

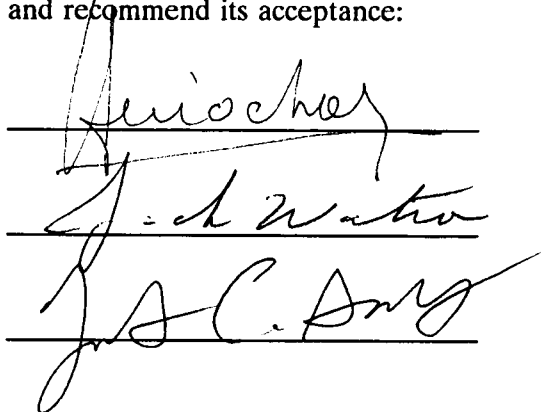
To the Graduate Council:

I am submitting herewith a dissertation written by Steven Jay Gerberding entitled "Preparative Ion Exchange Chromatography of Dairy Whey Proteins". I have examined the final copy of this dissertation for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Doctor of Philosophy, with a major in Chemical Engineering.



Dr. Charles H. Byers, Major Professor

We have read this dissertation
and recommend its acceptance:



Accepted for the Council:



Associate Vice Chancellor and
Dean of The Graduate School

**PREPARATIVE ION EXCHANGE CHROMATOGRAPHY
OF PROTEINS FROM DAIRY WHEY**

A dissertation

Presented for the

Doctor of Philosophy

Degree

The University of Tennessee, Knoxville

Steven Jay Gerberding

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ABSTRACT

The purpose of this work was to develop preparative liquid chromatographic methods for the separation of the four major proteins and lactose from dairy whey. Experiments using a new commercial anion exchange resin were carried out to determine the optimum conditions for initially separating the proteins alpha lactalbumin, beta lactoglobulin, bovine serum albumin, and immunoglobulin G 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 beta lactoglobulin from the feed mixture.

Following the anion exchange separation, its breakthrough curve was processed using a new commercial cation exchange resin to further recover the valuable immunoglobulin G. The whey output from an east Tennessee cheese manufacturer was used as a feedstock for the preparative scale experiments and as a reference in scaling to an economically optimized production level operation.

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LIST OF SYMBOLS

a	interfacial area, cm^2/cm^3
a	Langmuir constant, g/liter, cm^3/g
a	surface area of resin per unit bed volume, cm^{-1}
$\tilde{a}_k$	parameter defined by eq 3.41, dimensionless
B	parameter defined in eq 3.64, dimensionless
Bi	Biot number, $k_f R_p / D_e$, dimensionless
$\tilde{b}_k$	parameter defined by eq 3.42, dimensionless
b	Langmuir constant, liter/g, cm^3/g
C_h	capacity factor defined in eq 3.63, dimensionless
C	fluid phase protein concentration, g/liter
C_i^M	maximum elution peak concentration of component i, g/liter, g/cm^3
C_T	exchanger capacity, [M(solid)]
c	protein phase concentration, dimensionless
c	solid phase concentration of specie, dimensionless
c^∞	adsorbate saturation capacity, dimensionless
D	molecular diffusion coefficient, cm^2/s
D	column diameter, cm^2
D	depreciation per year
D_e	effective diffusion coefficient, cm^2/s
D_{eff}	effective dispersion coefficient, cm^2/s
D_p	effective pore diffusion coefficient, cm^2/s
D_s	effective surface diffusion coefficient, cm^2/s
D_z	axial dispersion coefficient, cm^2/s
d_p	particle diameter, cm
d_i	impeller diameter, cm
d_v	diameter of stirred batch vessel, cm
F	mass transfer driving force operator notation, g/liter
G_i	Gunn axial dispersion correlation operator eqn. 3.29, dimensionless
H	height of a theoretical plate, cm
h	reduced height of a theoretical plate, H/d_p , dimensionless
I	counter-ion, dimensionless
K	linear distribution coefficient based on the solid resin volume, equilibrium distribution coefficient
K_e	size exclusion factor, dimensionless
$\overline{K}$	linear distribution coefficient based on entire resin volume; Freundlich Equilibrium Constant
K'	equilibrium constant for ion-exchange reaction, dimensionless
$k_o a$	overall mass transfer coefficient, s^{-1}
k_f	fluid phase mass transfer coefficient, cm/s
k	fluid-phase mass transfer coefficient cm
k'	solid-phase mass transfer coefficient, cm;
	retention factor for a protein, dimensionless
L	length of a chromatographic bed, cm

m_o	mass of solute in feed pulse, g
m_s	molality of solute, gmoles/gmole water
MW	Molecular weight of specie, Daltons
N	number of components
N_{l+}	adsorption rate scaled by convection, dimensionless
N_{L-}	desorption rate scaled by convection, dimensionless
N_f	ratio of film mass transfer rate to convection rate
N_p	ratio of diffusion rate to convection rate
	diffusion, dimensionless
n	effective charge of an adsorbed protein, dimensionless
n_p	number of transfer units for particle
	diffusion, dimensionless
p	pressure, psig
P	parameter in Gunn axial dispersion coefficient correlation, eqtn. 3.29 dimensionless
P	protein, dimensionless
P_T	mass of protein in column prior to salt step, g
Pe	Peclet number, $u_o L / D_z$, dimensionless
Q_E	eluent flow rate, cm^3/min
Q_F	feed flow rate, cm^3/min
Q_S	salt step feed rate, cm^3/min
Q_T	total flow rate, cm^3/min
q	adsorbed phase concentration based on the solid resin volume, g/liter, g/cm^3
$\bar{q}$	average adsorbed phase concentration based on entire resin volume, g/liter, g/cm^3
q_m	maximum concentration of ion-exchange sites in resin, mol/liter
$\bar{R}$	resolution, dimensionless
R	scaling operator
R_p	resin particle radius, cm
Re	Reynolds number, $2R_p \epsilon_b / \mu$
r	radial coordinate of resin particle, cm
$\tilde{r}$	dimensionless phase period, dimensionless
$\tilde{r}_F$	dimensionless feed period, dimensionless
S	mass of resin in stirred batch, g
Sc	Schmidt number, $\mu / \rho D$, dimensionless
Sh	Sherwood number, $k_f d_p / D$, dimensionless
t	time, s
t_e	elution time, s
t_F	feed time, s
t_L	lag time between protein feed and salt feed, s
t_R	Retention time of protein in column, s
t_S	feed time of salt step, s
u_o	superficial velocity, cm/s
u'	fluctuating velocity, cm/s
V	volume of stirred batch vessel, cm^3
v	interstitial velocity, cm/s
v_s	shock velocity, cm/s
v'	reduced velocity, $d_p v / D$, dimensionless

W	peak width, degrees
x	z/L , dimensionless column position
Y	Consumption rate of given species, dimensionless
Y_l	rate of adsorption of specie from pore phase to solid phase
Y_p	consumption rate of specie by reaction on sorbent surface
z	length coordinate of a chromatographic bed, cm
Z	ratio of eluent to salt valence, dimensionless

Greek symbols

α_1	smallest positive zero root of the Bessel Function J_0 , 2.405
α	parameter defined in eqtn. 3.93, dimensionless
β	parameter exponent defined in eqtn. 3.92, dimensionless
$\tilde{\beta}$	parameter defined in eq 3.50, dimensionless
γ_k	parameter defined in eq 3.43, dimensionless
γ	tortuosity defined in eq 3.28, dimensionless
ϵ_b	void fraction of the chromatographic bed, dimensionless
ϵ_p	void fraction of the resin particle, dimensionless
ϵ_p	inclusion porosity of the resin particle, dimensionless
	interaction parameter defined in eqtn. 3.87
Θ	Time, dimensionless
λ_k	parameter defined in eq 3.45, dimensionless
λ	parameter defined in eq 3.30, dimensionless
μ	fluid viscosity, g/cm-s
π	3.1416, constant
ξ	r/R , dimensionless radial position in a particle
ρ	fluid density, g/cm ³
σ_t	standard deviation, s
σ_Θ	standard deviation, degrees
σ_ϕ	standard deviation, dimensionless
ς	dimensionless length, z/Z , dimensionless
τ	tortuosity factor, dimensionless
τ	percolation time, L/u_o , s
ϕ	modulator concentration, g
Ψ_p	parameter defined in eq 3.79, dimensionless

Superscripts

<i>D</i>	displacer
<i>i</i>	interface
<i>M</i>	peak concentration
*	equilibrium
∞	sorbent saturation capacity

Subscripts

<i>Ad</i>	adsorption
<i>ave</i>	average
<i>b</i>	bulk fluid
<i>bio</i>	biomolecule
<i>c</i>	cycle
<i>D</i>	desorption
<i>D</i>	displacer
<i>D</i>	diameter
<i>E</i>	eluent
<i>e</i>	maximum expected inlet value, supernatant phase
<i>F</i>	feed
<i>f</i>	fluid, final
<i>I</i>	counter-ion
<i>i</i>	component i, initial
<i>j</i>	cycle number, component counter
<i>L</i>	Length
<i>l</i>	langmuir
<i>max</i>	maximum
<i>n</i>	effective charge on a protein
<i>N</i>	flowrate
<i>P</i>	protein
<i>p</i>	particle, pore
<i>pore</i>	pore phase
<i>S</i>	surface, solid phase
<i>salt</i>	salt molecule
<i>solution</i>	solution phase
<i>T</i>	total
<i>t</i>	time
0	initial condition
1	component or fraction 1
2	component or fraction 2
<i>new</i>	scaled up state
<i>old</i>	laboratory scale

CHAPTER I INTRODUCTION

1.1 General Description Of Dairy Whey And Purpose For Research

Dairy whey is characterized as the water-like liquid or serum that separates from the curds during cheese manufacturing (Kosikowski, 1979). 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 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. At this pH the curds formed are removed from the whey which, like the acid whey, has a specific composition and is classified under these conditions as sweet whey (Dybing and D.E. Smith, 1991). These two types of whey can be related to common cheeses in that sweet whey results

from the manufacture of Cheddar, Swiss, Gouda, and Emmental cheeses. Acid whey results from Cottage and similar cheeses and from acid casein manufacture (G. Smith, 1976). In general whey is a dilute liquid composed of lactose, a variety of proteins, minerals, vitamins, and fat. Whey contains about 6 wt.% solids of which 70% or more is lactose and about 0.7% is proteins (Zall, 1984). Whey is a liquid mixture rich in 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 caseins or the major milk proteins have been used. This problem can be further grasped by considering that in the manufacture of cheeses or casein products only 10 to 20% of the raw milk is utilized to obtain the final product; 80 to 90% of the raw milk yields whey as a waste product (Sienkiewicz and Riedel, 1990). Thus the problem of whey utilization is that a tremendous amount of whey is produced worldwide each year that is rich in proteins and other valuable biochemicals and yet is in very dilute form. Table 1.1 gives the compositional breakdown of cheddar cheese whey (sweet whey) and cottage cheese whey (acid whey) (G. Smith, 1976). Table 1.2 gives the data for the production of liquid whey in the U.S, North America, and Worldwide for 1987 and calculated for 1995.

For comparison purposes data is also listed for whey production at the Dairyman Inc. plant in Greeneville, Tennessee. This plant produces cheddar cheese products for Kraft foods and will serve as a model system for this research project. That is, the purpose of this project is to develop fixed bed liquid chromatographic methods to separate the valuable compounds out of the whey from the Dairyman Inc. plant whey waste stream. Worldwide the amount of cheese produced and hence whey produced increases each year by about 3% and hence data for 1995 can be

Table 1.1
Composition of Cheese Wheys
(G. Smith, 1976)

Component	Cheddar	Cottage
Protein	0.62	0.7
Non-protein nitrogen	0.19	0.0
Fat	0.04	0.0
Lactose	4.6	4.5
Ash	0.56	0.6
Total solids	6.1	6.5
Water	87.9	87.7

Table 1.2
Cheese Whey Production Levels - 1987, 1995, in 1,000 tons
(Sienkiewicz and Riedel, 1990)

Year	1985	1995
World	90,757	121,969
N. America	21,692	29,152
U.S.	18,712	25,148
Greenville, Tn. Dairy	85.0	114.0

calculated from these tables.

There are three main types of whey utilization: animal feedstocks, lactose production, and the production of whey powders (Sienkiewicz and Riedel, 1990). Each of these will now be discussed in some detail so as to provide a background of how whey is utilized, to what extent, and with this in mind what this project hopes to accomplish.

Liquid whey has been used for centuries as an animal feedstock, particularly to pigs. Typically the practice in the past and even today is to take untreated whey direct from the dairy plant to the farm site and feed it to the pigs. The attitude of many dairies is that the whey is a nuisance by-product and that they are very happy to find a farm that will accept the untreated whey for feeding purposes, usually at little or no cost to the farmer (Holt, 1992). Because farms are decreasing in number and growing in size, a trend reflected by cheese manufacturers, the distance between both is increasing such that transportation costs for liquid whey are becoming prohibitive. As a result animal feedstocks of whey are preferred in powder form which lasts longer than liquid whey which will degrade with exposure to high temperature or microbially (Sienkiewicz and Riedel, 1990). Of course whey powders require on site drying facilities which necessitate capital expenditures generally ruling out such operations for small dairies and cheese factories. These facilities will continue to be interested in returning whey to farms in liquid form while larger cheese facilities can afford to look into drying facilities.

Whey animal feedstock in liquid form has been extensively studied and shown to be a good feed source for pigs. Whey powder is also a suitable feedstock provided that certain upper limits on the level of lactose in the powder are not exceeded. During the process for obtaining dry powder the lactose content will decrease. When

the powder is fed to cows this can adversely affect the milk production of the cows if there is not enough lactose in the feed (Sienkiewicz and Riedel, 1990). The production of lactose from whey has increased consistently worldwide since 1940. The pharmaceutical and food industries represent permanent markets for lactose utilization in the manufacture of drugs, baby foods, pastries, and confectioneries. In the pharmaceutical industry it is extensively used in the tableting of drugs. It is also used in the manufacture of infant foods and milk replacers to mimic the lactose content of mother's milk. Lactose for these products is primarily obtained from rennet type or sweet whey. Usually lactose is processed on site at the dairy facility or at other lactose refining plants. Chemical processes have been long established for manufacturing lactose from whey and are thoroughly described elsewhere (Sienkiewicz and Riedel, 1990). For the infant food and pharmaceutical industries the lactose must be refined by crystallization processes to obtain a high purity product. In general industrial lactose is 90 to 92% lactose, edible grade lactose is 98%, and pharmaceutical grade is 99.5 to 99.9% lactose (Sienkiewicz and Riedel, 1990). Table 1.3 gives data on the end uses of lactose from whey for human foods and products. The data is for 1990 with 1995 figures calculated assuming that an annual whey growth rate of 3% applies.

Whey is also processed into several different powders and concentrates for various end use specifications. These include partially and totally dry powders, dry whey products where the lactose or mineral content has been reduced, whey protein concentrates where the protein content is from 35 - 75%, or whey protein isolates where the protein content is at least 90% (Morr, 1992). These different forms of whey powders/concentrates are used extensively to manufacture a wide variety of food products. Included are powdered drink mixtures, champagnes, wines,

Table 1.3

U.S. End uses of Lactose from Whey in Human Foods and Products, in mm lbs.

(Graf, 1992)

Product	1990	1995
Infant Foods	41.4	48.0
Chemicals and Pharmaceuticals	16.4	19.0
Bakery Products	11.5	13.3
Prepared Dry Mixes	7.8	9.04
Ice Cream and other Dairy Products	6.8	7.9
Soft Drinks, Special Dietary Foods	1.6	1.9
Confectionary	8.3	9.6
All other Food Products	3.2	3.7
Total	99.0	112.4

non- alcoholic beverages, whey butters, ice creams, and cheeses. Also, bakery and confectionery products utilizing whey powders, gelified products, pasta preparations, and a host of others. Primary biochemicals in whey and which this study will look at in detail are known to promote some areas of baking processes. For example, lactose assists in crust browning and fresh keeping effectiveness while alpha lactalbumin affects browning qualities. Table 1.4 gives production levels in the U.S. from 1975 - 1990 for the different forms of bulk whey powders and concentrates. An overview of consumption data for 1976, 1981, and 1990 is shown in Table 1.5 for uses of dry whey in human foods and products in the U.S. Table 1.6 gives uses of whey constituents for other products (Sienkiewicz and Riedel, 1990).

Because whey is such a rich source of many biochemicals it has been studied extensively for many years worldwide. Its uses are numerous and the reader is referred to Sienkiewicz and Riedel's text (1990) for information on whey and its uses. Because these biochemicals are of relatively high value it is desirable to utilize the whey as a feedstock for obtaining them. Unfortunately the compounds are in dilute form and necessitate separation and other processes to extract them from the whey. As with all industrial processes it is the economics of the operation that is the driving force and this certainly applies to the end use markets for recoverable whey proteins. In 1990 the annual dollar value of dry whey powder for human food products was \$138 million dollars and \$24 million dollars for animal feed products. The measure of profitability of these powders is the average annual net margin and it was 2.22 cents per pound for human food products and 1.01 cents per pound for animal feeds (Graf, 1992). Also interesting is the fact that from 1976 - 1990 there was a 132 million pound increase or 35% in the use of dry whey powder in human food products. In 1990 99 million pounds of whey lactose, 43.4 million pounds

Table 1.4
(Graf, 1992)

U.S. Bulk Dry Whey Powdered Products Production, 1975 - 1990, mm lbs.

Year	Animal	Human	Total	Modified ^(a)	WPC ^(b)
1975	439	157	596	172	8
1980	534	156	690	193	4
1985	812	175	987	201	105
1990	953	190	1143	263	168

^(a) Modified Dry Whey Products : Reduced Lactose and Minerals Whey

^(b) Whey Protein Concentrate : 35 - 75 % protein content

Table 1.5

U.S. End Uses of Dry Whey in 1976, 1981, and 1990, in mm lbs.

(Graf, 1992)

Products	1976	1981	1990
Dairy	131.9	108.8	160.6
Bakery	93.5	110.3	117.9
Dry Mixes	21.3	30.9	75.0
Confectionary	10.3	13.4	7.0
Blends	41.2	60.1	90.0
Meat Processing	1.9	2.1	1.0
Soups	0.7	1.7	2.0
Beverages	0.6	0.1	3.2
Institutional	0.2	1.2	0.1
Chemicals	0.2	11.4	2.4
Margarines	0.3	0.3	4.4
Other	71.0	149.2	40.5
Total	373.1	489.5	505.0

Table 1.6**Uses of Whey and Whey constituents in unique products****(Sienkiewicz and Riedel, 1990)**

Whey Product	Use	Year of Introduction
Rennet whey powder	chocolate flavoring	1978, 1980
Whey proteins	Cosmetics, skin creams	1982, 1986
Whey	Spicing agent	1986
Rennet	Emulsifiers	1984
Whey	Starter cultures	1988
Lactalbumin	Food additives	1989
Condensed whey	salad dressing	1972

of reduced lactose and minerals whey, 13.2 million pounds of concentrated whey and 65 million pounds of whey protein concentrate were utilized for human food products (Graf, 1992). Table 1.7 gives historical data on whey powders selling price, processing costs, and net profit margin data for human food products. Table 1.8 gives data likewise for animal feed products. Table 1.9 gives data on the price that the purified proteins of whey command by the Sigma Chemical Company (1995) for a perspective as to their value. With the amount of whey increasing by about 3%, it can be proposed that with improved unit operations for whey proteins recovery and with widening markets and increasing feedstock, there should be continuing profitability. These issues will be discussed later in the economic analysis chapter.

It is felt that there is significant opportunity for improvements in the recovery of whey proteins and this is the focus of this project. With this in mind it is the purpose of this research project to develop fixed bed liquid chromatographic methods for separating lactose and the four major proteins from whey. The proteins of interest are: alpha lactalbumin, beta lactoglobulin, bovine serum albumin, and immunoglobulin G. Chromatographic methods have been researched by others in the past and utilized somewhat on an industrial scale, however, not using new improved process grade resins such as the ones utilized in this study. Two new ion-exchange resins were chosen for use. This choice was made for several reasons. A goal of this project was to study protein separation with the aim toward developing a process for proteins production. Since ion-exchange resins are used for up to 75 % of all protein separations industrially, it was logical to use such resins in this study (Bonnerjæe et al., 1986). Additionally, ion-exchange chromatography offers several practical advantages. Two of the key operating variables which control the attraction between the ion-exchange resin and the proteins are pH and salt con-

Table 1.7**Average Annual Net Profit Margins On Human Food Products From Dry Whey Powder****(1975 - 1990)****(Graf, 1992)**

cents per pound			
Year	Avg. Price	Proc. Cost (a)	Net Margin (b)
1975	7.54	6.72	+0.82
1980	14.35	9.99	+4.36
1985	15.20	12.50	+2.70
1990	14.44	14.03	+0.41

(a) Whey Processing Cost: Based on USDA determined cost of 12.5 cents per pound in 1985 for converting liquid whey into dry whey powder

(b) Column 1 minus Column 2

Table 1.8**Average Annual Net Profit Margins On Animal Feed Products From Dry Whey Powder****(1975 - 1990)****(Graf, 1992)**

cents per pound			
Year	Avg. Price	Proc. Cost (a)	Net Margin (b)
1975	6.02	6.72	-0.70
1980	11.91	9.99	+1.92
1985	13.86	12.50	+1.36
1990	12.88	14.03	-1.15

(a) Whey Processing Cost: Based on USDA determined cost of 12.5 cents per pound in 1985 for converting liquid whey into dry whey powder

(b) Column 1 minus Column 2

Table 1.9

Prices for the Purified Major Proteins and Lactose in Whey, \$/g
(Sigma Chemical Company, 1995 Product Catalog)

Constituent	\$/g
Lactose	0.0430
Alpha Lactalbumin	169.0
Beta Lactoglobulin	33.50
Bovine Serum Albumin	1.34
Immunoglobulin G	612.0

centration. These variables are relatively easy to control and are readily monitored experimentally.

1.2 Dairy Whey Protein Chemistry

Dairy whey is a fascinatingly complex mixture of many different biochemicals, and inorganic and organic compounds. The study of whey, its chemical composition, and its utilization, has been very active since the early 1900's. Whey provides an excellent and challenging system for the protein chemist to investigate because of its diversity and complexity. The utilization of whey as a source of biochemicals for industrial and food uses has also been very actively researched and exploited on an industrial scale. No single report including this one can come close to describing in great detail the whole subject of whey. However, a discussion of whey and its chemistry is necessary as a foundation for this dissertation.

Whey is classified according to two types: sweet whey and acid whey. Table 1.10 gives a detailed composition comparison between the two. As can be seen, characteristic differences between sweet and acid whey exist. Sweet whey has slightly greater protein content and less water than acid whey. Also, sweet whey contains greater lactose content and a lower amount of ash than acid whey. Because acid whey has a much lower pH than sweet whey, its utilization is limited due to resistance by consumers. Products derived from acid whey tend to have a bitter or acidic flavor thus leading to non-acceptance by consumers (Kosikowski, 1979). Table 1.11 gives a comparison between published sweet whey composition values for various components and the values measured for the sweet whey from the Dairyman Inc. cheese plant in Greeneville, Tennessee. This plant processes its sweet whey by Reverse Osmosis to raise the solids content from approximately six percent by

Table 1.10

Composition of Rennet and Acid wheys in wt. %
(Sienkiewicz and Riedel, 1990)

Parameter, component	Rennet whey	Acid whey
Water	93.3	95.58
Dry matter	6.7	6.42
Density at 15 C	1.026	1.025
Protein	0.60	0.53
Total N (mg/g)	1.30	1.19
NPN (mg/g)	0.34	0.34
Protein	0.60	0.53
Soluble N (mg/g)	1.30	1.18
NPN (mg/g)	0.34	0.34
Lactose	5.0	4.40
Ash	0.52	0.60
Lactic Acid	0.14	0.47
Citric Acid	0.10	0.0
Calcium	0.05	0.0
pH	6.1	4.7

Table 1.11

Comparison Between "Literature" Sweet Whey Composition
and Greeneville, Tn. Dairy Plant Values in wt.%
(Sienkiewicz and Riedel, 1990)

Parameter, component	Sweet Whey	Greeneville, Tn. Whey ^(a)
Water	93.3	95.58
Dry matter	6.7	6.42
Density at 15 C	1.026	1.025
Protein	0.60	0.53
Total N (mg/g)	1.30	1.19
NPN (mg/g)	0.34	0.34
Protein	0.60	0.53
Soluble N (mg/g)	1.30	1.18
NPN (mg/g)	0.34	0.34
Lactose	5.0	4.40
Ash	0.52	0.60
Lactic Acid	0.14	0.47
Citric Acid	0.10	0.0
Calcium	0.05	0.0
pH	6.1	4.7

^(a) Before Reverse Osmosis Processing

weight to approximately eighteen percent by weight in order to facilitate economical transportation of it. Table 1.12 compares the Greeneville plant's sweet whey before and after Reverse Osmosis processing.

For this project all samples utilized were taken directly from a whey holding tank in-line immediately following the Reverse Osmosis step and before the plant's Pasteurization process. Table 1.13 gives a compositional breakdown of the whey protein fraction, irrespective of whey type. Whey is also rich in minerals and vitamins having on average 10 metal elements and 9 vitamins. Table 1.14 gives the mineral analysis for acid and rennet wheys while Table 1.15 displays average trace elements and vitamins compositions.

As diversified and interesting is the makeup of whey, so is the chemical nature and function of the proteins that comprise it. For example, alpha lactalbumin is known to participate in the synthesis of lactose. It is known to consist of three genetic variations labelled as A, B, and C. However, only variant B is observed in Western cattle while variants A and C are found in African and Indian cattle respectively. Alpha lactalbumin is known to consist of 123 amino acid residues with distinctions in the placement of the residues resulting in the different variations (Eigel et.al, 1984).

The protein Beta Lactoglobulin is the major protein component of bovine milk with a typical composition of 2 - 3 g/l. It is known to have 7 genetic variants labelled A - G and 162 amino acids (Eigel, G. Smith, 1984, 1976). The function of Beta Lactoglobulin is thought to be an aid to the transport of Vitamin A and the binding of Retinol (Dybing and D.E. Smith, 1991). Bovine Serum Albumin (BSA) is a protein that is physiologically and immunologically very similar to serum albumin found in human plasma. It is a complex molecule with at least 400 amino

Table 1.12**Composition of Greeneville, Tennessee****Dairy Plant Sweet Whey Before and After Reverse Osmosis Processing**

in wt. %

(Sienkiewicz, 1990)

Parameter, component	Before RO	After RO
Alpha Lactalbumin	16.0 of Total Protein	15.9 of Total Protein
Beta Lactoglobulin	73.4 of Total Protein	68.7 of Total Protein
Bovine Serum Albumin	2.14 of Total Protein	1.99 of Total Protein
Immunoglobulin G	4.64 of Total Protein	5.76 of Total Protein
Lactose	3.70	11.03
Other Protein	3.82 of Total Protein	7.65 of Total Protein
Total Protein	1.12	1.91
Ash	0.49	1.37
Fat	0.15	0.21
Sodium	0.054	0.088
pH	6.04	5.5
Total Solids	5.9	14.7
Density (g/ml)	1.024	1.0573

Table 1.13**Whey Protein Composition and Molecular Weights**

(Dybing and D.E. Smith, 1991)

Protein	% of Total Whey Protein	Mol. Wt.
Alpha Lactalbumin	19.4	14,175
Beta Lactoglobulin	56.5	18,277
Bovine Serum Albumin	7.1	66,267
Immunoglobulins	13.4	
IgA	0.5	300,000 - 500,000
IgG	12.4	150,000 - 170,000
IgM	0.5	900,000 - 1,000,000
Proteose -peptones	3.6	4,100 - 22,000

Table 1.14**Mineral Composition of Whey in Wt.%**

(Demler, 1968)

Mineral	Acid Whey	Rennet Whey
Total Ash	0.60	0.56
Calcium	86.0	22.5
Phosphorous	63.0	42.5
Magnesium	8.9	5.8
Sodium	40.0	35.0
Potassium	133.0	109.0

Table 1.15
Trace elements and Vitamins in Whey
(Blanc, 1969)

Constituent	mg/100 g dry whey
Iron	1 - 7
Copper	0.5 - 5.0
Zinc	5 - 9
Manganese	0.01 - 0.04
Vitamin A	100
Thiamin	4 - 6
Pyridoxin	6 - 10
Riboflavin	7 - 30
Calcium Pantothenate	30 - 70
Biotin	0.2 - 0.3
Cobalamin	0.01 - 0.05
Vitamin C	30 - 50

acid residues and 17 intramolecular disulfide bridges. These disulfide bonds are common to proteins and connect one amino acid chain to another. The molecule is thought to exist in 3D space as two large double loops and one small double loop giving a shape of an ellipsoid (Eigel et. al, 1984). Bovine serum albumin or BSA accounts for about 6% of the total whey proteins but the amount depends on the stage of lactation in the animal (G. Smith, 1976). BSA is thought to facilitate the transport of ions and fatty acids (Dybing and D.E. Smith, 1991).

The immunoglobulins are classified as a family of large molecular weight proteins ranging in weight from 150,000 to 1,000,000 (Dybing and D.E. Smith, 1991). Immunoglobulins are biologically known as antibodies. In mammals there exist five classes of immunoglobulins. Only four are found in mammals milk: IgG, IgA, IgM, and IgE. All are made up of polymer or monomer units consisting of a basic unit of four polypeptide chains. Of course each differs by its amino acid sequence. IgG is the principal class found in bovine milk (G. Smith, Eigel, 1976, 1991). Within this class are subclasses IgG1, IgG2, IgG3, and IgG4. Overall, IgG amounts to 13.5% of the total whey proteins with 0.5 % for IgA and IgM (Dybing and D.E.Smith, 1991). IgG represents 75-80% of the immunoglobulins in the human body and is the only immunoglobulin that can cross the placenta during pregnancy. IgG is the second antibody produced in an immune system response to an antigen.

IgA represents about 13% of the immunoglobulins in the human body. Its primary function is to defend exposed organ surfaces from attack by microorganisms and other foreign antigen containing factors. It is the IgA in a mother's milk that assists in the protection of newborns against infections prior to the development of their own antibodies. IgM has the greatest valence (antigen-combining capacity) with 10 sites to bind with antigens. It is the first antibody produced in an immune

system response to an antigen. IgD is the least understood of the immunoglobulins. It is found on the surface of white blood cells accompanying IgM. IgE is the least common immunoglobulin. It is thought to mediate immune system responses to allergies (Hiatt, 1993). Specific disease states may result from deficiencies or imbalances of one or more of the subclasses of IgG (Hiatt, 1993).

The immunoglobulins are highly valued for their antimicrobial activity having applications in neonate feeds and health care products (Donnelly and Mehra, 1990). According to an April 1993 issue of Pharmacy Times, the use of intravenous immunoglobulins (IVIG) in the healthcare industry will likely increase significantly in the 1990's, as well as its cost. In 1993 it was anticipated that IVIG usage would surpass one million dollars (Hiatt, 1993). Original use of immunoglobulins in the 1950's was for primary humoral immuno-deficiency and was limited to intramuscular injection. Problems with this method included unpredictable adsorption of the immunoglobulins into the body's system. Since then improvements in the purification of IgG from the other antibody classes has enabled the use of intravenous injection for present day use. This is very important since recent research suggests that not all immunoglobulins may possess antibody function (Hiatt, 1993).

The proteose - peptone protein fraction of whey consists of several molecules thought to be derived from caseins (Humphrey, 1984). In general it is thought that there are five major peptones all amounting to 3.5% total of the whey protein fraction. The function of these proteins is not clear nor is their role in affecting the functionality of a composite whey protein fraction.

Lactose which amounts to between 4 and 5 % of whey is also a molecule of value commercially. Lactose is the lightest of the major biochemicals in whey and is often separated from the protein fraction by exploiting this fact. It exists as two

chemical forms: alpha and beta with the alpha form most dominant although the difference between the two is very subtle. Crystals of lactose are in the alpha form with one molecule of water attached. Of the two forms the beta variant is sweeter and has a greater solubility but demand for such is low. Interestingly, lactose is the only major carbohydrate of animal origin used for foods for humans. And yet, many people, particularly non-Caucasians have an intolerance for lactose even though it is an important biochemical in the diet. The reason for the intolerance is that the person lacks the enzyme lactase which aids in the digestion of lactose.

The desirable properties of whey proteins and hence commercial usage of them requires efficient separation and purification methods. These methods will necessarily exploit some difference in the physico-chemical properties of the different molecules. It is, therefore, appropriate to include a brief discussion of the characteristics of proteins in general and how they react to physicochemical changes to their local environment. Excellent overviews of the subject are available in biochemistry texts and readers seeking additional understanding are referred there (Lehninger, 1982; Stryer, 1981).

Proteins are large and complex molecules with molecular weights ranging from a few thousand to over 1,000,000. These compounds are composed of polypeptide chains of amino acids. Some twenty naturally occurring amino acids are found in proteins. Both the types of amino acids present and the manner in which they are assembled play key roles in the way a protein behaves. The great variety of proteins is due to the nearly endless number of combinations into which these amino acids can be arranged.

The structure of proteins is commonly described in four ways. A protein's primary structure refers to the sequence in which the amino acids are assembled

within a chain. The secondary structure refers to the way in which the amino acids are positioned relative to one another in space. For example, the amino acids may be formed into helixes or sheets. The protein may be further described in terms of its tertiary structure. This describes the way in which the chain is positioned in space. If a protein contains multiple chains, the position of the chains relative to one another is referred to as its quaternary structure.

Proteins may be further classified into one of two groups; fibrous and globular. Fibrous proteins have simpler structures than do globular proteins and tend to be insoluble in water. In nature they serve primarily by providing external protection, support, shape and form to organisms. Globular proteins tend to function in a more dynamic manner than do fibrous proteins. These complex molecules serve organisms in such capacities as catalysis, transport and in other regulatory roles. All of the proteins used in this study are globular proteins and only their properties will be discussed further.

Globular proteins are so named because their chains tend to be folded into tight globular shapes. In general, their more hydrophilic amino acids tend to be located on the exterior of the molecule, rendering the protein more soluble in water. The shape of globular proteins is maintained by hydrogen bonding, ionic charges and covalent crosslinkages.

While globular proteins normally exist in their "minimum energy "or "native state ", their shape is dynamic and may change to adapt to the environment in which they are present. If the environment is changed drastically the protein's structure may change to the point where the protein loses certain desirable characteristics. At this point the protein is said to be "denatured ". While some proteins are able to return to their native state or "renature ", denaturation is frequently an

essentially irreversible process. This characteristic of proteins makes them sensitive to extreme process conditions and increases the care which must be taken in their manufacture. Thus, separation and purification techniques involved in production must be sufficiently mild so as not to harm the protein.

The complex structures of proteins and their ability to adapt to changes to their local environment certainly apply to whey proteins. These proteins are sensitive to heat and other processing changes that they might undergo in a commercial operation. In the manufacture of Whey Protein Concentrate (WPC) containing 35 - 75% protein the proteins are subjected to chemical and physicochemical modifications during whey fractionation. These modifications cause loss of protein solubility and negate desired functional attributes of the WPC for certain food applications (Morr, 1987). In general processed whey is classified as Whey Protein Concentrate (WPC) having 35 - 75% protein level, and Whey Protein Isolate (WPI) having at least 90% protein content (Morr, 1992).

During the manufacture of WPC the whey proteins will undergo heat induced denaturation and protein - protein interactions involving the interchange of organic chemical groups on the protein molecules. The whey constituents will as a consequence then coagulate or form a gelatinous material depending on the pH, protein composition, and temperature. The changes brought about affect the WPC's viscosity and gelatinous structure which in turn affects its use in restructured food products. In general it is desirable to maintain the integrity of the whey proteins in WPC's or WPI's. The heat stability of whey proteins in commercial products is usually related to that of Beta Lactoglobulin since it is the protein of greatest amount in whey. It has been found that Beta Lactoglobulin can be stabilized against heat denaturation by increasing the lactose content present, adjusting the

pH to 3.0, or increasing the solids content. On the other hand increasing the calcium content or raising the pH to 8.6 will de-stabilize Beta Lactoglobulin against heat denaturation. Nearly opposite conditions of these will affect the stability of Alpha Lactalbumin. Interestingly Alpha Lactalbumin is reversibly heat denatured but only if Beta Lactalbumin or calcium is not present. For the major whey proteins the order of heat stability from most sensitive to most stable has been found to be: Alpha Lactalbumin, BSA, IgG, and Beta Lactoglobulin. Generally, if the whey proteins are exposed to temperatures between 60 - 70 C there will be reversible denaturation and some unfolding of protein structure. For temperatures greater than 70 C the proteins will increasingly irreversibly denature (Dybing and D.E. Smith, 1991).

A survey of commercial WPC's and WPI's found variations in overall composition, individual protein composition, physicochemical properties, functionality, and flavor of commercially manufactured WPC's and WPI's greatly limits their acceptance by the food industry as functional food ingredients (Morr, 1992). Morr and Foegeding (1990) propose that standardization of methods to manufacture WPC's and WPI's be developed to increase whey utilization and acceptance into the marketplace. One such benchmark for WPC and WPI characterization is the PER or Protein Efficiency Ratio. This ratio is a measure of growth and expresses the weight gain of an adult animal which 1 gram of food protein will effect. Typically whey has a PER of 3.2, compared to milk caseins with a value of 2.8 and a whole egg with a value of 3.9. WPC's can have PER's of 3.3 to 3.4. These values are generally independent on the source of the whey (Sienkiewicz and Riedel, 1990). Suffice it to say that for the commercial manufacture of WPC's and WPI's it is the objective to minimize protein denaturation and modification in order to preserve their

inherent solubility and functionality (Morr, 1987). Further reviews on the subject of whey protein functionality and the effects of modification are found elsewhere (Morr and Foegeding, 1990), (Morr, 1987), (Morr, 1992) (Huginin 1987), (Morr, 1984, 1985, 1987, 1989, 1990). Because whey proteins are very large in size and molecular weight they possess low diffusion coefficients in solution. Typical diffusion coefficients for globular proteins in aqueous solutions are in the range of 1×10^{-6} to $1 \times 10^{-8} \text{ cm}^2/\text{sec}$ (CRC Handbook of Biochemistry, 1970). For comparison, the diffusion coefficient for NaCl in water is about $1.5 \times 10^{-5} \text{ cm}^2/\text{sec}$ (CRC Handbook of Chemistry and Physics, 1986). Larger proteins, in general, diffuse more slowly than do those with lower molecular weights.

Because proteins are composed of amino acids, many of which have ionizable side groups, they possess interesting charge characteristics. A schematic of the assembly of a portion of a protein molecule is shown in Figure 1.1. The R groups of the amino acids shown in this figure give the protein its unique properties. For example consider the case of the amino acid lysine which is shown in Figure 1.2. At low pH the R group of lysine possesses a positive charge, while at high pH it bears a negative charge. In addition to the influence of the pendant R groups of the amino acids, a protein's charge may be influenced by the ionizable amino-terminal and carboxyl-terminal groups located at the ends of the polypeptide chains.

The overall charge of a protein is determined by the global behavior of the amino acids in its makeup. At a given pH, the protein will contain groups which may be positive, negative or neutral. The charges of these groups will combine to give the protein's overall charge. Additionally, the density of these charges will vary depending on the protein's structure. Some pH, in fact, will exist at which the protein will have no net charge. This pH is known as the protein's isoelectric point.

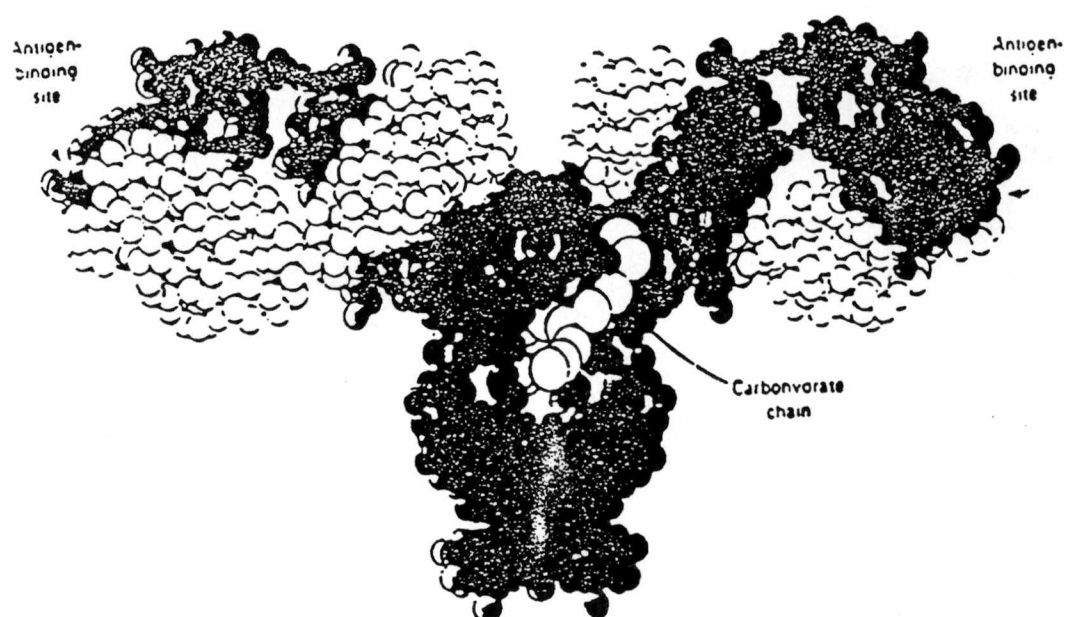


Figure 1.1 Schematic demonstrating the structure of a protein molecule. The structure shown is Immunoglobulin G, one of the proteins in this study (Stryer, 1981).

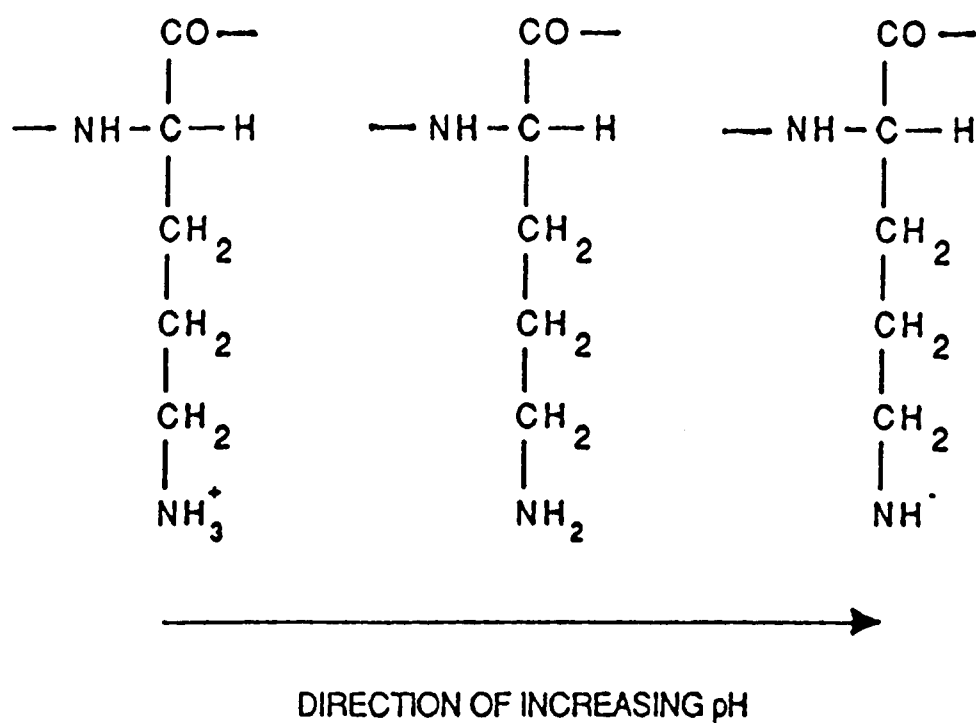


Figure 1.2 The effect of pH on the charge of the lysine R Group

As a demonstration of these traits, consider the charge characteristics of bovine serum albumin (BSA). BSA is a globular protein with a molecular weight of 68,500 and an isoelectric point of 4.9. At pH 7, BSA has 100 negative and 82 positive groups giving it a net charge of negative 18 (van der Wiel and Wessenlingh, 1989). The dependence of charge on pH is seen by observing that at pH values of 5 and 6 BSA has a net charge of negative 2 and negative 12, respectively. A model of the BSA molecule is shown in Figure 1.3. As this figure shows, the charges are not distributed evenly across the molecule but rather they are concentrated in certain regions. In the case of BSA, the carboxy- terminal end of the molecule is the most highly charged region. In summary, proteins are very large and complex molecules. The amino acids make up of a protein and their configuration determine the protein's behavior. A protein's solubility, diffusivity, and charge characteristics are determined by its structure. The complexity of proteins makes their production in pure form a difficult task which is further complicated by the fact that the protein may be irreversibly denatured if process conditions become too harsh.

