4.2 Bioseparations of Milk Proteins

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Milk is a complex biological fluid consisting of lipids, phospholipids, carbohydrates, proteins, sugars, salts, and vitamins. Casein proteins exist in the milk serum (non-fat milk) as micellar structures stabilized by colloidal calcium phosphate and protein surfactant. Raw milk also contains microorganisms and somatic cells, which contribute small amounts of proteolytic enzymes and nucleic acids to milk in the course of processing. Since milk is a suitable carrier for a diverse range of biological molecules, its fractionation is of considerable interest. Knowledge of these fractionation methods may further facilitate recovery of proteins of therapeutic value or of enhanced nutritional qualities from milk. The purpose of this chapter is to show separation strategies aimed at recovering selected molecules found in milk serum, and to indicate possible approaches for larger scale fractionations.

Production of milk and milk products is a large industry in the United States, with production of 142.5 billion pounds in 1987. Cultured products (cheese, yogurt, 9.6%), frozen products (8.3%), and dried products (4.2%) account for a large portion of dairy product consumption (by weight, not milk equivalents). The processes leading to these products often produce waste streams of high BOD which need to be treated further and may contain useful chemicals to be recovered. Improvements in fractionation techniques for these streams may help improve the economics of the overall process itself.

1. Introduction

In this article, we emphasize the handling and processing of milk in the context of traditional chemical engineering. The properties of proteins and protein solutions
will be addressed, in terms of their effect on separation interactions. The phase and charge properties are important to consider when developing an appropriate fractionation scheme, as are the kinetics of various reactions or interactions upon which the separation depends. In this context, we will address fractionation of caseins, whey proteins, calcium chloride, and lactose.

2. Casein Protein Separations

The majority of the proteins in milk are caseins. The major casein proteins are $\alpha_s$-casein (12.15 g/100 ml), $\beta_s$-casein (3.4 g/100 ml), $\beta$-casein (9.11 g/100 ml) and $\kappa$-casein (2.14 g/100 ml). Two other caseins, $\gamma$ and $\lambda$, are common by-products of casein processing. Another peptide, protease peptone, or glycylamidropeptide, is a cleavage product of $\kappa$-casein, produced during cheese making. Most of the caseins in milk exist as casein micelles, which are spherical aggregates of the casein proteins, stabilized by colloidal calcium phosphate.

These casein micelles are the important kinetic and structural units in the cheese making process. Cheese making begins with the addition of a bacterial culture to milk which provides part of the characteristic flavor. This step is followed by the addition of a milk-coagulating enzyme (rennet) to milk which attacks the Phe$\rightarrow$Met bond of $\kappa$-casein, destabilizing the micelles and resulting in their coagulation. The coagulated micelles form a gel, which is cut, resulting in curd and whey. The curd is further processed to the final cheese product. A review of milk proteins is given by Swaisgood et al., while a review of casein micelles can be found in Raettigmann and Ladisch.

Separations techniques have been used to help deduce the structure and composition of the micelles as well as to study their behavior under different environmental conditions. Casein micelles found in milk have a broad size range of 20-600 nm and electron microscopy studies have shown that the micelles themselves are composed of smaller units known as submicelles. The micelles disintegrate into the submicelle units when the colloidal calcium phosphate (CCP) is removed by dialysis, indicating the importance of CCP in stabilizing the micelle structure. Additionally, the micelles are not uniform in their casein composition with the smaller micelles having a larger proportion of $\kappa$-casein, indicating that $\kappa$-casein resides mainly on the micelle surface. The hydrophobities of $\alpha_s$, $\beta_s$, and $\kappa$-caseins have been calculated to be 1.2, 1.32, and 1.20 kcal mole$^{-1}$, respectively. The combination of such high hydrophobicities with relatively low molecular weights (23,600, 24,000, and 19,000) allows for strong hydrophobic interactions among the proteins. These hydrophobic interactions favor the association of the caseins, which can complicate separation procedures.

2.1 Precipitation Techniques

The casein proteins can be separated from milk by isoelectric precipitation at pH 4.6 and 20 °C. Acid precipitation of casein is an important process in itself, which leads to manufacture of sodium caseinate and cottage cheese and is usually
the first step in casein and whey fractionation. A second method is to fractionate the casein micelles from milk using ultracentrifugation at 45,000 g for 90 min at 37 °C. The caseins can also be separated into two protein fractions based on their solubility in the presence of calcium. Milk is adjusted to 0.25 M CaCl₂ at pH 7 and 37 °C. The insoluble fraction (calcium-sensitive) contains αs₁-, αs₂- and β-caseins. The soluble fraction (calcium-insensitive) contains α₁-, β-, and α-caseins.

