of the preparative columns used, 20-l Nalgene polypropylene tanks for feed, buffers, cleaning solutions and fractions collection, a pumping system, associated piping, a diode array ultraviolet spectrophotometer and a pH meter, both in-line. A schematic diagram of the experimental apparatus is shown in Fig. 1.

Stainless steel tubing was used for all process piping, except for the connections between the tanks and the main supply line, which were thick wall, reinforced polyethylene, for viewing fluid flow. The pump for the apparatus was a Milroyal Model DC-1-175R capable of pumping up to 140 ml/min. A pressure gauge was placed in-line upstream of the column to monitor the back pressure across the column.

The ion-exchange column used was a Pharmacia Biotech Model XK 50/30 laboratory column. It had a diameter of 5 cm and an overall length of 30 cm, 25 cm of which was used for packing. Eluate from the column was passed immediately through a pH probe, Model G-05662-90, a sealed Ag/AgCl probe with a stainless steel cell, Cole-Parmer Model G-05662-50, and detachable cable, Model G-05662-57 with a US standard connector. All three items were from Cole-Parmer (Niles, IL, USA). The output from the pH probe was sent to an Orion research Model 701A digital ionalyzer for visual digital display of

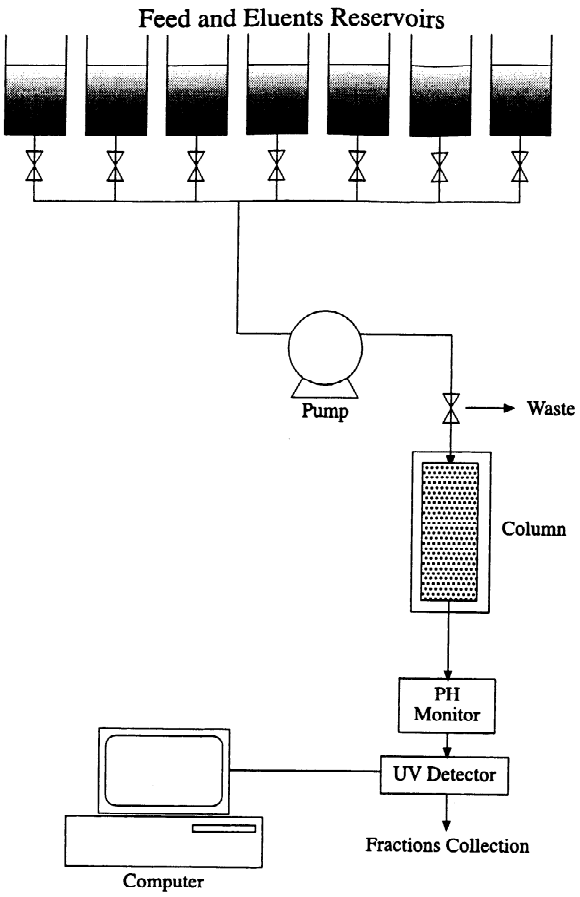

Fig. 1. Schematic diagram of chromatographic set-up.

the eluate pH. After the eluate passed through the pH probe, it immediately proceeded through a Hewlett-Packard HP8452A diode array UV spectrophotometer and its flow cell, Hewlett Packard model P 5061-3397. The spectrophotometer's software package operated on a Sony Trinitron CPD-1430 multiscan HG monitor. The spectrophotometer was operated at a wavelength of 280 nm.

The chromatographic resins chosen for this study were Pharmacia's Q- and S-Sepharose anion- and cation-exchange resins. These resins are composed of a highly cross-linked agarose matrix with the ion-exchange groups chemically attached to it through ether bonds, with a nominal cross-linkage of 6%. The resins are referred to as macroporous type and possess strong anion- and cation-exchange functionalities. That is, they maintain their charge capacities over a wide range of pH. The resins have a nominal particle size of 100–300 μm.

2.3. Research approach

The research approach that was taken in this study was to develop efficient and economical chromatographic processes for separating the lactose and proteins from the whey. In general the research involved laboratory scale fixed-bed studies to determine the chromatographic separation performance of the process-grade anion-exchange resin, Q-Sepharose Big Beads. The technique of anion-exchange chromatography was chosen as the initial step to separate the proteins from the whey and one another due to their differences in isoelectric points. The isoelectric point, p*I*, is the pH of the solution at which the net charge on the protein is zero. The isoelectric points and molecular masses for the four proteins of interest in the study are shown in Table 2. If a protein of interest has a p*I* above the local solution pH, the protein will bind to a cation resin. If the protein has a p*I* below that of the local solution pH, it will bind to an anion resin.

