Extraction of Immunoglobulin-G from Colostral Whey by Reverse Micelles

Chia-Kai Su* and Been Huang Chiang†

*Department of Leisure, Recreation, and Tourism Management, Southern Taiwan University of Technology, Tainan, Taiwan, Republic of China

†Institute of Food Science and Technology, National Taiwan University, Taipei, Taiwan, Republic of China

ABSTRACT

Separation of immunoglobulin G (IgG) from the other colostrum whey proteins was carried out by reversed micellar extraction. The colostrum whey was diluted to 5 times its original volume with 50 mM phosphate buffer at pH 6.35 containing 100 mM of sodium chloride. The aqueous solution was then mixed with an equal volume of isooctane containing 50 mM bis-(2-ethylhexyl) sodium sulfosuccinate (AOT), and shaken at 200 rpm and 25°C for 10 min. After extraction, the mixture was separated to the aqueous phase and the reversed micellar phase by centrifugation. This procedure extracted most of the non-IgG proteins to the reversed micellar phase and recovered more than 90% of the IgG in the aqueous phase. The IgG in the aqueous phase had a purity of 90%, and still possessed immunological activity. AOT was not detectable in the aqueous phase. (**Key words:** immunoglobulin G, colostrum, reverse micelle)

Abbreviation key: AOT = anionic surfactant bis-(2-ethylhexyl) sodium sulfosuccinate.

INTRODUCTION

Newborns are more resistant to infection when suckled than when fed formula. The resistance of breast-fed infants to infection is often ascribed to the immunoglobulins in human milk. Similar to milks from other species, bovine colostrum is rich in immunoglobulins. Hilpert et al. (1977) reported that the immunoglobulins of cow's milk could be added to infant formula to give similar immunological properties and performance as human milk. Bovine IgG has also been found to enhance the bacteriostatic effect of bovine lactoferrin (Moreau et al., 1983) and may play a role in the immunomodulatory effects of whey proteins (Wong and Watson, 1995). Im-

munoglobulins from cow's milk have been used as a health promoting additive in infant formula and other functional foods.

A number of methods have been proposed for isolating IgG from milk and whey, and most of them by chromatography (Al-Mashikhi et al., 1988; Donnelly and Mehra, 1990; Koneeny et al., 1994; Huang et al., 1995; Cochet et al., 1996; Hahn et al., 1998). Although chromatography has been demonstrated to be a feasible method for highly specific protein purification, it is not well suited for large-scale operations (Luong et al., 1988). The reversed micellar extraction, on the other hand, is an alternative to chromatographic procedures, since the system is easily scaled up, and in principle, allows for continuous separation processes, similar to the liquid-liquid extraction processes, which are commonly used in the chemical industry.

Reverse micells are thermodynamically stable, nanometer-scale aggregates that form spontaneously in the organic phase by the clustering of polar head groups of a surfactant around an inner core of water. The micelles could solubilize proteins and other biochemicals in the small water pool by the electrostatic interaction with the surfactant (Goklen and Hatton, 1987; Luisi et al., 1988; Dekker et al., 1989, 1991; Krei and Hustedt, 1992). A number of protein molecules are extracted by reverse micelles, including α -chymotrypsin (Jolivald et al., 1990; Marcozzi et al., 1991); lipases (Aires-Barris and Cabral, 1991; Taipa et al., 1992); peroxidase (Motlekar and Bhagwat, 2001); proteases (Rahaman et al., 1988); α -amylase (Krei et al., 1995; Brandani et al., 1996; Lazarova and Tonova, 1999); trypsin (Chang and Chen, 1995) and lysozyme (Lye et al., 1995; Naoe et al., 1995; Chou and Chiang, 1998; Jarudilokkul et al., 2000). Factors affecting the separation efficiency of reversed micellar extraction include the pH, ionic strength, and protein concentration of the aqueous phase, and the concentration of surfactant in the organic phase. In this study, the reversed micellar extraction procedure was investigated to separate IgG from the bovine colostrum whey. The objective was to establish a proper procedure and optimal conditions for an effi-

Received July 4, 2002.

Accepted August 22, 2002.

Corresponding author: B. H. Chiang; e-mail: bhchiang@ntu.edu.tw.

cient separation of IgG, and obtain a purified product with immunological activity.