2.2 Chromatography

The casein proteins undergo strong protein-protein interactions, as evidenced by the micelle and submicelle formation and by the self-association of caseins. These protein associations must be disrupted in order to separate the individual caseins. Urea is normally used to dissociate the casein complexes. Differential solubility of casein proteins in urea can be used to fractionate whole casein into β-caseins, γ-caseins, and a mixture of αs₁-, α₁-, and β-caseins. The mixture of αs₁-, α₁-, and β-caseins can then be separated by differential solubility in acid solutions of urea using trichloroacetic acid or sulfuric acid. The caseins have also been separated using differential solubility in ethanol.

The casein proteins have been fractionated using ion exchange chromatography on DAE-cellulose using urea as the dissociating agent with the proteins being eluted using a sodium chloride gradient. Chromatography on hydroxyapatite uses a phosphate buffer to elute the proteins, which desorb in order of their increasing phosphate content (α₁-, β-, αs₁-) for an increasing gradient. Fast protein liquid chromatography (FPLC) using ion exchange columns has been an effective method to separate and quantify the casein proteins. Barroso et al. used FPLC with a Mono-Q (Pharmacia) column. The caseins were dissolved in a 0.01 M imidazole buffer at pH 7.0 containing 3.3 M urea and 0.01 M 2-mercaptoethanol and eluted from the column with a 0.0-0.5 M NaCl gradient. Detection of protein was via absorbance at 280 nm using a uv monitor. These conditions resulted in very good resolution of the caseins, with the proteins eluting in order of increasing phosphate content: α₁-, β-, αs₁-, and αs₂-caseins.

Dalgleish used FPLC over Mono-Q and Mono-S to study the effects of α-caseins with different degrees of glycosylation on rennet kinetics as well as to determine the distribution of glyco-α-casein from different sized micelles. Dalgleish found that the glycosylation of α-casein did not affect its distribution in differently sized micelles and also that enzymatic hydrolysis of α-casein was independent of micelle size. Chromatography has also been used to follow the enzymatic hydrolysis of α-casein during renneting. Van Hooydonk and Ohman used HPLC over TSK 2000 SW columns to separate and quantify the amount of glycomacropeptide (GMP) released during various stages of renneting. GMP is the peptide (MW = 8000) cleaved from α-casein by enzymatic attack. Possible interference from whey proteins was avoided by removing them by adjusting the solutions to 8° trichloroacetic acid. About 40 min were required to complete the separations.

Recently, reverse phase chromatography has been shown to effectively separate the casein proteins. Barreto et al. used a C8 HR 5/10 column and eluted the proteins with a 30-40% acetonitrile gradient. The proteins eluted in order of
their hydrophobicities, except for α-casein (due to the presence of glycosylated residues): β-casein > α-casein > α₂-casein > β₂-casein. Carles 10 also used reverse phase HPLC to separate the caseins with an octadeccylsil stationary phase and a phosphate-buffered aqueous propan-2-ol mobile phase containing sodium dodecyl sulfate. This system not only resolved the four major caseins, but also separated two versions of the genetic variant β-casein A. The previously unidentified version had an amino acid substitution of proline for leucine. This separation shows the ability of RP-HPLC to resolve similar proteins that differ slightly in hydrophobicity.

The previous methods have been developed for separations of small samples. Larger quantities (gram amounts) of the caseins can be prepared using a method described by Wee and Whitney 111. The researchers adsorbed the proteins on DEAE cellulose and separated γ-, κ-, β, α₂, and α₁-caseins by successive extractions with increasing concentrations of sodium chloride. The process was performed batchwise in 3.3 M urea at pH 7.4.

Casein micelles themselves have been fractionated using chromatography on controlled pore glass 12,13,14,15. The fractionation of the micelles has aided in determining micelle size distributions and in determining the composition and structure of micelles of different sizes. Gel chromatography has been used to separate the submicelles which make up the casein micelles. The study by Ozó and Takaçi 16 found that the submicelles could be fractionated by size into four distinct classes, showing that the submicelles are not homogeneous. The two major fractions of submicelles had molecular weights of 500,000 and 200,000 or 100,000 (dependent upon concentration).