Following this step, the breakthrough curve from the anion-exchange step was further processed by cation-exchange chromatography using the process-grade cation-exchange resin SP-Sepharose Big Beads. The purpose of this step was to separate the valuable protein IgG from the other components of the anion-exchange breakthrough curve.

To develop efficient anion- and cation-exchange processes, experiments were conducted at several flow-rates and column volume loadings of feed and eluents. The techniques of pH, salt and simultaneous pH and salt step gradient elution were investigated to determine which one yielded the greatest resolution

Table 2
Molecular masses and isoelectric points for whey proteins

Protein	M_r	p*I*
α-Lactalbumin	14 000	4.2–4.5
β-Lactoglobulin	18 300	5.35–5.49
BSA	69 000	5.13
IgG	160 000–1 000 000	5.5–8.3

for the peaks at a given flow-rate. Due to a maximum pressure limit of 42 p.s.i. on the resin, the highest flow-rate permissible was 50 ml/min (1 p.s.i.=6894.76 Pa).

2.3.1. Cleaning of ion-exchange column

For both the anion- and cation-exchange processes it was necessary to incorporate a column cleaning method into the respective cycle to remove any unbound matter from the column and to prepare the column's fixed charge groups ionically for the start of the next process cycle. For both the anion- and cation-exchange cycles the cleaning steps were identical and implemented after each experiment run.

The cleaning method consisted of passing one column volume (490 ml) of 1 *M* sodium hydroxide followed by one column volume of deionized water to wash out residual sodium hydroxide. After this two and a half column volumes of 0.1 *M* acetic acid was passed through to lower the pH in the eluate. This was followed by one column volume of deionized water and finally two column volumes of the starting state buffer, 0.01 *M* sodium acetate, pH 5.8, was passed through the column to adjust the ionic strength in the column. The pH of the whey feed taken from the dairy plant was 5.8 with surprising frequency over the course of many months of experimentation and did not have to be adjusted by acid or base addition prior to loading onto the column.

2.3.2. Anion-exchange cycle for column

The strategy for the anion-exchange separation was to develop an elution schedule that would bind the proteins α-lactalbumin, β-lactoglobulin and BSA when loaded onto the column and yet allow the IgG to pass through the column non-adsorbed during the loading stage. The IgG would then end up in the breakthrough curve and would be subsequently recovered in a cation-exchange step. At the flow-rate of 50 ml/min, it was found that one column volume of whey feed, 490 ml, was optimum for obtaining a well-resolved chromatogram.

The elution schedule for the anion-exchange process is shown in Table 3. It was found that two step changes, simultaneous in pH and salt concentration were necessary to carry out the anion-exchange separation. A 0.01 *M* sodium acetate buffer, pH 5.8, was used for the starting state or feed loading buffer. After the whey feed was loaded onto the column, one column volume of this buffer was passed through to wash out any material that did not bind to the resin, including the IgG. Next, two column volumes of 0.05 *M* sodium acetate, pH 5.0, were passed through the column to desorb those proteins whose p*I* values were above 5.0. This includes the β-lactoglobulin and BSA. This was then followed by two column volumes of 0.1 *M* sodium acetate, pH 4.0, to finally desorb the α-lactalbumin whose p*I* range is 4.2–4.5, and thus above that of the passing pH wave of 4.0. After this second step change, the cleaning cycle was then implemented to prepare the column for the next run.

2.3.3. Cation-exchange cycle for column

The elution schedule for the cation-exchange process is shown in Table 4. It was found that one step change in pH was appropriate to carry out the cation-exchange separation. The buffer used was 0.05 *M* sodium acetate, pH 5.5, as the starting state

Table 3
Elution schedule for the anion-exchange process

Process step	No. of column volumes	Time (min)
Whey feed, pH 5.8	1	9.8
0.01 *M* NaOAC, pH 5.8	1	9.8
0.05 *M* NaOAc, pH 5.0	2	19.6
0.1 *M* NaOAc, pH 4.0	2	19.6
1 *M* NaOH	1	9.8
Deionized water	1	9.8
0.1 *M* HOAc	2.5	24.5
0.01 *M* NaOAc, pH 5.8	2	19.6

One column volume is 490 ml.