1.3 Review Of Technologies Available For Separating Proteins From Whey

The separation and purification of whey proteins has for a long time presented a research problem to the dairy industry. Because of the relatively high value placed on 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. Suffice it to

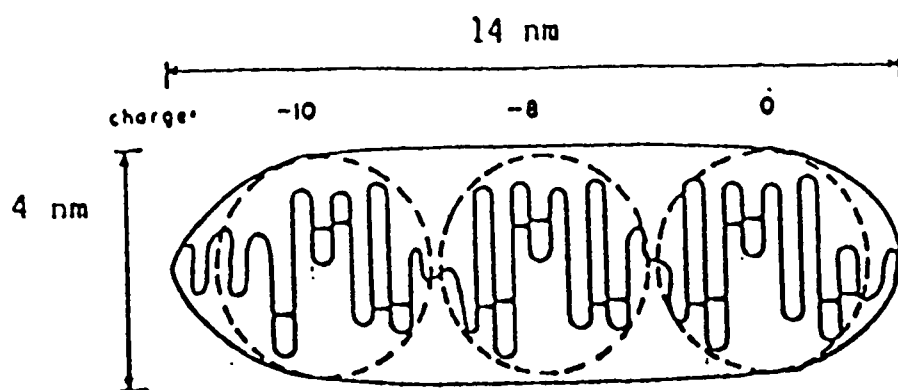


Figure 1.3 Model of BSA molecule. The charge distribution is shown as it exists at pH 7.0 (van der Wiel and Wesselingh, 1989).

say that there are many different separation methods that have been developed over the years to attempt to efficiently and economically recover proteins from whey or produce whey protein concentrates or isolates. This discussion cannot exhaustively cover all these processes. A very thorough report on whey separation methods is by Sienkiewicz and Riedel (1990) and the reader should consult it for additional details. Here, a brief discussion on past and present technologies will be presented.

To produce whey protein concentrates (WPC'S), a variety of techniques have been developed and utilized industrially. Again, a WPC contains 35 - 75% whey proteins. Table 1.16 gives the various techniques under general separation headings.

The physical operation of heat precipitation, also known as heat denaturation, is stated to be the most extensively studied (Sienkiewicz and Riedel, 1990), in whose book the process is detailed. However, precipitation of proteins by heat causes them to become insoluble or at best of low solubility. The proteins also will lose their native functionality and desirable properties depending on the conditions employed. This is due to irreversible denaturation of the proteins and other physico-chemical modifications taken on by the proteins during the precipitation process. These changes then negate certain food applications for the WPC (Morr, 1987a, Sienkiewicz and Riedel, 1990). Morr (1987a) states that the objective for manufacturing WPC's should be to minimize denaturation and modification of the whey proteins so as to maintain their native solubility and functionality. Because the goal is then to maintain the solubility and native functionality of the whey proteins, only certain separation methods have found commercial success. Ultrafiltration is the most widely used commercial method of whey fractionation offering considerable diversity in manufacturing a wide range of protein concentrates. However, it suffers from the drawback of high capital and operating costs, membrane fouling, incom-

Table 1.16

Techniques used for the manufacture of Whey Protein Concentrates
(Sienkiewicz and Riedel, 1990)

General Technique	Specific Technique
Membrane Fractionation	Ultrafiltration
	Reverse Osmosis
	Microfiltration
Precipitation or Complexation	Metaphosphates
	Carboxymethylcellulose
	Alcohol
Physical and Chromatographic Separation	Gel Filtration
	Ion - Exchange
	Inert adsorbents
	Heat precipitation

plete removal of low molecular weight 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 (Morr, 1987a). Ultrafiltration is a membrane separation process which fractionates whey proteins from lactose, minerals, and other solutes and water on a molecular weight basis. The process uses a pressure gradient and a semipermeable membrane to carry out the fractionation (Sienkiewicz and Riedel, 1990; Morr, 1987a).

Ultrafiltration is employed when the molecular weight cutoff point for solutes from proteins is 500 or greater. Below 500 molecular weight reverse osmosis is used (Sienkiewicz and Riedel, 1990).

Typically Ultrafiltration or UF is used to produce WPC'S with different protein concentrations as follows: 1. UF to a 4% protein content followed by evaporation and spray drying to a 35% content. 2. UF to a 4% protein content, diafiltration to a 16% content, and then evaporation and spray drying to a 50 - 75% protein content. Because various pretreatments are imposed on the whey before ultrafiltration and because of the evaporation and spray drying processes, the WPC's obtained will have protein solubilities ranging from 75 - 95% of their original values (Morr, 1987a).

It has been reported that, in 1981, 8% of the world's whey was processed was by ultrafiltration and this surely has grown (Matthews, 1990). In the U.S. in 1985 47,700 tons of whey protein concentrate or WPC was obtained by ultrafiltration using 35 commercial plants (Sienkiewicz and Riedel, 1990). Dybing and D.E. Smith (1991) reports that in 1987 that 100 mm pounds of WPC were produced in the U.S. by UF. 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 (Dybing and D.E. Smith, 1991).

Also tied into the success of UF is the pretreatment(s) used. Here the whey is filtered or centrifuged to remove suspended materials and fat. Prior removal of such enhances the performance of the UF unit (Matthews, 1990).

In conclusion it can be summarized that UF is the method of choice for large scale production of Whey Protein Concentrates of varying protein content. Its use for protein - protein separations does not appear to have been realized to date (Donnelly and Mehra, 1990).

Reverse Osmosis is a pressure driven process with a semipermeable membrane very similar to UF. As stated, the cutoff point for low molecular weight solutes is below 500 for RO as it is known. It has been reported that as of 1985 150 RO units were in use worldwide with 100 in the U.S. (Sienkiewicz and Riedel, 1990). Usually RO is used in conjunction with ultrafiltration or gel filtration for the concentration and partial demineralization of whole whey up to 28% dry matter and for concentration of the membrane permeate from UF units (Sienkiewicz and Riedel, 1990). The greatest problem with RO in the concentration of whey is the deposition of material, both low and high molecular weight, onto the membrane. This then necessitates in-place cleaning / sanitation procedures and thereby decreases the attractiveness of RO. Generally RO is considered viable only when small increases in the dry matter content of the whey are required. When higher values are required RO is used in combination evaporation (Sienkiewicz and Riedel, 1990).

Microfiltration is a relatively new whey processing technique. Here the pores of the membrane are slightly larger than those of UF membranes. This enables microorganisms to pass through thereby conducting an in-place sterilization of the membrane without heat treatment (Pearce and Marshall, 1991). The pore size used, 1.4 or 0.8 micrometer, allows nearly complete exclusion of microorganisms. 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 WPC'S. Microfiltration is also valuable in removal of residual lipid materials from separated whey. This is important because this material will concentrate with the main whey proteins thereby limiting their achievable content to about 75% (Pearce and Marshall, 1991). It is accepted that Microfiltration must be used in combination with UF and fat or cream removal methods to further enrich the proteins (Sienkiewicz and Riedel, 1991).

Gel filtration is a chromatographic method that has been extensively studied and utilized to a certain degree commercially. Here the whey proteins are fractionated from the low molecular weight solutes such as water and lactose by gel filtration chromatography. The large molecular weight proteins are excluded by size from the gel matrix and therefore elute ahead of the low molecular weight solutes (Morr, 1987). This process has been used at the laboratory scale by column and basket centrifuge methods (Morr, 1973). The use of Sephadex brand gels seems to be the most actively researched gel and several papers report preparative or commercial usage (Lindqvist and Wallin, 1973; Delaney and Mehra, 1973; Forsum, et al., 1973, Horton, 1972, De Koning, 1962, and Ek, 1968). The particular gel used was Pharmacia Sephadex G-25 coarse having a particle diameter of 100 - 300 microns and a molecular weight fractionation range of 1,000 to 5,000. The gel was contained in a Pharmacia Sephamatic column and Lindquist and Wallin (1973) give a process flowsheet and details of a commercial operation in Sweden that processed up to 3,700 liters/hr to produce 75% whey protein powders. Delaney et. al (1973)

also give details about the Sephadex process and state that preconcentration of the whey is necessary to increase throughput on the gel. They state that using Reverse Osmosis with an optimal 3:1 preconcentration ratio was best and that this gave a feed to the gel filter of 18 - 20% solids. They also state that the use of RO as a preconcentration step removes the necessity of a separate fines removal step, no heat damage to the whey proteins occurs, and the expense of a preconcentration step is minimized. Finally, they report that the gel filtration process yields two streams - one of low molecular weight solutes such as salt and lactose, and one of the high molecular weight solutes, namely the proteins. They state that the high molecular weight fraction recovery is very good - on the order of 95% from a single run through the gel. This was after the RO preconcentration step (Delaney et. al, 1973). Morr (1973), reports that WPC produced by the Sephadex gel filtration processes was highly soluble and functional and that the proteins were for the most part in their native undenatured state. It appears from the literature that there was considerable research into gel filtration for commercial usage in the early 1970's up to about 1974. Sienkiewicz and Riedel (1990) states that as of the middle of the 1970's gel filtration has been utilized for laboratory and preparative means only. Drawbacks with the gel filtration process include generation of large volumes of low molecular weight protein effluent, difficulty in sanitizing the gel and cleaning it, and that the gel is expensive for production scale quantities (Dybing and D.E. Smith, 1991, Sienkiewicz and Riedel, 1990). Matthews (1990) states that gel filtration appears to no longer be in commercial operation, though it has been used for isolating immunoglobulins and lactoferrin from whey (Sienkiewicz and Riedel, 1991).

Ion - Exchange adsorption and chromatography is another process that has been developed and utilized successfully on a commercial scale. Two processes have

been developed and used industrially.

They are the Vistec process (Palmer, 1977) and the Spherosil process. The Vistec process uses a cellulose based exchanger in a stirred tank reactor. The process involves pH adjustments of the whey and sequential elution of lactose and proteins. Subsequent ultrafiltration, evaporation, and spray drying yield a WPC containing 95% protein. The process is utilized by the Davisco Company of LeSuer, Minnesota. Because of the success of the process, the exchanger resin and other process details are kept secret. The Vistec process WPI has proteins that are 100% soluble and hence contributes to its success. The other process is the Spherosil process using cationic Spherosil S or anionic QMA resins. The process is capable of handling either acidic or sweet wheys. This process is described in the literature (Cueille and Tayot, 1985). Morr (1987a) reports that the Spherosil process yields WPC with only 60 - 65% protein content and a high ash content, up to 20%. The concentrates also displayed poor functionality and solubility.

Problems in general with the ion exchange processes are production of large volumes of rinse, chemical solutions and deproteinized whey that must be treated and disposed of. These solutions can become up to 2.5 times the volume of original whey. The reason for this is that the time required for the proteins to diffuse into the beads and back out is on the order of a hour. The rate of diffusion into the beads is limited by the rate which the beads and the whey become reach equilibrium in terms of the adsorption step. For large beads this is pronounced where for smaller beads the diffusion time is smaller but the time for liquid drainage from the column is increased as is the pressure drop across the bed. Also, the dilute eluate protein fraction requires concentration by UF, evaporation and drying. Finally the cycle times for each fractionation are long and the column requires in-place cleaning

and sanitation (Morr, 1987, Sienkiewicz and Riedel, 1990). Dybing and D.E. Smith (1991) reports that usually the whey is preconcentrated by UF to reduce the volume of liquid that the ion - exchange process needs to handle.

One new promising 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 (Zietlow and Etzel, 1995). Possible drawbacks of the process are fouling of the membrane, high capital costs, and difficulty in scaling up.

Table 1.17 summarizes the separation processes of Ultrafiltration, Reverse Osmosis, Microfiltration, Gel Filtration, and Ion-Exchange Chromatography along with their advantages and disadvantages.

1.4 Liquid Chromatography

Adsorptive separation processes are the method of choice in today's world where separation steps require the highest degree of selectivity. This is usually in the form of differential liquid chromatography where a small load of solute to be recovered is eluted from an adsorbent bed. Adsorptive separations such as liquid chromatography are also the method of choice for the concentration of dilute materials from solution and subsequent purification. These methods are also replacing crystallization in the purification of synthetic drugs because the drugs being developed today will often not crystallize (Lightfoot et al., 1992). The most important

Table 1.17

Characteristics of Separation Techniques used in Whey Protein Recovery

(Sienkiewicz and Riedel, 1990)

Technique	Advantages	Disadvantages
Ultrafiltration	Fractionation with	30 C temp. limit
	simultaneous concentration	pH limits 3 - 8
	low production costs	membrane fouling
Reverse Osmosis	Concentration of	No Fractionation
	all constituents	
	low production costs	max. conc. about 22%
Microfiltration	Separation of	Init. Cream Sep. Required
	microorganisms and fat	
Gel Filtration	Complete separation of	Pre and after
	low molecular wt.	concentration necessary
	constituents in 1 stage	
Ion Exchange	long life span of gel	high investment costs
	Removal of salts	Regeneration of
		resins necessary
		No concentration of proteins

adsorptive separation technique is liquid chromatography. Under the general heading of liquid chromatography there can be listed three principal types classified as recovery techniques in which a solute to be recovered establishes an equilibrium between a solid phase medium and the liquid solution. These three types are adsorption, ion exchange, and affinity chromatography. In adsorption chromatography the solute to be recovered is preferentially bonded to the solid phase medium by weak chemical bonds and subsequently eluted off from the solid phase. In ion exchange the solute to be recovered is done so by the exchange of ions between the liquid and solid phases. In affinity chromatography the solute to be recovered is selectively bound to a solid matrix with ligands that are covalently attached to the solid (Dechow, 1989).

1.4.1 Fundamentals Of Liquid Chromatography

As in any separation process, chromatography exploits differences in the physicochemical properties of the components of a mixture to effect the separation. In chromatography, the basis for separation is the differing affinities of the mixture components for a stationary phase. This stationary phase is normally a solid which may contain specific functional groups. Chromatographic materials are designed to take advantage of differences in molecular properties such as size, hydrophobicity, ionic charge, or even binding to a biospecific chemical group.

Chromatographic bioseparations are traditionally carried out in columns. The column contains the chromatographic medium, which is known as the stationary phase. This stationary phase is usually made up of small spherical particles. A fluid phase, usually a buffer solution, flows through the chromatographic column, typically continuously. This is referred to as the mobile or fluid phase. The mixture

to be separated is introduced at the top of the column and then eluted by the mobile phase. If there is a difference in the affinities of the mixture components for the stationary phase each species will move down the column at a different velocity causing the components to emerge from the column at different times. The separated components will then be recovered in a purer form at the bottom of the column.

The development of a chromatographic separation of a four component mixture according to this principle is shown in Figure 1.4. The initial injection of the mixture is followed by the continuous flow of the mobile phase causing the components to begin to move down the column. Since the individual components are retained to a different extent, they move through the column at different rates. Thus, continued flow of the buffer results in the elution of each component from the column at a different time.

To this point, we have considered only the effects of a compound's equilibrium affinity for the resin. While equilibrium between the protein and the resin is typically of primary importance, other factors affect chromatography. In addition to the sorption equilibrium, secondary equilibria may be important. Mixture components may react or otherwise interact. Equilibrium, both sorption and secondary, affects the loading of the components on the resin, the general shape of the peaks, as well as the time required to elute the components from the column.

Factors other than equilibrium also work to spread the peaks. Mass transport limitations may occur through slow pore diffusion or film mass transfer in the boundary layer surrounding the particles. Slow binding or desorption may contribute to kinetic limitations within the column. Additionally, hydrodynamic effects, such as non-uniform flow and viscous fingering may spread the peaks and

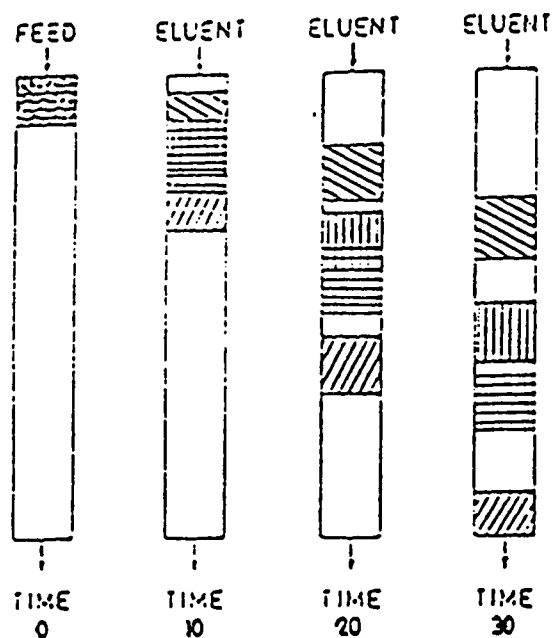


Figure 1.4 The development of a chromatographic separation of a four component mixture in a column chromatograph.

limit chromatographic performance. These factors must all be considered when designing a chromatographic separation. One of the reasons why chromatography is a popular bioseparation technique, is that it is an ideal medium to take advantage of the diversity present in many protein mixtures. Size exclusion resins can be used to separate protein mixtures characterized by a wide range of molecular weights. Reverse phase resins take advantage of differences in hydrophobicity between proteins. Affinity chromatography uses ligands to which only a specific protein will bind as a means of separating it from a mixture. In ion-exchange chromatography, resins are designed to take advantage of the unique charge characteristics of proteins. Charged groups are fixed to the solid matrix and thus provide adsorption sites for the oppositely charged proteins.

1.4.2 Size Exclusion Chromatography

Size exclusion chromatography is unique among the several different forms of liquid chromatography in that the solutes to be fractionated - usually large molecular weight compounds or macromolecules are partially to totally excluded from the solid phase medium. This is in direct contrast to other chromatography modes where the solutes are differentially attracted to the solid phase. The rejection of the large molecular weight molecules occurs because the solid phase is porous in nature with the pores small enough that the macromolecules fit in awkwardly or not at all. Commercial solids or polymeric resins used for size exclusion chromatography have a defined pore size range and corresponding fractionation range in molecular weights of molecules that will pass through the pores and ones that will not. Giddings (1991) states that when a molecule awkwardly fits inside a porous network severe limitations on its spatial motion are imposed. This limitation of freedom of

motion is associated with a loss of entropy. Loss of entropy is considered a factor thermodynamically unfavorable for the partitioning of the solute inside the porous network. In other words, the largest molecules in solution lose the most entropy because they cannot fit into the pores at all and are thus totally excluded. These molecules elute first in size exclusion chromatography. Because of this nature of size exclusion, it is used for the separation of low molecular weight molecules from large molecular weight ones. In this research project, it will be investigated for use for the separation of lactose and minerals from the proteins in whey.

Under the classification of size exclusion chromatography are two additional modes: Gel filtration chromatography for when the technique is carried out in aqueous solution, and Gel permeation chromatography when it is carried out in non-aqueous solution (Giddings, 1991). It appears from the literature that gel filtration is the predominant method of size exclusion. As for the solid medium to be used, the ideal gel filtration medium is inert, hydrophilic, porous, and rigid (J.C. Janson and P. Hedman, 1982). Any interaction of the gel with the solution is undesirable. The porosity will dictate the size of the sample to be used and the selectivity in fractionating large and small molecules. Rigidity will govern the average particle diameter that can be used and the maximum flowrate that can be handled. Also, as the particle diameter decreases, the pressure drop across a column will increase. When scaling up a process pressure drop and flowrate are critical parameters.

Common gel filtration media used are cross-linked dextran gels such as Sephadex brand by Pharmacia Biotech Inc. and cross-linked polyacrylamide gels or Bio-Gel P by BioRad Laboratories Inc., available in a wide range of porosities. These gels possess most of the properties listed above except that their rigidity is diminished

when the porosity approaches values useful for protein fractionations. Because of this these gels are limited in use to desalting of protein solutions (J.C. Janson and P. Hedman, 1982).

1.4.3 Ion-Exchange Chromatography

Sources are available which give good histories of ion-exchange chromatography (Helferich, 1962; Heftmann, 1975; Yamamoto et al., 1988). The first scientific study concerning ion-exchange can be traced to the 1850's work of Thompson and Way with natural ion-exchange in soil (Thompson, 1850). Approximately a century later, attempts were made to apply ion-exchange chromatography to biological compounds. Due to limitations of the resins available at that time, these efforts were met with limited success. Since that time, however, resins have been developed which allow ion-exchange chromatography to be applied to proteins. Because of their large size, proteins require different resin characteristics than those used for separating mixtures of low molecular weight compounds. These sorbents must have a very open structure to allow proteins to penetrate and access the binding sites. Additionally, the backbone matrix of the resin must not interact strongly with the protein. For these reasons, the sorbents used in the separation of low molecular weight compounds, such as the highly cross-linked gel-type polystyrene resins, are unsuitable for use in protein purification.

Adsorbents can generally be divided into three categories; inorganic adsorbents, synthetic adsorbents, and composite adsorbents (van der Wiel and Wesselingh, 1989). Activated carbon and silica are examples of inorganic adsorbents. These adsorbents, which are generally produced by physical processes, have only limited use in protein purification due to their low specificity and small pore size. Synthetic

adsorbents are normally hydrophilic cross-linked polymers produced by chemical synthesis for protein separations. Synthetic resins with large pore structures have been developed. Composite adsorbents combine the rigid structure of an inorganic support with the biocompatibility of hydrophilic polymers to create sorbents with both good structural stability and favorable adsorption characteristics. Of these, the resins based on agarose and dextran have the most widespread use.

As previously mentioned, approximately 3/4 of all industrial protein separations performed chromatographically utilize ion-exchange resins. Reasons for this popularity have been summarized by Asenjo (1990). Ion-exchange resins generally have high capacities, good durability and are relatively inexpensive. Additionally, they are highly selective and generally do not require potentially denaturing or toxic solvents.

The adsorptive behavior of an ion-exchange sorbent is determined to a large extent by the nature of the ionized groups attached to its matrix. Table 1.18 contains a listing of some of the common ionizable groups used in ion-exchange. As the table indicates, resins are normally classified according to the strength of their acid or base groups and by the charge of the species they attract. It is important to note that the strong acid and base groups are nearly always ionized and thus allow these resins to be used at most pH values. For this reason, it is usually advantageous to use resins based on either strong acids or strong bases (J.C. Janson and L. Ryden, 1989). These ionizable groups are always balanced charge-wise by a counter-ion.

The degree to which a protein adsorbs is determined primarily by two factors: the pH of the solution and the concentration of competing counter-ions. Other factors such as temperature may also influence adsorption equilibria, but usually to a lesser extent. The effect of solution pH on adsorption is a result of the amphoteric

Table 1.18

Common Ionic Groups in Organic Polymeric Ion-Exchangers

(Unger, 1990)

Cation Exchange Group		
Strong acid	Weak acid	
$-\text{SO}_3^-\text{H}^-$	$-\text{CO}_2\text{H}$	$-\text{PHO}_2\text{H}$
	 -CH	$-\text{AsO}_2\text{H}_2$
	$-\text{PO}_3\text{H}_2$	$-\text{SeO}_2\text{H}$
	 -SH	

Anion Exchange Group	
Strong base ^a	Weak base ^b
$-\text{NR}_3^+$	$-\text{NR}_2$
$-\text{PR}_3^+$	$-\text{NHR}$
$-\text{SR}_2^+$	$-\text{NH}_2$

^a Stored and often used as the halide form rather than the OH^- form because of poor stability of the latter.

^b The anion exchange site is produced when treated with a strong acid such as HCl to form the hydrochloride salt.

nature of proteins described earlier. As the pH of a protein solution is adjusted further away from the protein's isoelectric point, the protein becomes more highly charged. If the pH is below the isoelectric point the protein has a net positive charge and is thus attracted to the negatively charged groups of the cation exchange resin. Conversely, if the pH is above the pI, the protein has a net negative charge and is attracted by an anion exchange resin.

The salt concentration of the solution also plays a key role in the adsorption equilibria of proteins on ion-exchange resins. The adsorptive process can be viewed with the following stoichiometric relationship



This equation will be developed more fully later in this dissertation but is valuable here as an aid to understand the competitive nature of ion-exchange equilibria. Here, I represents the salt counter-ion, P represent the protein which may have multiple charges, and R represents the ionized groups of the ion-exchange resin. Because proteins are large molecules, they may adsorb on more than one site. This physical reality is taken into account by n , which represents the number of sites or functional groups occupied by an adsorbed protein. It is clear from this stoichiometric relationship that as the salt concentration is raised, *i.e.* I is increased, equilibrium is driven to the left and the protein is desorbed.

1.4.4 Modes Of Operation In Liquid Chromatography

Three modes of operation are commonly found in liquid chromatography and are useful for protein separations. These modes, defined by Giddings (1965), are: differential chromatography or elution analysis, frontal analysis, and displacement

chromatography. They are shown graphically in Figure 1.5. Each of these modes possess properties which make them preferable for use in certain separations. While the modes are operationally different and yield different results, they possess several unifying characteristics.

First, they are all carried out with the same columns, resins, and other equipment. Secondly, each is affected by equilibrium and mass transfer. Thirdly, all can be described using the same mathematics.

Elution analysis or differential chromatography includes two techniques; isocratic elution in which the eluent is unchanged during the process and gradient elution in which a change is made in the eluent during the elution process. Isocratic elution is characterized by good separation of mixture components and dilution of product streams. Conditions are usually adjusted to yield linear sorption isotherms. Typically only low solute loadings are utilized and the solute spreads in a Gaussian concentration distribution. During the process the majority of the column is free of solute and thus is wasteful of solvent and resin. This becomes increasingly true the greater the difficulty in separation (Lightfoot et al., 1992). Because of such conditions, the process is rather inefficient and isocratic elution finds its greatest usefulness in analytical applications of chromatography.

A technique which allows for a more efficient use of the column is gradient elution. In this case, the eluent used initially allows the protein to load heavily on the resin. The eluent is then changed, either in a step or in a smooth gradient which allows the mixture components to desorb and move at a more rapid pace through and out of the column. With ion-exchange chromatography, the change can be made in either the pH, in the counter-ion concentration, or in both simultaneously. Of these, the latter two are generally preferred because it is easier to control (Yamamoto et

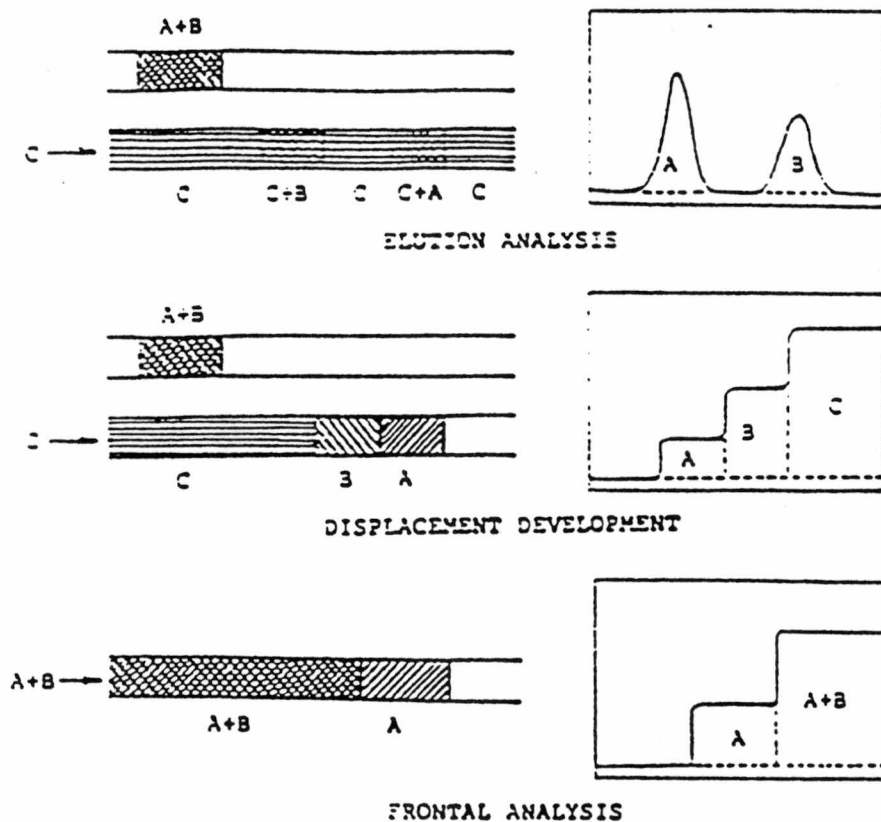


Figure 1.5 Modes of chromatographic separation: (A) elution analysis, (B) displacement development and (C) frontal analysis. The left-hand side represents a schematic of the column with a sample passing through it. The right-hand side shows the corresponding concentration history. A and B are sample components while C is either the eluent or the displacer (Ettre, 1980).

al., 1988). By carefully designing the application of the gradient, it is possible to achieve good separation of the mixture components without dilution of the products. In fact, it is possible to achieve both separation and concentration within a single step. Because of this, gradient elution is often used for preparative-scale separations.

Frontal analysis allows for the separation of one component from a mixture. This component is rarely diluted by the process. It is most often applied when the objective is to remove strongly retained components from an unretained product. Frontal chromatography has the advantage of allowing a relatively large volume of the product stream to be processed before impurities begin to break through.

Displacement development utilizes competitive adsorption for the resin exchange sites to obtain a simultaneous separation and concentration. In this technique, the mixture to be separated is fed into the top of the column. Conditions are such that the mixture components are strongly and competitively adsorbed by the resin. After loading the protein mixture on the resin, a component known as the "displacer" which has a greater affinity for the resin than any of the proteins in the mixture, is fed into the column. As this component moves down the column, the components of the protein mixture are displaced from the resin, concentrating in the mobile phase and eventually moving through the column at the same velocity of the displacer. The net result is that a separation of components is achieved while at the same time, all the components are concentrated, often by a large amount. For these reasons displacement chromatography is a very attractive technique for preparative-scale separations. One of the primary difficulties in implementing displacement chromatography, particularly in protein separation, is in finding suitable displacers. Advances have, however, been made in this area (Horvath, 1985; Frenz

and Horvath, 1985; Subramanian et al., 1988; Subramanian et al., 1989). In the literature survey for this research project no past work on displacement chromatography of whey proteins was found. Also, because of the primary goal of obtaining whey proteins in their native undenatured form and of high purity, it was deemed that a suitable displacer of food grade quality and one that would not adversely affect the proteins, could be found.

1.5 Continuous Liquid Chromatography

Various techniques have been developed to improve chromatographic separations. One area in which engineers have tried to improve chromatography, is to convert it from a batch process to a continuous processes.

Two general methods are used to make the conversion by establishing a relative motion of mobile and sorbent phases. One method is to move the adsorbent relative to the feed point. The second is to move the feed points of the mixture or mobile phase relative to a stationary bed. Several authors have summarized some of these methods (Seigell et al., 1986; DeCarli, 1989; Gordon and Cooney, 1990). Various devices utilizing either of these two methods have been developed as briefly discussed below.

One device for continuous chromatography has been developed by Sussman and his coworkers (Sussman et al., 1967; Sussman et al., 1974). In this device, shown in Figure 1.6, two disks are coated with a thin layer of solvent. The disks are placed close together, sharing the same axis. Both the eluent and mixture to be separated are fed at the center of the device and the disks are rotated while the eluent flows radially outward. The resulting separation occurs continuously. In the schematic of the process shown in Figure 1.6, Product A is the least strongly retained component

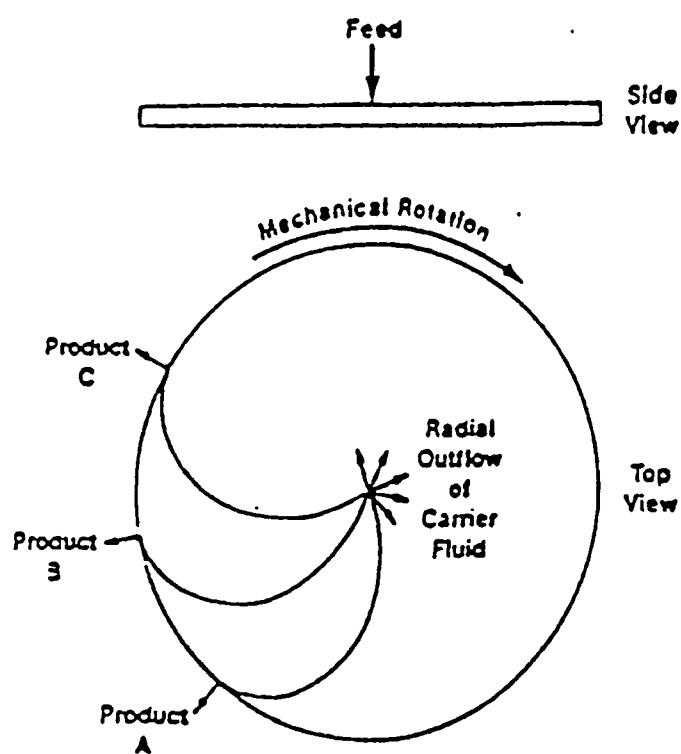


Figure 1.6 Continuous chromatography: mechanically rotated radial flow separation device (Siegele et al., 1986).

followed by products B and C.

Hughes and Charm (1979) have developed a device in which the adsorbent is moved by a belt through a series of chambers to effect a continuous separations as is shown in Figure 1.7. This mechanically complex device was developed for use in the purification of enzymes using an immunosorbent.

Seigell and his coworkers have developed a cross-flow magnetically stabilized bed (MSB) chromatograph (Seigell et al., 1986). The operating principle of this device is shown in Figure 1.8. The adsorbent used in this process is magnetizable. This allows the mixing characteristic of fluidized bed to be eliminated with the application of a magnetic field. The adsorbent is cycled through the device as the feed is injected continuously at the base of the device. The movement of the adsorbent and upward flow of the fluidizing mobile phase effect the separation.

A different approach has been described by Tuthill (1970) and is shown in Figure 1.9. The resin is held in a rectangular slab and the mixture to be separated is fed continuously into a corner of the device. In operation, the direction of the mobile phase is periodically altered by 90 degrees at periodic time intervals. The resulting separation occurs semicontinuously with the separated products exiting at different position at the end of the bed. In Figure 1.9, component A which is more strongly retained, exits the bed at a position closer to the feed point.

Another semicontinuous process known as the simulated moving bed process (Sorbex) has also been developed (Broughton, 1968; Sussman and Rathore, 1975). The process utilizes a rotating progression of fixed beds to simulate the behavior of a true countercurrent hypersorption process. It should be recognized that while the process is very efficient for separating binary mixtures, the separation of N components requires N - 1 Sorbex units. Thus, the process is not "chromatographic

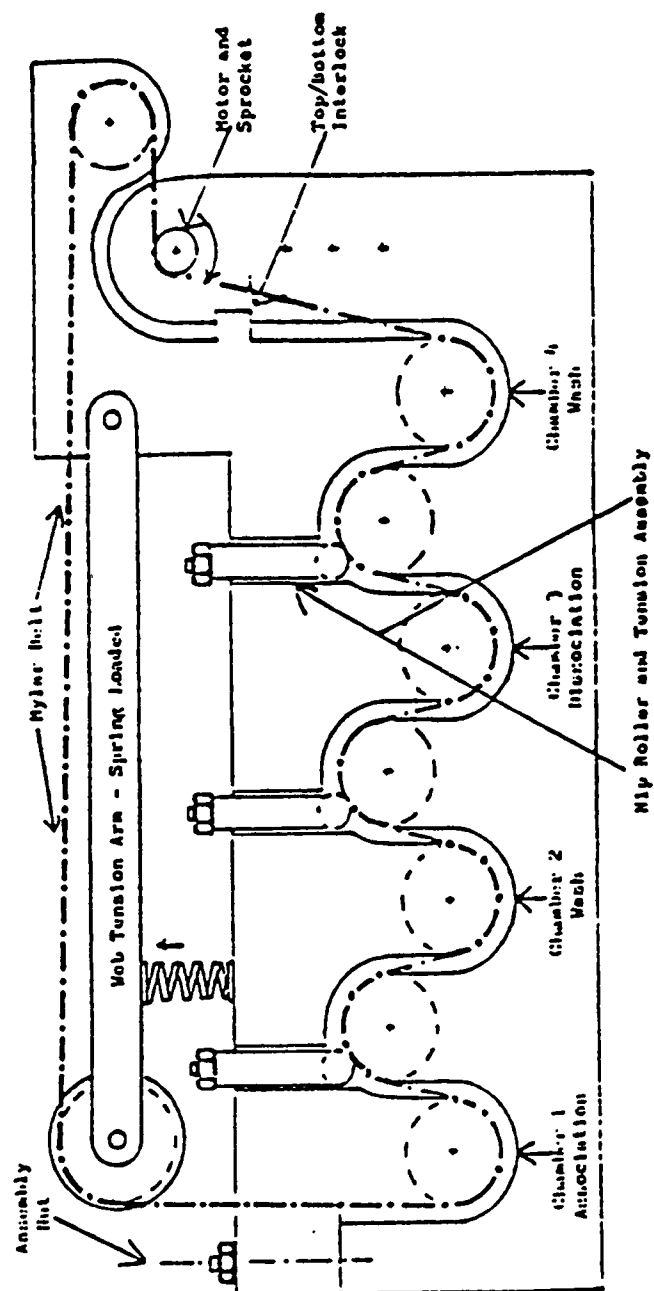


Figure 1.7 Continuous chromatography: moving belt separation device (Hughes and Charm, 1979).

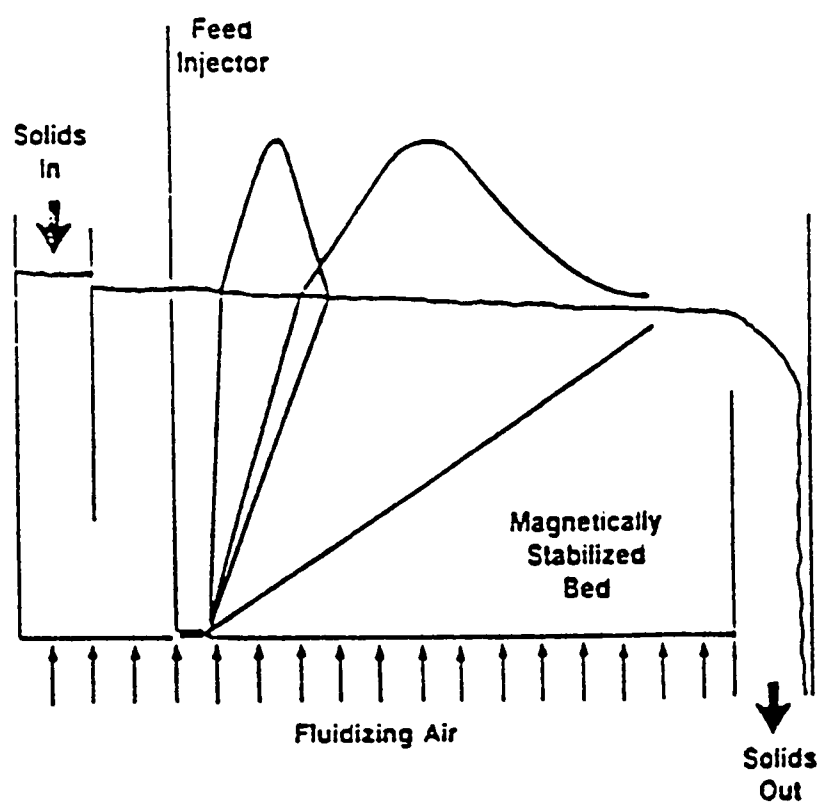


Figure 1.8 Continuous chromatography: mechanically stabilized bed (Siegel et al., 1986).

"in the sense that multicomponent separations are not effected.

1.5.1 Continuous Annular Liquid Chromatography

An alternative apparatus which is considered in this study, is the Continuous Annular Chromatograph (CAC), shown schematically in Figure 1.10. The CAC uses a sorbent placed in the annulus formed between two concentric cylinders. The protein solution to be separated is introduced through a feed port at the top of the column. This feed port remains at a fixed point in space while the entire bed assembly is rotated slowly about its vertical axis. When operating isocratically, the eluent is distributed uniformly about the unit circumference and flows from top to bottom. The components which are less strongly retained by the sorbent elute at a steeper angle than do the more strongly retained components. As a result, the greater the affinity a protein has for the resin, the greater the angular distance it will cover before exiting at the bottom of the unit. Thus, the traditional one dimensional batch-wise process is converted into a two-dimensional, continuous steady-state process.