2.3 Summary

It is clear that several separation techniques have been used to separate the casein proteins. These techniques have played an important role in determining the structure and composition of casein micelles, the properties of the casein proteins and how they interact with themselves and their environment. Continued development of these techniques may lead to the separation of the individual genetic variants of each of the caseins. The separation of these very similar proteins can be carried out now using gel electrophoresis. Further refinement of column-based separation technology may allow the use of faster and more convenient methods for separating casein components.

Chromatographic and precipitation techniques are useful for separating the very hydrophobic caseins. Most chromatographic techniques rely on the differing amounts of phosphorylation each molecule has. This is because such strong dissociating conditions are required to treat casein molecules individually (3.3 M urea, mercaptoethanol, sodium dodecyl sulfate) that normal hydrophobic adsorption is not useful.

The precipitation technique, in which Ca⁺⁺ ion is used to separate the caseins, gives insight into the role of calcium in stabilizing casein in milk. The results indicate that the more phosphorylated caseins are more sensitive to Ca⁺⁺. This corresponds with the biological importance of colloidal calcium phosphate in micelle stabilization 17.
3. Whey Protein Separations

Whey is the fluid which remains unincorporated in cheese after casein micelles have been coagulated. The major constituents of whey are water, lactose, salts, and globular proteins. Of the proteins, β-lactoglobulin makes up the largest proportion (2.4 g/l skin milk), and α-lactalbumin (1.15 g/l) and bovine serum albumin (0.1-0.4 g/l) make up most of the rest. Small amounts of immunoglobulins (0.6-1 g/l), mostly IgG) and ferritin (<0.1 g/l) also exist in whey. In colostrum (milk taken in the first day after calving) immunoglobulin is approximately 75%, of the total non-casein protein, but this proportion quickly decreases after the first day.

There are several methods for recovering or concentrating proteins in whey. The choice of a technique usually depends on the intended end use of the protein and the amount of sugar in the whey. The proteins may be separated from each other, free of salts and sugars, by chromatography or ultrafiltration. If a whole protein fraction is to be recovered in an active state and in a concentrated solution free of sugar and salt, adsorption (or ion exchange) or filtration may be used. Alternatively, the protein, sugar, and salt may be desired in a dry bulk form (for livestock feed) in which case the water is evaporated, although filtration can also be used to complement evaporation in dewatering processes because of energy efficiency. The strategies are shown in Fig. 1. A review of industrial preparations and applications of whey protein concentrate has been given.

Knowledge of the whey composition gives clues to the composition of the rest of the milk. For instance, β-lactoglobulin (β-lg) has two genetic variants in North American herds, called A and B, which differ by two out of 162 amino acids. However, cows homozygous in one variant or the other have been shown to produce milk with different cheese processing properties, as shown in Table 1.

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**Fig. 1.** Separation strategies for whey constituents. The operation used (color) depends on the product desired (right).
Table 1. The effects of genetic polymorphism of β-lactoglobulin on processing properties of milk. Animals homozygous in variant A are represented by AA, homozygous in B are represented BB.

<table>
<thead>
<tr>
<th>Property</th>
<th>Increased by</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-lg concentration</td>
<td>AA</td>
</tr>
<tr>
<td>Heat processing stability</td>
<td>AA</td>
</tr>
<tr>
<td>Fat concentration</td>
<td>BB</td>
</tr>
<tr>
<td>Casein concentration</td>
<td>BB</td>
</tr>
<tr>
<td>Casein clotting time</td>
<td>BB</td>
</tr>
<tr>
<td>Cord firmness</td>
<td>AA</td>
</tr>
</tbody>
</table>

β-Lactoglobulin also binds and transports retinol (vitamin A), a photo-oxidizable vitamin important in vision and has interesting heat and acid stable properties, which make it attractive in certain food additive applications as an emulsifying or foaming agent.

Many alternatives are available for the separation of whey proteins using chromatography or electrophoresis. Nearly every mode of chromatographic separation has been used successfully to separate the four β-lg A and B, α-lactalbumin (α-la) and bovine serum albumin (BSA)) major proteins of whey, including ion exchange, reverse phase, size exclusion, and chromatofocusing. Native gel electrophoresis is also effective. In comparison, SDS-PAGE and isoelectric focusing have not been as widely reported for this type of separation.