Table 4
Elution schedule for the cation-exchange process

Process step	No. of column volumes	Time (min)
Anion breakthrough curve	1	9.8
Fraction feed, pH 5.5		
0.05 *M* NaOAC, pH 5.5	1	9.8
0.05 *M* NaOAc, pH 8.5	2	19.6
1 *M* NaOH	1	9.8
Deionized water	1	9.8
0.1 *M* HOAc	2.5	24.5
0.05 *M* NaOAc, pH 5.5	2	19.6

One column volume is 490 ml.

or feed loading buffer. Through a series of experiments it was found that one column volume loading of the anion-exchange breakthrough curve fraction was optimum for loading onto the cation-exchange column. After the anion-exchange breakthrough curve fraction was loaded onto the column, one column volume of the initial buffer was passed through to wash out any material that did not bind to the resin. Next a step change in pH was implemented to elute the bound IgG. This was accomplished by passing two column volumes of the buffer, 0.05 *M* sodium acetate, pH 8.5. As the pH wave of this buffer passed through the cation bed it initiated the elution of the IgG because the upper value of its p*I* range is 8.3. After this pH step change the cleaning cycle was then implemented.

3. Results and discussion

The chromatograms resulting from the optimization of the anion- and cation-exchange process cycles are shown in Figs. 2 and 3, respectively. The analysis results of the respective peaks in each chromatogram are shown in Table 5 for the anion process and Table 6 for the cation process. As is seen from these chromatograms, very good resolution was achieved between the different peaks for each process. From the analysis tables it is seen that for the anion-exchange process, peak 2 is the most successful in terms of purity and recovery of protein. In this peak the β-lactoglobulin is obtained at a mass fraction 49.9% (w/w) with a recovery of 93.4% (w/w) of the protein that was originally in the feed.

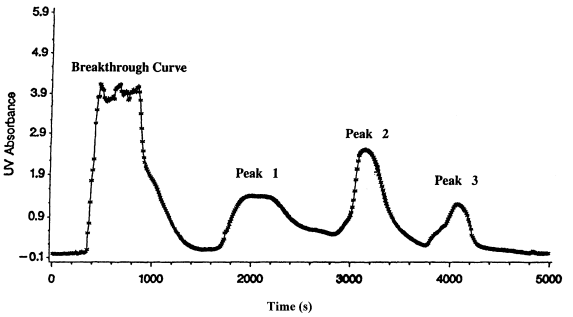

Fig. 2. Preparative anion-exchange chromatogram. One column volume feed, 50 ml/min. Pharmacia XK 50/30 column: 30×5 cm. Buffers: 0.01 *M* NaOAc, pH 5.8; 0.05 *M* NaOAc, pH 5.0; 0.1 *M* NaOAc, pH 4.0.

As discussed earlier, the anion breakthrough curve is subsequently processed later via cation-exchange to recover the IgG. Peak 1 of the anion-exchange chromatogram is a mixture of all four proteins of interest and would have to be further processed by ultrafiltration and spray drying to obtain a saleable product in the form of a whey protein concentrate with a protein concentration of 35–75% (w/w). All of the lactose in the whey feed ended up in the anion-exchange breakthrough curve.

The cation-exchange process was found to only be partially successful at transferring the IgG into its own peak. It was found that 83.1% of the IgG in the feed to the cation process went into the cation-exchange breakthrough fraction and only 16.9% of it went into peak 4. Future studies should investigate how to increase the transfer of the IgG into peak 4, perhaps by a change of cation-exchange resin and

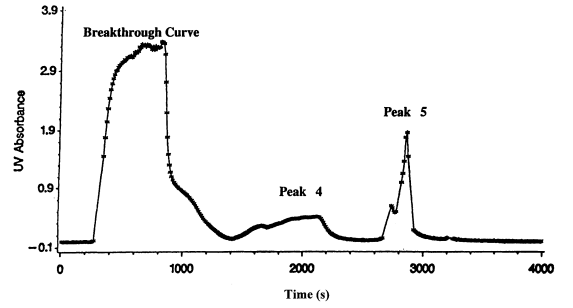

Fig. 3. Preparative cation-exchange chromatogram. One column volume of breakthrough curve from anion-exchange, 50 ml/min. Pharmacia XK 50/30 column: 30×5 cm. Buffer system: 0.05 *M* NaOAc, pH 5.5; 0.05 *M* NaOAc, pH 8.5.