MATERIALS AND METHODS

Materials

Bovine colostrum, obtained from a pool of different cows raised in a local dairy farm, was centrifuged at $4000 \times g$ at 4°C to remove fat. Colostral whey was then prepared by adding 1 *N* HCl to the skim milk to adjust the pH to 4.6 at 30°C , and centrifuged at $10,000 \times g$ for 15 min to remove casein precipitate. Immunoglobulin G, α -lactalbumin, β -lactoglobulin, lactoferrin, BSA, monoclonal anti-bovine IgG (unconjugated from mouse), polyacrylamide, Coomassie brilliant blue R-250, mono-, di-, and tri-sodium phosphates and Tris-HCl were purchased from Sigma Chemical Co. (St. Louis, MO). Isooctane (2,2,4,trimethylpentane) and the anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) were from Fluka Chemie AG Industriestrasse (Buchs, Switzerland).

Reversed Micellar Extraction

The phase-transfer experiments were carried out in tightly stoppered 20-ml vials. For the model system study, 5 ml of standard protein solution in phosphate buffers of various pH containing (1 mg each) IgG, BSA, lactoferrin, α -lactalbumin, and β -lactoglobulin, with ionic strength of 0.05 or 0.55, were mixed with an equal volume of micellar solution of 50 mM AOT in isooctane. For the real system, 5 ml of the aqueous solutions were prepared by adding various amounts of colostrum whey (0.2 to 5.0 ml) to 50 mM sodium phosphate buffer at various pH (4.0 to 7.0) and containing various concentrations of NaCl (0 to 200 mM). The aqueous solutions were mixed with 5 ml of the isooctane solutions containing 50 mM of AOT. The mixtures were shaken at 200 rpm for 10 min at room temperature, and the two phases were separated by centrifugation at $500 \times g$ for 30 min. The recovery of whey protein in the reversed micellar phase (organic phase) or in the aqueous phase after extraction was calculated as the ratio of protein concentration in the reversed micellar phase or in the aqueous phase after extraction to the initial protein concentration in the aqueous phase before extraction. The purity of the immunoglobulin G in the aqueous phase after extraction was the ratio of IgG concentration to the total protein concentration in the aqueous phase.

Analytical

SDS-PAGE was performed according to the methods of Laemmli (1970) after modification. The stacking gel

Figure 1. SDS-PAGE profile of the bovine colostrum whey and the aqueous phases after the whey were extracted by reverse micelles using an initial whey protein concentration of 4 mg/ml, anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) concentration 50 mM, NaCl concentration 50 mM, and various pH. Lane A, bovine colostrum whey; lane B, aqueous phase after extraction at pH 6.0; lanes C and D, aqueous phase after extraction at pH 7.5; lanes E and F, aqueous phase after extraction at pH 9.1; Lanes G and H, aqueous phase after extraction at pH 10.0. LF, lactoferrin; BSA, bovine serum albumin; IgG (HC), immunoglobulin G (heavy chain); IgG (LC), immunoglobulin G (light chain); LG, β -lactoglobulin; LA, α -lactalbumin.

was 4.5% polyacrylamide in 0.125 *M* Tris-HCl buffer at pH 6.8. The separation gel was 12.5% polyacrylamide. Samples were prepared in 0.0625 *M* Tris buffer at pH 6.8 containing 1% SDS and 2.5% β -mercaptoethanol, and heated at 100°C for 2 min. Coomassie brilliant blue R-250 was used for staining. The protein bands were analyzed by scanning the gel with a densitometer (model SLR-ID/2D, Biomed Instruments Inc., Fullerton, CA), and the protein concentrations were determined by using a calibration curve obtained by running standard protein solutions through SDS-PAGE and scanning with the densitometer. A typical SDS-PAGE profiles of the colostrum whey proteins during reversed micellar extraction is illustrated in Figure 1.

Total nitrogen content in the aqueous phase was analyzed by the Kjeldahl procedure, and a factor of 6.38 was used to estimate the total protein concentration (AOAC, 1984). The water content of the reversed micellar phase was determined with a Karl-Fischer moisture titrator. The amount of water in the reverse micelles (W_0) was calculated as the molar ratio of water to the surfactant (AOT) in the reversed micellar phase, $W_0 = [\text{H}_2\text{O}]/[\text{AOT}]$.