3.1 Electrophoresis

Aschaffenberg and Drewry made the initial discovery of β-lactoglobulin genetic variants on starch gel electrophoresis. Since then, Bell has developed a starch gel technique whereby 4 variants of β-lg, two variants of α-la, BSA, Ig and ferritin are all simultaneously resolved. Current analytical techniques are based on the

![Fig. 2. Native polyacrylamide gel (13%.) electrophoresis of whey proteins. Acrid dye prepared from skim milk by adjusting the pH to 4.6 (for left) is run next to β-lactoglobulin A, β-lactoglobulin B, α-lactalbumin and bovine serum albumin standards (from left to right). The standards correspond to the major bands in acid whey.](image-url)
polyacrylamide technique of Darling and Butech, which gives resolution of A and B variants of β-lg, α-la, BSA and Ig. These polyacrylamide techniques offer better resolution of smaller samples in shorter times than the DHA starch techniques of Bell. A native polyacrylamide gel is shown in Fig. 2. β-Lactoglobulin A migrates the furthest in the polyacrylamide gels, followed closely by β-lg B, whose mobility is about 5% less. α-Lactalbumin has a mobility about half that of the β-lg's. Bovine serum albumin has a very low mobility in the gels, but it is not clear whether this is due to its larger size or its smaller charge, or both. Immunoglobulins are effectively excluded from polyacrylamide gels of this concentration.

3.2 Selective Precipitation

Proteins can often be separated by varying the temperature, pH, or ionic strength of the solution. Selective enzymatic hydrolysis is also an effective means of eliminating or precipitating some proteins from a solution. As mentioned earlier, whey is formed by one such precipitation reaction, where casein micelles are precipitated by either a change to mildly acidic conditions, or with coagulation by a renneting enzyme. McKenzie gives a protocol for separating the whey proteins by salting out with ammonium sulfate. A method which uses trichloroacetic acid is also popular, especially for β-lg separation. Trichloroacetic acid is added to acid whey (pH 4.6) to a 3% solution. The precipitate contains α-la and BSA, while β-lg's remain soluble. Polyacrylamide gel electrophoresis of the precipitate and supernate is shown in Fig. 3. Heat treatment has also been used to separate β-lg from α-la, resulting in a very pure α-la precipitate, but a supernate with α-la contamination.

![Fig. 3. Acid whey which has been further precipitated with trichloroacetic acid is compared to acid whey by polyacrylamide gel electrophoresis. Trichloroacetic acid treatments of 3%, 2.5%, 2.0%, and 1.5%, (from left to right) all appear to be free of albumin fraction compared to acid whey and whey protein standards (β-lg A, β-lg B, α-la, BSA, left to right). β-Lactoglobulins appear stable across the acid range.](image)

3.3 Chromatography

As with the casein fraction of milk serum, the whey fraction may be fractionated by chromatographic methods. Chromatographic separations rely on a host of
protein properties, such as size, charge, and hydrophobicity, to affect separations. Some examples follow.

Size Exclusion Chromatography

Size exclusion chromatography may be used to separate whey proteins. Poor resolution is usually achieved, except when analytical type inorganic resins are used. Bican and Blanc \(^{29}\) were able to resolve Ig (100 kDa), BSA (67 kDa), β-lg (36 kDa), and α-la (14 kDa) (in that order, as expected) using TSK 300S5W (Toyopearl). Gupta got better resolution using the same method but a larger column \(^{29}\). A typical size exclusion result from a gel resin (Sephadex G-75) \(^{29}\) is shown in Fig. 4. Immunoglobulin and BSA elute together in the first peak, while the β-lg's and α-la elute together in the second. Riboflavin is in the third peak.

![Size exclusion chromatographic separation of acid whey proteins on Sephadex G-75. A 30 cm column resolves BSA, but does not separate the β-lactoglobulins from α-lactalbumin](image)

**Fig. 4.** Size exclusion chromatographic separation of acid whey proteins on Sephadex G-75. A 30 cm column resolves BSA, but does not separate the β-lactoglobulins from α-lactalbumin.

Ion Exchange Chromatography

Resolution of four major whey proteins (BSA, α-la, β-lg A and B) has been demonstrated by Manji et al. \(^{40}\), on a Mono Q ion exchange column. A stepwise gradient was run from distilled water to 0.7 M sodium acetate, with each step eluting a protein. α-Lactalbumin eluted at the lowest step (0.14 M sodium acetate), BSA eluted at 0.24 M sodium acetate, β-lg B at 0.39 M and β-lg A at 0.7 M. The separation is achieved over 55 min, but the time could be shortened with a faster responding column. Earlier, Humphrey and Newsome \(^{41}\) had resolved these four proteins in pyridine buffer (pH 6.0) with a 0.05 M NaCl buffer. The elution order was the same as that obtained by Manji et al.