The concept of continuous annular chromatography was first proposed in 1949 by Martin (1949). Research has been conducted by several workers since that time, primarily in the late 1960's and early to mid 1970's, but because gravity feed systems were used, none of these studies resulted in a device that allowed a suitably high throughput (Moskvin, 1974a, 1974b, 1975; Fox et al., 1969; Dunnill and Lilly, 1972; Byalyi and Ganitshii, 1969). Scott et al. (1975) are generally credited to be the first to have developed a successful high-throughput prototype of the CAC. Most of the early research with this device has been conducted on isocratic metal ion separations using ion-exchange resins (Scott et al., 1976; Cannon and Sisson, 1978;

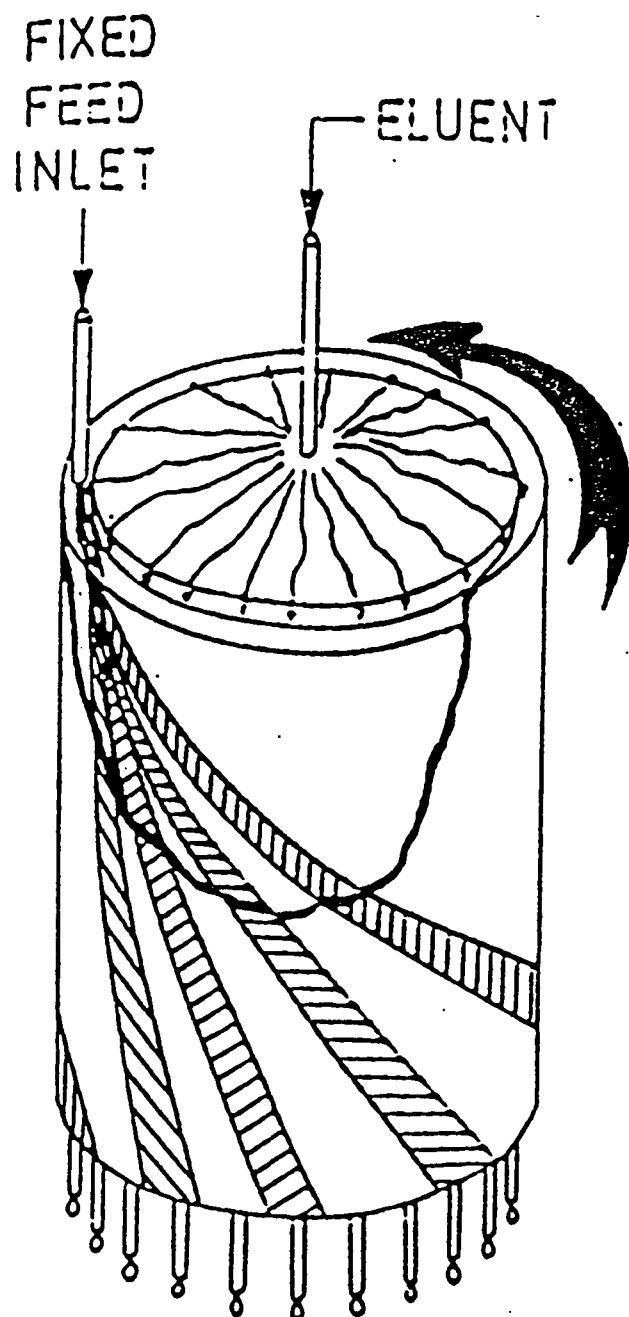


Figure 1.10 Continuous chromatography: continuous annular chromatograph (DeCarli, 1989).

Cannon et al., 1980; Begovich et al., 1983).

Several other separations were later investigated. Howard et al. (1988) have studied the separation of dilute sugar mixtures. This research also laid the groundwork for a more fundamental approach to modelling the system allowing the development of scale-up criteria for concentrated sugar mixtures (Byers et al., 1989). DeCarli et al. (1988) studied the implementation of step elution for metal ion separations, arriving at the development of models to predict the performance based on equilibrium and mass transfer data. Further work was also carried out to develop a continuous displacement chromatography apparatus based on the CAC concept (DeCarli et al., 1988; DeCarli, 1989). This apparatus was used for the displacement separation of amino acid mixtures. In general these studies demonstrated that, for the systems studied, parameters obtained from fixed bed operations could be used to model CAC operations. The CAC was typically capable of performing continuous separations with the same separating efficiency as fixed beds provided that operating conditions were rationally selected. Whether this is possible or not for any given system, depends upon the relative importance of dispersion and mass transfer effects (Carta and Byers, 1991). Thus, a fundamental study of these phenomena would provide data necessary to assess the validity of continuous operation with the CAC for each specific system.

With this review of liquid chromatography and the types of processes available, the methods of step elution ion-exchange chromatography and gel filtration were chosen for investigation experimentally at the preparative scale to determine their feasibility for separating lactose and the four major proteins from sweet dairy whey and from one another. From this research effort conclusions were then drawn about the possibility of effectively scaling up these methods to a process scale operation.

An outline of the research plan for accomplishing this is described in the next chapter.

CHAPTER II

RESEARCH OUTLINE

2.1 Objectives of Research

Many different types of liquid chromatography are utilized industrially for separation and purification of protein mixtures. These include size exclusion, ion-exchange, adsorption, and affinity chromatographic processes. Within the last decade there has been increased 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. This research needs to continue with specific research into industrially significant problems like multicomponent protein mixtures separation and purification. The methodology for the scale up of fixed bed and optimization of

large scale separations needs to be further defined.

The purpose of this research project is to investigate the potential of three new process grade resins, two of which are ion-exchange and the other gel filtration, for separating lactose and the four major dairy whey proteins from sweet dairy whey and one another, at the preparative scale. The four major proteins studied are: alpha lactalbumin, beta lactoglobulin, bovine serum albumin, and immunoglobulin G.

The objectives of this research are stated as follows:

1. To investigate the effectiveness of step elution techniques for the isolation of lactose and the four major dairy whey proteins from sweet dairy whey by preparative anion exchange chromatography followed by cation exchange chromatography for further recovery of immunoglobulin G.
2. To investigate the effectiveness of preparative gel filtration for the isolation of lactose and the four major dairy whey proteins from sweet dairy whey and one another; also, its potential in further purification of these compounds from the resultant peaks of the preparative anion exchange process.
3. To conduct an efficient scale-up of the optimized preparative anion and cation exchange processes and if viable, the gel filtration process. The next level of scale is generally classified as market development plant scale, the size of which will be determined in an economic optimization of the combined processes.
4. To layout a preliminary process for implementing the scaled-up ion-exchange processes.

2.2 Whey Protein System

To conduct these studies an actual dairy whey protein system was utilized. As discussed in chapter one this study is centered around the waste whey stream

produced at the Dairyman Inc. plant in Greeneville, Tennessee. This plant produces cheddar cheese products for Kraft Foods and consequently about 115 million pounds per year of sweet whey. A research agreement was made between the author and the plant management to facilitate this study. The plant provided whey samples to carry out the chromatographic studies. In return results of the research were provided to the plant in a report format.

Samples of the whey stream were collected and analyzed by two independent laboratories for lactose, proteins, and minerals content. Kendrick Labs of Madison, Wisconsin analyzed samples for the proteins and Silliker Laboratories of Stone Mountain, Georgia analyzed samples for lactose, minerals, and total solids content. It is important to note that the whey samples were taken from the dairy process after being concentrated with reverse osmosis from about 6 wt. % to 15-18 wt.% total solids content. An analysis of the whey before and after reverse osmosis was presented in Table 1.12. This composition set is representative of the plant's whey stream even though there may be slight variations from day to day production due to different cheeses being produced and the raw milk coming from various sources. The advantage of the reverse osmosis step is that a pre-concentration step has already been conducted. A previous study revealed this to be highly advantageous for size exclusion separations (Delaney et.al, 1973).

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 small scale fixed bed studies to determine the chromatographic separation performance of a new process grade anion exchange

resin, Q Sepharose Big Beads from Pharmacia Biotech Inc.

Following this step, the resultant peaks were then processed by preparative gel filtration using Pharmacia Biotech Inc.'s new gel filtration resin, Superdex 75 prep grade. As will be shown in Chapter 7, the results of this step were not satisfactory for the stated intention due to increased solids content in the isolated protein peaks, and so the use of this gel filtration resin was not investigated further.

Instead the breakthrough curve from the anion exchange step was further processed by cation exchange chromatography using Pharmacia Biotech Inc.'s S Sepharose Big Beads. The purpose of this step was to separate the valuable immunoglobulin G from the other components of the anion exchange breakthrough curve. The approach to these chromatographic studies will now be discussed.

2.3.1 Anion Exchange Chromatography - Step Gradient Elution Techniques for Initial Proteins Separation

1. Experiments with a preparative Pharmacia Biotech Inc. column were conducted at several flowrates and column volume loadings of feed. The column was 5 cm in diameter and 30 cm in length with a bed height of 25 cm. The feed to the column was fresh sweet dairy whey obtained from the Dairyman Inc. plant in Greeneville, Tennessee. The techniques of pH, salt, and simultaneous pH and salt step gradient elution were investigated in order to determine qualitatively which one yielded the greatest resolution for the peaks at a given flowrate. The buffer system used was sodium acetate in water at several concentration levels and pH levels, which are described in Chapter 6. Mass transfer parameters and bed properties were found experimentally once the conditions for maximum resolution and the largest permissible flowrate were determined.

2. Analytic and linear equilibrium mathematical models of the separation process at this scale were then utilized for scaling up the process to several larger column sizes available from Pharmacia Biotech Inc. as part of an economic optimization for the project.

2.3.2 Gel Filtration Studies For Further Isolation of Proteins From Anion Exchange Chromatography

The potential for further isolation of the lactose and the four major whey proteins from the resultant peaks of the anion exchange process was investigated. The preparative gel filtration column used was from Pharmacia Biotech Inc. and was 1.6 cm in diameter by 60 cm in length. Low loading levels of samples from the anion exchange peak fractions were chromatographed on the gel filtration column in the isocratic elution mode. The buffer system used was per recommendation from Pharmacia Biotech Inc., and was a combination of sodium chloride and sodium phosphate anhydrous. Details on the buffer system are given in Chapter 7.

2.3.3 - Cation Exchange Chromatography - Step Gradient Elution Techniques For Further Recovery Of Immunoglobulin G

1. Experiments with a preparative Pharmacia Biotech Inc. column were conducted using a new process grade resin also from Pharmacia Biotech Inc. - S Sepharose Big Beads. The feed to the cation exchange column was the breakthrough curve fraction from the anion exchange step. The goal of the step was to recover the valuable IgG in the breakthrough curve. The technique of pH gradient elution at specific salt buffer concentrations was investigated in order to determine qualitatively which salt concentration yielded the greatest resolu-

tion for the resultant peaks. The pH step changes implemented were designed to span the isoelectric point range of immunoglobulin G so as to initially adsorb the IgG and then desorb it. The buffer system used was sodium acetate in de-ionized water. Details are described in Chapter 8.

2. Analytic and linear equilibrium mathematical models of the separation process at this scale were then utilized for scaling up the process to several larger column sizes available from Pharmacia Biotech Inc. as part of an economic optimization for the project.

CHAPTER III

THEORETICAL DISCUSSIONS

3.1 Mathematical Analysis Of Fixed Bed Liquid Chromatographic Processes

The movement of a mixture component through a fixed-bed chromatographic column may be described mathematically. Numerous modeling approaches exist, as reviewed for example by Gu (1990), Lightfoot (1986), Lightfoot (1992), Rasmuson (1980), Ruthven (1984), Suwondo et al. (1991), and Whitley (1990). The column is characterized as having a uniform flow of carrier or eluent fluid through the interstices of a bed of identical spheres which is composed of two coexisting phases: a discontinuous solid matrix phase in a continuum of intersecting pores. Ideally this is represented mathematically by the following equations:

Mobile Phase

$$\frac{\partial c_{bi}}{\partial \theta} = \frac{1}{Pe_{bi}} \frac{\partial^2 c_{bi}}{\partial x^2} - \frac{\partial c_{bi}}{\partial x} + Y_{bi} - N_{fi}(c_{bi} - c_{pi, \xi=1}) \quad (3.1)$$

$$x = 0, \frac{\partial c_{bi}}{\partial x} = Pe_{bi}(c_{bi} - c_{fi}) \quad (3.2)$$

$$x = 1, \frac{\partial c_{bi}}{\partial x} = 0 \quad (3.3)$$

$$\theta = 0, c_{bi} = c_{bi}(0, x) \quad (3.4)$$

Pore Phase

$$K_{ei} \left(\epsilon_p \frac{\partial c_{pi}}{\partial \theta} - \epsilon_p Y_{pi} \right) + \left(\frac{\bar{C}_{Ti}}{C_{ei}} \right) Y_{li} = N_{pi} \frac{1}{\xi^2} \frac{\partial}{\partial \xi} \left(\xi^2 \frac{\partial c_{pi}}{\partial \xi} \right) \quad (3.5)$$

$$\xi = 0, \frac{\partial c_{pi}}{\partial \xi} = 0 \quad (3.6)$$

$$\xi = 1, \frac{\partial c_{pi}}{\partial \xi} = Bi_i(c_{bi} - c_{pi}) \quad (3.7)$$

$$\theta = 0, c_{pi} = c_{pi}(0, \xi) \quad (3.8)$$

Solid Phase

$$\frac{\partial \bar{c}_{pi}}{\partial \theta} = Y_{li} + \bar{Y}_{pi} \quad (3.9)$$

$$\theta = 0, \bar{c}_{pi} = \bar{c}_{pi}(0) \quad (3.10)$$

These equations and associated boundary conditions comprise a general rate model for fixed bed chromatographic separation processes. The equation set is in dimensionless form and is solved numerically by the method of orthogonal collocation on finite elements. This numerical method will be further discussed later. The software package that simulates this equation set has been developed by researchers at Purdue University Berninger et al. (1991). It is known as VERSE-LC and is designed to simulate frontal, elution, and displacement chromatography in isocratic or gradient modes of operation. The model is also capable of simulating non-equilibrium adsorption and solution and solid phase reactions with a wide range of isotherm models to choose from (Whitley, 1990).

In this equation set, the mobile, pore, and solid phase concentrations for a component i are scaled by their respective maximum expected values:

$$c_{bi} = \frac{C_{bi}}{C_{ei}} \quad (3.11)$$

$$c_{pi} = \frac{C_{pi}}{C_{ei}} \quad (3.12)$$

$$\bar{c}_{pi} = \frac{\bar{C}_{pi}}{\bar{C}_{Ti}} \quad (3.13)$$

In these equations, C_{ei} is the maximum possible inlet concentration for component i which is likely to be the feed concentration for component i . $\overline{C}_{Ti}$ is the maximum capacity of the adsorbent material for component i . Likewise, space and time variables are scaled by characteristic values:

$$x \equiv \frac{z}{L} \quad (3.14)$$

$$\xi \equiv \frac{r}{R} \quad (3.15)$$

$$\theta \equiv \frac{t}{\tau} \quad (3.16)$$

$$\tau \equiv \frac{L}{u_0} \quad (3.17)$$

where τ is the time for one bed volume of solution to percolate through the column.

The terms Y_b and Y_p represent the consumption of a component i by reaction in the bulk and pore phases, respectively. The term Y_{li} in the pore phase equation represents the net loss of component i in the pore phase by adsorption to the solid phase particles.

The mathematical modeling aspects of the chromatographic process can be broadly classified into two groups: linear and non-linear adsorption/desorption. In linear chromatography the equilibrium distribution coefficient, the rate constant for adsorption, and the fluid properties are considered to be independent of concentration. This is reasonable for dilute samples or low loadings, and for some special cases such as sugars.

Because of the complexities associated with the solution of the general rate model for fixed bed calculations, simplified representations of the transport rate

are often used. The most popular expression is the so called linear driving force approximation (Glueckauf, 1955) which relates component mass transfer between the pore liquid and the solid phase. Instead of relating the concentration of a component in the pore phase liquid, at the particle surface, to the pore and solid phase transport equations, it is assumed that there is a linear equilibrium between the pore phase liquid and the solid phase for the component of interest. In this case there is a single transport equation, shown here in dimensional form for clarity:

$$\frac{\partial C}{\partial t} = \epsilon_b D_z \frac{\partial^2 C}{\partial z^2} - (1 - \epsilon_b) \frac{\partial q}{\partial t} - \epsilon_b v \frac{\partial C}{\partial z} \quad (3.18)$$

where q is the sorbent phase component concentration, C is the fluid phase component concentration, D_z is the z direction axial dispersion coefficient, and v is the interstitial velocity.

In this equation, the term $(1 - \epsilon_b) \frac{\partial q}{\partial t}$ is part of a subsequent equation that expresses the mass transfer of component to and from the solid phase in terms of a global mass transfer coefficient, $k_o a$ and a driving force $F(q, C)$, shown by (Howard, 1987):

$$(1 - \epsilon_b) \frac{\partial q}{\partial t} = k_o a F(q, C) \quad (3.19)$$

If it is proposed that transfer of component to and from the particle experiences two film resistances, fluid and solid, then $k_o a$ can be equated to a two-film mass transfer resistance model with resistances in series (Howard, 1987):

$$k_o a = \left(1/ka + 1/k'K \right)^{-1} \quad (3.20)$$

and

$$F(q, C) = (C - C^*) \quad (3.21)$$

where Howard (1987) gives:

k = fluid-phase mass transfer coefficient

k' = solid-phase mass transfer parameter

K = equilibrium distribution coefficient

C^* = solid-phase equilibrium concentration

a = interfacial area

Therefore,

$$(1 - \epsilon_b) \frac{\partial q}{\partial t} = k_0 a (C - C^*) \quad (3.22)$$

For linear equilibrium, C^* is related to q by:

$$q = KC^* \quad (3.23)$$

The initial and boundary conditions for this model are:

I.C., for all z :

$$t = 0, q = C = 0;$$

B.C.'S

$$z = 0, C = C_f, 0 < t < t_f; C = 0, t > t_f; \quad (3.24)$$

For spherical particles with intraparticle diffusion control, $k_0 a$ is related to the effective diffusivity by (Ruthven, 1984):

$$k_o a = \frac{15D_e}{R_p^2} \quad (3.25)$$

The effective diffusivity is further related to the pore and surface diffusion coefficients for a component by

$$D_e = D_p + D_s \frac{\partial q}{\partial C_p} \quad (3.26)$$

Therefore, if the effective diffusivity is known or is used as a fitting parameter, then the linear driving force model can be solved to obtain breakthrough curves. This of course assumes that the linear equilibrium coefficient K is known for the component concentration range of interest. The model can be solved numerically by the software package PDECOL (Madsen and Sincovec, 1979), or for infinitely small pulses, analytically, as described in Sherwood, Pigford, and Wilke (1975). Since the feed pulses used in this work are substantial in volume, only numerical simulations of models will be investigated.

For situations where high loadings of component(s) take place onto a column, the assumption of a linear equilibrium distribution is likely not to be valid; nor is the assumption that the adsorption parameters and fluid properties are independent of concentration. This situation is demonstrated for the ion-exchange chromatography of proteins as will be discussed later. In this case the general rate model of equations 3.1 - 3.10 are required to be solved numerically with the appropriate isotherm inserted into the model.

For the numerical solution of the equation 3.1 - 3.10, the VERSE-LC software package uses the method of orthogonal collocation on finite elements. From a survey of the literature on preparative chromatography modeling and simulation, this method seems to be the frequently applied and efficient for solving such

partial differential equation systems. Sometimes the model of the preparative chromatography process is referred to as a two region model, one region being the axial dimension related terms and the other the radial dimension of the particles related terms (Costa and Rodrigue, 1989). The finite element method is a well known technique that is explained in detail by several authors (Baker, 1983; Finlayson, 1980; and Villadsen and Michelsen, 1978;).

The method of orthogonal collocation on finite elements converts a system of partial differential equations into a system of ordinary differential and algebraic equations. This system is then solved with the time integrator package DASSL, a Differential/Algebraic System Solver (Petzold, 1982). The method of orthogonal collocation on finite elements, coupled with this time integrator package, has been shown to be an efficient method for solving the detailed rate model of preparative chromatography (Whitley, 1990).

As can be seen by the general rate model equation set, there are several non-dimensional parameter coefficients in front of the various algebraic and partial differential terms. These non-dimensional parameters represent ratios of the different rate processes taking place in a column during a chromatographic separation. Such rate processes are axial dispersion, film mass transfer, intraparticle diffusion, adsorption and desorption, and reaction. One or more of these may be rate controlling depending on the particular chromatographic process taking place. The more important rate ratios are broken down as follows: The ratio of axial dispersion to convection is $\frac{1}{Pe_{bi}}$, where Pe_{bi} is the Peclet number for component i in the bulk or mobile phase. The ratio of film mass transfer to convection is $\frac{3Lk_{fi}(1-\epsilon_b)}{Ru_o\epsilon_b}$, and is given the symbol N_{fi} . The ratio of film mass transfer to intraparticle diffusion is $\frac{k_{fi}R}{D_e}$, and is given the symbol Bi_i , the Biot number for component i . Finally,

the ratio of intraparticle diffusion to convection is $\frac{L}{R} \left(\frac{1}{Pe_{pi}} \right)$, and is given the symbol N_{pi} . Ratios for adsorption and desorption to convection, film mass transfer, and intraparticle diffusion are listed in the table of symbols. Since solution and solid phase reactions are not anticipated to occur in this project, they, along with their respective rate ratios will not be discussed. The value of these rate ratios becomes evident when attempting to optimize an existing preparative-scale separation and then scaling up to a larger column operation. By using experimentally determined parameters and those obtained from correlations for the respective rates, the dimensionless parameters can be determined. Comparison of these dimensionless rate ratios will then reveal which rate process is controlling.

3.2 Mass Transfer Parameters

The three types of mass transfer considered in this rate model are: axial dispersion, film mass transfer, and intraparticle diffusion. Each of these is characterized by a dimensional parameter which is then used in the dimensionless rate ratios discussed above. These types of mass transfer will now be discussed in further detail.

Axial dispersion is the mixing of component(s) in the bulk flowing phase along the vertical or axial direction of a packed column due to the tortuous path a component(s) must undergo from the point of initial injection at the top of the column to exiting at the bottom of the column. It also results from column wall effects as the component(s) molecules flow adjacent to the column's wall and also very close to the sorbent particles in the column. This mixing is undesirable since it reduces the concentration differences and hence gradients taking place in the column for a particular component. Axial dispersion therefore contributes to the spreading of a

component's band profile as it exits the column because the component(s) molecules spend additional time in the column. This in turn causes dilution of the component of interest. In this general rate model, the flow pattern is assumed to be plug flow with axial dispersion imposed onto it. Typically in modelling liquid chromatography systems, the contributing causes of axial dispersion are lumped together into one characteristic parameter, the axial dispersion coefficient (Ruthven, 1984). Much research has been conducted into axial dispersion phenomena for flows through packed beds (Gunn, 1987). From this research three main correlations have been developed to predict the axial dispersion coefficient or the Peclet number which it is a part of. They are:

Chung and Wen (1968)

$$Pe_{bi} = \frac{L}{2\epsilon_b R} (0.2 + 0.01 Re^{0.48}) \quad (3.27)$$

Gunn (1987)

$$\frac{1}{Pe_{bi}} = G_i(1-p)^2 + G_i^2(1-p)^3 \left(\exp\left(\frac{-1}{G_i p(1-p)}\right) - 1 \right) + \frac{\epsilon_b}{\gamma Re Sc_i} \quad (3.28)$$

where

$$G_i = \frac{Re Sc_i}{4\alpha_1^2(1-\epsilon_b)}; p = 0.17 + 0.33 \exp(-24) \quad (3.29)$$

and α_1 is the smallest positive zero root of the Bessel function J_0 , and is equal to 2.405, and γ is the tortuosity factor, equal to 1.4.

Koch and Brady (1985)

$$\frac{1}{Pe_{bi}} = \frac{3}{4}\epsilon_b + \frac{\pi^2}{6}\epsilon_b(1-\epsilon_b)\ln(Re Sc_i) + \frac{\epsilon_b}{Re Sc_i} \quad (3.30)$$

If the reciprocal of the bulk phase Peclet number is plotted against the Reynolds number, it is seen that the Gunn and Koch and Brady correlations show a greater dependence on the Reynolds number than the Chung and Wen correlation. All three correlations are available in the VERSE-LC model and only use of each in a given simulation will reveal which is the appropriate one to use.

Film mass transfer or the resistance to such by an external fluid film surrounding the sorbent particles in the packed bed, is determined by the hydrodynamic conditions present. The fluid mechanical boundary condition of no slip of molecules at a solid boundary means that each particle is surrounded by a laminar sublayer, through which the mass transfer of a component occurs by molecular diffusion. The thickness of this sublayer and hence the mass transfer coefficient for the flux of component through it, is determined by the hydrodynamic conditions (Ruthven, 1984). For low Reynolds numbers, between 0.0015 and 55, the following correlation has been found to be useful (Wilson and Geankoplis, 1966):

$$\frac{k_{fi}}{u_o \epsilon_b} = \frac{1.09}{\epsilon_b} Re^{-2/3} Sc^{-2/3} \quad (3.31)$$

where k_{fi} is the mass transfer coefficient for component i . If the definitions for the Reynolds and Schmidt numbers are inserted, the following is obtained:

$$k_{fi} = \frac{1.09 u_o (D_i)^{2/3}}{(2 R_p u_o \epsilon_b)^{2/3}} \quad (3.32)$$

This equation shows that the film mass transfer coefficient for a component is a weak function of the superficial velocity through the chromatographic bed and is more dependent on the component's molecular diffusivity. Simulations with a linear equilibrium model will be shown later in chapter five which confirm this.

The third type of mass transfer, intraparticle diffusion, is discussed in the following sections.

3.3 Pore Diffusion Modeling

In the preparative-scale chromatography of proteins, pore diffusion is found to be the dominant mass transfer resistance leading to peak spreading. A knowledge of intraparticle transport is therefore essential to an understanding of their chromatographic behavior.

There are four mechanisms by which a compound may be transported through the pores of a chromatographic resin. These mechanisms are: molecular diffusion, Knudsen diffusion, Poiseuille flow, and surface diffusion (Ruthven, 1984). Knudsen diffusion is dominant in systems in which the mean free path between colliding molecules is larger than the pore diameter. It is, therefore, more typical of low pressure gaseous separations using resins with small pore diameters. Poiseuille flow occurs when a significant pressure drop across a particle exists. This pressure drop is generally small when particles are packed in a bed and may be considered negligible unless the pores are very large ($> 2,000 \text{ \AA}$). Thus, two mechanisms commonly account for transport of proteins through the resin pores: molecular diffusion and surface diffusion.

Molecular diffusion occurs in the liquid phase of the pores and may be written as an effective pore diffusivity which takes into account the tortuosity of the resin pores, τ_p , and the volume occupied by the solid portions of the resin. This relationship may be expressed as

$$D_p = \frac{D\epsilon_p}{\tau_p} \quad (3.33)$$

where D is the molecular diffusivity of the component.

Surface diffusion provides a mechanism by which the intraparticle transport may be increased. It is the mechanism which describes the flux of adsorbed molecules along the pore walls. Surface diffusion coefficients are usually an order of magnitude smaller than molecular diffusion coefficients. In spite of this, surface diffusion may be a significant mechanism of intraparticle transport when the adsorbed phase concentration is significantly higher than the mobile phase concentration. It should also be noted that, for large components such as proteins, under some circumstances, high loadings may result in "hindered" diffusion within the particle. This results when the proteins are large enough relative to the pore size to restrict flow through the pores upon their adsorption. Intraparticle diffusion is the process by which a component i enters a particle's porous network and diffuses to interior adsorbent sites. This porous network is dependent on the type of sorbent particle and can be composed of one or more pore radii distributions. The tortuous path a component takes to reach the particle interior from its surface represents a resistance to the diffusion of the component. For large components such as proteins, this is a major resistance as the large molecule must attempt to enter substantially smaller pores in the particle. It is in fact usually the one that controls chromatographic mass transfer processes for proteins separations. The coefficient of the flux for a component into a particle is the effective intraparticle diffusion coefficient, D_e . This coefficient can be further broken down into two components: a pore diffusion term and a surface diffusion term.

$$D_e = D_p + D_s \frac{\partial q}{\partial C_p} \quad (3.34)$$

The adsorbed phase concentration, q , may be defined in terms of the entire

resin volume or in terms of the solid volume of the resin. These definitions are related by

$$\bar{q} = \frac{3}{R_P} \int_0^{R_P} [\epsilon_p C_p + (1 - \epsilon_p)q] r^2 dr \quad (3.35)$$

where $\bar{q}$ is the average adsorbed phase concentration base on the entire resin volume, q is the solid phase concentration based on the solid resin volume, C_p is the pore phase concentration, R_P is the particle radius and ϵ_p is the particle porosity.

By writing a differential mass balance on the resin particle we obtain

$$\epsilon_p \frac{\partial C_p}{\partial t} + (1 - \epsilon_p) \frac{\partial q}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 \left(D_p \frac{\partial C_p}{\partial r} + D_s \frac{\partial q}{\partial r} \right) \right] \quad (3.36)$$

where D_p and D_s are the effective pore and surface diffusion coefficients, respectively. If we use the definition of the effective intraparticle diffusivity, equation 3.34, we may write the following equation for protein uptake from a liquid bath:

$$\epsilon_p \frac{\partial C_p}{\partial t} + (1 - \epsilon_p) \frac{\partial q}{\partial t} = D_e \frac{1}{r^2} \frac{\partial}{\partial r} \left(r^2 \frac{\partial C_p}{\partial r} \right) \quad (3.37)$$

As was previously pointed out, D_p is typically an order of magnitude larger than D_s . Thus, for conditions of weak adsorption, when $\partial q / \partial C_p$ is relatively small, intraparticle diffusion is dominated by pore diffusion. If, on the other hand, the adsorption is strong and, thus, $\partial q / \partial C_p$ is high, surface diffusion may be the dominant mechanism of transport. This dependency of effective intraparticle diffusivity on loading offers a means of elucidating its two components as is shown in the following two sections.

3.3.1 Intraparticle Diffusion For Weak Adsorption Conditions

Under weak adsorption conditions such as those found in linear elution chromatography, pore diffusion is the dominant mechanism of intraparticle transport. Thus by examining the protein elution curves of linear elution chromatography, it is possible to determine the effective pore diffusivity of the protein.

For compounds exhibiting linear isotherms it is possible to obtain analytical solutions to the equations governing chromatographic behavior. Much effort has been made in deriving solutions of to models describing breakthrough curves. Many of these have been summarized by Ruthven (1984). The earliest of these solutions was determined by Anzelius (1926) who based his solution on an external mass transfer resistance model. Extensions to this solution have been derived by Lapidus and Amundson (1952) whose model included axial dispersion effects and by Rosen (1952, 1954) who developed a solution to a model with intraparticle diffusion control. Solutions to the chromatographic response to pulse injections also exist, though they are less common than those predicting breakthrough behavior. Solutions have been developed for infinitesimal injection pulses and these work well for analytical-scale applications. Carta (1988) has developed an analytical solution to a model which accounts for "rectangular" feed injections applied in a periodic manner such as may be encountered in process chromatography. This model includes both external film and intraparticle diffusional mass transfer resistances. Boundary conditions are written at the column entrance in which the component concentration is assumed to vary in a periodic manner to yield

$$c_f(0, t) = c_F \text{ for } (j-1)(t_F + t_E) < t < j(t_F + t_E) - t_E \quad (3.38)$$

$$c(0, t) = 0 \text{ for } j(t_F + t_E) - t_E < t < j(t_F + t_E) \quad (3.39)$$

where c_F is the protein feed concentration, t_F and t_E are the duration of the feed and elution pulses and j represents the cycle number. The resulting series solution is,

$$\begin{aligned} \frac{c_f}{c_F} = \frac{\check{r}_F}{2\check{r}} + \frac{2}{\pi} \sum_{k=1}^{\infty} \left[\frac{1}{k} \exp\left(-\check{n}_p \frac{N_f}{N_p} \check{a}_k\right) \times \sin\left(\frac{k\pi r_F}{2r}\right) \right. \\ \left. \times \cos\left(\frac{k\Psi_p}{\check{r}} - \frac{k\pi\check{r}_F}{2\check{r}} - \frac{k\check{\beta}\check{n}_p}{\check{r}} - \check{n}_p \frac{N_F}{N_p} \check{b}_k\right) \right]. \end{aligned} \quad (3.40)$$

where

$$\check{a}_k = \frac{(\gamma_k - \lambda_k)[\gamma_k - (1 - N_F/N_p)\lambda_k] + \check{n}_k^2}{[\gamma_k - (1 - N_F/N_p)\lambda_k]^2 + \check{n}_k^2} \quad (3.41)$$

$$\check{b}_k = \frac{(\gamma_k - \lambda_k)\check{n}_k + [\gamma_k - (1 - N_F/N_p)\lambda_k]\check{n}_k}{[\gamma_k - (1 - N_F/N_p)\lambda_k]^2 + \check{n}_k^2} \quad (3.42)$$

$$\gamma_k = \sqrt{\frac{k}{2\check{r}}} \left(\sinh\sqrt{\frac{2k}{\check{r}}} + \sin\sqrt{\frac{2k}{\check{r}}} \right) \quad (3.43)$$

$$\check{n}_k = \sqrt{\frac{k}{2\check{r}}} \left(\sinh\sqrt{\frac{2k}{\check{r}}} - \sin\sqrt{\frac{2k}{\check{r}}} \right) \quad (3.44)$$

$$\lambda_k = \cosh\sqrt{\frac{2k}{\check{r}}} - \cos\sqrt{\frac{2k}{\check{r}}} \quad (3.45)$$

and

$$\check{n}_p = \frac{3(1 - \epsilon_b)D_e/R_p^2}{u} Z \quad (3.46)$$

$$N_p = \frac{3(1 - \epsilon_b)D_e/R_p^2}{u} Z \quad (3.47)$$

$$N_f = \frac{3(1 - \epsilon_b)k_f/R_p}{u} Z \quad (3.48)$$

$$\Psi_p = \frac{D_e/R^2}{[\epsilon_p + (1 - \epsilon_p)K]} t \quad (3.49)$$

$$\beta = \frac{\epsilon_b}{3(1 - \epsilon_b)[\epsilon_p + (1 - \epsilon_p)K]} \quad (3.50)$$

$$\theta = \frac{3(1 - \epsilon_b)k_f/R_p}{[\epsilon_p + (1 - \epsilon_p)K]} t \quad (3.51)$$

In eqs 3.40 through 3.50, D_e is the effective intraparticle diffusivity, R_p is the particle radius, Z the column length, k_f the external film mass transfer coefficient, u_o the superficial velocity, ϵ_b and ϵ_p the bed and particle porosities respectively, $\check{r}$ and $\check{r}_f$ are dimensionless phase periods for the elution and feed periods calculated by means of eq 3.51 for the period of interest and K the linear distribution coefficient. For most chromatographic applications of interest in preparative protein chromatography, this series solution converges rapidly giving excellent results using 10 terms.

The external mass transfer coefficient may be estimated from empirical equations. Kataoka et al. (1973), for example, indicate that for beds of small ion-exchange particles

$$\frac{\epsilon_b k_f}{u} = 1.85 \left(\frac{\epsilon_b}{1 - \epsilon_b} \right)^{1/3} (Re)^{-2/3} (Sc)^{-2/3} \quad (3.52)$$

where Re and Sc are the Reynolds and Schmidt numbers, respectively. Note, however, that because in protein chromatography pore diffusion is the dominant mass transfer resistance, the value of k_f is not very important to the model calculations.

By using D_e as a fitting parameter, the effective diffusivity and thus the pore diffusivity may be determined by matching eqs 3.40 to the transient elution profile of a protein pulse through a fixed bed under linear isotherm conditions, provided that other bed properties are estimated independently.

3.3.2 Intraparticle Diffusion For Strong Adsorption Conditions

When the conditions are such that the adsorption is strong, surface diffusion may be a significant mechanism of intraparticle diffusion. Under such conditions equilibrium isotherms are nonlinear, elution profiles non-Gaussian and elution times may be quite long. For these reasons, the fixed bed experiments, which are useful in determining pore diffusivity for weak adsorption, are ineffective in the study of surface diffusion and stirred batch experiments are preferred. In these experiments the resin is contacted with the protein solutions in a well mixed vessel. Two approaches that are commonly used to model the protein uptake behavior in such a vessel are described as follows.

3.3.3 Differential Model

A mass balance on a protein in a well-stirred vessel of volume V may be written as

$$V \frac{dC}{dt} = -ak_f V(C - C^i) \quad (3.53)$$

where C and C^i are the protein concentration in the vessel and at the resin-solution

interface, respectively, a is the resin interfacial area per unit volume of the batch and k_f is the external mass transfer coefficient.

It should be noted that eq 3.52 used to predict the external mass transfer coefficient was developed for particles packed in a column. Empirical correlations also exist for estimating the coefficient in stirred vessels. One such correlation, for small particles, is given by Levens and Glastonbury (1972) as

$$Sh = 2.0 + 0.47 Re^{0.62} \left(\frac{d_i}{d_v} \right)^{0.17} Sc^{0.36} \quad (3.54)$$

where $Sh = (k_f d_p)/D_f$ is the Sherwood number, $Re = (d_p u' \rho)/\mu$ is the Reynolds number, $Sc = (\mu \rho)/D_f$ is the Schmidt number, d_i the impeller diameter, d_v the vessel diameter, and u' the turbulent fluctuating velocity.

As before, a material balance on the spherical resin particle yields,

$$\epsilon_p \frac{\partial C_p}{\partial t} + (1 - \epsilon_p) \frac{\partial q}{\partial t} = \frac{1}{r^2} \frac{\partial}{\partial r} \left[r^2 \left(D_p \frac{\partial C_p}{\partial r} + D_s \frac{\partial q}{\partial r} \right) \right] \quad (3.55)$$

Boundary conditions may be written as

$$\frac{\partial C_p}{\partial t}(0, t) = 0 \quad (3.56)$$

$$C_p(r = R_p, t) = C_{bf}(t) \quad (3.57)$$

and

$$\left(D_p \frac{\partial C_p}{\partial t} + D_s \frac{\partial q}{\partial t} \right)_{r=R_p} = k_f (C_b(t) - C_{bf}(t)) \quad (3.58)$$

Equations 3.55 through 3.58 may be combined with an appropriate isotherm and solved numerically to yield the uptake of a protein as a function of time. In using

these equations, the value of D_p can be determined from fixed bed experiments carried out for low loading conditions. The surface diffusivity may then be used as a fitting parameter to match the model to experimental data. These equations can then be solved numerically using the method of orthogonal collocation on finite elements.

3.4 Irreversible Isotherm Model

As the uptake equilibrium isotherm of a protein becomes increasingly non-linear, numerical solution of the differential model becomes increasingly difficult, and, as a result of long computational time, eventually becomes impractical. Approximations may be made which allow simplification of the mathematics and analytical solutions are often obtained. An approximation often used to describe extremely non-linear isotherms is known as the irreversible isotherm. This isotherm may be expressed as

$$\bar{q}_i = \bar{q}_{i,max} H(C) \quad (3.59)$$

where $H(C)$ is the Heavyside unit step function. Thus, the adsorbed phase concentration is at its maximum when any amount of component is present in the fluid phase.

McKay (1984) has developed an analytical solution to the batch uptake problem based on the use of the irreversible isotherm. Mass transfer within the pores is assumed to occur by either pore or surface diffusion. This model is based on the unreacted shrinking core theory developed by Levenspiel (1972). This solution may be written as

$$\tau' = \frac{1}{6C_h} \left[\ln \left(\left| \frac{x^3 + \tilde{a}^3}{1 + \tilde{a}^3} \right|^{2B-1/\tilde{a}} \right) + \ln \left(\left| \frac{x + \tilde{a}}{1 + \tilde{a}} \right|^{3/\tilde{a}} \right) \right] + \quad (3.60)$$

$$+ \left[\frac{1}{(\tilde{a}\sqrt{3})C_h} \left(\tan^{-1} \frac{2 - \tilde{a}}{\tilde{a}\sqrt{3}} - \tan^{-1} \frac{2x - \tilde{a}}{\tilde{a}\sqrt{3}} \right) \right] \quad (3.61)$$

where τ' is a dimensionless constant given by

$$\tau' = \frac{D_e t}{R_P^2} \frac{C_0}{\bar{q}_{max}} \quad (3.62)$$

C_h is a capacity factor defined by

$$C_h = \frac{S\bar{q}_{max}}{VC_0} \quad (3.63)$$

$$B = 1 - \frac{1}{B_i} \quad (3.64)$$

$$x = (1 - \eta)^{1/3} \quad (3.65)$$

and

$$\tilde{a} = \left(\frac{1 - C_h}{C_h} \right)^{1/3} \quad (3.66)$$

In these equations, $B_i = k_f R_P / D_e$, is the Biot number for mass transfer and η is the dimensionless solid phase concentration, $\bar{q}/\bar{q}_{max}$. C_0 is the initial fluid phase concentration in the vessel, ρ_s the particle density, and S the mass of resin. As previously defined, R_P , D_e , and t represent the adsorbent particle radius, the effective intraparticle diffusivity and time, respectively.

3.5 Correlation Model For Effective Intraparticle Diffusivity

Another method of obtaining the effective intraparticle diffusivity is through a recent correlation of Coffman (1994). This correlation relates the intraparticle diffusivity to the molecular weight of the protein, the radius of the particle's pores and the bulk phase diffusivity of the protein:

$$D_{pore}/D_{solution} = \frac{1 - \frac{1.80MW^{0.33}}{R_{pore}}}{2.02} \quad (3.67)$$

where MW is the molecular weight of the protein in Daltons and R_{pore} is in Angstroms. The author states that it is representative of data to within eight percent on average and a maximum deviation of thirty percent. It is best suited for proteins that are globular and spherical. A comparison of this method with those describe earlier will be made in the experimental results chapter.

3.6 Ion Exchange Equilibria Of Proteins

The relationship that describes the equilibrium between a protein and an ion-exchange resin is, in many systems, the key factor which determines chromatographic performance. The manipulation of this equilibrium is, in fact, the basis for the various elution techniques employed in chromatography.

From the literature it appears there are three main methods employed to determine single component equilibrium isotherm parameters. These are: (1) Batch experiments, (2) Frontal Analysis Method, and (3) Impulse Response Method. Each technique is discussed in the following sections.

3.6.1 Batch Experimental Method To Obtain Isotherm Data

In the batch experiments method, the dissolved component and adsorbent are

brought into contact in a closed vessel and allowed to reach equilibrium. The time required to reach equilibrium is not fixed, although several authors have found that 12 to 24 hours is sufficient (Bloomingburg, 1992; Whitley, 1989a). For protein systems, aqueous solutions are prepared with a buffer that will be used in the process at various concentrations and pH levels that are anticipated for the particular protein. Some researchers have employed shaking flask experiments in an isothermal water bath to bring the solution and sorbent into equilibrium (Bloomingburg, 1992), or a slowly rotating end-over-end test tube apparatus (Whitley, 1989a). Bloomingburg (1992) found that the shaking bath approach resulted in denaturation of the proteins and foaming of the solution due to agitation at the air-liquid interface.

It appears that the best approach for this method is either the test tube method or to occasionally gently swirl the closed vessels by hand over the course of a 24 hr period. After the equilibration period, the supernatant liquid is analyzed for the protein concentration. By knowing the initial protein concentration, the initial liquid volume, and measuring the supernatant volume, the amount of protein adsorbed can be calculated as follows:

$$C_s = \frac{(C_0V_0 - C_eV_e)}{V_0 - V_e} \quad (3.68)$$

where C_s is the solid phase concentration of protein, C_0 is the initial concentration, C_e is the supernatant concentration, V_0 is the initial liquid volume, and V_e is the supernatant liquid volume. The difficulties with this method are in estimating how much sorbent to add to the liquid solution, ensuring that the amount of sorbent to be added is indeed the amount transferred to the solution, and measuring the supernatant liquid volume. The problem with measuring this volume is in draining off the supernatant from the sorbent. Gu (1990) used a very fine membrane

filtration apparatus to further remove the supernatant from the sorbent, but such was not attempted in this work. Experience with this method, as will be shown, is that it yields data that has considerable scatter and is unreliable, while also being considerably time consuming and tedious. If isotherm data points for more than one component are required, the procedure is substantially more time consuming. This is definitely not the method of choice for obtaining isotherm data points.