Table 5
Composition of peaks for one column volume loading of whey feed to the anion-exchange column

	α-Lactalbumin	β-Lactoglobulin	BSA	IgG	Lactose
Fraction	Feed	Feed	Feed	Feed	Feed
Fraction (%, w/w)	1.68	7.31	0.40	0.82	9.72
Loaded (g)	1.4	6.1	0.33	0.69	51.051
Fraction	Breakthrough	Breakthrough	Breakthrough	Breakthrough	Breakthrough
Fraction (%, w/w)	1.07	0.45	0.31	0.78	61.60
Recovery (%)	63.6	6.066	78.8	94.2	100.0
g	0.89	0.37	0.26	0.65	51.051
Fraction	Peak 1	Peak 1	Peak 1	Peak 1	Peak 1
Fraction (%, w/w)	10.91	0.64	0.16	0.86	0.0
Recovery (%)	36.43	0.49	2.2	5.80	0.0
g	0.51	0.03	0.0073	0.04	0.0
Fraction	Peak 2	Peak 2	Peak 2	Peak 2	Peak 2
Fraction (%, w/w)	–	49.9	0.55	–	–
Recovery (%)	–	93.4	19.0	–	–
g	–	5.7	0.063	–	–

operating conditions, but this was not investigated further. Peaks 3 and 5, of the anion- and cation-exchange chromatograms, respectively, were found to contain ash, fat and other minor components not of interest. These components, while of no value, did nonetheless bind to the resins and were removed only as a result of the cleaning step of each process cycle.

For the peaks of commercial value, final processing steps of ultrafiltration and spray drying would be required to obtain purified whey protein concentrates or proteins as in peak 2 for β-lactoglobulin and the cation-exchange breakthrough curve and peak 4 for the IgG. The cation-exchange breakthrough curve and peak 4 would not be spray dried as the IgG denatures at the temperatures employed in spray drying. Rather the protein would be sold as a liquid product. Inspection of the anion-exchange chromato-

Table 6
Composition of peaks for one column volume loading of anion-exchange breakthrough curve to the cation-exchange column

	α-Lactalbumin	β-Lactoglobulin	BSA	IgG	Lactose
Fraction	Feed	Feed	Feed	Feed	Feed
Fraction (%, w/w)	1.12	0.46	0.42	0.82	57.99
Loaded (g)	0.89	0.37	0.26	0.65	51.051
Fraction	Breakthrough	Breakthrough	Breakthrough	Breakthrough	Breakthrough
Fraction (%, w/w)	1.32	1.58	0.26	1.29	0.17
Recovery (%)	61.8	97.3	42.3	83.1	14.3
g	0.55	0.36	0.11	0.54	7.30
Fraction	Peak 4	Peak 4	Peak 4	Peak 4	Peak 4
Fraction (%, w/w)	0.0	0.018	0.0	2.62	0.0
Recovery (%)	38.2	2.7	57.7	16.9	85.7
g	0.34	0.01	0.15	0.11	43.75

gram reveals that the elution order of the α-lactalbumin and β-lactoglobulin is reversed in comparison to theory. The α-lactalbumin eluted first when it should have eluted after the β-lactoglobulin according to their respective isoelectric points. The reason for this reversal could be due to incomplete binding of the α-lactalbumin due to competition from the other minor components in the whey feed and a greater selectivity of the resin for the β-lactoglobulin. This reversal did not affect the resulting resolution of these two proteins.

3.1. Scale-up of preparative experiments

The operating conditions for the optimized anion- and cation-exchange preparative chromatography cycles were scaled-up as part of an economic optimization to a proposed production-level operation. This economic optimization was conducted using actual economic operating data from the dairy plant. Details of the economic optimization will not be discussed here but can be obtained from the author.

To carry out the scale-up calculations it was necessary to know which mass transfer resistance was controlling each ion-exchange process. Typically intraparticle diffusion is the controlling mass transfer resistance for protein chromatography. To check this hypothesis for the process being investigated, the number of external film mass transfer and particle diffusion transfer units, N_f and N_p, were calculated, as discussed in Ref. [9]. The equations for calculating these are:

$$N_f = 3(1-\epsilon_b)k_f Z/(R_p u) \quad (1)$$

$$N_p = 3(1-\epsilon_b)D_e Z/(R_p^2 u) \quad (2)$$

Here ϵ_b is the bed void fraction, k_f is the fluid phase mass transfer coefficient, cm/s, Z is the packed column length, cm, R_p is the radius of the ion-exchange beads in cm, u is the superficial velocity in cm/s, and D_e is the effective intraparticle diffusivity, cm^2/s. Values for all the parameters were readily obtained from experimental conditions except for k_f and D_e. k_f was calculated according to [10]:

$$k_f = \frac{1.09u(D_i)^{2/3}}{(2R_p u\epsilon_b)^{2/3}} \quad (3)$$

where D_i is the molecular diffusion coefficient for a component of interest. For this parameter, the molecular diffusion coefficient of β-lactoglobulin was used with a value of $3.4\cdot10^{-7}$ cm^2/s [11]. D_e was calculated from the correlation provided by Ref. [12]:

$$D_e/D_{solution} = \frac{1-1.80M_r^{0.33}}{\frac{R_{pore}}{2.02}} \quad (4)$$

where $D_{solution}$ is the diffusion coefficient of the protein of interest in solution, M_r is the molecular mass of the protein of interest and R_{pore} is the radius of the pores of the ion-exchange resin. This correlation was used assuming that surface diffusion was not a contributing component to the effective diffusion coefficient, which can be a function of both pore and surface diffusion contributions. The radius of the pores was specified as 200 Å by the manufacturer (Pharmacia Biotech, Uppsala, Sweden). The bed void fraction, ϵ_b, was determined in the usual manner by passing a pulse of 1.0 g/l Blue Dextran through the resin bed. Blue Dextran is a large polysaccharide with a molecular mass of 2 000 000. This solute is excluded from the resin pores due to its size. A value of 0.40 was obtained from this experiment.

The reason that β-lactoglobulin was chosen as the protein of interest for the parameters requiring a specific protein transport property was that it was the most separated and resolved protein in the preparative studies.

According to Ref. [13], if the number of particle diffusion mass transfer units is less than the number of external film mass transfer units, then intraparticle diffusion is the controlling resistance. In this case scale-up can be accomplished by maintaining the column bed length, superficial velocity and particle size [13].

Indeed, for the anion-exchange process, the number of particle diffusion mass transfer units was calculated to be 19, while the number of external film mass transfer units was 202. Therefore, scale-up, as part of the economic optimization, was carried out by maintaining the preparative column length, the superficial velocity and the particle size constant.

The results of the economic optimization revealed that the most economically favorable scale-up situation is to process 22.5% (w/w) or 28 575 000 lbs per year of the $130 \cdot 10^6$ lbs per year of whey produced at the Greeneville, TN cheese plant by the anion-exchange process. To accommodate this processing requirement a total of nine anion-exchange columns were required, which would run simultaneously with an 85% operating schedule yearly. For this processing situation the anion-exchange breakthrough curve is not processed further by cation-exchange chromatography due to uncertainty about the market selling price of the IgG for the amount being produced in this scaled-up design. In the future if the pricing data and demand for this protein would become more well defined, then its processing by cation-exchange chromatography could be considered in the economic optimization.

Each column is commercially available with a scaled-up size of 140 cm in diameter and 30 cm in length. For this processing situation the anion-exchange breakthrough curve fraction and peak 1 is to be further processed by ultrafiltration and spray drying to produce saleable whey protein concentrates. Peak 2 is also to be further processed by ultrafiltration and spray drying to produce a saleable product in the β-lactoglobulin.

4. Conclusions

This research project has investigated the preparative chromatographic separation of the four major proteins and lactose from sweet dairy whey. The types of chromatography investigated were preparative anion- and cation-exchange chromatography. Following the experimental portion of the project to determine the optimum combination of these chromatographic methods and the processing cycle steps required, a detailed economic optimization was conducted to scale-up the preparative results to a production-scale operation. For all experiments and as a basis for the economic optimization, sweet dairy whey from the Dairyman, Inc., Greeneville, TN, USA dairy plant was utilized. From the results of the experimental program and the optimization studies, several conclusion can be made:

1. The direct processing of sweet whey by anion-exchange chromatography through simultaneous pH and salt step elution techniques is effective for partially separating the four major dairy whey proteins and lactose from the whey itself. Furthermore, it is effective in partially separating the β-lactoglobulin from the other proteins into its own peak.
2. It was found that the processing of the anion-exchange breakthrough curve fraction by cation-exchange chromatography and pH step elution techniques is partially effective for separating a portion of the IgG from this fraction into its own peak.
3. The fractions which contain all of the proteins of interest to some degree would require further processing by ultrafiltration and spray drying to produce powdered products high in protein concentration and lactose free, before they could be sold in the marketplace. Whether whey protein concentrates or individual protein products would be produced depends on the performance of the ultrafiltration and spray drying unit operations and the economics of the operation. The exception to this is the IgG in the cation-exchange breakthrough curve and peak 4 which cannot tolerate spray drying due to denaturation by heat.
4. Due to the number of steps required in both ion-exchange processes, long cycle times are required. Any modifications to the processing cycles which can reduce the time required without sacrificing performance will improve the economic picture of the scaled-up plant. The issues of the lifetime of each resin and the necessary frequency of implementing the cleaning portion of each process cycle should be investigated in future studies to determine how these impact the total time for each process cycle.
5. The economic optimization revealed one favorable processing option which could merit further attention and this is the anion-exchange processing of the sweet whey.

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