The chromatographic results indicate a stronger binding, and thus a larger concentration of positive charge on the β-lg's than on BSA and α-la around neutral pH. Skudder \(^{42}\) also confirms this with frontal chromatography of these four proteins on Spherosil QMA. In this experiment, β-lg A served as the ultimate displacer.
causing concentration "roll up" in the other three protein elution profiles. Skudder's experiment showed that at pH 6.5, β-lg A is the most strongly adsorbed protein of the four. This is the opposite of the order these proteins run in alkaline (pH 8.4) electrophoresis gels, with the exception of the transposition of BSA and α-la. This reflects that total charge must be highest for the β-lactoglobulins, and that the mobility of BSA might be reduced more than the mobilities of other proteins by sieving effects in the gel matrix.

Chromatofocusing (where a pH rather than a salt gradient is used to elute protein) has been reported by Pearce and Shanley. All four peaks are resolved, over a gradient of 5.2 to 4.2, with BSA eluting first, followed by β-lg B, α-la, and β-lg A. This would seem to indicate that the isoelectric point of α-la is between that of the two β-lg's. However, the values as reported by Swaisgood indicate that α-la has a lower isoelectric point (4.8) that β-lg A (5.26) or β-lg B (5.34), and should have eluted later than either β-lg. The results from chromatofocusing indicates some mechanism of binding other than ion exchange for all three proteins.

Reverse Phase Chromatography

An example of reverse phase chromatography of whey proteins has been reported in the literature. In this, Pearce used a 7.5 cm column packed with Spherisorb S5 C6 to separate whey proteins using a gradient from 0.15 M NaCl/HCl (pH 2.1) to 36% acetonitrile in 0.15 M NaCl/HCl. Again, β-lg A and β-lg B were the most strongly retained proteins, followed by α-la and BSA. This result is not in conflict with the result for ion exchange. The relative hydrophobicity of a protein is not related to the relative charge, which is pH dependent. It also suggests that some hydrophobic interaction between the lactoglobulins and ion exchange resins (nonspecific adsorption) may have contributed to the retention times of these proteins. Protein hydrophobicity appears to be most strongly affected by aromatic, rather than aliphatic amino acid groups (Huyakawa and Nakai).

3.4 Summary

It has been shown that the various constituents of whey protein may be fractionated. Crude separations are attainable with salt, pH, and heat precipitation techniques, while more refined separations are possible when electrophoretic or chromatographic techniques are employed. In developing these techniques, important insights into the properties of the molecules can be made. The β-lactoglobulins appear to be more hydrophobic in general, and have higher charge around neutral pH (6.9) than the other major whey proteins. Bovine serum albumin appears to be slightly more charged than α-la, but its size becomes a factor when separating BSA from the much smaller lactalbumins and lactoglobulins. β-Lactoglobulin is also the most acid stable protein, which may be part of its physiological function as a retinol (vitamin A) carrier. β-Lactoglobulin likely keeps retinol soluble as it passes through the highly acidic environment of the stomach, so it may be adsorbed in the intestine.
4. Lactose/Calcium Chloride Separation

Lactose is a disaccharide of glucose and galactose present in milk serum. Its presence in whey contributes to high BOD values that make whey costly to dispose of. An essentially protein free, lactose rich liquor is produced in the manufacture of whey protein concentrate by ultrafiltration (see Fig. 1). Therefore an economic method for handling the lactose is a final consideration in milk protein purification and utilization. Lactose is responsible for indigestion in some individuals who develop lactose intolerance due to a deficiency of the lactase enzyme in their intestines \(^4\). This limits lactose's value as a sweetener. However, lactose is valuable in pure form, as USP lactose for the pharmaceutical industry. Lactose may also be hydrolysed by treatment with \(F\)-galactosidase to yield glucose and galactose, which are valuable as sweeteners \(^5\). Glucose may be further processed to fructose \(^6\) for additional sweetness. The enzymes required for these sugar conversions are sensitive to the other components of the lactose mixture, especially ions, so methods of purifying lactose are important as the last step in processing these components of milk.

Lactose is usually purified by a crystallization step. This step is complex; it involves pH adjustment and centrifugation of very fine particles (0.5 to 1.5 mm) \(^7\). Chromatographic steps may also be taken to deash whey.