3.6.2 Frontal Analysis Method To Obtain Isotherm Data

The Frontal Analysis method to obtain isotherm data points is perhaps the most efficient and reliable one available. It has been reviewed by Jacobson et al (1984, 1987), and Seidel-Morgenstern and Guiochon (1993). In this method successive step changes in the eluent composition at the column inlet are performed. From the successive breakthrough curves, one point of the isotherm can be calculated by solving the mass balance equation (Seidel-Morgenstern and Guiochon, 1993). This mass balance equation is:

$$t_R = t_0 \left(1 + F \frac{q(C_E) - q_0}{C_E - C_0} \right) \quad (3.69)$$

where q_0 is the amount of protein adsorbed at equilibrium before the step change from C_0 to C_E , the next concentration level at the column inlet, is performed. t_0 is the retention time of a nonretained component, F is the ratio of solid packed volume to bed porosity, and t_R is the retention time of the protein in the breakthrough curve. The isotherm data point is $q(C_E)$. According to Seidel-Morgenstern (1993), if the isotherm is known to be Langmuir in type, then the concentration steps ought to be positive while if the curvature of the isotherm is opposite to that of the Langmuir type, the steps should be decreasing in concentration. This method was

not used in this work due to equipment limitations.

3.6.3 Impulse Response Method To Obtain Isotherm Data

In the impulse response method, as described in detail by Whitley (1989b), the chromatography column is discretized into N stages of equal volume. Each stage is assumed to be well mixed with the column flowrate Q taken to be equal through each stage. A pulse of a single component at a specific concentration is applied to the column and the effluent history is obtained. This experimental curve is then matched to the model curve through three key parameters. The model parameters are the Langmuir coefficients a and b , and the overall mass transfer coefficient k_L . Whitley's approach further utilized the routine DUNLSF from the International Mathematical and Statistical Libraries (IMSL, Houston, Texas), to update estimates of the three parameters and to minimize the error between the model effluent curve and the experimental one. The transport of a single component into the sorbent and through the column is modeled by accounting for film mass transfer and pore diffusion as a linear driving force weighted by an overall mass transfer coefficient. The equilibrium relationship is assumed to follow the common two parameter Langmuir type. The resulting mass balance equations are ordinary differential equations, due to the approximation of the column as a series of discrete stages. The resultant set of equations is then amenable to solution with the ordinary differential - algebraic equation solver DASSL (Petzold, 1982).

The value of this approach is in obtaining the equilibrium parameters a and b , although the value of b is strongly dependent on the pulse concentration and volume. Whitley (1989b), explains in his article a strategy for determining the appropriate pulse concentration and volume which involve use of contour plots of

the experimental data, and the reader is directed to consult the original article for further details. Since the effects of film mass transfer, pore diffusion, pulse size and concentration, and column contributions to dispersion, are all lumped into the single mass transfer coefficient k_L , the usefulness of the impulse response method is limited for scale-up work.

A further extension of this method would be to use equation 3.18 coupled with a Langmuir isotherm and solve the model with PDECOL (Madsen and Sincovec, 1979), and then update the isotherm parameters with the DUNSLF routine (IMSL, Houston, Texas), in order to match the model and experimental curves. The major improvement with this approach is that not all of the dispersive effects are lumped into the overall mass transfer coefficient but rather into the traditional axial dispersion term. Or, if the VERSE-LC model discussed in section 3.1.1 could be coupled with the DUNSLF routine, then a very detailed parameter matching method would be available for determining non-linear isotherm parameters from impulse responses to a column.

3.7 Adsorption Isotherm Models

The previously discussed transport rate models are quite useful for describing the phenomena encountered in preparative liquid chromatography of biomolecules, whether the adsorption conditions are weak such as for linear chromatography or strong for non-linear chromatography. Nevertheless, these models are not very useful at all unless they are supplied with accurate adsorption isotherm equations which describe the thermodynamic equilibria between the biomolecule in the flowing bulk phase and the stationary phase. Therefore, to obtain accurate equilibria data for the component system and the subsequent modeling of the data, it is of great

importance to conducting simulations of the chromatographic process. If more than one component is in the experimental system, the complexity and time required for obtaining this data and modeling it becomes increasingly great. Adsorption isotherm models will be discussed in general followed by discussions on the different models available.

Equilibrium isotherms are generally classified as either favorable, linear, or unfavorable. In linear isotherms the distribution coefficient, defined as the ratio q/C_p , is constant. In favorable and unfavorable isotherms this ratio is a function of C_p . In unfavorable isotherms this ratio is an increasing function with C_p , while with favorable isotherms the ratio decreases with C_p . In ion-exchange chromatography, proteins typically exhibit favorable isotherms at high loadings and low salt concentration and linear isotherms at low loading and high salt concentrations.

Many different approaches have been proposed to model the uptake equilibrium of proteins by ion-exchange resins. Among these are those described by Yamamoto et al. (1988) based on (1) the law of mass action, (2) the Donnan potential and (3) empirical or semiempirical equations. Also, excellent reviews are given by Bellot and Condoret (1993) and James and Do (1991). The models utilized the most will now be reviewed.

3.7.1 Langmuir Isotherm

The Langmuir isotherm is the most common theoretical model available to describe the adsorption of a single component onto the surface of an adsorbent. It was originally proposed by Langmuir in 1931. The model is based on the assumption that the adsorbed molecule is held at localized sites and that the energy of adsorption is constant over all sites, and that the layer of adsorbed molecules

is monolayer James and Do (1991). Even though in some experimental situations these restrictive assumptions are not valid, due to the mathematical structure of the equation, the model has been found to show good agreement with many gas-solid and liquid-solid equilibria systems and so finds widespread use Bellot and Condoret (1993).

The Langmuir model is, for a single component:

$$q = \frac{q_{max}bC}{1 + bC} \quad (3.70)$$

where the traditional symbol q is the same as $\bar{c}_p$ used earlier and q_{max} is the saturation capacity of the resin. Likewise, the symbol C is the bulk phase concentration and is the same as c_b . Another formulation of the model replaces q_{max} and b with a , and a and b are known as the Langmuir isotherm parameters. At very low component concentrations, the Langmuir model becomes a quasi-linear curve, $q = aC$, the molecules being sparingly distributed onto the sorbent surface. In this case the adsorbed phase concentration is directly proportional to the bulk phase concentration. At high concentrations of component, this is not true. Here the probability of molecules meeting each other on the sorbent surface is greater, causing the isotherm to curve at the higher concentrations and finally become asymptotic with $q = a/b$. At this point the adsorption sites are saturated Bellot and Condoret (1993).

For multi-component systems, the single component Langmuir model is extended to the multi- component system with the additional assumption that all components have identical saturation capacities for the sorbent. This model is:

$$q_i = \frac{a_i C_i}{1 + \sum_{j=1}^N b_j C_j} \quad (3.71)$$

where the a_i and the b_i are the individual Langmuir isotherm parameters, and there

are j components in the system. This model is known as a competitive isotherm because it takes into account the competition of the components for adsorption sites. It is also thermodynamically inconsistent when the component saturation capacities different Bellot and Condoret (1993). Nonetheless, it is found to be able to describe multi-component adsorption behavior generally well and is an easy to use model.

In the case where non-equilibrium conditions are present, that is, where the rates of adsorption and that of desorption are slow enough to be rate controlling for the mass transfer process, the Langmuir model takes on the following form:

$$Y_{li} = N_{li,+} c_{pi} \left(1 - \sum_{j=1}^{N_c} \bar{c}_{pj} \right) - N_{li,-} \bar{c}_{pi} \quad (3.72)$$

where $N_{li,+}$ is the adsorption rate scaled by convection and $N_{li,-}$ is the desorption rate scaled by convection. This equation is in turn used by eq. 3.71 and its associated boundary conditions. In these equations surface diffusion is not accounted for since research has shown that it does not appear to be significant for large molecules such as proteins. For fast adsorption and desorption rates, the rate constants are related to the equilibrium Langmuir isotherm through the following (Whitley, 1990):

$$a_i = \frac{l_{i,+}}{l_{i,-}} \bar{C}_{Ti}; b_i = \frac{l_{i,+}}{l_{i,-}} \quad (3.73)$$

Naturally, the question arises as to when does adsorption and desorption become rate controlling for the mass transfer process. Whitley (1990) states that for the case of a single component breakthrough curve, if the two rates are assumed equal, symbolized as $N_{li,\pm}$, then for $N_{li,\pm} = 100$, local equilibrium is reached between the solid phase and the pore phase solution. This values means that the kinetic rate is 100 times faster than the convection rate. For $N_{li,\pm} = 10$, the

breakthrough curve for a single component is nearly the same as that of the local equilibrium case, but when $N_{li,\pm} = 1$, the breakthrough curve front shows asymmetric broadening or the typically seen sigmoidal shape. Further decreasing the $N_{li,\pm}$ value below 1 will result in loss of capacity of the sorbent for the component(s). This is because the adsorption process is so slow that there is not enough residence time for adsorption to occur.

According to the definitions for the dimensionless adsorption and desorption parameters, $N_{li,+}$ and $N_{li,-}$ a definite way to affect these rates is to adjust the superficial velocity and hence the volumetric flowrate through the column. By decreasing the flowrate, these dimensionless parameters are increased which means that the ratio of adsorption or desorption to convection is increased.

Unfortunately, the dimensionless adsorption/desorption rate parameters are a function of the respective adsorption/desorption rate constants, which are not easily measured parameters. Therefore, if a chromatographic process is suspected to be controlled by kinetics it can be confirmed numerically by using the general rate model along with a non-equilibrium isotherm. The kinetic rate constants will likely have to be determined by fitting them so that the numerical and experimental chromatograms match. Then the dimensionless rate adsorption/desorption rate parameters can be calculated and checked to see if they are greater than 10 or closer to 1, thereby discounting or confirming that kinetics control the process.

3.7.2 Freundlich And Freundlich-Langmuir Isotherms

Another isotherm model of practical application is the Freundlich isotherm which is derived from the Langmuir isotherm but allows for a distribution of adsorption energies for the sorbent surface sites. This distribution of energies follows

an exponential decay function and results in the following formulation (James and Do, 1991):

$$q = KC^{1/\eta} \quad (3.74)$$

where K is the Freundlich equilibrium constant. For values of η greater than 1, the isotherm shape is favorable, convex, and for values of η less than 1, the isotherm shape is unfavorable, concave. When η equals 1, the isotherm is linear (James and Do, 1991).

One limitation of this isotherm is that it predicts that the amount of component adsorbed will increase indefinitely with increasing concentration in the solution. To overcome this flaw, the Freundlich and Langmuir isotherms may be combined into a single isotherm model containing two fitting terms. According to James and Do (1991), this should allow a better approximation for modeling adsorption of a heterogeneous nature. The combined isotherm is:

$$q = \frac{q_{max}bC^{1/\eta}}{1 + bC^{1/\eta}} \quad (3.75)$$

For the multicomponent case the model becomes:

$$q_i = \frac{q_{max_i}b_iC_i^{1/\eta_i}}{1 + \sum b_jC_j^{1/\eta_j}} \quad (3.76)$$

This isotherm model has been found to provide a good empirical correlation of binary equilibria data over limited concentration ranges. Extensions to non-equilibrium situations are not seen in the published literature; the model is applicable to equilibrium cases where salt concentration and pH or salt concentration alone are varied at different levels and it is desired to correlate the resulting data to a single isotherm (James and Do, 1991). This is demonstrated for the adsorption

of a biomolecule such as a protein in the presence of a salt which is treated as a binary equilibria system as follows (James and Do, 1991):

$$q_{bio} = \frac{q_{max} b_{bio} C_{bio}^{1/\eta_{bio}}}{1 + b_{bio} C_{bio}^{1/\eta_{bio}} + b_{salt} C_{salt}^{1/\eta_{salt}}} \quad (3.77)$$

3.7.3 Counter-Ion Modulator Isotherm

In elution liquid chromatography a sample feed mixture is fed to the top of a column and its components are eluted by a continuous stream of an eluent. This eluent adsorbs to the stationary phase by physical or chemical forces less strongly than any of the feed mixture constituents, known as elutes. The elution process is called isocratic when the nature of the eluant is kept constant. This means that its chemical composition and characteristics such as pH are constant. Typically this is achieved by maintaining a constant ionic strength or pH of the eluent stream. For biochemical separations such as protein purifications, a buffer is employed with the eluent stream to maintain the pH at some desired value. In gradient elution chromatography the chemical nature of the eluent is varied in an increasing or decreasing manner. This means that the ionic strength or pH is positively or negatively varied in some prescribed manner to effect the separation. Possible types of gradient elution include linear, stepwise, and nonlinear. The most common and simplest gradient method is the linear version in which the eluent ionic strength is varied in a positive or negative manner with time (Synder, 1980). However, stepwise and nonlinear are more efficient at effecting separation in less time (Ghris and Synder, 1988). The tradeoff is that these latter two methods are more difficult to implement.

The key to implementing a gradient elution is to manipulate an additive in the

eluent chemical system positively or negatively as discussed for the desired type of gradient. This additive is known as the mobile phase modulator. By varying the modulator concentration in the mobile phase (eluent) in a prescribed manner, all of the feed components which were initially fed to the column are separated to some degree in a single pass of the eluent wave. In ion exchange chromatography applied to the separation of proteins the eluent is an aqueous buffer system containing an inorganic salt. The salt is the modulator component. Because the protein mixture to be separated will probably be composed of proteins of different types and molecular weights, each protein type will be retained by the ion exchange stationary phase to varying degrees with the result a wide range of protein retentivity by the resin.

The modulator can affect the retentivities of the elutes primarily in two different ways. First, the modulator can compete with the elutes for the binding sites on the ion exchange resin. Then the modulator is treated as a competing component in the multicomponent isotherm utilized together with the elutes. Secondly, the modulator affects the retentivity of an elute primarily by changing its adsorption equilibrium constant Gu et al. (1992). Several different models exist for the relationship between the retention factor for a protein and the molality of the modulator in the eluent. In their model for gradient elution chromatography, they chose the model by Melander et al. (1989). Their relationship used is:

$$\log k' = A - B \log m_s + C m_s \quad (3.78)$$

Here, k' is the retention factor for a protein, A is a data fitting parameter, B is termed the electrostatic interaction parameter, C is the hydrophobic interaction parameter, and m_s is the molality of the modulator in the eluent. This relationship was developed by Melander et al. (1989) after investigating ion exchange chromatog-

raphy from a theoretical and experimental approach. The equation is formulated by proposing that the modulator effects the retention factors of the elutes (proteins), but that it is negligibly adsorbed onto the ion exchange resin. This relationship between the modulator and the elutes is termed the interaction type. Details are found in their paper. As already mentioned, the modulator can affect the retentions of the proteins or their respective adsorption equilibrium constants. Also, changes in the modulator concentration can cause changes in the conformation and extent of ionization of the proteins, and in the number of accessible binding sites on the resin. Furthermore modulator concentration changes can alter the type of interaction mechanism between the modulator and the elutes, Melander et al., 1989). The above relationship has been found satisfactory for describing the interaction mechanism between a salt modulator and a protein system.

The retention factor for a protein, k' , can be determined from retention data in linear chromatography experiments and subsequently the Langmuir isotherm equilibrium constant b can be found from (Gu, 1992):

$$k' = \phi C^\infty b \quad (3.79)$$

where C^∞ is the same as q_{max} the sorbent saturation capacity for the component, and ϕ is the modulator concentration. According to Gu (1992), the isotherm parameter b can be used in the nonlinear concentration range if the isotherm is Langmuir type in that range. Once the equilibrium constant b is determined, the equilibrium constant a can be found from $a = q_{max} b$. Then the counter-ion modulator isotherm model can be put together to yield the following result (Whitley, 1990):

$$q_i = \frac{a_i \phi^{-Z_i} C_{pi}}{1 + \sum_{j=1}^N b_j \phi^{-Z_j} C_{pj}} \quad (3.80)$$

where q_i and C_{pi} are the solid and pore phase concentrations of component i respectively in dimensional form, N_c is the number of components in the system, Z is the ratio of eluent to salt valence, and ϕ is again the modulator concentration. Though the effort required to obtain the experimental parameters is involved, especially for a multicomponent system, this model yields fairly good agreement to a wide range of experimental data (Whitley, 1990). An improvement of the model allows for competition of the salt modulator with the other components in the system (Whitley, 1990).

3.7.4 Mass Action Law

Still another approach to modeling ion-exchange equilibria is through the mass action law. This method was first applied to such by Gans (1913). Gans based his model on the mass action law in its simplest form, without activity coefficients and assuming univalent ions. Helfferich (1962) has reviewed further development of the mass action law approach to ion-exchange equilibrium. Kielland (1935), who included the use of solid phase activity coefficients in his model, was the first to extend this approach to more complex systems. More general treatments have been made, for example, by Gaines and Thomas (1953) in which the ion-exchange process is viewed as a heterogeneous reaction of "electrolytes" and "resinates", the Gibbs-Duhem equation is utilized to calculate the activity coefficients and the thermodynamic equilibrium constant from experimental data taken in a variety of conditions.

While adsorption isotherms based on the mass action law have been in existence for many years, examples of the application of these laws to protein uptake equilibrium data are few. A mass action law relationship which accounts for the multiple

charges of proteins is developed here for comparison to experimental uptake data.

A mass action law model of protein uptake may be derived taking into account the multifunctionality of a protein at a given pH. The uptake is assumed to involve a stoichiometric exchange of protein and counter-ions,



In this equation, R represents the resin, I the counter-ion, P the protein, and n the effective charge on the protein. The effective charge is the number of charged groups of the protein which participate in binding to the ion-exchange sites. Thus, a protein with an effective charge of n will displace n counter-ions from the resin. The effective charge of a protein is dependent on pH.

Based on eq 3.81, an equilibrium constant, K' , may be defined as

$$K' = \frac{q_P C_I^n}{q_I^n C_P} \quad (3.82)$$

A balance on the resin exchange sites may be written to yield

$$q_m = nq_P + q_I \quad (3.83)$$

with q_m representing the maximum number of ion-exchange sites available in the resin. The maximum protein uptake capacity, q_{max} is given by $q_{max} = q_m/n$.

Combining eqs 3.82 and 3.83 we obtain the following relationship describing protein equilibrium:

$$\frac{q_P/q_{max}}{C_P} = \frac{K' n^n q_{max}^{n-1}}{C_I^n} \left(1 - \frac{q_P}{q_{max}} \right)^n \quad (3.84)$$

The equilibrium uptake behavior described by this equation as a function of counter-ion and protein concentration is shown in Figure 3.1 for typical values of the parameters q_{max} , n and K' . As seen from this figure, the equation is capable of representing the saturation behavior of the Langmuir isotherm, as well as predicting in a qualitatively correct way the effects of counter-ion concentration. At low protein loadings, as is the case when the counter-ion concentration is high

$$\frac{q_P}{q_{max}} \ll 1 \quad (3.85)$$

and eq 3.84 reduces to,

$$\frac{q_P}{C_P} = K' \left(\frac{q_{max} n}{C_I} \right)^n = K \quad (3.86)$$

yielding a constant distribution coefficient dependent upon the counter-ion concentration. When the log of K is plotted against the log of C_I over a range of salt concentrations for which $q_P/q_{max} \ll 1$, two important pieces of information can be obtained. The slope of this plot yields n , the effective charge of the protein. The intercept of this line represents the constant $K'(q_{max} n)^n$. Thus, once q_{max} is known K' may be determined and may be used to predict uptake equilibrium over the entire range of salt and protein concentrations.

3.8 Relationship Of Single Component Isotherm Parameters To Competitive Isotherms

The relationship of the above mentioned single component isotherm parameters to their role in competitive isotherm models must be discussed. Each of the previously mentioned multicomponent isotherm models has the sole purpose of representing multicomponent adsorption behavior from single component adsorption

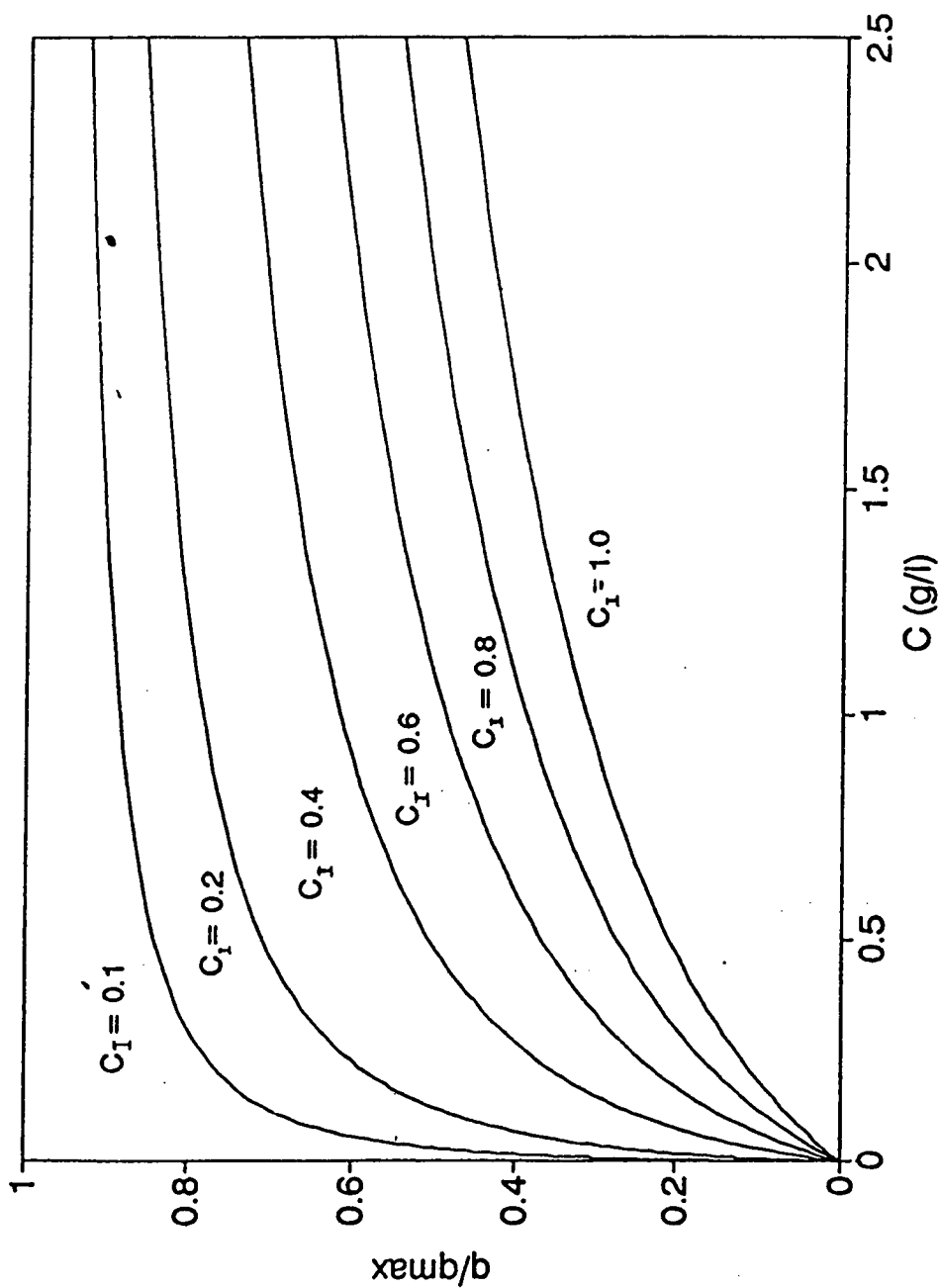


Figure 3.1 Equilibrium uptake behavior as predicted by the law of mass action. (Parameter values: $q_{\max} = 0.007 \text{ mol/liter}$, $n = 2.0$, $K' = 1000$. The counter-ion concentrations, C_I , are given in mol/liter.)

data statistically fitted to the multicomponent model. Bellot and Condoret (1993) state that better accuracy may be obtained by extracting additional coefficients from the experimental competitive isotherm data. Schay (1957) demonstrated this with a modified Langmuir isotherm. The modification was an interaction parameter, η , which is a characteristic of each component in the mixture to be adsorbed and also depends on the concentrations of the other components. This modified Langmuir model is shown as:

$$q_i = \frac{a_i C_i / \eta_i}{1 + \sum_{j=1}^N b_j C_j / \eta_i} \quad (3.87)$$

The η_i are estimated from experimental competitive adsorption data. Yon and Turnock (1971) and Matthews (1975) have applied this approach to other isotherm models derived from the Langmuir model, and Fritz and Schluender (1974) and McKay (1990) have extended the approach to Freundlich isotherm models.

In this work all multicomponent isotherms were constructed from single component data fitted to the multicomponent models. For completeness the above mentioned approach was discussed but due to difficulty in measuring competitive isotherm data, it was not investigated for this work.

3.9 Theoretical Developments For Scaleup

For scale-up of gradient elution ion-exchange liquid chromatographic processes, there are two major methods to utilize: analytic scaling rules and numerical simulations. It must be stated that in the past scale-up was based on the HETP approach or height equivalent to a theoretical plate. This evolved from analytical column experiments where the separation to be carried out was optimized and the HETP was then calculated. From the analytical separation's chromatogram reten-

tion time and bandwidth, the number of plates can be calculated. Then, dividing the column length by the number of plates yields the HETP.

In the analytic scaling rules approach, developed by Wankat (1992), equation 3.18 is put into dimensionless terms. This is the equation that was coupled earlier with the linear driving force approximation. In the analytic scaling rules approach, the linear driving approximation is replaced by an appropriate term that accounts for the controlling resistance in the process. The two resistances considered in the method are pore diffusion and film diffusion. The approach makes the following assumptions: negligible axial and radial dispersion, constant particle and bed porosities, laminar flow, isothermal operation, constant physical properties, and rapid adsorption kinetics (Wankat, 1992). Also, it is inherently assumed that different size columns can be packed with equal efficiency and that distribution of liquid in different size columns is equally good.

From this dimensionless partial differential equation with the respective boundary and initial conditions and the appropriate controlling resistance, it is seen that the dimensionless solution to the equation set for particular values of the dimensionless variables will be equal to another dimensionless solution if the variables have the same values. Therefore, scaling rules can be developed by solving a series of algebraic equations that keep the dimensionless variables constant. The method centers around ratios of the key variables in the chromatography process, such as superficial velocity, column length, particle diameter, mass of adsorbent, and column diameter. The ratio operator for a key variable is the value of the variable at the scaled-up state to that at the existing state. The analytic scaling rules approach keeps process throughput, pressure drop, and separation performance constant (Wankat, 1992).

Rather than derive the equations of the analytic scaling rules approach, only

the results for the two controlling resistances cases will be presented.

For the case of pore diffusion as the controlling mass transfer resistance, the superficial velocity should be kept constant when scaling up so that the ratio of the superficial velocity at the scaled-up state to that of the original scale is one:

$$R(u_F) = 1 \quad (3.88)$$

where R is the ratio operator for scaled-up state to original scale for a variable, and u_F is the superficial velocity. Also, the ratio of column diameters can be related to the ratio of flowrates by:

$$R_D = R_N^{1/2} \quad (3.89)$$

where D is the column diameter, and N is the flowrate to the column in units of choice. Usually, when separations are optimized at the preparative scale, the column length is kept constant when scaling to a larger scale. If, however, the column length needs to be altered from that at the preparative scale, then the ratio of column lengths is related to the process cycle time by:

$$R_L = R(t_c) \quad (3.90)$$

where L is the column length, and t_c is the process cycle time. This is also related to the flowrates ratio, and the column diameters ratio by:

$$R_L = \frac{R_N R(t_c)}{R_D^2} \quad (3.91)$$

For the case of film mass transfer as the controlling resistance, the ratio of column diameters is related to the ratios of flowrates and process cycle times by:

$$R_D = R_N^{1/2} (R(t_c))^{-\beta/8(1-\beta)} \quad (3.92)$$

where β is the parameter exponent of the Reynolds number in the following correlation for the mass transfer coefficient:

$$Sh = \frac{\alpha}{\epsilon} Re^\beta Sc^\gamma \quad (3.93)$$

where Sh is the Sherwood number, Re is the Reynolds number, and Sc is the Schmidt number, further defined as (Wankat, 1992):

$$Re = \frac{\rho_f u \epsilon d_p}{\mu} \quad (3.94)$$

where ρ_f is the fluid density, ϵ is the bed porosity, d_p is the particle particle diameter, and μ is the fluid viscosity.

$$Sc = \frac{k_f d_p}{D_m} \quad (3.95)$$

where k_f is the film mass transfer coefficient, and D_m is the fluid phase diffusion coefficient for the component of interest. Wilson and Geankoplis (1966), determined values for α as 1.09, and β as 0.33 for Reynolds numbers between 0.0016 and 55, and α as 0.25 and β as 0.69 for Reynolds numbers between 55 and 1500 for liquid systems. The parameter γ was found to be 0.33 for all Reynolds numbers.

Unfortunately, the analytic scaling rules approach does not work well when considering mass transfer resistances in series, such as film mass transfer and pore diffusion, due to over constraining the algebraic equation system (Wankat, 1992). However, since pore diffusion is most likely to be the controlling mass transfer resistance for protein systems of interest, the results presented here for this case will be used later on in chapter seven for scale-up calculations.

For the situation of using numerical simulations to the detailed mathematical models of liquid chromatography for scale-up issues, two cases were explored in this work. First, a Basic computer program, written by Carta (1988), for simulation of equations 3.38 - 3.51, was utilized. This model accounts for the mass transfer effects of pore diffusion and resistance in the external film around the sorbent particles. The model is for systems exhibiting linear equilibrium and only requires parameters which are readily available. Once the preparative scale ion-exchange separation is successfully simulated, scale-up can be predicted by changing the superficial velocity, the mass transfer coefficient, and if required, the column length. This method of scale-up assumes that the equilibrium parameter remains constant when scaling up, along with the bed, particle, and fluid properties.

The other means of numerical simulation is to use the general rate model, equations 3.1 - 3.10, and the VERSE-LC software package. This is a very detailed model of the ion-exchange process and the computer package is equally detailed. To use the simulation of this model for scale-up issues, first the controlling mass transfer resistance should be determined, as well as whether or not the process is kinetically controlled. This was discussed in section 3.8.1. Once these aspects are determined, then the dimensions of the scaled-up column and its associated flowrate can be entered into the model and the simulation for the scaled-up column can be conducted. This method also assumes that the equilibrium parameters, along with the bed, particle, and fluid properties, remain constant when scaling up. Once the simulation for the scaled-up state is obtained, it can be compared to that for the preparative scale to see if there are any gross differences in the two chromatograms.

As has been seen in this discussion of the theoretical aspects of preparative chromatography, the framework of mathematical modeling ranges from fairly sim-

ple to quite complex. The use of the available models for numerical simulation and scale-up issues is highly dependent on the incorporation of an isotherm model which satisfactorily describes multicomponent protein adsorption behavior. The acquisition of protein equilibria is not an easy task. The results of experiments to obtain isotherm data are presented in the next chapter.

CHAPTER IV

EQUIPMENT AND PROCEDURES

4.1 Preparative Chromatography Apparatus

The centerpiece of this research project was a preparative chromatography apparatus, constructed by the Plant and Equipment trade crafts group at Oak Ridge National Laboratory. A photograph of the apparatus is shown in Figure 4.1. The apparatus consists of the preparative columns used, 20 liter Nalgene tanks for feed, buffers, and cleaning solutions, and for individual isolated solutes collection, a pumping system, and associated piping. Also, a diode array ultraviolet spectrophotometer and a pH meter, both in-line.

Stainless steel tubing was used for all process piping, except for the connections between the tanks and the main supply lines, 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

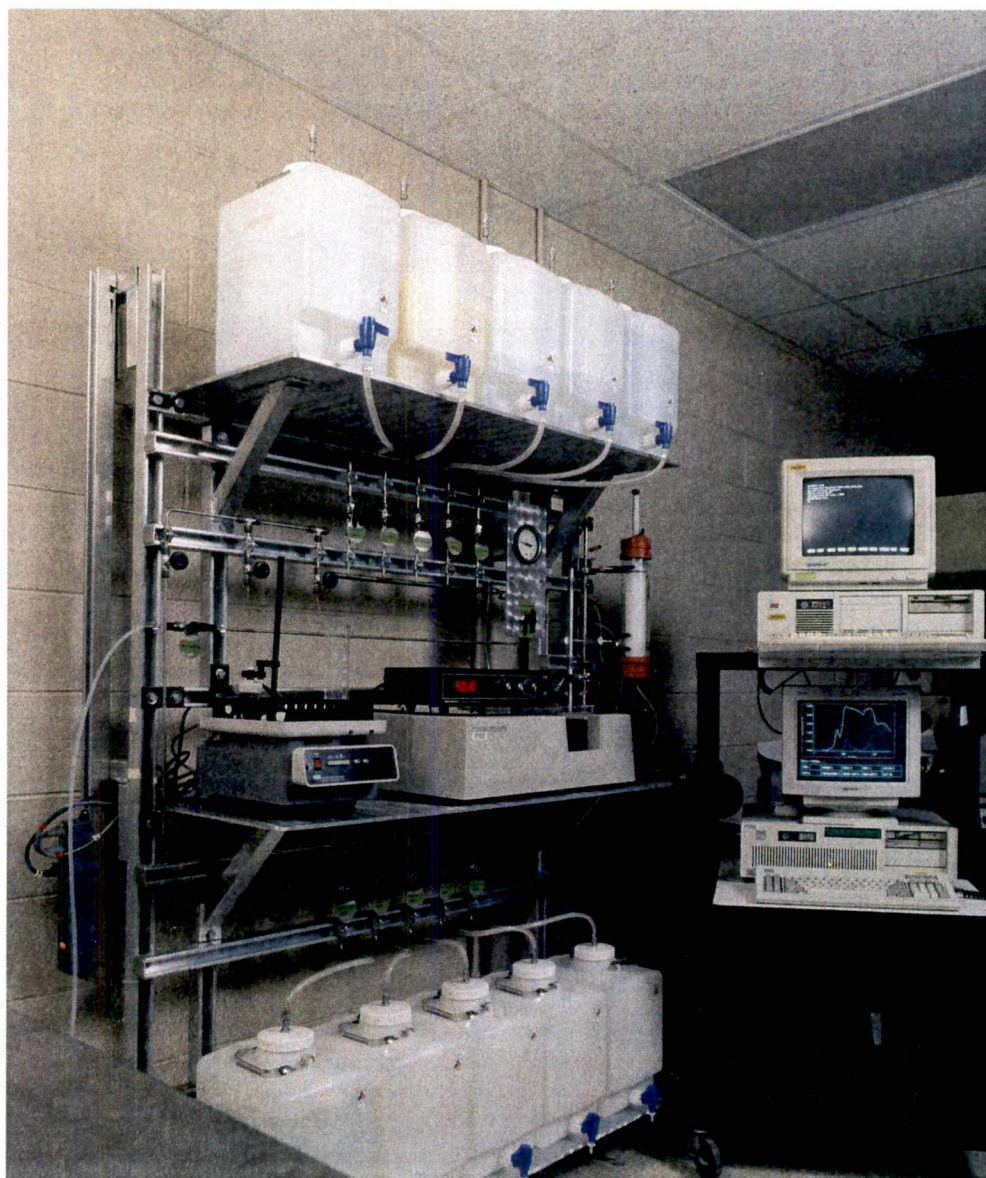


Figure 4.1 Preparative Chromatography Apparatus at Oak Ridge National Laboratory.

place in-line upstream of the column to monitor the pressure across the column.

The ion-exchange column used was Pharmacia Biotech Inc.'s 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. Eluant 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 U.S. Standard connector. All three items were from the Cole-Parmer Instrument Company, Niles, Illinois. The output from the pH probe was sent to an Orion Research Model 701A digital Ionalyzer for visual digital display of the eluant pH.

After the eluant 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.

Eluant flowrates were determined by closing off the process piping to the product storage tanks by a valve and opening a valve upstream to the collection tanks to allow eluant to flow into a 250 mL graduated cylinder and measuring the time required to fill 50 mL. This valve was after the in-line UV spectrophotometer. Several determinations of the eluant flowrate were made for each experimental run and it was found that the pump was steady and reliable.

One of the original intentions of the project was to collect the resolved peaks of the preparative chromatograms and to analyze them in an analytical size exclusion chromatography system. This system was constructed early in the project and consisted of a Waters Associates WISP 701B automated sample injection unit which

was upgraded to a model 712 with a new injection assembly and software during the project. Three size exclusion columns were purchased from Synchrom Inc., model GPC 100, 30 cm in length and 7.8 mm in diameter, packed with a proprietary silica gel. Also, a guard column, model GPC 100, 50 mm in length and 7.8 mm in diameter was used, packed with the same lot of gel as the main columns. This gel had a pore size of 100 Angstroms and a molecular weight cutoff of 160,000.

The eluant from the size exclusion columns was passed through a Perkin-Elmer LC-95 Ultraviolet Light Detector and then immediately through a Perkin-Elmer LC-25 Refractive Index Detector. The UV detector was set at 280 nm wavelength like the preparative system's one, and was used to measure the proteins absorbance. The refractive index detector was used to measure the relative lactose quantity in each sample. Output from each detector was sent to a dedicated computing integrator, Perkin-Elmer model LCI-100. The pump used for the analytical system was a Perkin-Elmer series 410 LC pump. A photograph of the analytical system is shown in Figure 4.2.

Much effort was expended in developing the analytical system and optimizing it in terms of sample processing times, computing integrator parameters, and the development of individual protein's concentration standards curves. The goal of this system was to be able to efficiently process small samples of the resolved chromatogram peaks from the preparative column. The WISP unit was capable of processing 48 samples in one run; it was found that each sample required 30 minutes run time.

Unfortunately, the analytical system's performance was found to be unsatisfactory for quantitation purposes. The size exclusion resin was not able to resolve bovine serum albumin from immunoglobulin G, even though there is significant dif-

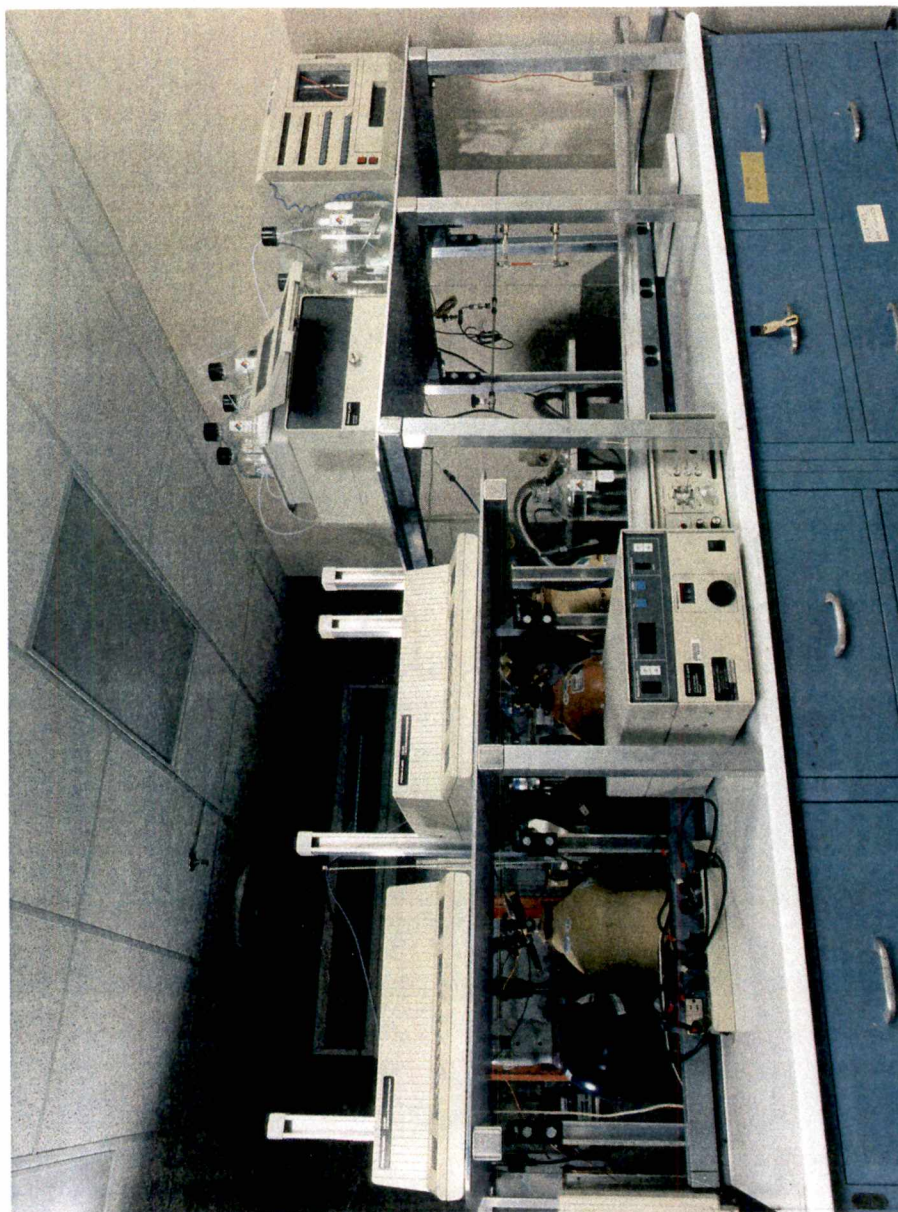


Figure 4.2 Analytical System for Preparative Chromatography System.

ference in their molecular weights. In fact the immunoglobulin G did not exit the columns as a peak but rather as a small plateau. In a telephone discussion with the manufacturer, they stated that the IgG tends to partially adsorb or stick to the column as it passes through, instead of doing so unretained. A new gel from SynChrom Inc. has in fact been developed to overcome this problem.

Another problem with the system was that the peak elution times as recorded on the computing integrators would vary from run to run as compared to the recorded times for pure protein pulses put through the system. Also, there were problems with the WISP injection unit in terms of mechanical breakdown including breakage of the injection needle system.

It was therefore deemed that for reliable quantitation of the chromatogram peaks from the preparative chromatography unit, another analytical method was needed. The same two companies that analyzed the sweet whey feed from the Dairyman Inc. plant, as displayed in Figure 1.12, were utilized for analysis of the optimized chromatogram peaks. Kendrick Laboratories of Madison, Wisconsin analyzed the peaks by two dimensional electrophoresis to quantitate the individual and total proteins content, and Silliker Laboratories of Stone Mountain, Georgia analyzed the peaks for lactose and total solids content, and density. These laboratories were selected based on recommendations from the dairy industry.

For the gel filtration studies, a Pharmacia Biotech Inc. XK 16/60 pre-packed column containing Pharmacia Biotech Inc.'s Superdex 75 Prep Grade resin was used. This column is 1.6 cm in diameter and 60 cm in packed length. It, like the ion-exchange column, was limited to an operating pressure of 42 psi. Eluant from this column was passed through the pH and UV system as with the ion-exchange column.

4.2 Chromatographic Resins

The chromatographic resins chosen for this study were Pharmacia's Q and S-Sepharose anion and cation exchange resins, and the gel filtration resin Superdex 75 Prep Grade. 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 in type and possess strong anion and cation exchange functionalities. That is, they maintain their charge capacities over a wide range of pH. The gel filtration resin is a composite of cross-linked agarose and dextran. Some of the properties of these resins, as provided by the manufacturer, are given in Tables 4.1 and 4.2.