The residual AOT content in the extract (aqueous phase) was analyzed with a HPLC system (ICI Instrument Co., Victoria, Australia). The extract was separated on a 5 μm , 220×4.6 mm Spheri-5 RP-8 column (Perkin Elmer Co., Wellesley, MA) at 30°C , and eluted at 1 ml/min with methanol/deionized water (78:22, vol/vol) containing 2 mM tetrabutylammonium bromide.

Table 1. The compositions of colostrum and normal milk whey proteins.

Composition	Molecular Weight*	pI ¹	Colostrum		Normal milk	
			Conc. (mg/ml)	%	Conc. (mg/ml)	%
IgG	150,000	~6.8	10.3	53.1	0.7	13.0
Lactoferrin	80,000	~8.0	1.0	5.1	N	N
BSA	68,000	4.7	1.0	5.2	0.4	5.6
β -lactoglobulin	18,300	5.2	6.0	30.9	3.2	58.2
α -lactalbumin	14,500	4.8	1.1	5.7	1.1	19.8

N: negligible.

¹The molecular weights and pI values for IgG and lactoferrin were from McArthur et al. (2000), and the values for BSA, β -lactoglobulin and α -lactalbumin were from Wong et al. (1983).

Aliquot of 20 μ l was injected into the system, and analyzed with a RI detector at 40°C.

Activity Assay

Double gel diffusion was performed on a glass slide. The slide was precoated with 0.5% agarose and overlaid with 2% agarose in 0.015 M barbitone buffer, pH 8.2. The central well was filled with 3 μ l of the anti-bovine IgG and the peripheral wells were filled with the aqueous phase after reversed micellar extraction. The precipitation reaction was proceeded at 37°C for 12 h.

RESULTS AND DISCUSSION

Experiments with Standard Protein Solutions

The composition and some properties of colostrum whey protein are shown in Table 1. The major protein was IgG, which accounted for 53.1% of the total protein. Although β -lactoglobulin is the most abundant protein found in the normal cow's milk whey, it only occupied 30.9% of the total colostrum whey protein. Other proteins including lactoferrin, bovine serum albumin, and α -lactalbumin also existed as minor components.

The experiments with various standard protein solutions were conducted at a fixed AOT concentration of 50 mM. The concentration of surfactant in a reversed micellar phase that is in equilibrium with an aqueous phase has little effect on the size and structure of the reverse micelles (Dekker et al., 1989), and the partition behavior for various proteins between two phases is independent of the surfactant concentration (Fletcher and Parrott, 1988). Although the extent of protein uptake from the aqueous phase increases in proportion to the surfactant concentration in the reversed micellar phase, too much surfactant in the system will induce emulsion formation and destroy the two-phase system. Because the effect of protein concentration in the aqueous phase on the separation performance was a vari-

able, the surfactant (AOT) concentration was fixed at 50 mM based on preliminary studies.

Preliminary studies were also carried out to determine the effect of ionic strength of the aqueous phase on the separation efficiency between the IgG and non-IgG proteins using standard protein solutions. It was found that when the ionic strength in the aqueous phase was very low, phase transfer did not occur. Instead, a stable emulsion appeared. This was possibly due to excessive water being transferred into the micellar solution (Leser et al., 1986; Marcozzi et al., 1991). When the ionic strength of the aqueous phase was controlled at 0.05, the extraction of immunoglobulin to the reversed micellar phase was higher than that of other proteins within the pH range of 5 to 8 (Figure 2). For large molecules, such as IgG, the extraction might be controlled not only by electrostatic interaction but also by surface charge distribution or hydrophobic interaction (Kamihira et al., 1994). In a recent study, Gerhardt and Dungan (2002) proposed that IgG may form clus-

Figure 2. Recovery of various colostrum whey proteins from the standard solutions at ionic strength of 0.05 by reverse micelles. The extraction was conducted at anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) concentration of 50 mM and various pH.