We have studied ion exclusion chromatography as a method for purifying lactose from inorganic salts such as calcium chloride, which constitute the major part of ash in lactose liquors. Ion exclusion chromatography utilizes resins in the same ionic form as the ion to be excluded, and works on the basis of a chemical and electrical field gradient which excludes ionic species, while its effect on non-ionic solute partitioning is small.

A resin which has been derivatized with negatively charged groups (such as a sulfonyl group) will exchange ions with an electrolyte solution. Negatively charged ions are largely excluded from the resin, according to a phenomenon known as Donnan equilibrium. The higher the charge on the negative species, the more strongly it will be excluded from the resin. Uncharged, or non-ionic species, may still partition between the resin and electrolyte phases.

4.1 Comparison of Ion Exclusion to Ion Exchange

The lactose/\(\text{CaCl}_2\) system gives a good example for ion exclusion separation. A cation exchange resin, initially in the \(\text{Ca}^{++}\) form (\(\text{Ca}^{++}\) ions are in equilibrium with the resin's bound negative charge) is used to adsorb lactose, while excluding \(\text{Cl}^–\). If a sample of lactose/\(\text{CaCl}_2\) is injected into a column of this resin in a dilute buffer (or distilled water), \(\text{Ca}^{++}\) ions may exchange freely with \(\text{Ca}^{++}\) previously on the resin, lactose can distribute freely between the phases, and \(\text{Cl}^–\) ions will be excluded by the immobilized negative charges in the resin, and elute at an earlier time. Since \(\text{Ca}^{++}\) is exchanging with \(\text{Ca}^{++}\) in this case, \(\text{Cl}^–\) will elute as \(\text{CaCl}_2\). This is known as ion exclusion. If the resin were initially in a \(\text{H}^+\) or \(\text{Na}^+\) form, \(\text{Ca}^{++}\) would displace these ions, and \(\text{Cl}^–\) would elute as \(\text{HCl}\) or \(\text{NaCl}\). If this is the desired result, the column would have to be periodically regenerated with acid.
or sodium salt in high concentration to displace the absorbed calcium as is the usual case for ion exchange.

The choice of a buffer or eluent for ion exclusion is critical for an efficient separation. Excluding an ion depends on an electric repulsive force which can be screened or compressed with increasing ionic strength. Therefore, the efficiency of exclusion will decrease with increasing buffer ionic strength. By contrast, partitioning of non-electrolytes like lactose are unlikely to be affected greatly by buffer ionic strength.

The screening effect of electrolyte on ion exclusion can be seen in the shape of the isotherm for CaCl₂ with cation exchange resin in the Ca⁺⁺ form. This shape is generally referred to as concave upward. Solutes with this shape isotherm give a characteristic elution profile with a sharp tail (right hand side of peak) and a diffuse front (left hand side of peak). Solutes whose partitioning is unaffected by concentration have Gaussian shaped chromatographic elution profiles. These two shapes can be seen for CaCl₂ and lactose, respectively, in Fig. 5 for a 2.54 cm i.d. x 119 cm long column at 57 °C.

![Diagram](image)

**Fig. 5.** Ion exclusion chromatography of lactose and CaCl₂ of different sample sizes (2, 4, 6, and 8 ml). The CaCl₂ peak shows a sharp tailing edge, indicating a concave upwards (unfavorable) isotherm. The lactose peak is essentially Gaussian, indicating linear equilibrium with the resin. HR 113.

5. Conclusions

The major milk serum molecules may be purified to varying degrees by a number of techniques. These techniques are developed based on a working knowledge of the varied properties of the different milk constituents. Casein micelles may be destabilized either by pH adjustment or enzymatic hydrolysis, to separate the caseins from the serum. The individual caseins may then be dispersed using urea and separated based on phosphorylation or glycosylation. Casein fractionations will help elucidate the role of casein in milk, as well as the cheese making properties of individual constituents.
The remaining serum may be fractionated by electrophoretic or chromatographic techniques associated with soluble and globular proteins. The behavior observed helps characterize the whey proteins, and suggests applications in which they might be used as food additives, either individually or as a whey protein concentrate. Sugars from the whey fractionation could be further processed to yield USP grade lactose by crystallisation or adsorption processes.

Milk is a source of a diverse set of biological molecules. The strategy of fractionation procedures for milk serum reflects general strategies available for fractionation of biological solutions. In this context the milk protein separation techniques briefly reviewed here may have parallels in other areas of the biotechnology industry.

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