4.3 Chemical Reagents

All chemical reagents used in this study for the preparation of buffers and cleaning agents were food and/or pharmaceutical grade in quality. 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 will 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 respective 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 respective ion-exchange process cycles, was purchased from J.T. Baker with a purity rating

Table 4.1

Properties of Sepharose Ion-Exchange Big Beads Resins[†]

Exchange capacity	0.18 - 0.25 mmol/ml
Exclusion limit	4×10^6 daltons
pH-Stability	2-14
Particle size	100-300 μm
Maximum Operating Back Pressure	42 Psi

[†] Data from the Manufacturer (Pharmacia Biotech Inc.).

Table 4.2

Properties Of Superdex 75 Prep Grade Gel Filtration Resin[†]

Molecular Weight Fractionation Range	3,000 - 70,000
Bead Size	24 - 44 Microns
pH-Stability	1-14
Maximum Operating Back Pressure	42 Psi

[†] Data from the Manufacturer (Pharmacia Biotech Inc.).

of N.F./F.C.C.

For the gel filtration studies, sodium chloride in combination with sodium phosphate anhydrous were used. The sodium chloride was purchased from J.T. Baker with a purity rating of U.S.P./F.C.C. The sodium phosphate anhydrous was a gift from the Monsanto Company and had a purity rating of F.C.C.

Before a set of column experiments was conducted, 20 liter 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 deionizing unit. Details on the different buffers used are in the subsequent chapters.

High purity proteins used for characterization studies in the gel filtration column experiments, batch isotherm experiments, and development of concentration standards curves, were purchased from the Sigma Chemical Company, St. Louis, Missouri. The proteins were kept in a lab refrigerator/freezer as directed by the Sigma Chemical Company to retard degradation.

CHAPTER V

FUNDAMENTAL STUDIES

5.1 Batch Equilibrium Studies

As discussed in section 3.6.1 of chapter 3, the batch equilibrium method to acquire single component equilibrium data was attempted. In this procedure, capped, 250 ml flasks containing on average 30 ml of a beta lactoglobulin solution, was shaken for 24 hours in a constant shaking water bath. The temperature of the bath was 22 C, the same temperature as that of the lab where the preparative experiments were conducted. The amount of anion exchange resin in each flask was 3 grams.

For a set of experiments, eight flasks of varying beta lactoglobulin concentration were carefully prepared with a set salt buffer concentration and pH. The concentration range was from 0 percent dilution to 90 percent dilution so as to span the concentration range of the beta lactoglobulin peak that came off the column in

the anion exchange process. This peak was the most resolved in the anion exchange process and had the greatest purity of any peak at 50 percent beta lactoglobulin. Details on the anion exchange process are discussed in chapter six. It was proposed that if accurate isotherm data for this peak could be obtained, then hopefully a successful numerical simulation of the anion exchange process could be conducted for simulation purposes.

After the flasks were shaken for the allotted time, the supernatant liquid was analyzed by using the UV spectrophotometer in the preparative apparatus at a wavelength of 280 nm, the supernatant concentrations being determined by using a concentration-standard curve previously constructed. By applying the mass balance method of section 3.6.1, chapter 3, the concentration of beta lactoglobulin adsorbed to the resin could be determined. Unfortunately, the data, when plotted, revealed much scatter, even when several repetitions of the same set of concentrations were made. One reason for this is the error introduced in measuring the supernatant liquid volume. It was impossible to separate the resin slurry from the liquid phase, even with very careful decantation. Another reason for the scatter may have been that the reagent, purchased from Sigma Chemical Company, might contain the two forms of beta lactoglobulin, A and B. The results of one set of batch experiments are shown in Figure 5.1. This scatter of data did not allow correlation statistically to one of the isotherm models discussed in section 3.7 of chapter 3, as was the original intention.

Another method to obtain single component isotherm data would be to use the previously described analytical system and utilize the frontal analysis method. However, because of the equipment limitations with this system as discussed, this option was also not available.

Beta Lactoglobulin Equilibrium Data

0.05 M NaOAc - pH = 5.6

Shaking Flask Equilibrium Method

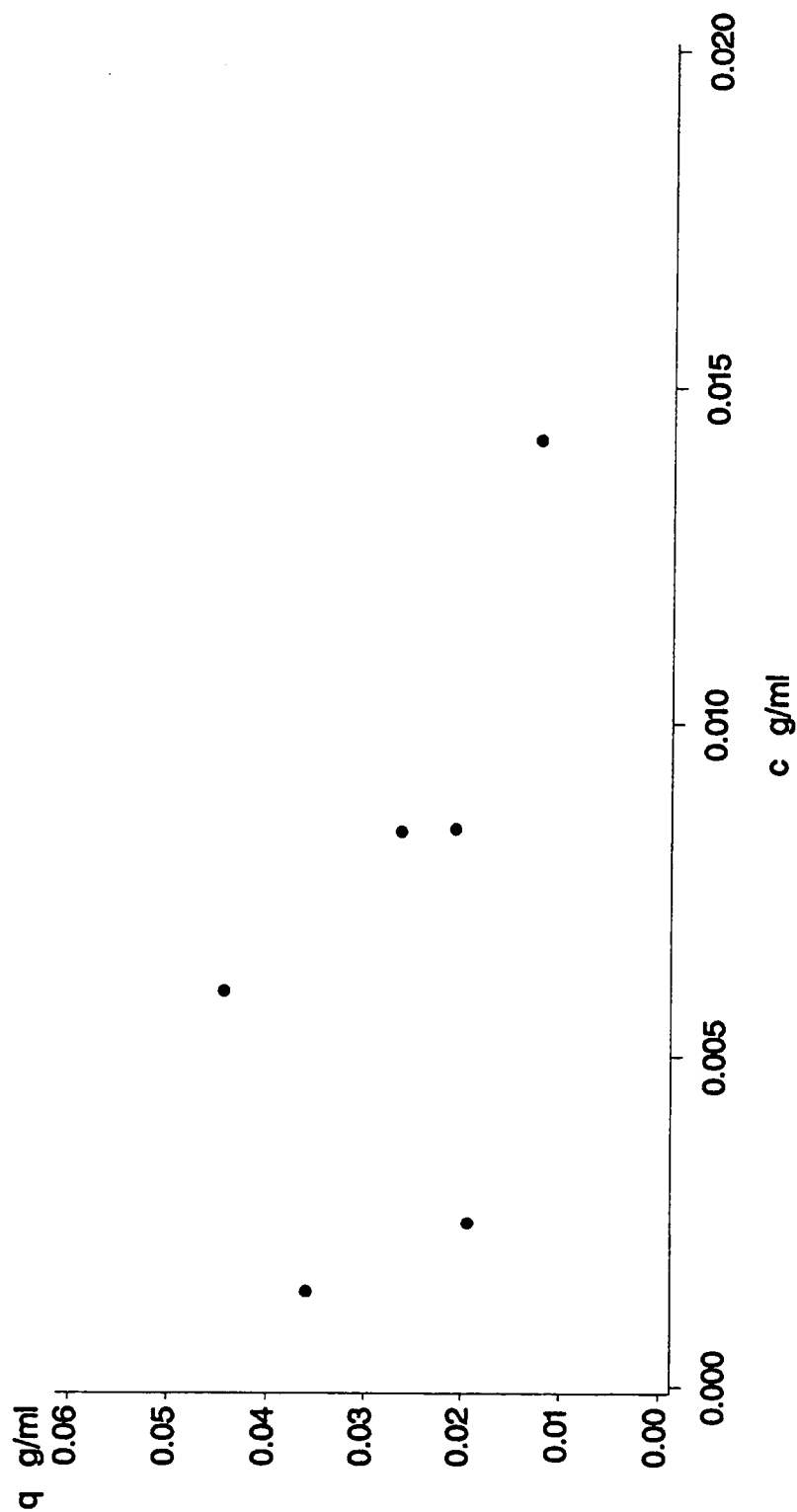


Figure 5.1 Equilibrium Data For Beta Lactoglobulin On Q Sepharose Big Beads.

Finally, probably the most effective method to determine the anion and cation exchange isotherm data for single components would be to carry out the process cycles as discussed in chapters five and six, but instead of applying whey feed pulses, apply pure component protein pulses. Then these single component isotherm data could hopefully be combined into multicomponent models using the method discussed in section 3.9 of chapter three. The problem with this method is that a large amount of each protein is required for the flowrate and column volumes utilized in the ion-exchange processes, thus requiring a substantial investment in proteins. As this was also not reasonable, the conclusion was made that no reliable isotherm data could be obtained in this project and so the linear equilibrium model, as discussed in section 3.3.1 of chapter three, was investigated for use in scale-up issues. This is discussed further in chapter nine.

5.2 Bed And Intraparticle Porosities

Fixed bed experiments were conducted to determine the bed and intraparticle porosities. These two key parameters are used in the detailed mathematical model describing the chromatographic process as described in equations 3.1 - 3.10.

To obtain these parameters experimentally, consider a mass balance on a component moving through a column packed with a porous resin under conditions where linear equilibrium prevails. Such an analysis yields:

$$K = \left[\left(\frac{ut_e}{Z} - \epsilon_b \right) \frac{1}{1 - \epsilon_b} - \epsilon_p \right] \frac{1}{1 - \epsilon_p} \quad (5.1)$$

where K is a linear distribution coefficient defined by

$$K = \frac{q}{c} \quad (5.2)$$

Here q and c represent the solute concentration in the resin and pore fluid phases respectively, u is the superficial velocity, Z the bed length, and t_e the elution time of a chromatographic peak. Note that the linear distribution coefficient is defined in terms of the solid volume of the resin. This value, of course, can be defined in terms of the entire resin volume, $\bar{K}$ obtaining

$$\bar{K} = \epsilon_p + (1 - \epsilon_p)K \quad (5.3)$$

If the solute passing through the column is not adsorbed, $K = 0$ and eq 5.1 may be rearranged to yield the particle porosity accessible by that solute

$$\epsilon_p = \left[\left(\frac{ut_e}{Z} \right) - \epsilon_b \right] \frac{1}{1 - \epsilon_b} \quad (5.4)$$

Finally, if the solute is too large to penetrate the pores of the resin eq 4.4 may be further rearranged to yield the bed void fraction as a function of elution time

$$\epsilon_b = \frac{ut_e}{Z} \quad (5.5)$$

Thus, by carefully choosing solutes and elution conditions, it is possible to obtain the desired bed and intraparticle porosities data.

Blue dextran, a large polysaccharide with a molecular weight of 2,000,000 was purchased from the Sigma Chemical Company, St. Louis, Missouri. This compound, with its large molecular weight, is classically used for determining the bed void fraction in chromatography columns. For this experiment, a 1.0 g/L solution of the blue dextran in de-ionized water was prepared. Then feed pulses of varying size, from 1 ml to 50 ml were applied to the preparative anion exchange column containing the Q Sepharose Big Beads. The output from the column was measured

at 280 nm UV. Unfortunately it was found that at any feed pulse size the blue dextran stuck to the resin and discolored it permanently. The effluent peak from the column was not at all Gaussian in nature and so the use of blue dextran to determine the bed void fraction was deemed not appropriate. The discolored resin was subsequently changed out. To obtain a reasonable value for the bed void fraction, necessary for obtaining the intraparticle porosity, the literature was reviewed for experimental values. It was found that typically the bed void fraction has a value from 0.36 to 0.40. A value of 0.40 was used for further calculations in this work.

The intraparticle porosity was determined using sodium nitrite, a small salt, which, should be able to penetrate the entire void volume of the particle. It was purchased from J.T. Baker company with a purity rating of U.S.P. For this experiment, a 10 g/L solution of the salt in deionized water was prepared. Previous experience with this salt found that this concentration level was appropriate (Bloomingburg, 1992). Before feed pulses of the sodium nitrite were applied to the column, several column volumes of 0.01 molar sodium acetate, pH=5.5, were put through the column to wash the resin and establish a straight baseline. The flowrate was 25 ml/min. and the eluant from the column was analyzed by the preparative apparatus UV at a wavelength of 208 nm. Fourteen experimental runs were conducted with various feed pulses of the sodium nitrite. It was found that 10 ml feed pulses yielded the best elution peaks visually and consistently. Using the bed void fraction of 0.40 and equation 5.4, an intraparticle porosity of 0.89 was calculated, in agreement with past research efforts (Bloomingburg, 1992). A chromatogram of a sodium nitrite experiment is shown in Figure 5.2.

Intraparticle Porosity Experiment

10 g/L Sodium Nitrite – 10 mL Pulse – 25 mL/min.

Pharmacia Biotech XK 50/30 Column: 5cm x 30 cm

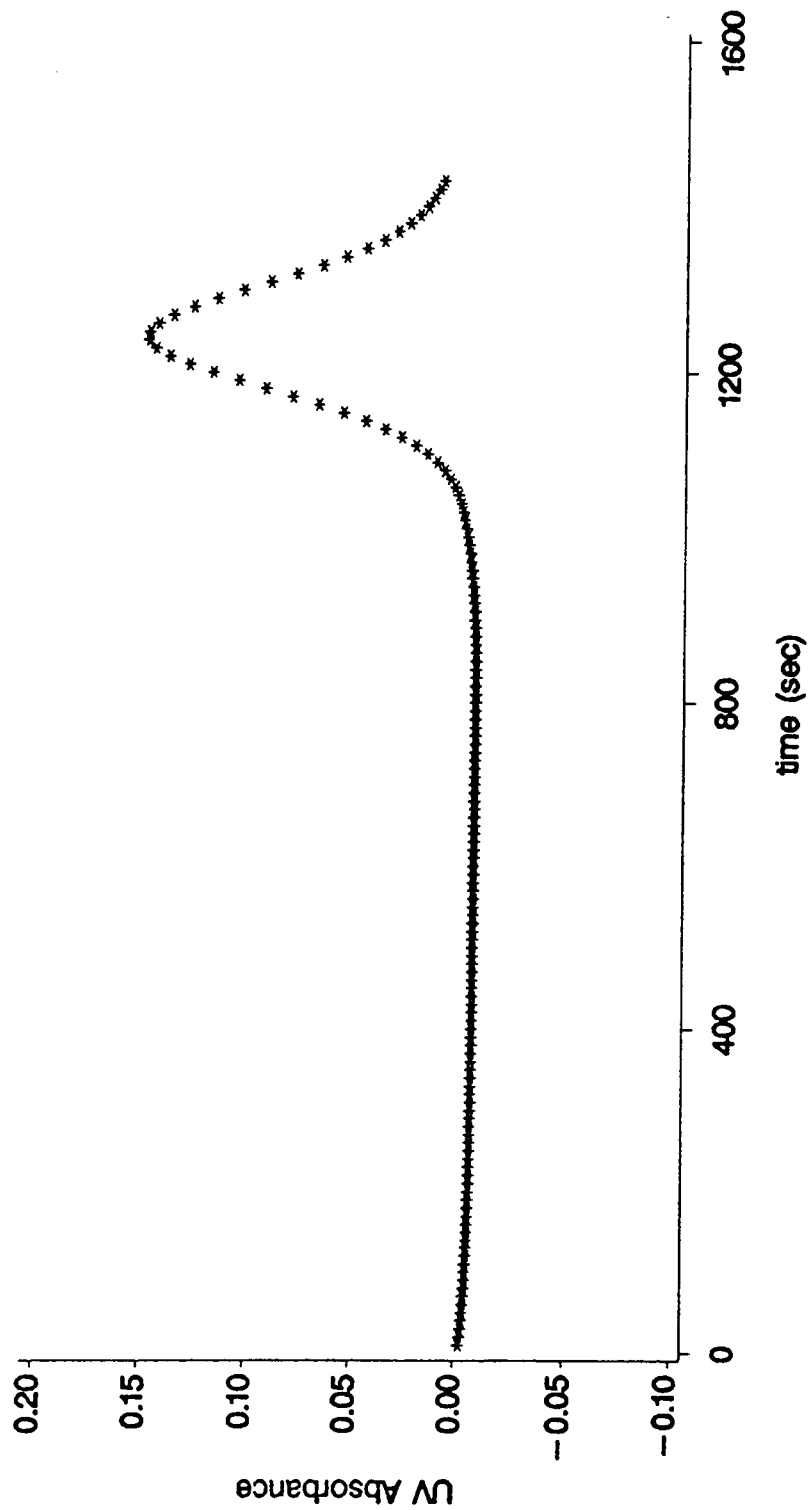


Figure 5.2 Sodium nitrite pulse experiment to determine intraparticle porosity.

CHAPTER VI

PREPARATIVE ANION EXCHANGE CHROMATOGRAPHY

INITIAL SEPARATION OF SWEET DAIRY WHEY PROTEINS

6.1 Introduction

As discussed in section 1.3 of chapter one, there are several technologies available for separating whey proteins from one another or to produce whey protein concentrates and isolates. In the case of concentrates and isolates, the proteins concentrations are increased; for a concentrate the proteins concentration is from 35 to 75 wt.%, for an isolate it is 90 wt.% and above. The selection of the various technologies for use in processing whey depends on the type of proteins product desired.

In this research project it was desired to separate the four major whey proteins and lactose from the sweet dairy whey and one another. The reason for this was that a review of the open literature did not reveal any previous research into in-

dividual whey proteins isolation. This project investigated the use of ion-exchange chromatography to see what degree of individual proteins isolation could be attainable. Then in the scale-up and economics optimization studies, discussed in chapters eight and nine, it would be proposed that the isolated protein fractions be sold individually or a portion of them be recombined to produce marketable protein concentrates and isolates, depending on the economics.

With this in mind, anion exchange chromatography was chosen as the first separation step to isolate the proteins and lactose from sweet whey. Since the most valuable of the four major proteins is immunoglobulin G (IgG), anion exchange and initial loading conditions were chosen so that the IgG would not bind to the resin upon loading but rather pass through in the breakthrough curve. The other proteins, alpha lactalbumin, beta lactoglobulin, and bovine serum albumin would bind to the resin due to their respective isoelectric points (pI's) and the initial loading conditions for the whey feed.

The isoelectric point is the pH for a protein where the sum of the distributed charges across the molecule are zero. That is, the net charge is zero at that pH. At a pH above a protein's pI, the protein has an overall negative charge and thus binds or adsorbs onto anion exchange resins which have negatively charged exchangeable counter ions. At a pH below a protein's pI, the protein has an overall positive charge and thus binds or adsorbs onto a cation exchange resins which have positively charged exchangeable counter ions. The fact that the four major whey proteins have unique pI's allows ion-exchange chromatography to be used to effect their separation through a combination of anion and cation exchange resins. The respective isoelectric points for the whey proteins are shown in Table 6.1.

In general, to effect a separation of proteins by ion exchange chromatography,

Table 6.1
Molecular Weights and Isoelectric Points for Whey Proteins

Protein	M.W.	pI
alpha lactalbumin	14,000	4.2 - 4.5
beta lactoglobulin	18,300	5.35 - 5.49
bovine serum albumin	69,000	5.13
immunoglobulin G	160,000 - 1,000,000	5.5 - 8.3

five sequential steps must be planned. These are: (1) Selection of initial pH and salt buffer concentration for equilibration of the ion exchanger to bring it to a starting state which allows the proteins of interest to bind to the resin. This is known as starting state elution. (2) Application of the feed pulse (or volume) to the ion exchange column and adsorption of the desired proteins to the resin. The protein molecules, with their respective pI's, displace the counter ions of the gel and thereby bind. (3) Once the proteins to be adsorbed are, the initial equilibration buffer is passed through the column in a prescribed volume to wash out any unbound proteins and other miscellaneous solutes. Here the IgG would be washed from the column. (4) Next, the proteins of interest that were bound ionically in step 2, are not selectively removed from the column by changing the salt buffer concentration and/or pH to elution conditions which are unfavorable for binding. The proteins are released from the column in order of how strong they are bound to the resin, the weakly bound ones released first. This process may require two or more step changes. (5) The fifth and final step is to regenerate the column with cleaning solutions and finally the initial equilibration buffer to prepare the column for another adsorption/desorption cycle.

The successful exploitation of this cycle, in combination with various salt buffer concentrations and solution pH levels, is discussed in the next section.

6.2 Experimental Approach

For the anion exchange studies, the inorganic salt buffer chosen was sodium acetate, per recommendations from Pharmacia Biotech Inc. (1994). This salt, when dissolved in degassed, deionized water, forms a buffer system useful for initially equilibrating the anion exchange resin, and for carrying out step changes by in-

creasing its concentration. In general, the buffer concentrations were varied from 0.01 molar to 0.2 molar sodium acetate. The buffer pH of the initial equilibration buffer ranged from 5.6 - 5.8, the same as the pH of the fresh whey feed. For the step change buffers, their pH's were always 5.5 and 4.0, in that order, to cross the pI ranges for the three major proteins bound to the column. Thus, the pH levels were chosen so that decreasing in pH from the initial equilibration buffer one, beta lactoglobulin should come off first since its pI range is from 5.35 - 5.49, close to that of the feed pH at 5.6- 5.8. Next, bovine serum albumin should come off with its pI at 5.13. Finally, alpha lactalbumin should come off the column as the elution pH is dropped to 4.0, going below alpha lactalbumin's pI range of 4.2 - 4.5. This elution strategy is sound in theory, however, as will be shown in the next section, the order of elution for the alpha lactalbumin and the beta lactoglobulin were found to be reversed in the optimized experiments. The reasons for this will be discussed shortly.

For cleaning and regeneration of the anion exchange column, Pharmacia Biotech Inc. recommends passing 1 molar sodium chloride through the column to knock off strongly, but reversibly bound material. For severe contamination, such as precipitated proteins onto the column, one column volume of 0.5 - 2.0 molar sodium hydroxide is recommended. For progressively built up contamination, after repeated separation cycles, a cleaning-in-place (CIP) procedure of 2 molar sodium chloride, 1 molar sodium hydroxide, and a 70% ethanol solution are recommended. Finally, for sanitization of the gel to reduce microbial contamination, passing 1 molar sodium hydroxide through the bed is recommended.

For this research experiment, solutions of 1 molar sodium hydroxide or 1 molar sodium chloride were passed through the column, in one column volume amounts

(491 ml), to clean the bed. When sodium hydroxide was used, the eluant pH was always over 12.0 and had to be reduced through use of a dilute acid solution step followed by several column volumes of the equilibration buffer. Since no quantitative methods were developed in this project to determine the frequency of column cleaning, the cleaning cycle was applied after each experimental run. Through discussions with Pharmacia Biotech Inc., it was learned that one column volume of 1 molar sodium hydroxide solution, followed by several column volumes of dilute acetic acid solution were appropriate to clean the column and lower the eluant pH. Acetic acid was suggested by Pharmacia Biotech Inc. because the acetate ion in acetic acid is also present in the sodium acetate buffer. By using acetic acid the process chemistry's exchangeable counter anion is restricted to the acetate ion. When the sodium hydroxide passes through the column, the high concentration of hydroxide ion displaces any other bound anions and therefore "cleans" the column. Following the acid step, the column is regenerated with several column volumes of equilibration buffer to replace the bound hydroxide anion with acetate anion.

The development of an optimal process cycle for the anion exchange separation is discussed in the following section.

6.3 Experimental Results

A total of 46 experimental runs were conducted for the anion exchange separation phase of the project. In general the experiments were conducted at various loadings of whey feed using different buffer concentrations and pH levels within the framework of the starting state elution strategy discussed in section 6.1. Each experiment was conducted on a separate day and used fresh whey feed from a specific production run. From experimental run to run it was found that the feed com-

position was quite similar, varying only slightly from batch to batch. In Figures 6.1 - 6.9 nine representative chromatograms are shown with pertinent details. The ultraviolet wavelength used for in-line monitoring of the eluting peaks was 280 nm. These chromatograms cover the range of experimental conditions investigated and are numbered according to their role in the effort to develop an optimized process. The anion exchange process was judged as optimized when the resolution between the four resultant peaks was qualitatively best or nearly baseline at the maximum flowrate permissible. Before the peak fractions from the optimum conditions run were analyzed by the commercial labs, samples of each from a duplicate run were processed by the analytical system shown in Figure 4.2 to qualitatively determine their composition. This gave a rough idea of the effectiveness of the separation.

In Figures 6.1 - 6.3 are shown resultant chromatograms for the first few attempts at separating the proteins from a 25 mL feed pulse at a flowrate of 25 mL/min. As shown on the figures, different concentrations of sodium acetate buffer were utilized with a common method of loading the whey pulse on at a pH of 5.5 and carrying out elution with decreasing pH and increasing salt concentration.

These peaks were analyzed by the analytical system shown in Figure 4.2 to roughly determine the effectiveness of the separation, which was found to be incomplete in terms of individual proteins fractionation.

With this experience gained, it was desirable to load greater quantities of the whey onto the column to utilize more of the column capacity, and to operate at a higher flowrate. As mentioned earlier, the resin is limited to an operating column pressure of 42 psi, above which crushing of the beads will occur. Incorporating a margin of safety, it was found that a flowrate of 50 mL/min. was the maximum possible in the pilot apparatus, and this flowrate was used in all further experiments.

Preparative Anion Exchange Chromatogram
Pilot Run #1 - 25 mL Feed Pulse - 25 mL/min
Pharmacia XK 50/30 Column: 5cm x 30 cm

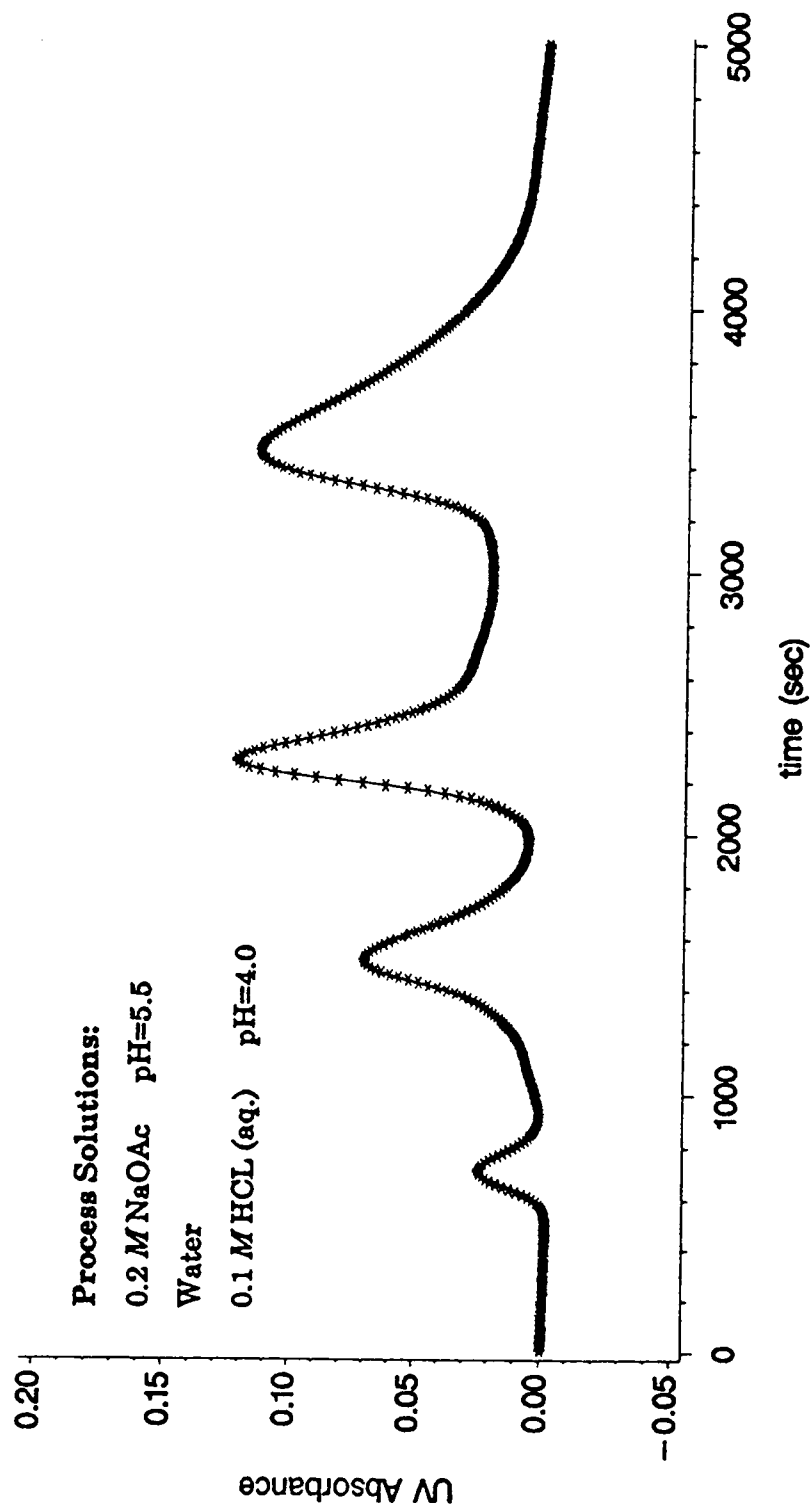


Figure 6.1 Preparative anion exchange chromatogram for a 25 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #9 - 25 mL Feed Pulse - 25 mL/min
 Pharmacia XK 50/30 Column: 5cm x 30cm

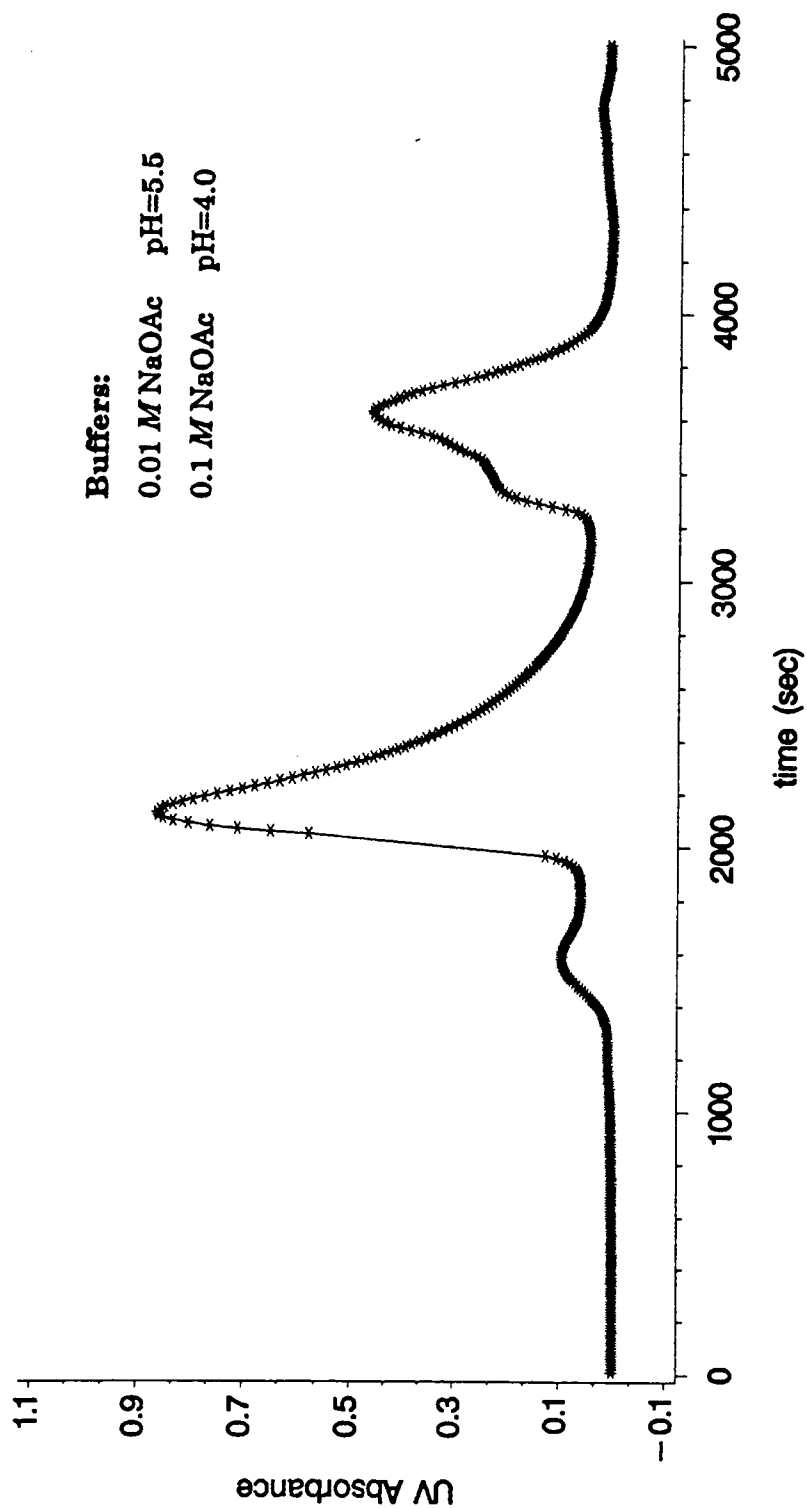


Figure 6.2 Preparative anion exchange chromatogram for a 25 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #11 - 25 mL Feed Pulse - 50 mL/min
Pharmacia XK 50/30 Column: 5cm x 30cm

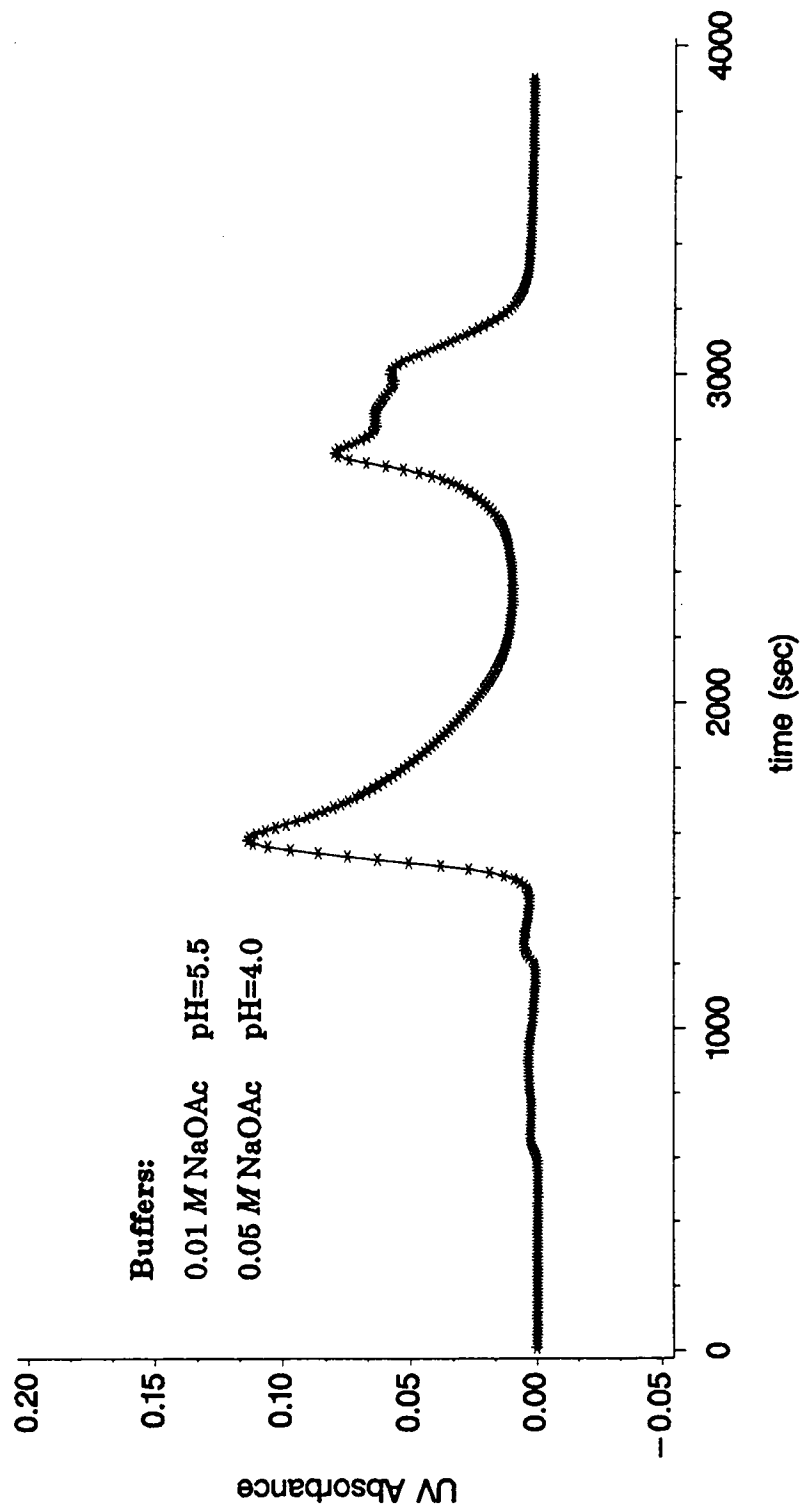


Figure 6.3 Preparative anion exchange chromatogram for a 25 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #24 - 392 mL Feed Pulse - 50 mL/min
 Pharmacia XK 50/30 Column: 5cm x 30cm

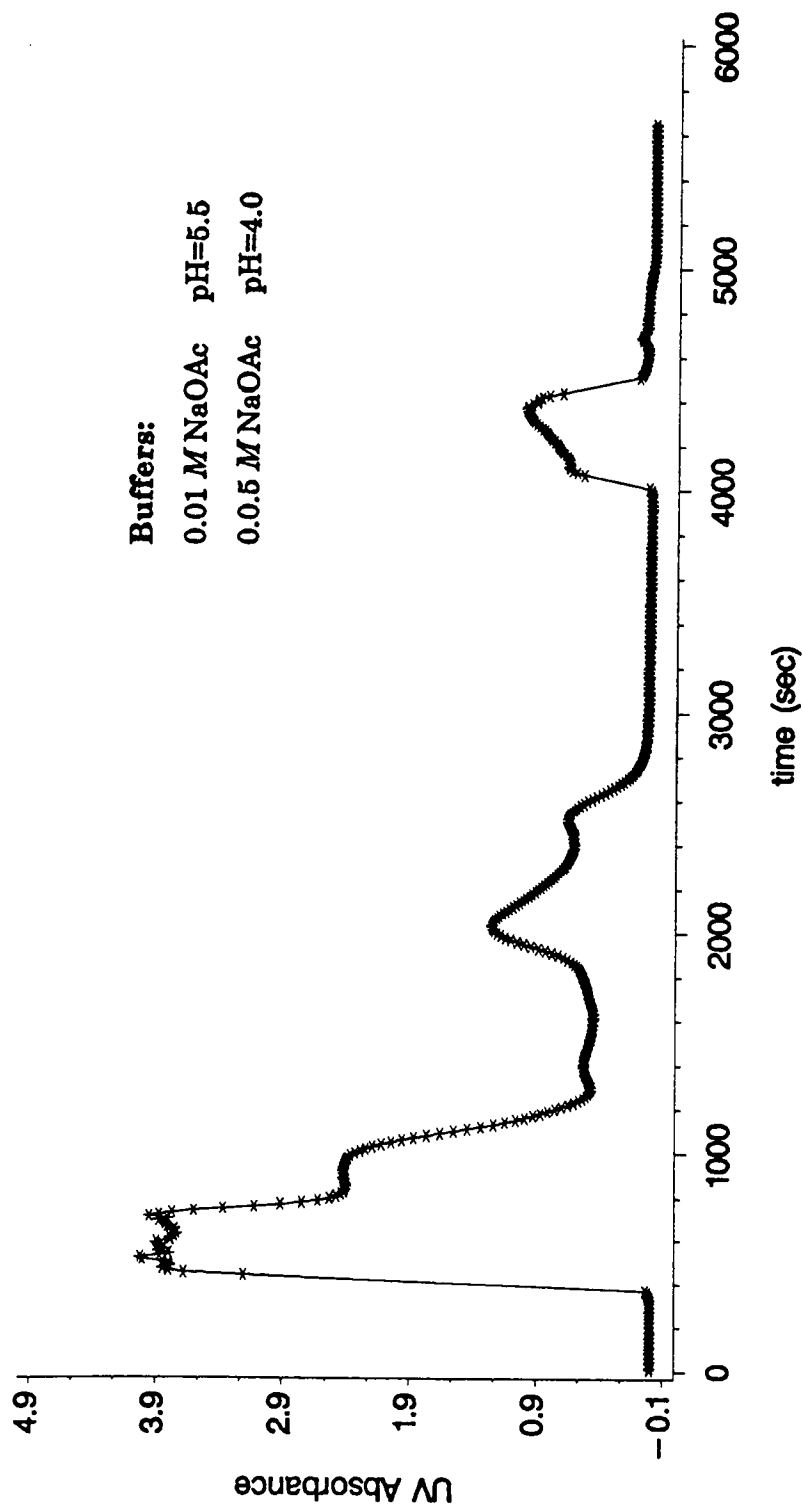


Figure 6.4 Preparative anion exchange chromatogram for a 392 mL whey feed pulse.