Figure 3. Recovery of various colostral whey proteins from the standard solutions at ionic strength of 0.55 by reverse micelles. The extraction was conducted at anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) concentration 50 mM and various pH.

ters with the micelles, and the protein's hydrophobic domains act as cross-links between the individual members, whereas hydrophilic domains contributed by the protein polar moieties, reside in the water pool. However, at high ionic strength ($I = 0.55$), the transfer of other whey proteins to the reversed micellar phase was higher than that of immunoglobulin (Figure 3), probably because these proteins possessed more hydrophilic regions than IgG. The hydrophilic protein surface would enable its solubilization through a layer of water, "connecting" the protein to the surfactant shell (Castro and Cabral, 1988). When the target compound was IgG, it appeared to be a good idea to extract the non-IgG whey proteins into the reversed micellar phase and leave the IgG in the aqueous phase. This procedure could eliminate the step of backward extraction and simplify the extraction process. In addition, this procedure reduces the opportunity for IgG to contact with the organic solvent, thus decreasing the chance of denaturation. Therefore, in the subsequent studies the process conditions for transferring a maximum amount of non-IgG proteins to the reversed micellar phase and leaving the IgG in the aqueous phase from colostral whey were investigated.

Effect of pH

The aqueous phase pH determines the ionization state of the charged groups of the proteins. When the overall charge of the protein molecule is opposite to the charge of the surfactant polar head group the electro-

static interactions would enable the protein molecules to transfer into the reverse micelles. Figure 4 shows the effect of pH of the aqueous phase on the reversed micellar extraction of colostral whey proteins. There were essentially no non-IgG proteins found in the aqueous phase when the pH was below 6.0. All of the non-IgG whey proteins have pI values around 5.0 (Table 1), and are most likely positively charged at low pH. Therefore, the non-IgG proteins could interact with the anionic surfactant AOT and transfer into the reverse micelles. However, it was also observed that significant amount of insoluble aggregate was formed at the interface between the reversed micellar phase and the aqueous phase when the pH of the aqueous phase was lower than about 6.0, suggesting that some protein molecules were denatured by the action of surfactant. Upon increasing the pH, the amount of protein precipitate at interface decreased and more proteins, especially IgG, appeared in the aqueous phase. Although IgG is also positively charged at low pH, it was more difficult to be extracted into the reversed micellar phase than the other whey proteins because of its large size, thus most of the IgG remained in the aqueous phase. It was observed that the non-IgG proteins did not appear in the aqueous phase until the pH increased to a value higher than 6.28. Considering the yield and purity of IgG, the pH value 6.35 of aqueous phase was chosen for the reversed micellar extraction process.

Effect of Protein Concentration

The effect of initial protein concentration in the aqueous phase on the recoveries of various colostral whey

Figure 4. Residual proteins in the aqueous phase after reversed micellar extraction of the colostral whey. The extraction was conducted at anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) concentration 50 mM, NaCl concentration 50 mM, and various pH.

Table 2. Effect of initial protein concentration on the recovery of bovine colostrum whey proteins in the aqueous phase during reversed micellar extraction. Aqueous phase: 50 mM NaCl, pH 6.35. Reversed micellar phase: 50 mM AOT in isooctane.¹

Initial protein concentration (mg/ml)	Immunoglobulin (%)	β -lactoglobulin (%)	α -lactalbumin (%)
20.0	80.3 ^a	51.5 ^a	43.8 ^a
16.0	75.6 ^a	45.0 ^a	23.1 ^b
12.0	79.4 ^a	21.1 ^b	13.0 ^c
4.0	75.8 ^a	18.0 ^b	0.0 ^d
3.2	71.7 ^a	12.3 ^c	0.0 ^d
2.4	68.3 ^a	10.3 ^c	0.0 ^d
1.6	75.8 ^a	10.6 ^c	0.0 ^d

^{a,b,c}Means within each column superscripted with different letters were significantly different ($P < 0.05$).

¹Other whey proteins were not found in the aqueous phase after extraction.

proteins during reversed micellar extraction is shown in Table 2. In general, the amount of residual proteins in the aqueous phase increased with increasing initial protein concentration. Because the surfactant concentration used in this study was fixed at 50 mM, it was expected that the initial protein concentration in the aqueous phase would not affect the amount of proteins being extracted into the reverse micelles when their concentrations reached certain levels. In fact, it was found that more cloudy aggregate appeared at the interface between the micellar and aqueous phases with higher initial protein concentration. It appeared that diluting the colostrum whey could reduce the interactions between the whey proteins, and facilitated the extraction of non-IgG whey proteins into the reverse micelles, and increased the purity of IgG in the aqueous phase. However, dilution of colostrum whey would increase its volume, and result in operation difficulty. The proper dilution should be evaluated based on the separation efficiency as well as operational feasibility. Nevertheless, an initial protein concentration of 4.0 mg/ml used throughout this investigation appeared to be proper for an efficient separation of IgG from the colostrum whey.