Preparative Anion Exchange Chromatogram *Pilot Run #33 – 2 Column Volumes Feed – 50 mL/min* Pharmacia XK 50/30 Column: 5cm x 30cm

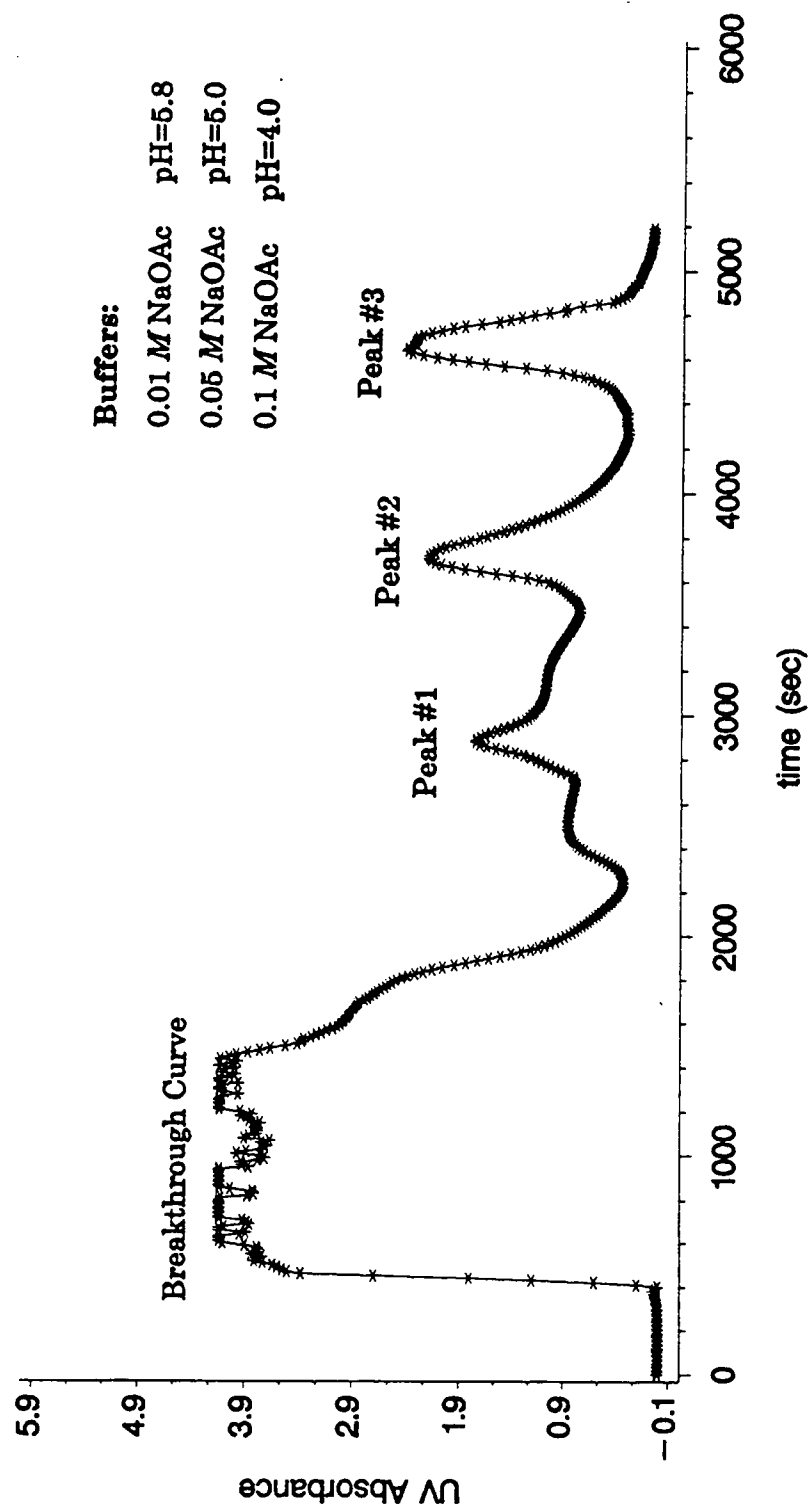


Figure 6.5 Preparative anion exchange chromatogram for a 982 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #37 - 1.0 Column Volume Feed - 50 mL/min
 Pharmacia XK 50/30 Column: 5cm x 30cm

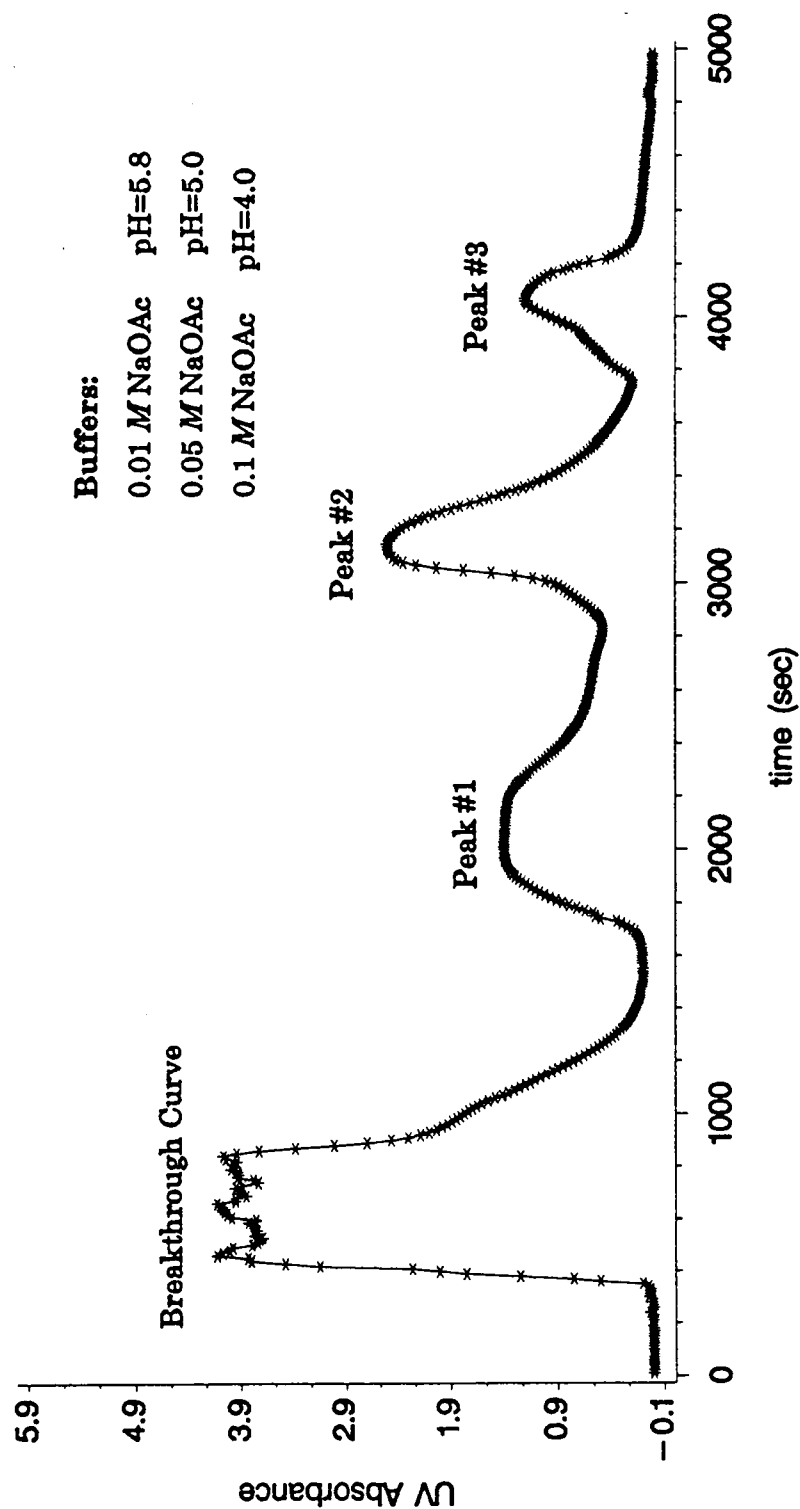


Figure 6.6 Preparative anion exchange chromatogram for a 491 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #39 – 0.5 Column Volume Feed – 50 mL/min
 Pharmacia XK 50/30 Column: 5cm x 30cm

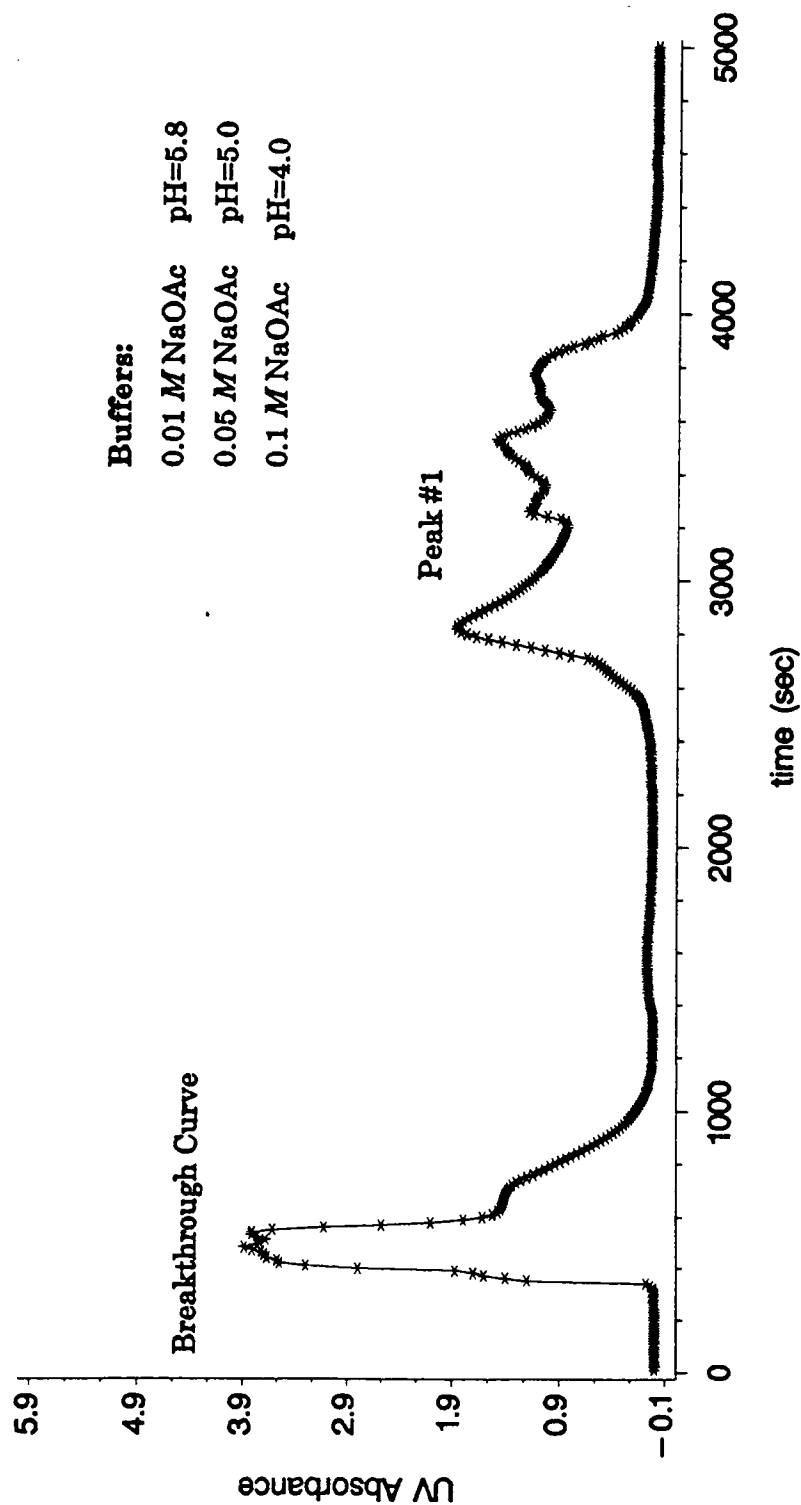


Figure 6.7 Preparative anion exchange chromatogram for a 246 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #40 – 0.75 Column Volume Feed – 50 mL/min
 Pharmacia XK 50/30 Column: 5cm x 30cm

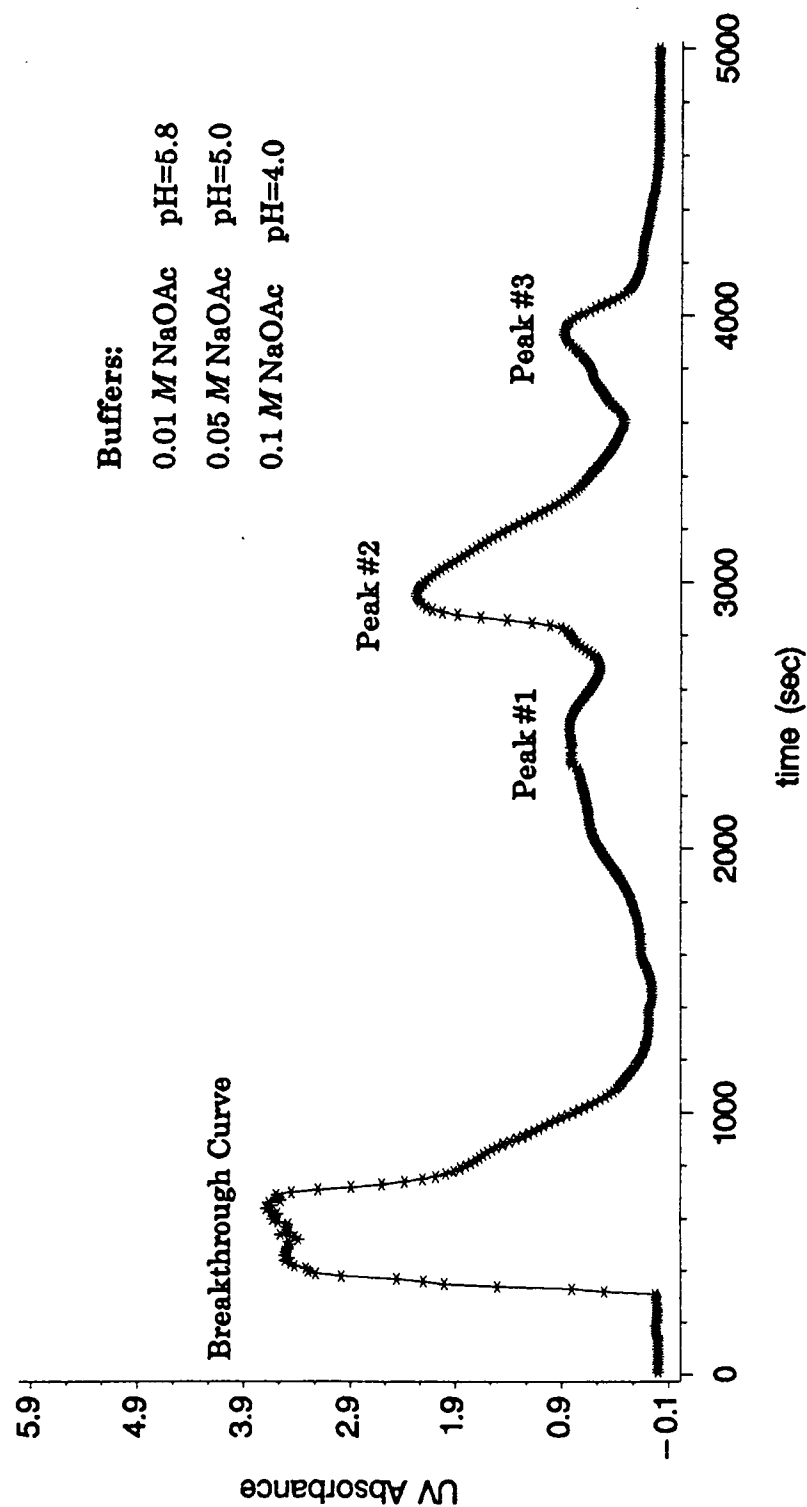


Figure 6.8 Preparative anion exchange chromatogram for a 368 mL whey feed pulse.

Preparative Anion Exchange Chromatogram
Pilot Run #41 – 1.5 Column Volumes Feed – 50 mL/min.
 Pharmacia XK 50/30 Column: 5cm x 30cm

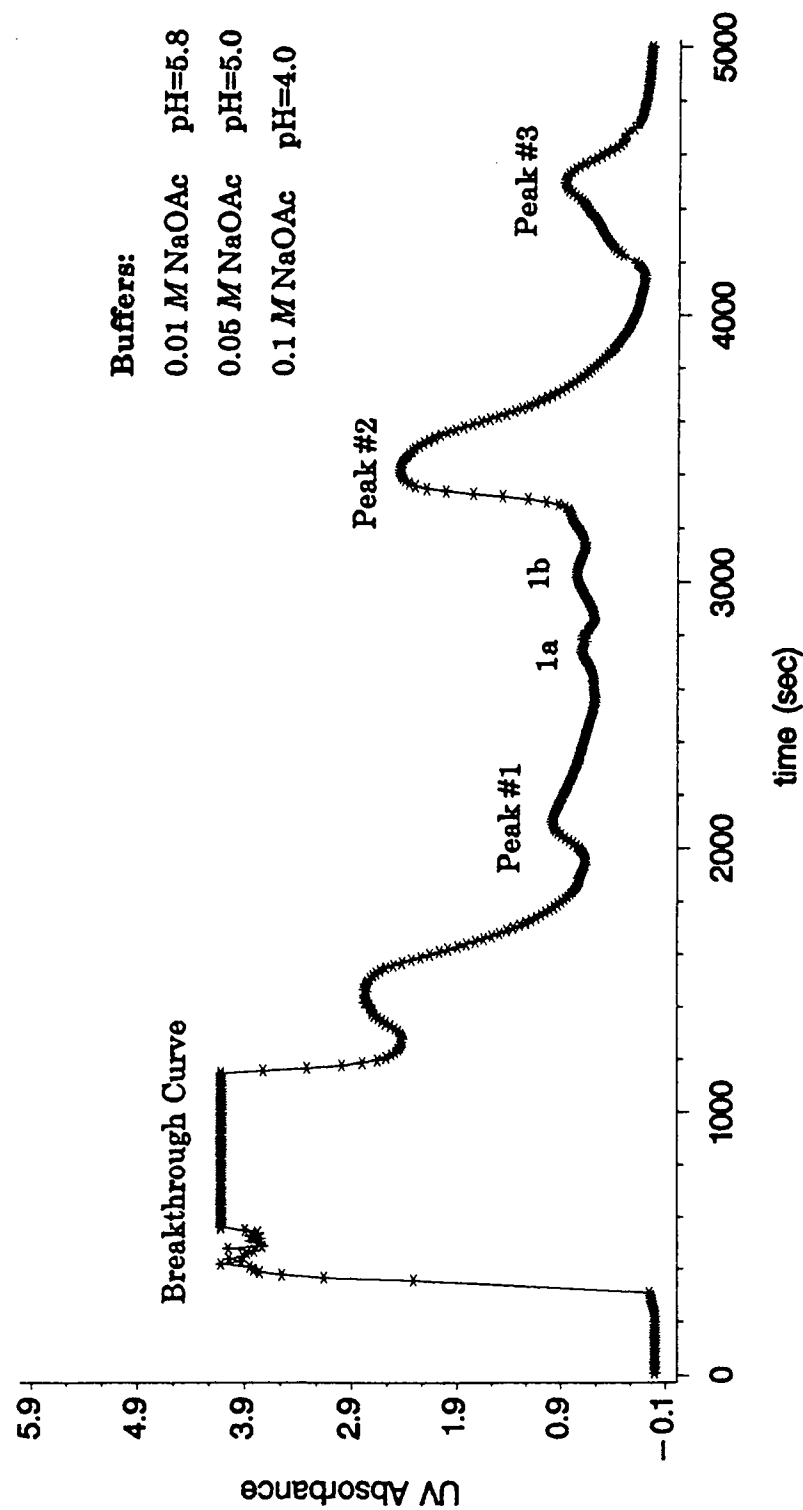


Figure 6.9 Preparative anion exchange chromatogram for a 737 mL whey feed pulse.

In Figures 6.4 - 6.9 are shown the results of using various column volumes or fractions thereof of whey feed while operating at 50 mL/min. It is observed from these chromatograms that as additional material is loaded onto the column the three individual peaks become separated. This is believed to be due to the so called displacement effect in chromatography. This effect proposes that a particular solute band in the column is accelerated and perhaps noticeably contracted in the presence of a large amount of another solute which is moving slower through the column. Peak 2 is the most retained of all the peaks of interest and also contains the greatest amount of mass of any of the fractions. Therefore as more and more feed is loaded onto the column, peak 2 tends to displace the other peaks assisting in their separation due to this effect and the column conditions providing for strong binding of the beta lactoglobulin in peak 2.

It is unclear why the beta lactoglobulin peak eluted after the peak containing the alpha lactalbumin, since the beta lactoglobulin should come off first as waves of decreasing pH solutions are processed in the column. This should be so because the beta lactoglobulin has a higher isoelectric point range than the alpha lactalbumin. It can only be proposed that the peak's elution order is reversed due to a combination of the presence of other contaminating solids and the processing conditions selected, i.e., the salt concentrations. The effect of having the beta lactoglobulin elute after the alpha lactalbumin does not appear to be detrimental since it is the peak separated the most with the highest purity of any protein of interest. Whether the alpha lactalbumin is detrimentally effected by eluting earlier is uncertain, since it is not well isolated in peak 1. Further studies on this phenomena in future studies would probably clarify this issue.

As experience was gained with the different loadings and buffer concentration

levels, gradually a process operating cycle was developed which involved two step changes simultaneous in salt concentration and pH. In each step change the salt concentration would increase and the pH level would be decreased. To aid in the development of this part of the cycle, samples of the peaks would be analyzed with the analytical system to obtain a rough estimate of the effectiveness of the separation for a particular experimental run. It was found that the first step change should be an increase in sodium acetate concentration from 0.01 M to 0.05 M with a decrease in pH from an initial 5.8 to 5.0. The second step change was found to be most effective with an increase in sodium acetate concentration from 0.05 M to 0.1 M and a decrease in pH from 5.0 to 4.0.

By viewing Figures 6.4 - 6.9 it is seen that qualitatively the optimal column loading is one volume or 491 mL, as shown in Figure 6.6 for experimental run 37. The conditions to achieve this chromatogram were subsequently used in scale up issues. Specifically, the cycle for obtaining this chromatogram involved the following, in this order: (1) Cleaning of the column with one column volume of 1 M sodium hydroxide. (2) One column volume of deionized water through the column to flush out the sodium hydroxide. (3) Two and a half column volumes of 0.1 M acetic acid to lower the pH of the liquid in the column and the eluant from it. (4) Two column volumes of 0.01 M sodium acetate, pH=5.8 to equilibrate the column in terms of acetate anion concentration bound to the resin sites to prepare for feed application. (5) Application of one column volume of whey feed at a pH of 5.8, directly from cheese plant production line. (6) One column volume of 0.01 M sodium acetate to wash out any material not bound to the resin from the feed step. (7) Two column volumes of 0.05 M sodium acetate, pH=5.0 to begin step elution of proteins bound to the resin. (8) Two column volumes of 0.1 M sodium acetate,

pH=4.0 to carry out final step change to elute remaining bound proteins. (9) Begin new cycle with cleaning step. A diagram of this operating cycle is shown in Figure 6.10. In Table 6.2 these eight steps of the process cycle are listed in terms of the time requirement for each. At the flowrate of 50 mL/min. it is calculated that one process cycle requires 122 minutes and 43 seconds.

Once the conditions for qualitatively optimizing the process cycle were set, it remained to be determined the effectiveness of the step changes on the separation of the proteins through quantitative methods. Experimental run 37 was repeated and each peak fraction was collected and sent to the commercial laboratories discussed in chapter two for analysis. It must be pointed out that peak 3 was not analyzed because previous analyses of this peak revealed that it did not contain any of the five components of interest. The results are displayed in Table 6.3. These results show that the anion exchange process is most effective in separating the beta lactoglobulin from the whey feed mixture and from the other proteins and lactose. It is seen that 93.4% of the beta lactoglobulin is recovered in peak 2 at a purity of 50%. The valuable immunoglobulin G for the most part ends up in the breakthrough curve, 94.2% of it being in that fraction. As for the alpha lactalbumin, it is distributed among the breakthrough curve and peak 1, while the BSA is distributed among the breakthrough curve, peak 1 and peak 2. Also, all of the lactose ends up in the breakthrough curve fraction. This makes sense since it is not bound to the resin in the feed loading step and so washes through in the breakthrough curve. As will be discussed in chapters 8 and 9, the breakthrough curve is processed further by cation exchange chromatography and ultrafiltration to recover the immunoglobulin G. It will be proposed in the scaled-up process that peak 1 be processed by ultrafiltration to increase the concentration of the four major proteins in the fraction through

Preparative Anion Exchange Column Process Operating Cycle

Flowrate = 50 mL/min.

1 Column Volume = 491 mL

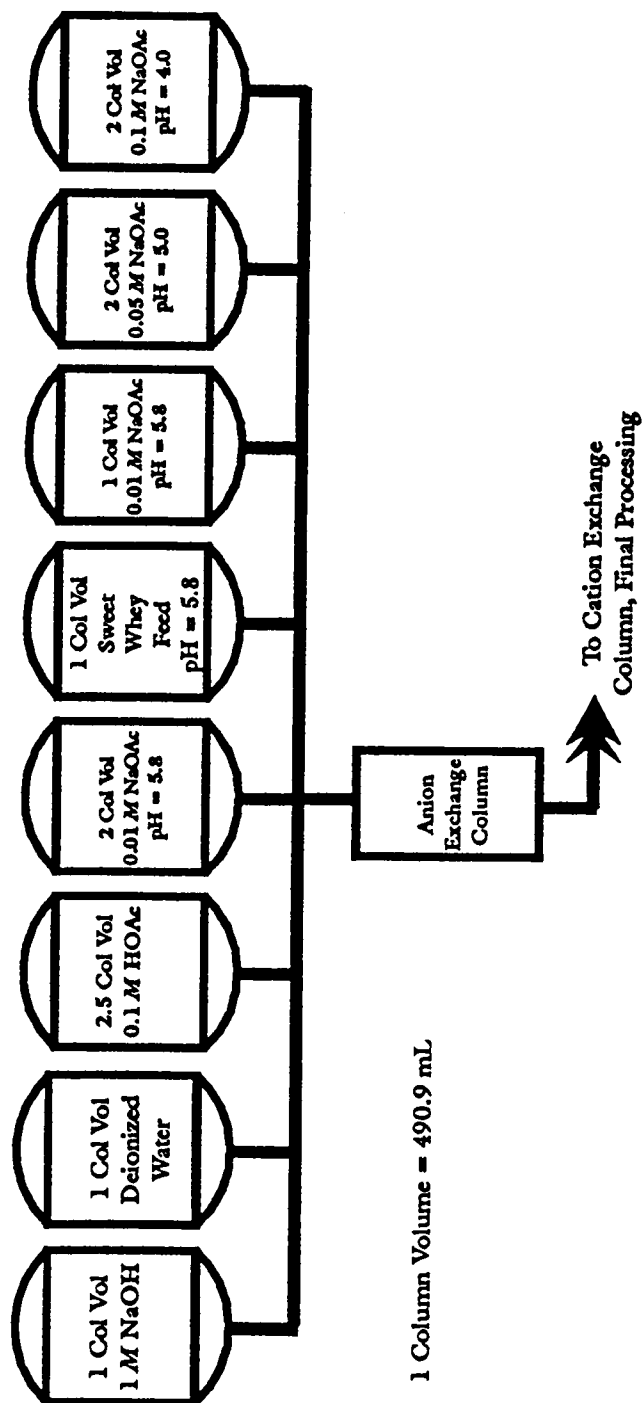


Figure 6.10 Preparative anion exchange column process operating cycle.

Table 6.2

Time requirements for each step in the anion exchange process

Experimental Run 37

Process Step	No. of Col. Vols.	Time (min.)	Cumul. Time (sec.)
whey feed, pH=5.8	1	9.8	589
0.01 M NaOAc, pH=5.8	1	9.8	1178
0.05 M NaOAc, pH=5.0	2	19.6	2356
0.1 M NaOAc, pH=4.0	2	19.6	3534
1 M NaOH	1	9.8	4123
D.I. H ₂ O	1	9.8	4712
0.1 M HOAc	2.5	24.5	6185
0.01 M NaOAc, pH=5.8	2	19.6	7362

Table 6.3
Composition of peaks for one column volume loading
of whey feed to the anion exchange column
Experimental Run 37

	$\alpha - LA$	$\beta - LG$	BSA	IgG	LAC	density ^(a)
fraction	feed	feed	feed	feed	feed	feed
%wt. fraction	1.68	7.31	0.40	0.82	9.72	1.07
%recovery	—	—	—	—	—	—
g loaded	1.4	6.1	0.33	0.69	51.051	

fraction	brkthrh.	brkthrh.	brkthrh.	brkthrh.	brkthrh.	brkthrh.
%wt. fraction	1.07	0.45	0.31	0.78	61.60	1.035
%recovery	63.6	6.066	78.8	94.2	100.0	—
grams	0.89	0.37	0.26	0.65	51.051	—

fraction	peak 1	peak 1	peak 1	peak 1	peak 1	peak 1
%wt. fraction	10.91	0.64	0.16	0.86	0.0	1.004
%frac./feed	36.43	0.49	2.2	5.80	0.0	—
grams	0.51	0.03	0.0073	0.04	0.0	—

fraction	peak 2	peak 2	peak 2	peak 2	peak 2	peak 2
%wt. fraction	—	49.9	0.55	—	—	1.004
%frac./feed	—	93.4	19.0	—	—	—
grams	—	5.7	0.063	—	—	—

^(a) Density in g/ml.

removal of low molecular weight components. This would be followed by spray drying. Details are given in these chapters.

CHAPTER VII

PREPARATIVE GEL FILTRATION CHROMATOGRAPHY

FURTHER SEPARATION OF SWEET DAIRY WHEY PROTEINS

7.1 Introduction

In chapter 6 the use of anion exchange chromatography was successful in partially separating the four major whey proteins and lactose from the whey mixture. However, to reach the desired goal of obtaining individual protein fractions through chromatographic methods, additional processing steps are necessary. To accomplish this goal, preparative gel filtration was investigated for its potential in separating the four major whey proteins and the lactose from one another and the other minor whey components in the anion exchange fractions.

In gel filtration, the sample to be chromatographed is loaded onto the column and eluted isocratically. In this elution mode, the same elution buffer is used throughout the process. This chromatographic process is characterized by very low

flowrates but with good resolution between resultant peaks.

7.2 Experimental Approach

For the gel filtration studies, the inorganic salt buffer chosen was a combination of sodium phosphate anhydrous and sodium chloride, per recommendations from Pharmacia Biotech Inc. (1994). These salts, when dissolved in degassed, deionized water, forms a buffer system useful for initially equilibrating the gel filtration matrix and subsequently carrying out elution of the feed sample isocratically. The concentrations of the salts were 0.05 M sodium phosphate anhydrous and 0.15 M sodium chloride, with the solution adjusted to a pH of 7.0 with 6 M hydrochloric acid. All reagents were of food and pharmaceutical grade quality. As discussed in chapter 4, the gel filtration column was 1.6 cm in diameter by 60 cm in pre-packed length as obtained from Pharmacia Biotech Inc. As with the ion exchange resins used in this project, the gel filtration column was limited to an operating pressure of 42 psi.

For cleaning and regeneration of the gel filtration column, Pharmacia Biotech Inc. recommends passing 1 molar sodium chloride through the column to knock off strongly, but reversibly bound material. For severe contamination, such as precipitated proteins onto the column, one column volume of 0.5 - 2.0 molar sodium hydroxide is recommended. For progressively built up contamination, after repeated separation cycles, a cleaning-in-place (CIP) procedure of 2 molar sodium chloride, 1 molar sodium hydroxide, and a 70% ethanol solution are recommended. Finally, for sanitization of the gel to reduce microbial contamination, passing 1 molar sodium hydroxide through the bed is recommended.

For these research experiments, solutions of 1 molar sodium hydroxide were

passed through the column, in one column volume amounts (491 ml), to clean the bed. Typically two bed volumes of the elution buffer had to be passed through the bed in order to reduce the eluant pH to the starting state of 7.0. Since no quantitative methods were developed in this project to determine the frequency of column cleaning, the cleaning cycle was applied after each experimental run. Through discussions with Pharmacia Biotech Inc., it was learned that one column volume of 1 molar sodium hydroxide solution, followed by several column volumes of dilute acetic acid solution were appropriate to clean the column and lower the eluant pH. However, acetic acid was not used in these experiments to assist in lowering the eluant pH but should function as expected as in the anion exchange experiments.

The use of the gel filtration column for further separation of the anion exchange fractions is discussed in the following section.

7.3 Experimental Results

Several experimental runs were conducted using the gel filtration column both with synthetic whey protein mixtures and actual dairy whey feedstock to determine the appropriate sample volume to be loaded onto the column. It was found that samples of 3 mL were optimal in terms of the resultant peaks and resolution between them. Manufacturer's recommendations are for sample volumes up to 5 mL. For all experiments the flowrates were 1 mL/min. and in-line monitoring of the process was accomplished with the same UV detection system as in the anion exchange experiments at a wavelength of 254 nm. In Figures 7.1 - 7.3 three representative chromatograms are shown with pertinent details for gel filtration processing of the breakthrough curve, peak 1, and peak 2 from preparative anion

Preparative Gel Filtration Chromatogram *Breakthrough Fraction From Pilot Run #33 – 1 mL/min.* Pharmacia XK 16/60 Column: 1.6cm x 60cm

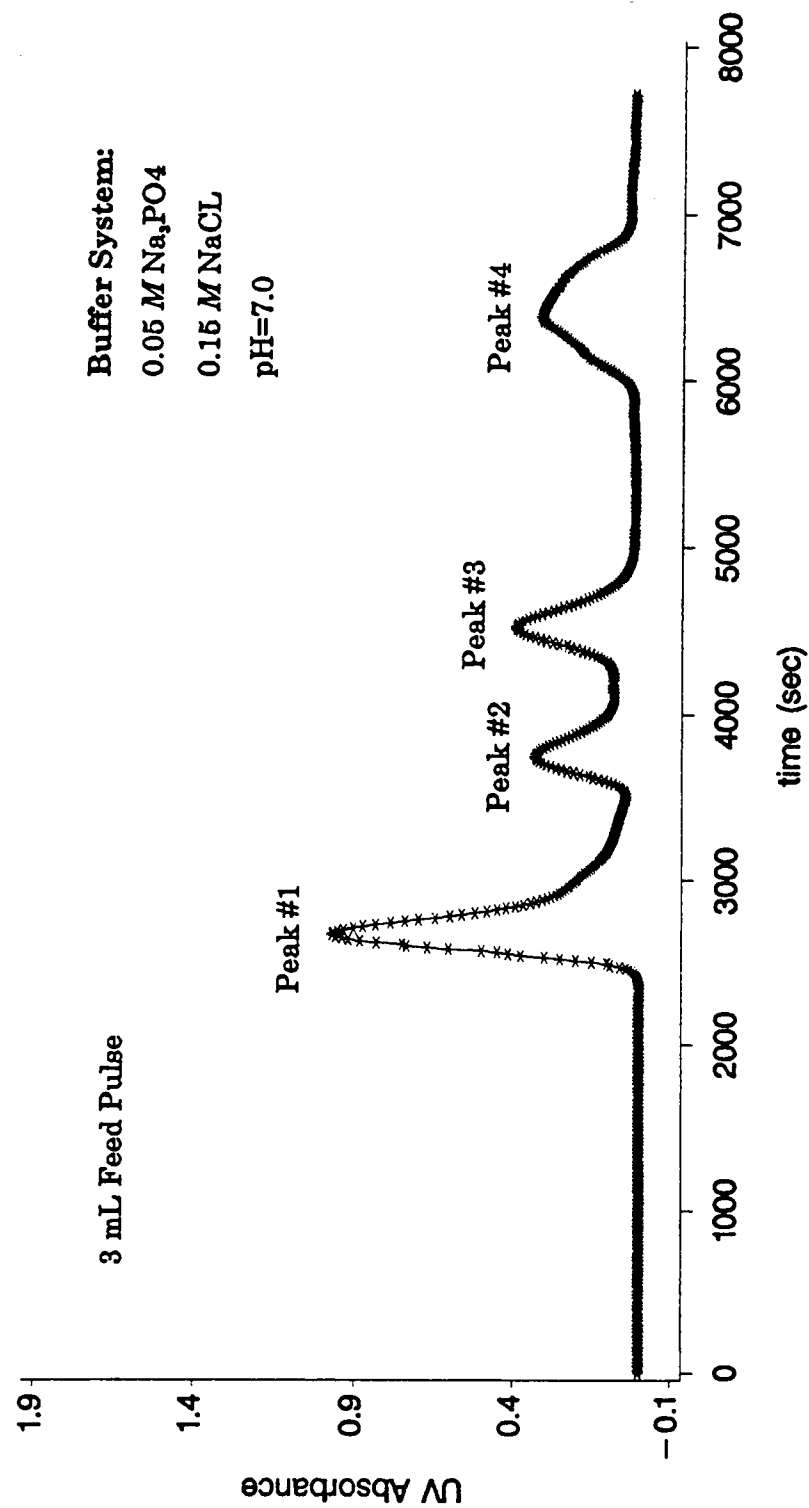


Figure 7.1 Preparative gel filtration chromatogram for a 3 mL pulse of breakthrough curve.

Preparative Gel Filtration Chromatogram

Peak #1 Fraction From Pilot Run #33 - 1 mL/min.

Pharmacia XK 16/60 Column: 1.6cm x 60cm

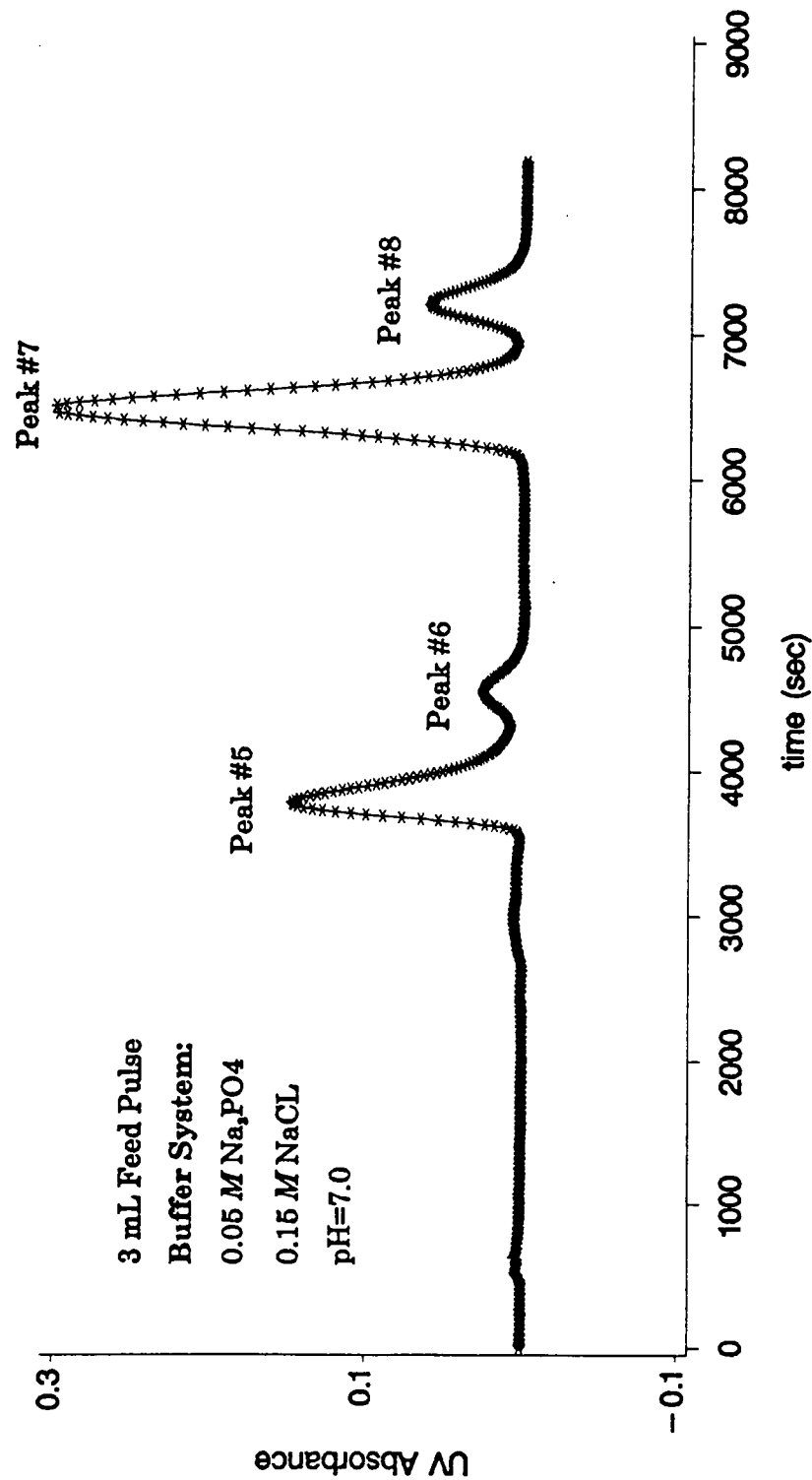


Figure 7.2 Preparative gel filtration chromatogram for a 3 mL feed pulse of peak #1.

Preparative Gel Filtration Chromatogram
Beta Lactoglobulin Fraction From Pilot Run #33 - 1 mL/min.
Pharmacia XK 16/60 Column: 1.6cm x 60cm

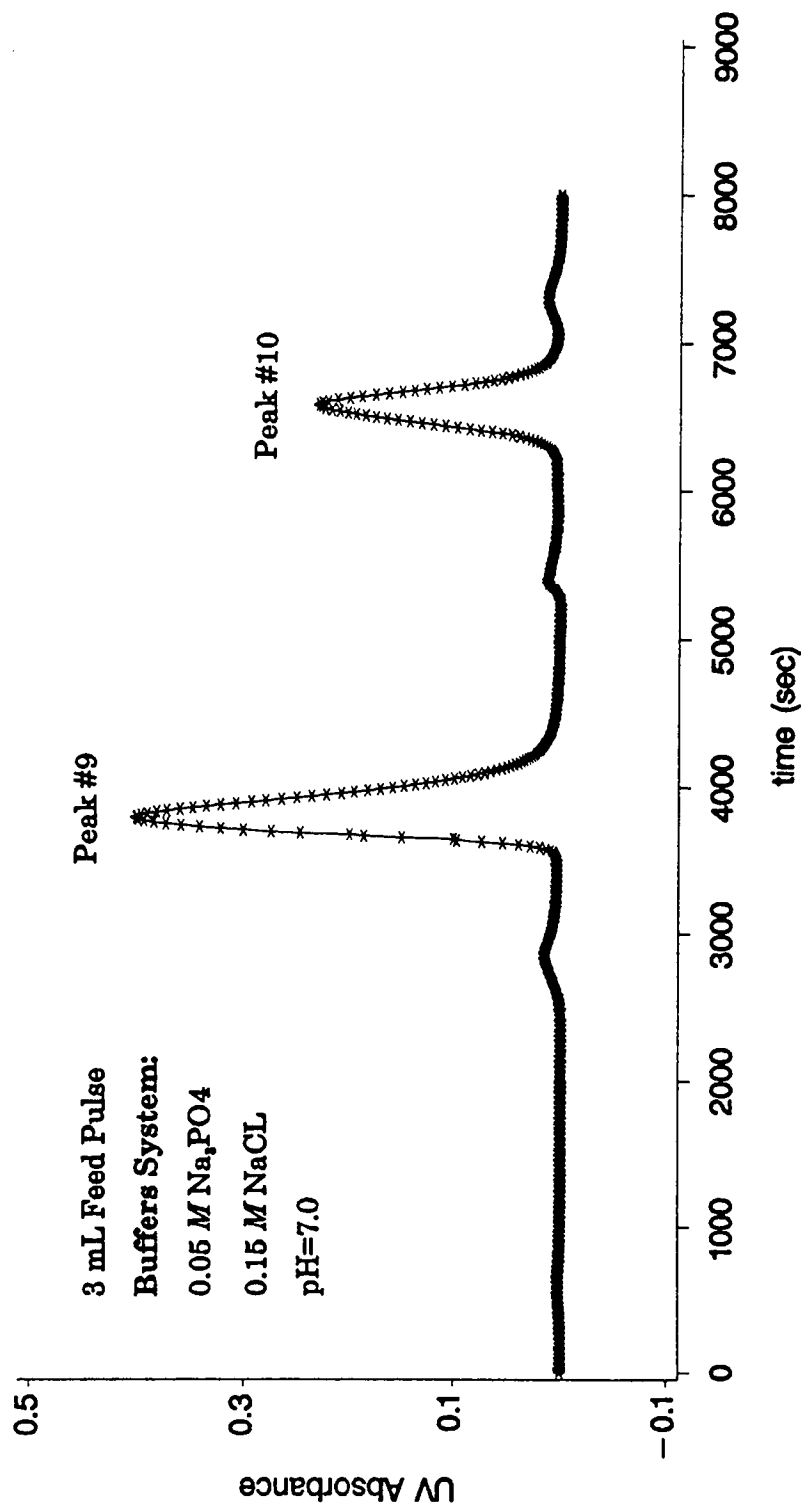


Figure 7.3 Preparative gel filtration chromatogram for a 3 mL feed pulse of beta lact.

exchange run 33. This anion exchange run was for two column volumes of whey feed. After anion exchange run 33 was conducted to obtain its respective fractions for gel filtration processing, the fractions were very gently swirled to ensure mixing of their components yet not agitated enough to cause foaming and consequently protein denaturation. As stated 3 mL of each anion exchange fraction were then applied to the gel filtration column in three separate experiments, one experimental run for each fraction.

As can be seen in Figures 7.1 - 7.3, there is excellent baseline resolution between the resultant peaks for each anion exchange fraction processed. The chromatograms were found to be reproducible as with the anion exchange ones. Before sending collected fractions of these peaks to the commercial laboratories for analysis, they were analyzed by the analytical system shown in Figure 4.2 to roughly determine their composition and the effectiveness of the gel filtration separation.

The results of the commercial laboratories analysis of the respective gel filtration peaks are shown in Tables 7.1 - 7.3. From these results it is clearly seen that the gel filtration process is most successful at separating the beta lactoglobulin from the other proteins and lactose but not at separating the bovine serum albumin, immunoglobulin G, or alpha lactalbumin from one another. Also, it is seen that there is tremendous dilution of each protein from its respective anion exchange fraction. And finally, the total solids concentration of each gel filtration peak is significant when compared to the concentration of the proteins and lactose in the different peaks.

In light of these analytical results, and keeping in mind that the gel filtration process has inherently slow flowrates, it was deemed that the potential for gel filtration as a secondary processing step was not realistic. As will be shown in chapter 9,

Table 7.1
Composition of peaks from gel filtration processing
of 3 mL sample of anion exchange breakthrough curve
from experimental run 33

	$\alpha - LA$	$\beta - LG$	BSA	IgG	LAC	tot. solids ^(a)
fraction	peak 1	peak 1	peak 1	peak 1	peak 1	peak 1
conc. mg/mL	0.0	0.0	0.039	0.064	0.0	16.22
%dilution	—	—	1,487.2	812.5	—	—
fraction	peak 2	peak 2	peak 2	peak 2	peak 2	peak 2
conc. mg/mL	0.0	0.98	0.0	0.0	0.0	17.07
%dilution	—	408.2	—	—	—	—
fraction	peak 3	peak 3	peak 3	peak 3	peak 3	peak 3
conc. mg/mL	0.4	0.038	0.0	0.0	0.0	16.38
%dilution	455.7	10,530	—	—	—	—
fraction	peak 4	peak 4	peak 4	peak 4	peak 4	peak 4
conc. mg/mL	0.0	0.0	0.0	0.0	0.35	28.51
%dilution	—	—	—	—	22,340	—

^(a) in g solute/L peak

Table 7.2
Composition of peaks from gel filtration processing
of 3 mL sample of anion exchange peak 1
from experimental run 33

	$\alpha - LA$	$\beta - LG$	<i>BSA</i>	IgG	LAC	tot. solids ^(a)
fraction	peak 5	peak 5	peak 5	peak 5	peak 5	peak 5
conc. mg/mL	0.0	0.45	0.0	0.0	0.0	15.91
%dilution	—	444.4	—	—	—	—
fraction	peak 6	peak 6	peak 6	peak 6	peak 6	peak 6
conc. mg/mL	0.018	0.0018	0.0	0.0	0.0	15.40
%dilution	277.8	111,100	—	—	—	—
fraction	peak 7	peak 7	peak 7	peak 7	peak 7	peak 7
conc. mg/mL	0.0	0.0	0.0	0.0	0.0	14.1
%dilution	—	—	—	—	—	—
fraction	peak 8	peak 8	peak 8	peak 8	peak 8	peak 8
conc. mg/mL	0.0	0.0	0.0	0.0	0.0	15.15
%dilution	—	—	—	—	—	—

^(a) in g solute/L peak

Table 7.3
Composition of peaks from gel filtration processing
of 3 mL sample of anion exchange peak 3
from experimental run 33

	$\alpha - LA$	$\beta - LG$	BSA	IgG	LAC	tot. solids ^(a)
fraction	peak 9	peak 9	peak 9	peak 9	peak 9	peak 9
conc. mg/mL	0.0	1.1	0.0	0.0	0.0	16.22
%dilution	—	481.8	—	—	—	—

	peak 10	peak 10	peak 10	peak 10	peak 10	peak 10
fraction	peak 10	peak 10	peak 10	peak 10	peak 10	peak 10
conc. mg/mL	0.0	0.0	0.0	0.0	0.0	14.32
%dilution	—	—	—	—	—	—

^(a) in g solute/mL peak

the scale of operation required for economic profitability is large and would therefore require many gel filtration columns to handle the anion exchange feed loads. This fact, and especially the preparative gel filtration results showing incomplete solutes separation, led to the decision to not investigate gel filtration any further for possible use in processing of the anion exchange fractions. In the next chapter, cation exchange chromatography will be discussed as a potential processing step for further separation of the anion exchange breakthrough fraction.

CHAPTER VIII
PREPARATIVE CATION EXCHANGE CHROMATOGRAPHY
FURTHER SEPARATION OF ANION EXCHANGE
BREAKTHROUGH CURVE PROTEINS

8.1 Introduction

In chapter 6 the use of anion exchange chromatography was successful in partially separating the four major whey proteins and lactose from the whey mixture. The processing strategy chosen was to select feed loading conditions such that the highly valuable immunoglobulin G protein would pass through the column in the feed loading stage and end up in the breakthrough curve. The other proteins of interest would bind to the resin and then be eluted by step gradients.

In order to recover the valuable immunoglobulin G from the anion exchange breakthrough curve, and therefore proceed towards developing an economically attractive whey separation process, the use of cation exchange chromatography was

investigated. As discussed in chapter 4, Pharmacia Biotech Inc.'s S Sepharose Big Beads resin was chosen for this work. This step is opposite to the anion exchange step in that now the processing conditions are such that the immunoglobulin G is bound to the cation exchange resin during feed loading while hopefully the other components in the anion exchange breakthrough curve pass out of the column. The results of these experiments are discussed in the next chapter.

8.2 Experimental Approach

As with the anion exchange experiments, the inorganic salt buffer chosen was sodium acetate, per recommendations from Pharmacia Biotech Inc. (1994). However, in these experiments the buffer salt concentration was kept constant while the pH of the buffer solution was changed in stepwise fashion. A single change in pH was made from the initial loading pH of 5.5 to the final state of 8.5. In this manner the entire range of isoelectric points for immunoglobulin G, 5.5 - 8.3. The cation exchange processing cycle was as follows: (1) Clean column with one column volume of 1 M sodium hydroxide. (2) Pass one column volume of deionized water through the column to wash out residual sodium hydroxide. (3) Pass 2.5 column volumes of 0.1 M acetic acid through the column to lower the column liquid and eluant pH. (4) Equilibrate the column with two column volumes of 0.05 M sodium acetate, pH=5.5. (5) Load one column volume of the breakthrough curve fraction from anion exchange run 37 onto the column. Prior to the start of the run the breakthrough curve fraction was adjusted in pH from 5.8 to 5.5 with 0.3 mL of 17.5 M commercial acetic acid, food grade. This slight pH adjustment was necessary in order to load the feed onto the column at the lower level of the IgG isoelectric point range. (6) Pass one column volume of 0.05 M sodium acetate, pH=5.5 through the

column to wash out any material that did not bind in the feed loading step. (7) Pass two column volumes of 0.05 M sodium acetate, pH=8.5 through the column to elute the IgG bound to the resin. (8) Begin cleaning cycle. As discussed in chapter 4, the column was the same as the one used for the anion exchange experiments, 5.0 cm in diameter by 25 cm in packed length. Also, the cation exchange resin was limited to an operating pressure of 42 psi. A diagram of this processing cycle is shown in Figure 8.1 while in Table 8.1 these seven steps are shown in terms of the time requirements for each. At a flowrate of 50 mL/min. it is calculated that the entire cation exchange process cycle requires 103 minutes and 5 seconds.

For cleaning and regeneration of the cation exchange resin, Pharmacia Biotech Inc. recommends passing 1 molar sodium chloride through the column to knock off strongly, but reversibly bound material. For severe contamination, such as precipitated proteins onto the column, one column volume of 0.5 - 2.0 molar sodium hydroxide is recommended. For progressively built up contamination, after repeated separation cycles, a cleaning-in-place (CIP) procedure of 2 molar sodium chloride, 1 molar sodium hydroxide, and a 70% ethanol solution are recommended. Finally, for sanitization of the resin to reduce microbial contamination, passing 1 molar sodium hydroxide through the bed is recommended.

For these research experiments, solutions of 1 molar sodium hydroxide were passed through the column, in one column volume amounts (491 ml), to clean the bed. Since no quantitative methods were developed in this project to determine the frequency of column cleaning, the cleaning cycle was applied after each experimental run. Through discussions with Pharmacia Biotech Inc., it was learned that one column volume of 1 molar sodium hydroxide solution, followed by several column volumes of dilute acetic acid solution were appropriate to clean the column and

Preparative Cation Exchange Column Process Operating Cycle

Flowrate = 50 mL/min.

1 Column Volume = 491 mL

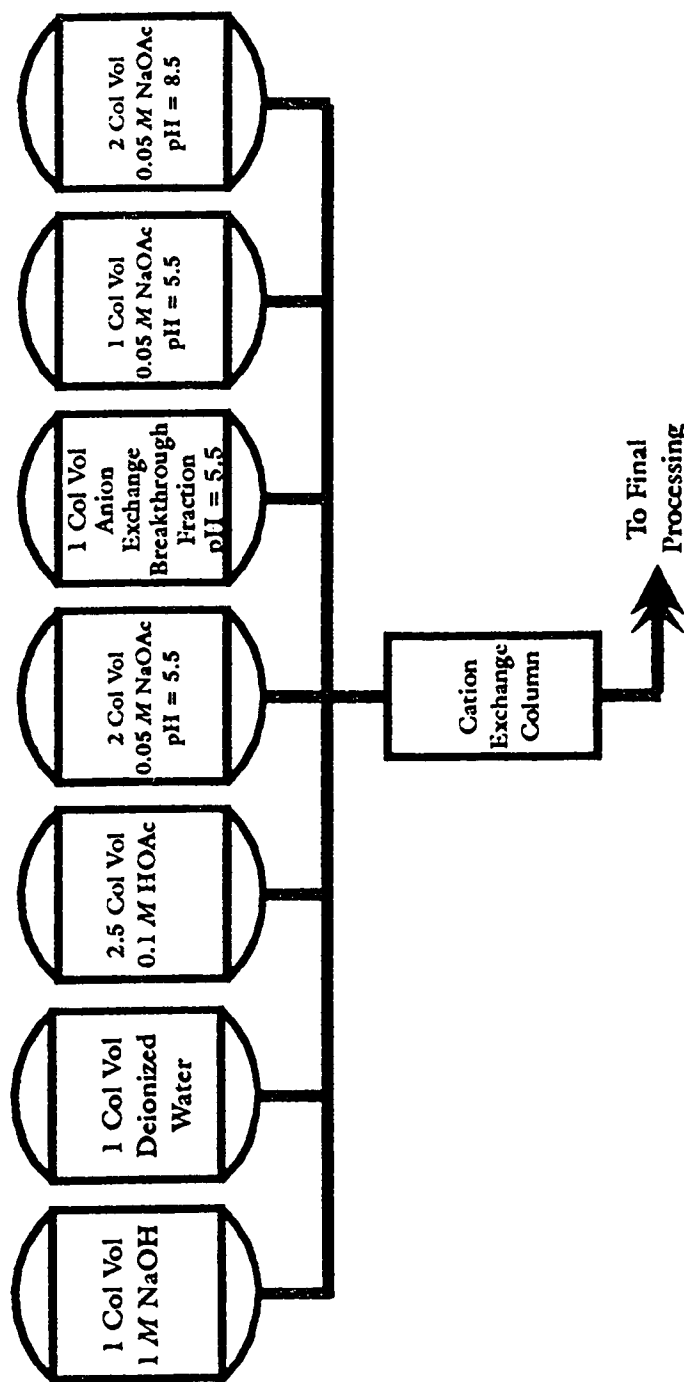


Figure 8.1 Preparative cation exchange column process operating cycle.

Table 8.1

Time requirements for each step in the cation exchange process

Experimental Run 46

Process Step	No. of Col. Vols.	Time (min.)	Cumul. Time (sec.)
whey feed, pH=5.5	1	9.8	589
0.05 M NaOAc, pH=5.5	1	9.8	1178
0.05 M NaOAc, pH=8.5	2	19.6	2356
1 M NaOH	1	9.8	2945
D.I. H ₂ O	1	9.8	3534
0.1 M HOAc	2.5	24.5	5007
0.05 M NaOAc, pH=5.5	2	19.6	6185

lower the eluant pH. As with the anion exchange experiments, 0.1 M acetic acid was used in these experiments to assist in lowering the eluant pH followed by several column volumes of the equilibrating buffer to prepare the bed for the next cycle.

The use of the cation exchange resin bed for further separation of the anion exchange breakthrough curve is discussed in the following section.

8.3 Experimental Results

Two experimental runs were conducted using the cation exchange resin column and anion exchange breakthrough curve fractions from repeated runs of experimental run 37 as this run was determined to be optimal in terms of column loading and resultant resolution. As with the anion exchange work, the flowrate was adjusted to 50 mL/min. and the in-line UV monitoring wavelength at 280 nm.

In Figures 8.2 and 8.3 two representative chromatograms are shown with pertinent details for cation exchange processing of the breakthrough curve from preparative anion exchange run 33. This anion exchange run was for two column volumes of whey feed.

As can be seen by comparing Figures 8.2 and 8.3, there is a larger peak following the cation breakthrough curve for one column volume loading than for a half column volume loading. This peak, 11 should contain the IgG of interest according to the proposed elution strategy. After the run with one column volume loading was conducted, the resultant breakthrough curve and peak 11 were collected and sent to the commercial laboratories for analysis. The results are shown in Table 8.2. Only the cation exchange breakthrough curve and peaks 11 and 12 were analyzed. It was found that none of the proteins of interest were in peak 12 while the lactose not found in the breakthrough curve and peak 11 ended up in peak 12. The peaks from run

Preparative Cation Exchange Chromatogram

1.0 Column Volume Of Breakthrough Curve From Anion Exchange – 50 mL/min
Pharmacia XK 50/30 Column: 5.0 cm x 30 cm

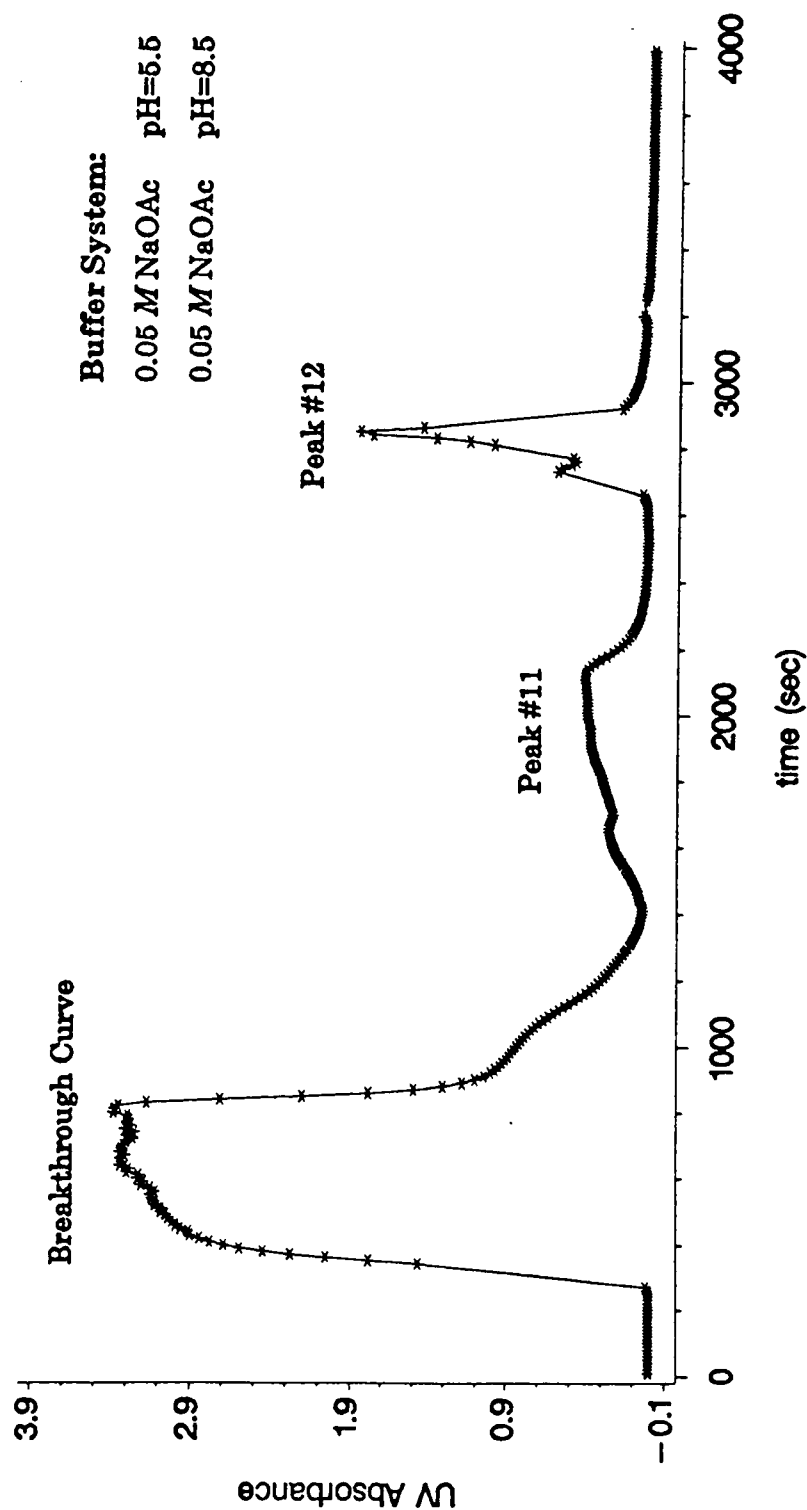


Figure 8.2 Preparative cation exchange chromatogram for one column volume loading.

Preparative Cation Exchange Chromatogram

0.5 Column Volume Of Breakthrough Curve From Anion Exchange – 50 mL/min

Pharmacia XK 50/30 Column: 5.0 cm x 30 cm

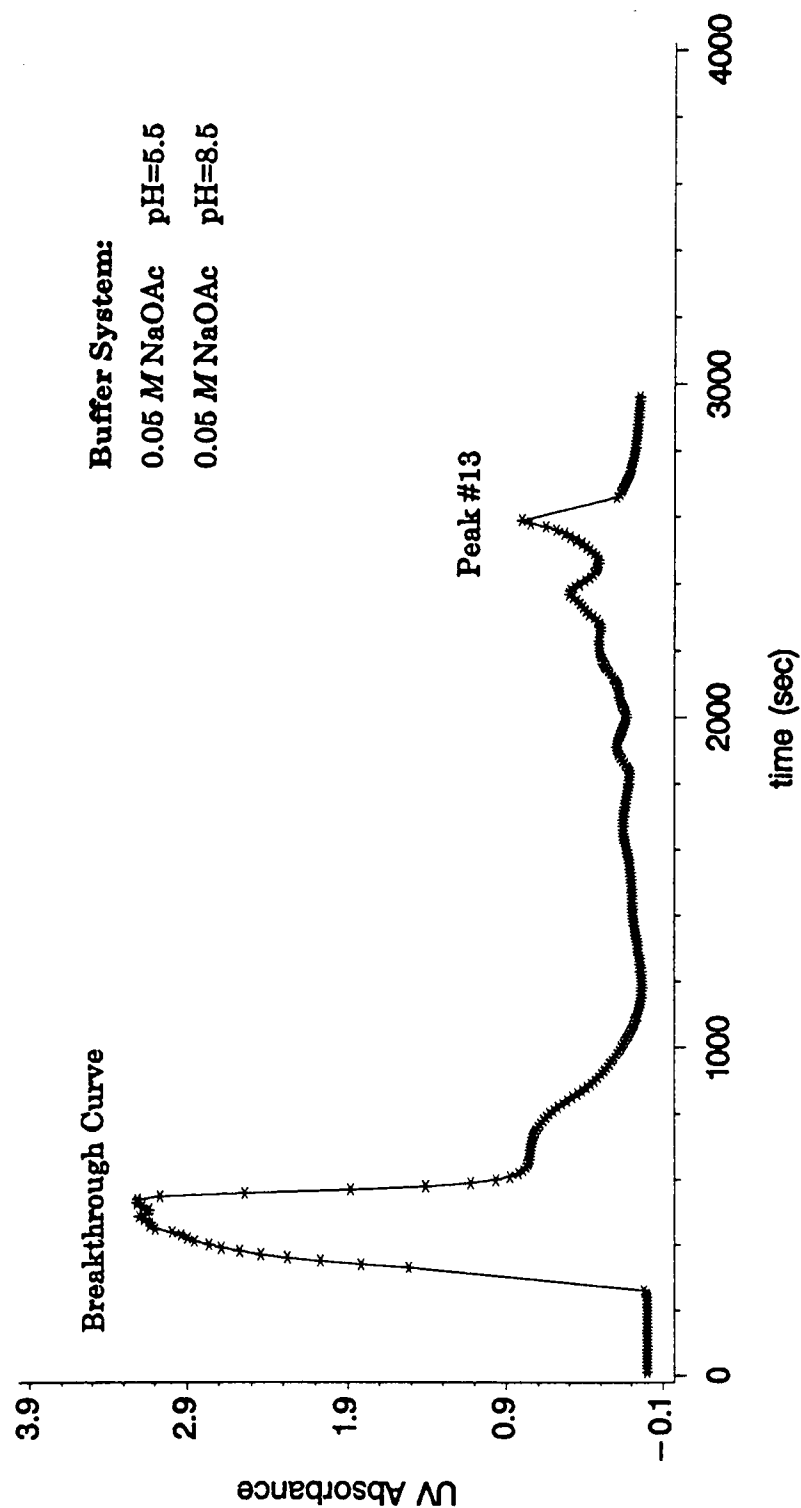


Figure 8.3 Preparative cation exchange chromatogram for one half column volume loading.

Table 8.2

Composition of peaks for one column volume loading
of anion exchange breakthrough curve onto cation exchange column
from experimental run 37

	$\alpha - LA$	$\beta - LG$	<i>BSA</i>	IgG	LAC	density ^(a)
fraction	feed	feed	feed	feed	feed	feed
%wt. fraction	1.12	0.16	0.29	2.33	13.2	1.06
%recovery	—	—	—	—	—	—
g loaded	0.89	0.13	0.23	1.85	11.62	

fraction	brkthrh.	brkthrh.	brkthrh.	brkthrh.	brkthrh.	brkthrh.
%wt. fraction	1.32	0.17	0.25	1.48	0.039	1.05
%recovery	61.8	53.08	44.8	33.5	14.3	—
grams	0.55	0.069	0.1	0.62		—

fraction	peak 11	peak 11	peak 11	peak 11	peak 11	peak 11
%wt. fraction	0.0	0.11	0.0	7.37	0.0	1.05
% recovery	—	46.9	—	16.8	—	—
grams in frac.	—	0.0044	—	0.31	—	—

^(a) Density in g/ml.

47 which processed one half of a column volume of anion breakthrough curve were not analyzed. From these results it is clearly seen that the cation exchange process is fairly successful at separating the immunoglobulin G from the other components in the anion exchange breakthrough curve. Unfortunately only about 17% of the IgG in the anion exchange breakthrough curve is recovered in peak 11 of the cation exchange process at a low weight fraction of 7.4%. These initial experiments with cation exchange reveal a potential for its use in further recovering the IgG. However, future experiments should address adjusting the processing strategy to bind more of the IgG in the feed loading step and the possible use of a combined salt and pH step change as in the anion exchange experiments.

For an initial look at how the IgG in the feed to the cation exchange process binds, an additional experiment was conducted in which the anion breakthrough curve fraction was augmented with peak number 11 from a previous experiment. The purpose of this experiment was to study the effect of spiking the anion breakthrough fraction with peak number 11 so as to hopefully bind more of the IgG in the feed to the cation exchange process to the resin and then elute it in peak number 11. That is, fresh whey was obtained and processed via the anion exchange process under one column volume loading. Two experimental runs were conducted to obtain sufficient anion breakthrough curve. Then one column volume of the breakthrough curve was processed via the cation exchange process to yield peak number 11 containing the IgG. This peak was then cut out and added to one column volume of the anion breakthrough curve fraction. Finally, the augmented mixture was adjusted to a pH of 5.5 with dilute acetic acid and then processed via the cation exchange process. The total volume of the augmented mixture was 1,108 mL, and was processed at 50 mL/min.

The resultant chromatogram is shown in Figure 8.4. The processing conditions for obtaining the chromatograms in Figures 8.2 and 8.4 were identical with the exception that the point in time at which the pH step gradient was implemented was different due to the fact that for the augmented case the amount of feed processed was 1,108 mL while for Figure 8.2 it was 491 mL. This translates to a point in time of 1,919 seconds at which the pH step gradient was implemented for the augmented case while for Figure 8.2 it was 1,178 seconds. A comparison plot of the two chromatograms is shown in Figure 8.5. From this plot it can be seen that the early portion of each peak number 11 is similar with a small shoulder. However, for the augmented case there is a significant peak as compared to the non-augmented case. It therefore seems reasonable that for the augmented case there is a greater amount of IgG in its peak due to the recycling of peak number 11 into the cation process feed and subsequently utilizing more of the resin bed capacity. This of course could be verified by analyzing the peak for IgG content. Future experiments should repeat this augmented feed study and have peak number 11 analyzed for the IgG content to measure the effectiveness of spiking the cation exchange feed. The plot also shows the effect of the time delay in implementing the pH step change for the augmented case.

For the scale up and economic optimization issues discussed in the next chapter, the cation exchange step was included in the analyses. As will be shown the cation exchange step at this point of development is costly and the possible improvements mentioned would positively affect the overall economic situation.

Preparative Cation Exchange Chromatogram

Pilot Run #51 – Anion Breakthrough Curve Augmented With IgG – 50 mL/min

Pharmacia XK 50/30 Column: 5cm x 30cm

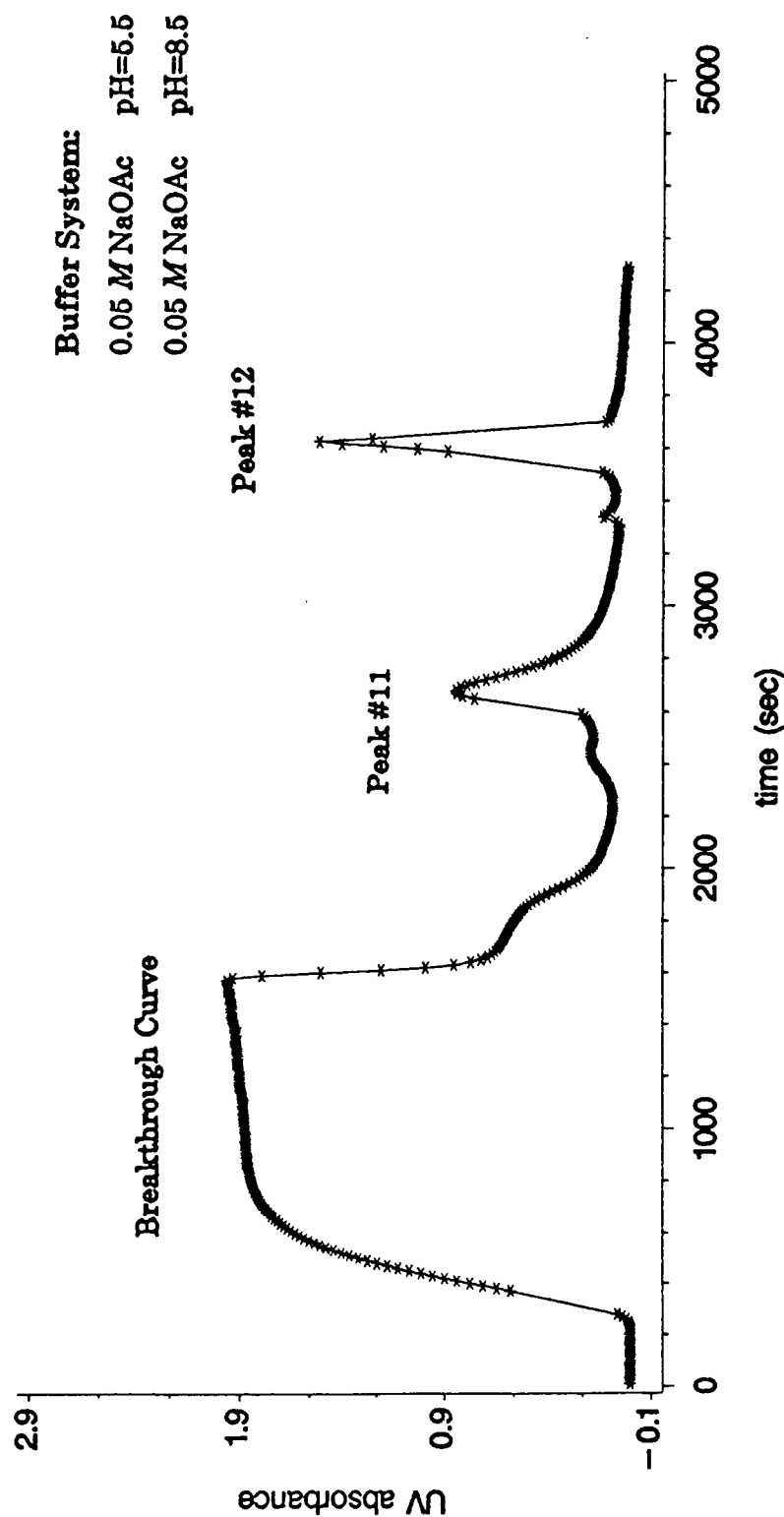


Figure 8.4 Preparative cation exchange chromatogram augmented with IgG.

Preparative Cation Exchange Chromatogram Comparison Between Pilot Run # 46 and IgG Augmented Pilot Run # 51 Flowrate = 50 mL/min.

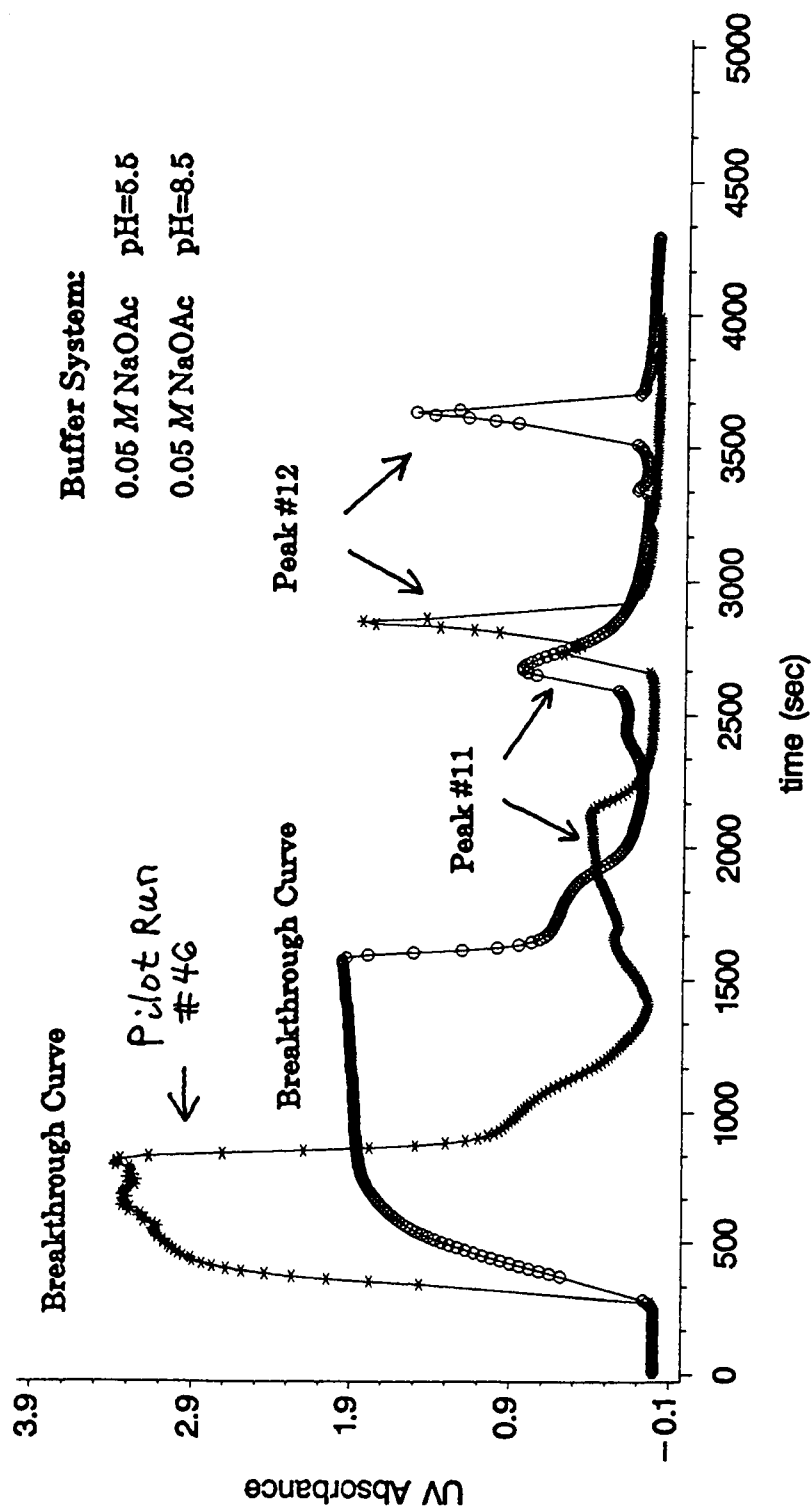


Figure 8.5 Comparison between pilot runs 46 and 51.

CHAPTER IX

SCALEUP AND ECONOMIC OPTIMIZATION OF WHEY ION EXCHANGE PROCESSES

9.1 Introduction

A major objective of this research project was to scale-up the optimized anion and cation exchange processes to an economically favorable industrial scale of operation. This is the logical extension to a chemical engineering research and development study. The goal of the project was to separate the proteins and lactose of interest from the dairy whey and one another and prepare them for final processing and sale.

To carry out a successful scale up and economic optimization, two key groups of information are required: (1) Which mass transfer resistance is overall controlling the separation processes. As discussed in chapter three, this will guide the scale up calculations. (2) Detailed economic information is needed for the process equipment

to be used in the scaled-up operation, the cost of raw materials, the cost for building a new grass roots plant, the various costs for operating the plant, and the anticipated selling prices for the whey proteins fractions obtained. Once these data are obtained the scaled-up equipment dimensions can be calculated for different whey processing rates, and an economic optimization performed. Details on these two issues are discussed in the following sections.

9.2 Determination of controlling mass transfer resistance

In reviewing the literature about mass transfer studies on preparative protein chromatography, it was learned that the usual controlling resistance for mass transfer in the protein chromatographic process is intraparticle diffusion. If this was the controlling resistance in the preparative ion-exchange processes, then the scale up methodology of Wankat (1992) could be utilized, as discussed in equations 3.89 and 3.90, and repeated here:

$$R(u_F) = 1 \quad (9.1)$$

Here R is the ratio operator for scaled-up state to original scale for a variable, and u_F is the superficial velocity. Also, the ratio of column diameters can be related to the ratio of flowrates by:

$$R_D = R_N^{1/2} \quad (9.2)$$

where D is the column diameter, and N is the flowrate to the column in units of choice. Usually, when separations are optimized at the preparative scale, the column length is kept constant when scaling to a larger scale. Thus if intraparticle diffusion does control the separation processes, then for scale up one needs to keep

constant the column superficial velocity.

To determine whether the intraparticle diffusion was the controlling mass transfer resistance, two experiments were conducted, one numerical and the other experimental. First for the numerical simulation, the model of Carta (1988) was utilized, as described in equations 3.38 to 3.51 in chapter three. For the optimum anion exchange chromatogram of pilot run 37 which processed one column volume of whey feed, the Carta model was fitted to the most separated peak in the process, peak 2 which contained the beta lactoglobulin. It was proposed that if the evolution of this peak could be successfully simulated and allow determination of the controlling resistance, then the results would also be valid at the next scale of operation.

To simulate the anion exchange beta lactoglobulin peak, the Carta model was written in a GW-Basic computer program by Carta (1988) and kindly supplied to the author. This model incorporates both film and intraparticle diffusion for linear equilibrium cases. The two mass transfer resistances are characterized by individual mass transfer unit variables whose numerical values, for the given experimental conditions and when compared, will reveal which resistance is controlling. That is, if the transfer unit variable for intraparticle diffusion is smaller than the one for film diffusion, then intraparticle diffusion is the controlling resistance.

Of all the parameters required for this simulation, only one was used as a fitting parameter, the linear equilibrium coefficient K . The bed void fraction, ϵ_b , was specified as 0.40 as discussed earlier in chapter five. A table of the parameters values used in the Carta model simulation are shown in Table 9.1 along with the calculated mass transfer units for film and intraparticle diffusion. A comparison graph of the simulated anion exchange beta lactoglobulin peak and anion exchange pilot run 37 are shown in Figure 9.1 for the case where the beta lactoglobulin peak

Table 9.1
Parameters used in Carta (1988) linear equilibrium model
simulation of anion exchange pilot run 37

Equilibrium Distribution Coefficient	60.0
Superficial Velocity	0.0425 cm/s
Column Length	25 cm
Particle Radius	0.0045 cm
Mass Transfer Coefficient	0.00086 cm/s
Particle Diffusivity ^a	0.0000218 cm ² /min. ^b
Bed Void Fraction	0.40
Particle Void Fraction	0.89
Number Of External Film Mass Transfer Units ^c	202
Number Of Particle Diffusion Transfer Units ^d	19.0

^a for beta lactoglobulin

^b calculated from equation 3.89

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

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

Preparative Anion Exchange Chromatogram
Comparison Between Pilot Run # 37 and Linear Equil. Model

Flowrate = 50 mL/min.

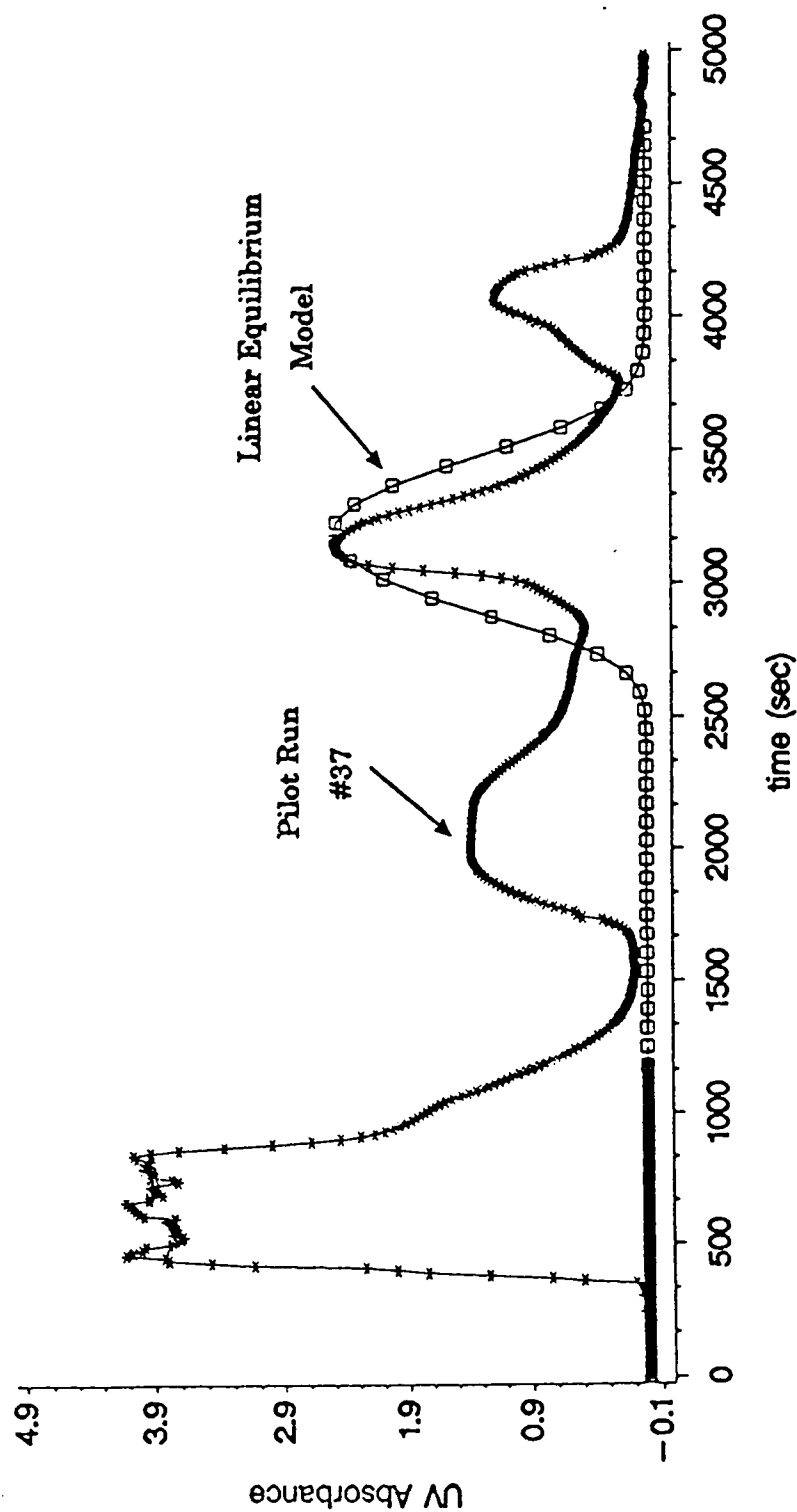


Figure 9.1 Comparison between experiment and model for intraparticle diffusion control.

is simulated considering that its band in the column migrates immediately after being loaded onto the column. That is, its simulation is from time equals zero. The other possibility for simulating the beta lactoglobulin peak is to consider that its band does not migrate through the column after being loaded until the second anion process step change is implemented and its wave desorbs the stationary band. For this case the simulation does not include the time for the first anion process step change but only the time for the feed, washout, and second step change. This situation is shown in Figure 9.2. As can be seen, there is a fair match between the simulated peaks and the experimental one for both situations. Also, it is seen that although the simulated peak is narrower for the case where the band is assumed to not migrate from time equals zero until the step change wave passes through it, there really is not much difference between the two cases. It can be concluded that the linear equilibrium model and its simulation is not completely satisfactory for simulating the anion exchange beta lactoglobulin peak due to its multicomponent makeup and likely non-linear adsorption behavior. Comparing the number of mass transfer units for film and intraparticle diffusion in Table 9.1 it is seen that the number of mass transfer units available for intraparticle diffusion is extremely less than those available for film mass transfer by a factor of 10.6. This observation does support the proposal that intraparticle diffusion is the controlling mass transfer resistance.

To further support this proposal, an experiment was conducted in which a single feed pulse of beta lactoglobulin was applied to the anion exchange column operating under the optimized cycle conditions as discussed in chapter six. As discussed in that chapter, the beta lactoglobulin peak, 2 in the anion exchange process, had a peak concentration of 7.9 g/L. For a feed volume of 491 mL, this amounts to 3.88

Preparative Anion Exchange Chromatogram Comparison Between Pilot Run # 37 and Linear Equil. Model Flowrate = 50 mL/min.