Effect of Ionic Strength

The effect of the electrolyte concentration on the extent of protein separation was further investigated. The experiment was performed with varying NaCl concentration, and the result is shown in Figure 5. It was found that increasing the salt concentration up to 100 mM resulted in an increase in the recovery of IgG in the aqueous phase. Further increases of the NaCl concentration, however, did not exert significant effect on the yield of IgG. The water contents (W_o) of the reversed micellar phase, defined as $W_o = [H_2O]/[AOT]$, at various sodium chloride concentrations is also shown in Figure 5. The water content decreased as the sodium chloride concentration increased. The positively charged sodium

ion could interact with the negatively charged surfactant head and enter the inner core of the reverse micelles. The sodium ion reduced the surfactant head group repulsions and led to the formation of reverse micelles with small sizes. Also, an increase of salt concentration may cause an electrostatic screening effect, which reduces the electrostatic interaction between protein and AOT and decreases the size of reverse micelle (Leodidis, 1990; Nishiki et al., 1993). It appeared that 100 mM of sodium chloride facilitated the formation of reverse micelles with proper size for accommodating the non-IgG whey proteins, but the ionic strength developed by 100 mM of sodium chloride was not suitable for transferring IgG to the reversed micellar phase, thus left IgG in the aqueous phase and resulted in a high yield.

It is also worth noting that a stable cloudy aggregate was observed at the interface between the micellar and aqueous phases when the salt concentration was lower than 50 mM. The precipitation at the interface was

Figure 5. Effect of NaCl concentration on the yield and purity of immunoglobulin G in the aqueous phase and the water content of the reverse micelles. The reversed micellar extraction of colostrum whey was conducted at anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) concentration 50 mM and pH 6.35.

Figure 6. Analysis of immunoglobulin G in the aqueous phase by immunoprecipitation with anti-bovine IgG. The central well was filled with anti-bovine IgG serum, and peripheral wells with aqueous phase extracted at pH 6.35, NaCl concentration of 100 mM and anionic surfactant bis(2-ethylhexyl) sodium sulfosuccinate (AOT) concentration of 50 mM. (a) aqueous phase without dilution; (b) aqueous phase with 2-fold dilution; (c) aqueous phase with 3-fold dilution.

most likely caused by the strong interaction between proteins and surfactant at low ionic strength. However, since the non-IgG whey proteins either being extracted by the reverse micelles or precipitated at the interface, the purity of IgG in the aqueous phase could be maintained at approximately 90% even without NaCl in the system.

Immunological Activity and Residual AOT in the Aqueous Phase

Although it would be difficult to quantitate the specific antigen-antibody reaction, the antiserum was used for double gel diffusion assay based on the principle that both reactants (antigen and antibody) move through an inert media and form a precipitin line (stable antigen-antibody complex). The characteristics of the positive immunoprecipitin lines may be interpreted in relation to a known antigen and/or antibody. In this study, the aqueous phase containing most of the IgG of the colostrum whey was tested, and precipitin lines were observed (Figure 6). This result indicated that the IgG purified by reversed micellar extraction was capable of forming complex with anti-serum, and therefore still possessed immunological activity.

Although the LD₅₀ of AOT is 1900 mg/kg, the residual AOT in the aqueous may still cause concern if the purified IgG is used for food applications. The HPLC employed for this study could detect AOT when its concentration was as low as 667 mg/kg. But it still could not detect any AOT in the aqueous phase. Therefore, it can be concluded that AOT was not present in the IgG solution obtained by reversed micellar extraction.

CONCLUSION

Reversed micellar extraction appeared to be a feasible method for separating and purifying IgG from the other

proteins in the colostrum whey. Under proper conditions, most of the non-IgG proteins could be extracted from the aqueous phase to the reverse micelles in the organic phase, and the immunoglobulin could be recovered in the aqueous phase. This procedure is simple, and reduces the opportunity for the immunoglobulin to contact with the organic solvent, thus can maintain its biological activity.

REFERENCES

- AOAC. 1984. Official Methods of Analysis of the Association of Official Analytical Chemists. 14th ed. Washington DC, USA.
- Aires-Barros, M. R., and J. M. S. Cabral. 1991. Selective separation and purification of two lipases from *Chromobacterium viscosum* using AOT reversed micelles. *Biotechnol. Bioeng.* 38:1302–1307.
- Al-Mashikhi, S. A., and S. Nakai. 1988. Separation of immunoglobulin and transferrin from blood serum and plasma by metal chelate interaction chromatography. *J. Dairy Sci.* 71:1756–1763.
- Al-Mashikhi, S. A., E. Li-Chan, and S. Nakai. 1988. Separation of immunoglobulins and lactoferrin from cheese whey by chelation chromatography. *J. Dairy Sci.* 71:1747–1755.
- Brandani, S., V. Brandani, G. Di Giacomo, and L. Spera. 1996. Effect of nonlinear equilibrium on the mass transfer rate of α -amylase extraction by reversed micelles. *Progress Biochem.* 31:249–252.
- Castro, M. J. M., and J. M. S. Cabral. 1988. Reversed micelles in biotechnological processes. *Biotechnol. Adv.* 6:151–167.
- Chang, Q. L., and J. Y. Chen. 1995. Reversed micellar extraction of trypsin: Effect of solvent on the protein transfer and activity recovery. *Biotechnol. Bioeng.* 46:172–174.
- Chou, S. T., and B. H. Chiang. 1998. Reversed micellar extraction of hen egg lysozyme. *J. Food Sci.* 63(3):399–402.
- Cochet, S., H. Pesliakas, Y. Kroviarski, J. P. Cartron, and O. Bertrand. 1996. Chromatography of bovine lactoserum on the immobilized metallized dye drimarene rubine R/K 5BL. Modulation of selectivity by metal exchange and application to purification of milk immunoglobulins. *J. Chromatogr. A.* 725:237–247.
- Dekker, M., R. Hilhorst, and C. Laane. 1989. Review: Isolating enzymes by reversed micelles. *Anal. Biochem.* 178:217–226.
- Dekker, M., K. Van't Riet, B. H. Bijsterbosch, R. B. G. Wolbert, and R. Hilhorst. 1989. Modeling and optimization of the reversed micellar extraction of α -amylase. *AIChE J.* 35:321–324.
- Dekker, M., K. Van't Riet, J. J. Van Der Pol, J. W. A. Baltussen, R. Hilhorst, and B. H. Bijsterbosch. 1991. Effect of temperature on the reversed micellar extraction of enzymes. *Chem. Eng. J.* 46:B69–B74.
- Donnelly, W. J., and R. K. Mehra. 1990. Fractionation of milk proteins. *Biochem. Soc. Trans.* 18:238–240.
- Fletcher, P. D. I., and D. Parrott. 1988. The partitioning of proteins between water-in-oil microemulsions and conjugate aqueous phase. *J. Chem. Soc. Faraday. Trans. 1.* 84(4):1131–1144.
- Gerhardt, N. I., and S. R. Dungan. 2002. Time-dependent solubilization of IgG in AOT-Brine-Isooctane microemulsions: Role of cluster formation. *Biotechnol. Bioeng.* 78:60–72.
- Göklen, K. E., and T. A. Hatton. 1984. Liquid-liquid extraction of low molecular-weight proteins by selective solubilization in reversed micelles. *Sep. Sci. Technol.* 22:831–841.
- Hahn, R., P. M. Schulz, C. Schaupp, and A. Jungbauer. 1998. Bovine whey fractionation based on cation-exchange chromatography. *J. Chromatogr. A.* 795:277–287.
- Hilpert, H., H. Gerber, J. J. Amster, A. Pahud, L. Ballabriga, F. Arcalis, E. Farriaux, E. DePeyer, and D. Nussle. 1977. Bovine immunoglobulins: their possible utilization in industrially prepared infant's milk formula. Page 182 in *Food and Immunology*. L. Hambræus, L. A. Hanson and M. Mcfarlane, eds. Almquist and Wiksel Int., Stockholm, Sweden.
- Huang, Y. C., Y. C. Tu, and T. Y. Lee. 1995. Purification of antibodies by zeolite A. *Enzyme Microb. Technol.* 17:564–569.