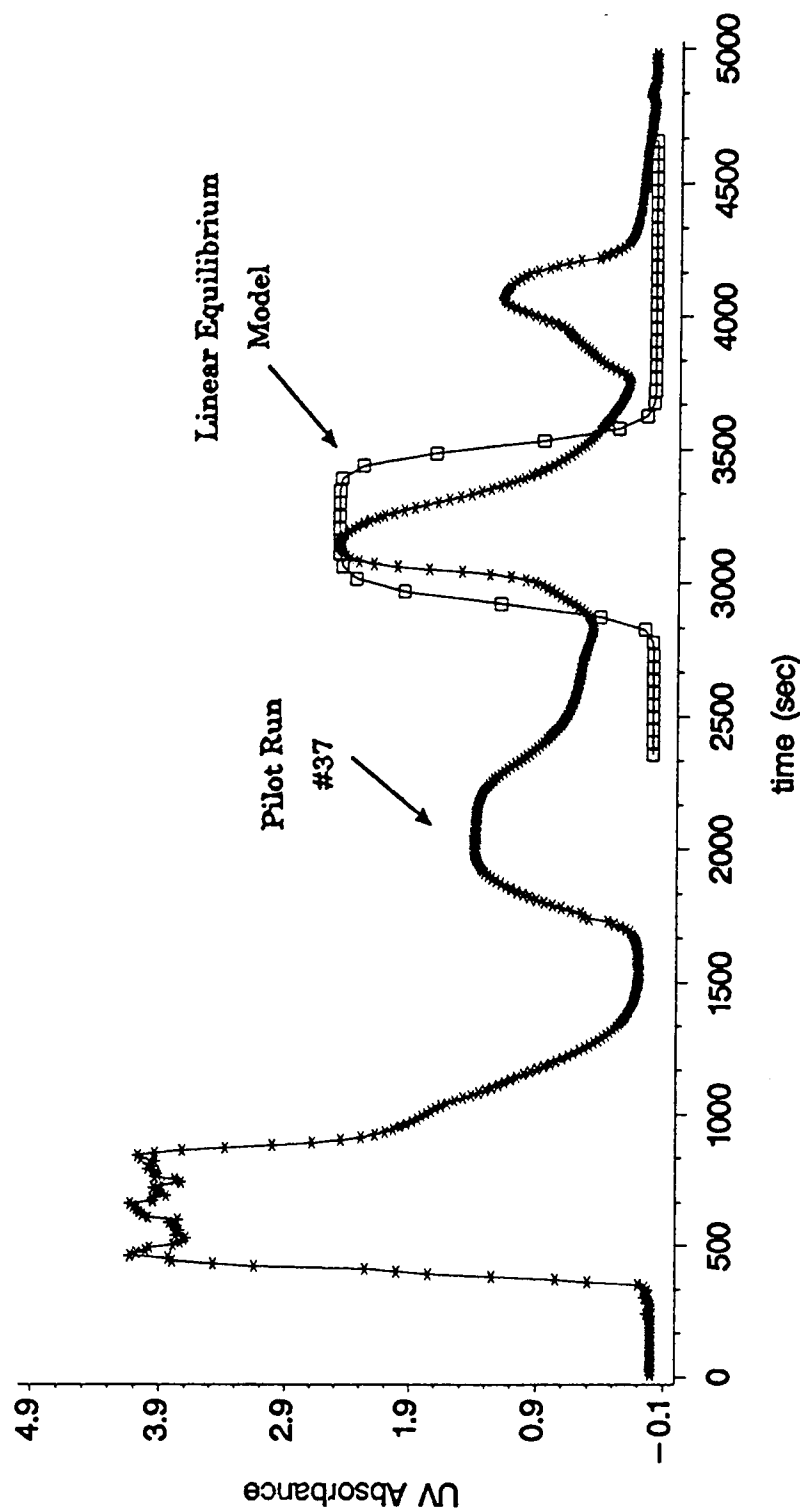


Figure 9.2 Comparison between experiment and model for Intraparticle diffusion control.

grams of beta lactoglobulin fed to the column. For the experiment conducted, a solution of 491 mL of 7.9 g/L beta lactoglobulin was fed to the column in place of one column volume of whey feed. The reason for carrying out this experiment was to investigate whether the beta lactoglobulin would elute from the column in the same time neighborhood as it did in anion exchange pilot run 37 which processed actual whey. If it did elute in the same time neighborhood and was Gaussian in shape, then it could be interpreted that the equilibrium relationship for the beta lactoglobulin binding to the anion exchange resin is linear as calculated in the Carta simulation. This would then provide support for the creditability of the Carta simulation results and thus for the proposal of intraparticle diffusion as the controlling mass transfer resistance.

As can be seen in Figure 9.3, which compares anion pilot run 37, the Carta model simulation for the case where the simulation is for the assumed initial non-migrating band and the single beta lactoglobulin peak experiment, the beta lactoglobulin peak does elute approximately in the same time neighborhood as that in pilot run 37 and that calculated by the Carta model simulation. Unfortunately the beta lactoglobulin peak is not at all Gaussian but rather displays significant tailing due to the moderately high concentration in the feed pulse, the low value of the mass transfer coefficient calculated, and the apparent Langmuir isotherm behavior exhibited. For the actual whey separation, it seems that, as the beta lactoglobulin band is contaminated with other solids not of interest to a degree of 50 wt.%, its adsorption characteristics are altered so as to elute with a Gaussian shape, indicating that the peak as a whole follows a linear equilibrium relationship. The presence of the contaminant solids dilutes the beta lactoglobulin peak and thereby acts to change its adsorption behavior from non-linear and Langmuirian to linear

Preparative Anion Exchange Chromatogram Comparison Between Pilot Run # 37, Linear Equil. Model And Single Beta Lactoglobulin Feed Pulse

Flowrate = 50 mL/min.

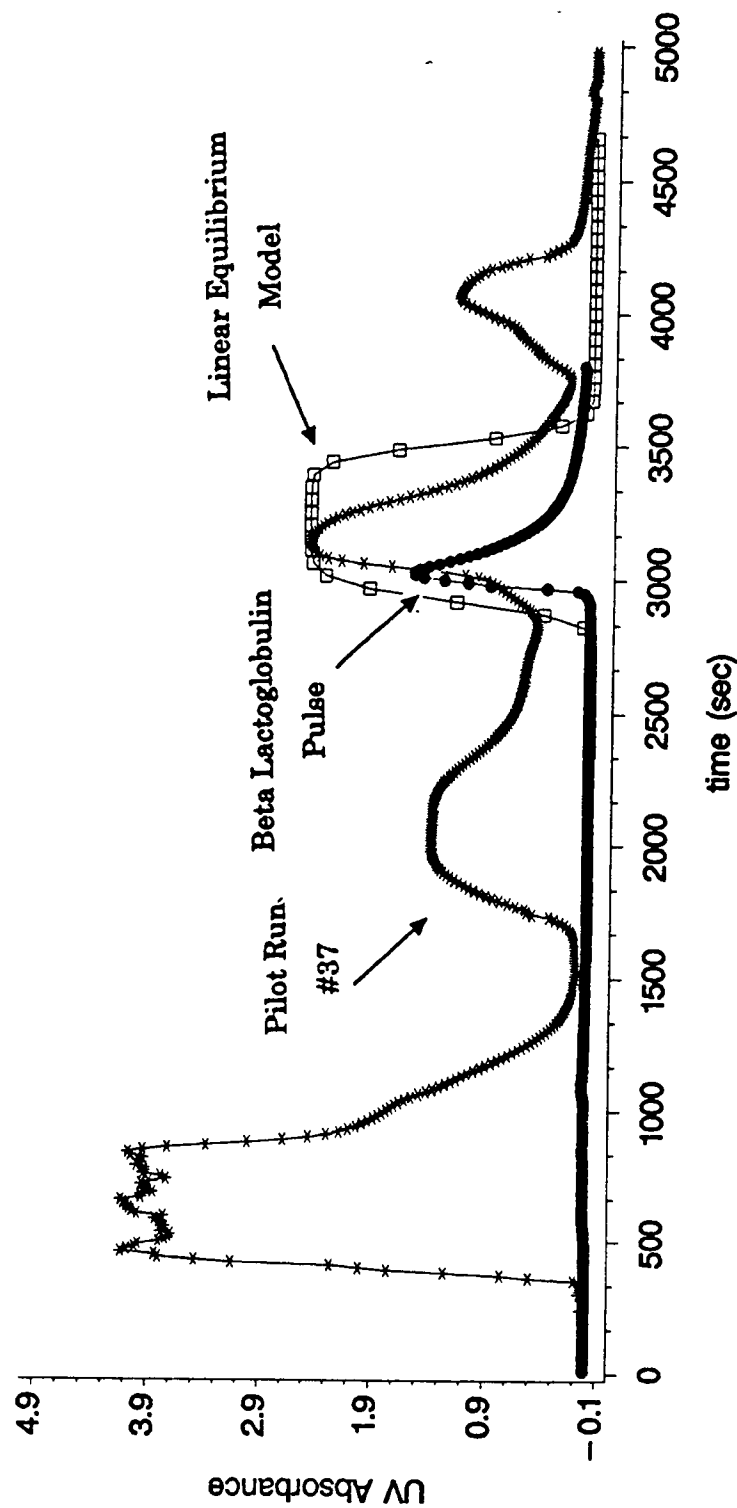


Figure 9.3 Comparison between experiment and model for intraparticle diffusion control.

and Gaussian. Whether the contaminant solids offer competition for adsorption with the beta lactoglobulin is not clear since the resultant peak shape is Gaussian. On the other hand, for the pure beta lactoglobulin peak, there are no other contaminant solids present in the eluting peak to speak of and the capacity of the column is more fully utilized for adsorption of the solute. In this case there is much more solute available for adsorption thereby leading to the non-linear isotherm behavior and subsequently peak tailing. In light of the results for the actual whey separation, the simulations, the pulse experiment, and particularly the comparison between the number of film and intraparticle diffusion mass transfer units, it was concluded that indeed the controlling mass transfer resistance was intraparticle diffusion for the anion exchange process. Such was also assumed for the cation exchange process. In this case the peak of interest is 11 shown in Figure 8.3, containing immunoglobulin G. Here, the peak shape is definitely non-Gaussian and the peak contains a high amount of contaminant solids. Due to a lack of additional information about the isotherm nature of immunoglobulin G in the presence of a large amount of contaminants, intraparticle diffusion control was assumed for this process also. Future work should attempt to verify this.

With intraparticle diffusion control as the controlling mass transfer resistance, scale up can now be conducted, keeping the column's bulk fluid phase superficial velocity constant from one scale to the next. The results of scale-up incorporating this underlying principle and the subsequent economic optimization are presented in the next section.

9.3 Economic optimization

To carry out a realistic economic optimization of a scaled-up process, a detailed

economic model of the proposed plant is required. For this project, the calculation outline presented by Peters and Timmerhaus (1980) is used. Here, the elements necessary for an economic optimization are broken down into calculation of the total capital investment cost and the total product cost, each consisting of many different cost elements. The cost elements for estimation of the total capital investment are shown in Table 9.2 and the cost elements for the estimation of the total product cost are shown in Table 9.3. The percentages listed for the different elements are for conducting economic analyses for chemical plant designs in general and not specific to any one type of process industry. However, for this project the calculational outline was deemed detailed enough to warrant its use.

In order to measure the possible success of the economic optimization, several key economic variables were utilized. These variables, as discussed in Peters and Timmerhaus (1980), are the following: annual cash flow or ACF; average annual profit, or AAP; average annual rate of return, or AAROR; revenue, or REV; total product cost, or TPC; fixed-capital investment, or FCI; total capital investment, or TCI; depreciation of Greeneville, Tennessee plant, or D; and payout period, pp, with no interest charge accounted for. To account for the interest in the payout period, the interest on the total project capital investment during the service life would have to be known, and is not for this work. The service life of the scaled-up plant for this project was defined as ten years with a 25% salvage value of the plant and its equipment. These economic variables are defined below:

$$ACF = REV - TPC \quad (9.1)$$

$$AAP = ACF - (FCI - 0.25(TCI))/(10) \quad (9.2)$$

Table 9.2

Total capital investment cost components estimates

Peters and Timmerhaus (1980)

Direct Costs	% of FCI ^a	% of TPEC ^b
purchased eqmt.	15-40	-
Installation	-	25-55
Instrumentation	-	6-30
Piping	-	10-80
Electrical	-	10-40
Buildings	-	10-70
Land	1-2	-
Indirect Costs	% of FCI ^a	% of Direct Costs
Engineering	-	5-30
Construction	-	6-30
Contingency	5-15	-

Fixed-capital Investment = Direct costs + Indirect costs

Working capital = 20% of total capital investment

Total capital investment = fixed-capital + working capital

^a FCI = fixed-capital investment

^b TPEC = total purchased-equipment cost

Table 9.3
Total product cost components estimates
Peters and Timmerhaus (1980)

Direct costs	% of TPC ^a	% of FCI ^b	% of TCI ^c
raw materials	10-50	—	—
operating labor	10-20	—	—
supervision	10-25 of labor	—	—
utilities	10-20	—	—
maintenance	—	2-10	—
supplies	—	0.5-1.0	—
lab charges	10-20 of labor	—	—
patents	0-6	—	—
Fixed charges	% of TPC ^a	% FCI	% of TCI ^c
depreciation	—	10	—
taxes	—	1-4	—
insurance	—	0.4-1.0	—
plant overhead	5-15	—	—
General Expenses	% of TPC ^a	% FCI	% of TCI ^c
administration	2-6	—	—
distribution	2-20	—	—
research	5	—	—
financing	—	—	0-10

Total product cost = Direct costs + fixed charges + general expenses

^a TPC = total product cost

^b FCI = fixed-capital investment

^c TCI = total capital investment

$$AAROR = (AAP)/(TCI)100 \quad (9.3)$$

$$pp = (TCI)/(AAP + D) \quad (9.4)$$

To provide as much realistic economic data to the optimization, discussions were held with the plant manager of the Dairyman Inc. plant in Greeneville, Tennessee. On behalf of his plant economic operating data was provided to this project. This data eliminated many of the estimates in the total capital and total product cost calculations. Specifically, only the following items were estimated as listed in Tables 9.2 and 9.3: For total capital investment, the installation, piping, and electrical costs, and the engineering, construction, and contingency costs. For the total product cost, the patents cost, the administrative, distribution, and research costs. For each of these cost components the largest percentage listed in the respective tables was used. Each cost component provided by the plant was from operating year 1993 and no contingency factor was multiplied to these data. Also, although the existing whey plant is moderate in size with respect to the dairy industry, the new plant may require additional amounts of each cost component that cannot be accounted for accurately at this time.

The data provided by the plant are shown in Table 9.4. Other data needed for the calculation of the total capital investment and total product cost estimates, such as purchased equipment and raw materials, was obtained through direct vendor quotes for the different types of equipment and materials needed. Specifically, in Table 9.5 are listed delivered costs for different sizes of stainless steel chromatography columns manufactured by Pharmacia Biotech Inc. In Table 9.6 are listed quotes for delivered Pfaudler Glasteel process storage tanks of different sizes. In

Table 9.4
Dairyman Inc. plant economic data
Greeneville, Tennessee - 1993

economic component	cost \$
no. of dairies in cooperative	3500
cost of building	75/sq.ft.
wage rate across plant	10.50/hr.
of employees	29 - no union
pounds of whey produced per year	127,000,000
total operating expenses including utilities	703,000
price for whey sold	0.60/lb.
maintenance costs	115,000 of labor
insurance costs	26,000
supplies costs	260,000
plant overhead costs	included in total operating expenses
depreciation costs	225,000
no rent charge	
total taxes	19,600
total cost for 2.5 acres of land adjacent to existing plant	62,500

Table 9.5

Delivered costs for Pharmacia Biotech Inc.
BioProcess Stainless Steel Columns - single column basis

Column Size - diameter x length (cm)	delivered cost \$ ^a
40 x 30	75,600
60 x 30	92,100
80 x 30	108,600
100 x 30	124,950
120 x 30	142,200
140 x 30	158,100

^a 10 % discount for six or more ordered, 15 % discount for 15 or more. Costs are for 60 cm length columns.

Table 9.6

Delivered costs for Pfaudler Glasteel process tanks
single column basis

Tank size - diameter x length (cm)	delivered cost \$ ^a
300 gallons	117,864
500 gallons	121,480
1,000 gallon	136,444
1,500 gallons	147,640
2,000 gallons	154,863

^a cost includes stainless steel sampler probe, temperature sensor, and pH probe.

Table 9.7 are listed quotes for the chemical components needed, delivered. In Table 9.8 are listed the delivered cost quotes for the anion and cation exchange resins, with a 10% additional resin quantity charge figured in for topping off the scaled-up columns. On this note, one aspect of this project that was not addressed is the life-time of the resins and how often the scaled-up columns would have to be re-packed. This was not addressed due to a lack of information. However, it is felt that there is sufficient contingency built into the working capital estimate and the initial resin cost estimate so that in the future when the scaled-up columns would have to be changed out, there would be enough capital available to do so.

In Table 9.9 are listed miscellaneous quotes for other necessary one-time purchased equipment such as ultrafiltration membranes, spray dryers, water purification equipment and water. For the water purification system, four pharmaceutical grade water supply systems were specified after discussions with U.S. Filter Corporation of Rockford, Illinois. These systems can supply 7 gpm each, with four units being sufficient for the projected water requirements of the scaled-up plant. Pharmaceutical grade and water for injection (WFI) quality were specified as the scaled-up plant would produce food stuff raw materials and also the immunoglobulin G which is a component in human injectable vaccines. Additional data on these equipment are described in appendix A. Shipping costs were figured into the equipment prices through quotes with the Roadway Trucking company of Morristown, Tennessee and calculated for final destination of Greeneville, Tennessee and its ZIP code. The origination ZIP code was dependent on where the equipment is manufactured and was also considered into the shipping charges.

It must be pointed out that for this research project, the sizing of the final processing equipment was not carried out due to the fact that pilot scale experiments

Table 9.7

Delivered costs for process chemicals
acetic acid, sodium acetate, and sodium hydroxide

chemical	delivered cost \$
glacial acetic acid	12909/7,200 lbs. ^a
sodium acetate	128,567/4,400 lbs. ^b
sodium hydroxide	18,473/10,010 lbs. ^c

^a shipped in 450 lb. drums from J.T. Baker company.

^b shipped in unknown container size from Acros chemical, supplier to Fisher Sci.

^c shipped in 110 lb. drums from J.T. Baker company.

Table 9.8

Delivered costs for Pharmacia Biotech Inc.

Q and S Sepharose Big Beads Resins

235.6 liter basis - equivalent to a 100 x 30 cm column

resin	delivered cost \$ ^a
Q Sepharose Big Beads	170,829/235.6 liters
S Sepharose Big Beads	170,929/235.6 liters

^a up to 10 % discount for volumes ordered over 1,000 liters. Cost includes 10% extra resin amount.

Table 9.9

Delivered cost estimates for ultrafiltration membranes
 spray dryers, pumps, process control systems, and water supply systems

equipment	delivered cost \$
ultrafiltration membranes	12,000,000 ^a
spray dryers	11,250,000 ^b
pumps	500,000 ^c
water supply systems	1,600,000 ^d
process control systems	500,354 ^e

^a four membrane units required - manufactured by Osmonics, Inc., Minnetonka, Minnesota.

^b three spray dryers required - manufactured by Niro Hudson Inc., Fond du Lac, Wisconsin.

^c pumps sizing and specification to be conducted at design stage.

^d four water supply systems required - manufactured by U.S. Filter, Rockford, Illinois

^e two process control systems required - manufactured by Pharmacia Biotech Inc.

using such equipment were not conducted. The reason for this was that the scope of this project was preparative chromatography and not inclusive of these other unit operations. Without exit stream concentration data for the proteins of interest from the spray dryers and ultrafiltration membranes, accurate sizing of these processes and hence costing cannot be accomplished. The cost estimates for the spray dryers were obtained from discussions with NIRO Hudson Inc. of Fond du Lac, Wisconsin, a leading manufacturer of fluidized bed dryers for the food and dairy products industry. The cost estimate for the ultrafiltration membrane modules is generous and admittedly, a guess, allowing three million dollars each for four units total. Phone discussions with the manufacturer, Osmonics Inc. of Minnetonka, Minnesota, failed to yield even an estimated cost because concentration / performance data at the lab / pilot scale are not available. Future experiments with their preparative ultrafiltration membranes processing the various peak fractions discussed earlier, should address this issue so that a more accurate cost estimate for the membranes could be obtained.

Finally, to conduct the economic optimization, the anticipated selling prices of the anion and cation exchange peak fractions is required, along with the degree of how much can be sold in the marketplace. For this project, it was assumed that all of each chromatographic fraction, after final processing, could be sold, regardless of the amount produced. As will be shown shortly, only a fraction of the Greeneville, Tennessee annual whey output was considered for ion-exchange processing.

To incorporate accurate selling prices for the protein fractions into the optimization, early in the project discussions were held with New Zealand Milk Products Inc., based in the Chicago area for their North American operations. They are one of the leading dairy products and whey processing companies in the world. Un-

fortunately, they were not willing to reveal their whey products pricing policies and other important economic data that would have been quite valuable to this project. Also, discussions were held with DAVISCO International, Inc., of LeSuer, Minnesota. This is the leading company in the United States for producing a whey protein isolate product called BiPRO through a proprietary ion-exchange process. These discussions were a little more fruitful, whereby it was learned that their product sells for \$6.50 per pound. However, the annual amount they produce was not disclosed.

With this paucity of selling price information, the only option available was to conduct the optimization using extremely discounted values of the prices the proteins command when sold by the Sigma Chemical Company, St. Louis, Missouri for the beta lactoglobulin rich fraction and the immunoglobulin G fraction. For the anion exchange breakthrough curve, anion exchange peak 1, and the cation exchange breakthrough curve, discounted values of the DAVISCO International Inc. whey protein isolate price were utilized. The range of discounted selling prices used for the economic optimization are shown in Table 9.10. It is felt that this range of selling prices is very reasonable considering that high value products are obtained after the final processing stage of the operation. In the next section the combination of selling prices to obtain a specified rate of return and in a reasonable payout period will be shown.

9.4 Economic optimization Results

The economic optimization consisted of six cases studies in which different combinations of the chromatographic peak fractions were considered marketable. Also, the effect of imposing a \$0.60 per pound of whey raw material cost was built

Table 9.10
Discounted selling prices for anion and cation exchange
peak fractions

fraction	range of selling prices \$/lb. fraction
anion breakthrough	1.625, 0.65, 0.325 ^a
anion peak 1	1.625, 0.65, 0.325, 0.065, 0.0325 ^b
anion peak 2	152.1, 76.05, 38.02, 15.21, 1.52, 0.76 ^c
cation breakthrough	0.65, 0.325, 0.065, 0.0325 ^d
cation peak 1	2,778.5, 1,389.2, 277.8, 138.9, 27.8, 13.9, 2.8, 1.4 ^e

^a corresponds to 25, 10, and 5 % discounted prices of DAVISCO International Inc. BiPRO product

^b correspond to 25, 10, 5, 1, and 0.5 % discounted prices of DAVISCO International Inc. BiPRO product

^c corresponds to 1, 0.5, 0.1, 0.05, 0.01, and 0.005 % discounted prices of pure beta lactoglobulin selling price/lb. from Sigma Chemical Company

^d corresponds to 10, 5, 1, and 0.5 % discounted prices of DAVISCO International Inc. BiPRO product

^e corresponds to 1, 0.5, 0.1, 0.05, 0.01, 0.005, 0.001, and 0.0005 % discounted prices of pure immunoglobulin G selling price/lb. from Sigma Chemical Company

into the model and the option of not including this charge was also considered. This is the price that the plant currently obtains for selling the whey to a pet food manufacturer in Ohio.

For each case study, 1 to 25 % of the plant's annual whey output in increments of 2.5 % was considered as a feedstock to the ion exchange processes. This ranges from 1,270,000 to 31,750,000 pounds per year of whey processed with the exception of case study two which required a whey utilization rate of 38 % or 48,260,000 pounds per year to achieve favorable economic indicators. Each case study considered an 85% operating rate. All columns were sized to maintain the same length as at the preparative scale, 30 cm. However, the cost for the columns was considered for a column double in length to allow for backwashing during the cleaning cycle. This has been shown to be advantageous for the removal of suspended matter that can accumulate in a column over a period of time. Expansion of the resin bed will take place during the backwashing step by up to 100%, therefore necessitating the doubling of the column length (DeChow, 1989). Therefore, the cost quotes for the 30 cm columns were multiplied by a factor of 1.5 to accommodate this doubling of length.

The six case studies are defined as: (1) for a given whey utilization rate, all whey is processed by the anion exchange process and all of the anion exchange breakthrough curve generated is processed by cation exchange. No raw material cost for whey is considered. (2) same as case one except raw material cost for whey is included. (3) for a given whey utilization rate, all whey is processed by anion exchange and there is no cation exchange processing of the anion breakthrough curve. No raw material cost for the whey is considered. (4) same as case three except a raw material cost for the whey is included. (5) for a given whey utilization rate, all

whey is processed by anion exchange and 15 % of the anion exchange breakthrough curve is processed by cation exchange. The remaining anion breakthrough fraction is sold after final processing. There is no raw material cost for the whey considered. (6) same as case five except a raw material cost for the whey is included. The optimization was conducted using a Lotus 1-2-3 spreadsheet model.

In Table 9.11 are shown the best selling prices for the five different chromatographic fractions obtained via the anion and cation exchange processes for a particular case study. These selling prices were determined as best based on the criteria that a minimum of a 20% average annual rate of return or AAROR be obtained at a payout period under five years, or half of the defined service life of the plant. The fractions are defined as: ANBKSP is the selling price of the anion exchange breakthrough curve. AP1SP is the selling price of peak 1 of the anion exchange process. AP2SP is the selling price of peak 2 of the anion exchange process. CABKSP is the selling price of the cation exchange breakthrough curve. CAP1SP is the selling price of peak 11 of the cation exchange process.

The results of the economic optimization for the six case studies are shown in Table 9.12 in terms of the annual revenue, AAROR, payout period, and whey utilization rate required to obtain the values of these key economic variables. In Table 9.13 are shown the values of the other important economic variables, such as total capital investment (TCI), total product cost (TPC), the total product cost per pound of whey processed, the total purchased equipment cost (TPEC), the annual cash flow to the project (ACF), and the average annual profit to the scaled-up plant (AAP) for the six case studies. And in Table 9.14 are shown the consumption data and costs for the raw chemical materials used to prepare the buffers used in both ion exchange processes. These include the acetic acid, the sodium acetate, the sodium

Table 9.11

Best peak fractions selling prices for six different case studies

prices in \$ per pound of fraction

case	ANBKSP ^a	AP1SP ^b	AP2SP ^c	CABKSP ^d	CAP1SP ^e
1	–	0.0325	7.60	0.0325	138.92
2	–	0.0325	7.60	0.0325	138.92
3	0.65	0.65	38.023	–	–
4	0.65	0.65	45.63	–	–
5	0.65	0.65	7.60	0.65	1,389.24
6	0.65	0.65	7.60	0.65	1,389.24

^a corresponds to selling price of anion exchange breakthrough curve^b correspond to selling price of peak 1 of anion exchange process^c corresponds to selling price of peak 2 of anion exchange process^d corresponds to selling price of cation exchange breakthrough curve^e corresponds to selling price of peak 1 of cation exchange process

Table 9.12

Values of key economic variables for six different case studies

case	whey rate ^a	REV ^b	AAROR ^c	payout period ^d
1	31,750,000	115.4	20.57	4.84
2	31,750,000	175.4	21.42	4.65
3	28,575,000	125.8	21.26	4.69
4	28,575,000	149.8	20.09	4.96
5	25,400,000	132.2	23.11	4.31
6	31,750,000	165.2	21.76	4.58

^a whey utilization rate is in pounds per year^b REV is the annual revenue to the plant in millions of dollars per year^c average annual rate of return^d payout period in years

Table 9.13

Values of other economic variables for six different case studies

case	TCI ^a	TCI/dairy ^b	TPC ^c	TPC/lb. ^d	TPEC ^e	AAP ^f	ACF ^g
1	247.2	70,618	50.7	1.6	37.2	50.83	64.4
2	265.1	75,741	1.04	2.2	40.0	56.8	71.4
3	270.8	77,385	53.0	1.9	40.9	57.6	72.5
4	270.8	77,385	80.2	2.8	40.9	54.4	69.3
5	274.7	78,473	53.3	2.1	41.4	63.5	78.6
6	286.0	81,710	87.0	2.7	43.2	62.2	78.0

^a total capital investment in millions of dollars^b total capital investment per 3500 dairies in Dairyman Inc. cooperative^c total product cost in millions of dollars^d total product cost per pound of whey processed^e total purchased equipment cost in millions of dollars^f average annual profit in millions of dollars^g annual cash flow in millions of dollars

Table 9.14

Raw materials consumption, costs, and number of columns required
for six different case studies

case	H ₂ O ^a	NaOAc ^b	HOAc ^c	NaOH ^d	Raw ^e	anion ^f	cation ^g
1	15.1	0.090	0.051	0.14	5.1	10	2
2	22.9	0.14	0.077	0.21	36.8	15	2
3	13.5	0.075	0.045	0.12	4.2	9	2
4	13.5	0.075	0.045	0.12	21.4	9	2
5	12.1	0.072	0.041	0.11	4.1	8	1
6	15.1	0.089	0.051	0.14	24.1	10	2

^a water consumption in millions of gallons annually

^b sodium acetate consumption in millions of pounds annually

^c acetic acid consumption in millions of gallons annually

^d sodium hydroxide consumption in millions of pounds annually

^e total raw materials cost in millions of dollars annually

^f total number of anion exchange columns required - 140 cm dia. x 30 cm length

^g total number of cation exchange columns required - 140 cm dia. x 30 cm length

hydroxide, and the water requirements. The acetic acid and water are expressed in gallons per year while the other components are in pounds per year. This table also shows the total number of anion and cation exchange columns required for a given case study.

Finally, a significant consideration for deciding whether or not to build a scaled-up whey proteins processing plant is the tremendous amount of waste that is generated annually in terms of peak 3 of the anion exchange process and peak 2 from the cation process. These peaks, in addition to other waste streams that would result from periodic cleaning of process tanks and piping, would represent a considerable impact on the local sewage treatment plant, the cost of which was not known for this economic study. These waste disposal loads are shown in Table 9.15 in terms of pounds and gallons per year to be disposed of. If capital, in addition to that specified for the Greeneville plant's utilities, was required for the scaled-up plant to accommodate sewage disposal costs, it could be obtained from the working capital fund of the project.

Upon analysis of the values of the economic variables for the six case studies, it is seen that they are considerably influenced by the selling price of cation peak 11, CAP1SP. This is the peak fraction that contains the most valuable immunoglobulin G thereby strongly dictating the profitability of a particular case study. As with each of the marketable protein fractions, should this process ever be constructed at one of the six case studies, a thorough marketing study needs to be conducted to determine the demand and pricing relationships for each fraction. This would then give a better picture of to what extent the immunoglobulin G fraction controls the plant's economics.

For the six case studies, it is felt that case study four is the most realistic for

Table 9.15
Waste disposal streams from ion exchange processes
for six different case studies

case	anion peak 3 ^a	cation peak 2 ^b	total disp. lbs. ^c	total disp. gals. ^d
1	18.8	3.1	21.9	2.4
2	28.5	4.7	33.2	3.6
3	16.9	—	16.9	1.8
4	16.9	—	16.9	1.8
5	15.6	0.37	15.9	1.7
6	19.4	0.46	19.9	2.2

^a anion peak 3 waste disposal load in millions of pounds annually

^b cation peak 2 waste disposal load in millions of pounds annually

^c total waste disposal load in millions of pounds annually

^d total waste disposal load in millions of gallons annually

processing of the whey by anion exchange and no cation exchange processing of its breakthrough curve. The reason for this is the uncertainty about the selling price of the IgG fractions and the fact that they control the overall economics to such an extreme. Also, at this stage of the process development, the cation exchange recovery of the IgG from the anion breakthrough curve fraction is not all that favorable. Future studies should address the issue of how to increase the IgG recovery via cation exchange and thereby favorably impact the economics.

Case study four incorporates a realistic \$0.60 per pound of whey raw material cost, yields a 20.09 % average annual rate of return and a payout period of 4.96 years. This case study processes 22.5 % of the Greeneville plant's annual whey output or 28,575,000 pounds of whey. The total capital investment, at \$270,846,816 is great, but if divided among the 3500 dairies in the Dairyman Inc. cooperative, amounts to \$77,385 dollars per dairy with the same rate of return and payout period. In this case the average annual profit to each dairy is \$15,543 which is also not all that bad considering the rather small initial investment. The selling prices of the anion exchange breakthrough fraction, peak 1 and peak 2 are respectively, \$0.65, \$0.65, and \$45.63 per pound of each fraction. Here, the economics are controlled by the price of peak 2, although this selling price seems to be reasonable also. The other two fractions, containing each protein of interest to a different degree, sell for 10 % of the selling price of the DAVISCO International Inc. BiPRO whey protein isolate product. All three fraction's selling prices are also before final processing by ultrafiltration and spray drying which would upgrade their concentrations and purities.

Obviously the profitability of case study four is dictated by the selling price of peak number 2 which contains the fairly well separated beta lactoglobulin. The

effect of its selling price on the payout period for this case study is shown in Figure 9.4. For this figure, the beta lactoglobulin selling price ranged from 1 % to 0.2 % of the Sigma Chemical Company selling price. This translates to a selling price range of \$ 152.1 to \$ 30.4 per pound of peak number 2 fraction. For selling prices of less than 0.2 % or \$ 30.4 per pound of fraction, the payout period becomes negative meaning that the plant loses money at all whey processing levels. In conclusion it is seen from the figure that for a payout period of a little over two years, the selling price of the beta fraction should be about \$ 61 per pound of fraction.

For this case study, it is seen from Table 9.14 that 9 anion exchange columns, measuring 140 cm in diameter by 30 cm in length would be required for continuous processing of the whey. Since the cation process only handles the anion breakthrough fraction, only two columns of the same dimensions would be required.

Beta Lactoglobulin Selling Price vs. Payout Period
25 % Whey Utilization Rate
Economic Case Study Four

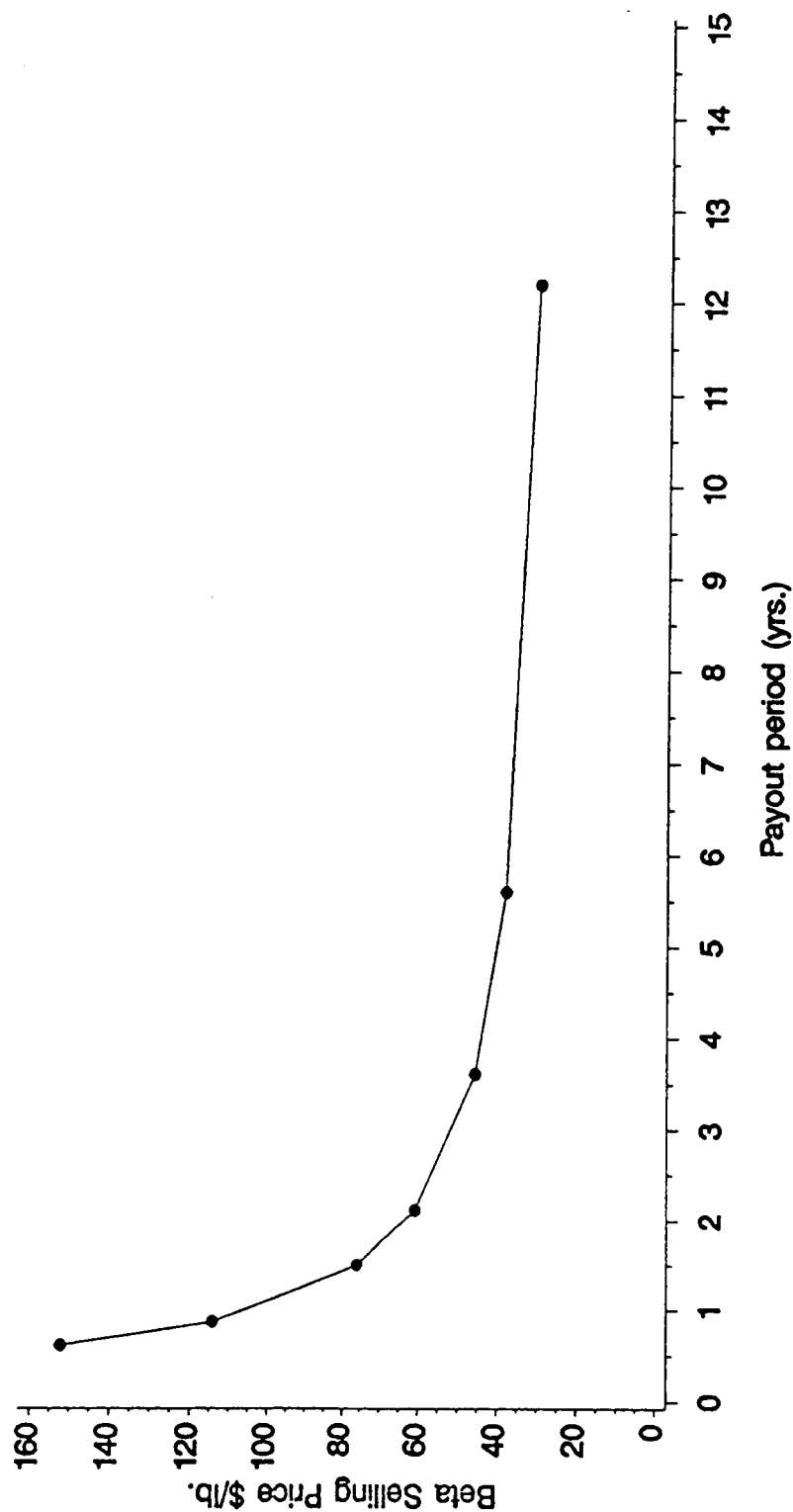


Figure 9.4 Beta Lactoglobulin selling price vs. payout period.

CHAPTER X

CONCLUSIONS AND RECOMMENDATIONS

This research project has investigated the preparative-scale separation of the four major proteins and lactose from sweet dairy whey. The types of chromatography investigated were gel filtration and anion- and cation-exchange. 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 preparative experiments and as a basis for the economic optimization, sweet dairy whey from the Dairyman Inc., Greeneville, Tennessee dairy plant was utilized. From the results of the experimental program and the optimization studies, several conclusions can be made:

1. The direct processing of sweet whey by anion exchange chromatography through 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 beta lactoglobulin from the other proteins into its own peak. This peak though, like the other ones, is contaminated with other solids, thereby necessitating further processing via ultrafiltration and spray drying. In conclusion about the anion exchange step, it can be said that it is most successful in separating the beta lactoglobulin from the whey and the other proteins and lactose of interest.

2. The use of gel filtration for further separating the proteins and lactose of interest resulting from the anion exchange process is partially successful; however, with the tradeoff of substantial dilution of the anion exchange peak's components. Furthermore, the very low flowrates enforced in gel filtration chromatography make it an unrealistic processing option for very large chromatographic separations such as has been proposed in this research project. Therefore, the use of gel filtration at this scale is not recommended for further consideration in the separation of the lactose and proteins.
3. The processing of the anion exchange breakthrough curve fraction via cation exchange chromatography and pH step elution techniques is effective for separating a portion of the immunoglobulin G from this fraction into its own peak. This peak though, like the other ones, is contaminated with other solids, thereby necessitating further processing via ultrafiltration.
4. The protein 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 concentrate or isolate products would be produced depends on the performance of the ultrafiltration

and spray drying unit operations. This issue would also need to be addressed. The exception to this is the immunoglobulin G fraction which cannot tolerate the spray drying step due to heat denaturation.

5. Both ion exchange processes require long cycle times due to the number of steps involved from the feed stage through elution and finally cleaning the column. 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.
6. The economic optimization revealed one favorable processing option which could merit further attention.

With these conclusions drawn about the research work conducted, several recommendations are suggested.

1. As a followup to this work, future experiments should investigate the preparative scale processing of the five different peaks by ultrafiltration and where appropriate, spray drying also. Such experiments would yield the outlet stream composition data from each unit operation and therefore reveal whether a protein concentrate or isolate product could be obtained from those fractions destined to become such. The isolate product would command a higher price than the concentrate. These experiments would also allow these unit operations to be scaled-up properly for each whey utilization rate and subsequently a better cost estimate of each could be built into the optimization spreadsheet.
2. Future experiments should also address the issue of how to increase the amount of immunoglobulin G that transfers from the anion exchange breakthrough curve into its own peak fraction in the cation exchange step. Increasing the IgG concentration in this peak would directly decrease the size and hence cost

of downstream ultrafiltration equipment for its final processing. Adjustments in the cation exchange step elution techniques may accomplish this.

3. A study should be conducted to determine the impact that the scaled-up process would have on a local sewage treatment plant and the costs involved. A tremendous amount of deproteinized whey fractions result from the process and could adversely affect the treatment plant's operation without modification to it or pre-treatment of the fractions. This study could then incorporate the associated costs into the optimization spreadsheet.
4. A study should be conducted to determine if the cleaning portion of each ion exchange is required every cycle or if it can be implemented once every certain number of cycles. Decreasing the number of cleaning steps each operating year will of course decrease the processing cycle times and positively affect the economic picture of the scaled-up plant. This could be determined by performing detailed analytical studies of the protein fractions as a function of cycle time while adjusting the cleaning steps frequency. After a certain number of processing steps, contaminant matter will build up in the column and determining when this occurs is vital. Furthermore, it should be investigated on how to conduct the cleaning cycle in the reverse flow or backwashing mode. The columns were priced to accommodate this.
5. Another study should address the issue of the lifetime possible for each resin. This important piece of information is not known. The lifetime of each resin will also impact the economic situation, with a couple of bed replacements likely during the defined ten year plant life. However, there should be enough capital available in the working capital fund to accommodate these changeouts.
6. Should industrial interest develop in using the results of this research project,

several issues need to be addressed. These include the marketability of the tremendous amount of whey proteins produced and the pricing structure defined in this project. Such an analysis should attempt to learn the supply and demand relationship in the US and in the world for these proteins and at what range of prices can they compete in the marketplace. Also, very detailed analytical chemistry studies should be performed on each of the five ion exchange protein fractions to determine the extent of protein denaturation resulting from the processes. In addition to this, the level of mineral content should be determined as well as the biological value (BV) and protein efficiency ratio (PER). A low mineral content is desirable for each fraction. These two characterization parameters apply to the fractions which would be final processed into a whey protein concentrate or isolate, depending on the performance of the ultrafiltration and spray drying steps. In order for the whey protein concentrate and/or isolate products resulting from this process to compete in the marketplace, these two parameters need to be in line with those resulting from direct ultrafiltration or crossflow microfiltration of whey processes. In the commercial proteins supplements industry, for example, the trend at the time of this writing, is for crossflow microfiltration processing of whey to obtain a high concentration, undenatured protein product. However, it may be that as the demand for whey proteins mixtures grows, as well as the demand for the isolated beta lactoglobulin and immunoglobulin G, the process described in this work may be suitable for large scale production.

In conclusion, a future marketing study would need to confirm the competitiveness of this process for producing these products before any further plans could be proposed for putting it into operation.

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