- Jarudilokkul, S., E. Paulsen, and D. C. Stuckey. 2000. Lysozyme extraction from egg white using reverse micelles in a Graesser contactor: Mass transfer characterization. *Biotechnol. Bioeng.* 69(6):618–626.
- Jolival, C., M. Minier, and H. Renon. 1990. Extraction α -chymotrypsin using reversed micelles. *J. Colloid. Interface Sci.* 135:85–96.
- Kamihira, M., H. Yanagisawa, K. Takahashi, and H. Takeuchi. 1994. Fractionation of IgG fragments using reversed micellar extraction. *J. Ferment. Bioeng.* 77(1):80–84.
- Kinugase, T., S. I. Tanahashi, and H. Takeuchi. 1991. Extraction of lysozyme using reversed micellar solution: Distribution equilibrium and extraction rates. *Ind. Eng. Chem. Res.* 30:2470–2476.
- Koneeny, P., R. J. Brown, and W. H. Scouuten. 1994. Chromatographic purification of immunoglobulin G from bovine milk whey. *J. Chromatogr. A.* 673:45–53.
- Krei, G. A. and H. Hustedt. 1992. Extraction of enzymes by reverse micelles. *Chem. Eng. Sci.* 47:99–111.
- Krei, G., U. Meyer, B. Burner, and H. Hustedt. 1995. Extraction of α -amylase using BDBAC-reversed micelles. *Bioseparation* 5:175–183.
- Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature* 227:680–685.
- Lazarova, Z., and K. Tonova. 1999. Integrated reversed micellar extraction and stripping of α -amylase. *Biotechnol. Bioeng.* 63(5):583–592.
- Leodidis, E. B. 1990. Amino acids in reverse micelles: 1. Determination of interfacial partition coefficients using the phase-transfer method. *J. Phys. Chem.* 94:6400–6411.
- Leser, M. E., G. Wei, P. L. Luisi, and M. Maestro. 1986. Application of reverse micelles for the extraction of proteins. *Biochem. Biophys. Res. Commun.* 135(2):629–635.
- Luisi, P. L., M. Giomini, M. P. Pileni, and B. H. Robinson. 1988. Reverse micelles as hosts for proteins and small molecules. *Biochim. Biophys. Acta* 947:209–246.
- Luong, J. H. T., K. B. Male, and A. L. Nguyen. 1988. A continuous affinity ultrafiltration process for trypsin purification. *Biotechnol. Bioeng.* 31:516–520.
- Lye, G. J., J. A. Asenjo, and D. L. Pyle. 1995. Extraction of lysozyme and ribonuclease-a using reverse micelles: Limits to protein solubilization. *Biotechnol. Bioeng.* 47:509–519.
- Marcozzi, G., N. Correa, P. L. Luisi, and M. Caselli. 1991. Protein extraction by reverse micelles: a study of the factors affecting the forward and backward transfer of α -chymotrypsin and its activity. *Biotechnol. Bioeng.* 38:1239–1246.
- McArthur, S. L., K. M. McLean, P. Kingshott, H. A. W. St. John, R. C. Chatelier, and H. J. Griesser. 2000. Effect of polysaccharide structure on protein adsorption. *Colloids Surfaces B: Biointerfaces* 17:37–48.
- Moreau, M. C., Y. Duval-Iflah, M. C. Muller, P. Raibaud, M. Vial, J. C. Gabilan, and N. Daniel. 1983. Effect of supplement of diet with bovine lactoferrin and IgG on the establishment of *Escherichia coli* in the digestive tract of gnotobiotic mice and human infants. *Ann. Microbiol.* 134B(3):429.
- Motlekar, N. A., and S. S. Bhagwat. 2001. Activity of horseradish peroxidase in aqueous and reverse micelles and back-extraction from reverse-micellar phase. *J. Chem. Technol. Biotechnol.* 76(6):643–649.
- Naoe, K., Y. Shintaku, Y. Mawatiari, M. Kawagoe, and M. Imai. 1995. Novel function of guanidine hydrochloride in reversed micellar extraction of lysozyme from chicken egg white. *Biotechnol. Bioeng.* 48:333–340.
- Nishiki, T., I. Sato, and T. Kataoka. 1993. Partitioning behavior and enrichment of proteins with reversed micellar extraction: I. Forward extraction of proteins from aqueous to reversed micellar phase. *Biotechnol. Bioeng.* 42(5):596–600.
- Rahaman, R. S., J. Y. Chee, J. M. S. Cabral, and T. A. Hatton. 1988. Recovery of an extracellular alkaline protease from whole fermentation broth using reversed micelles. *Biotechnol. Prog.* 4:218–224.
- Taipa, M. A., M. R. Aires-Barros, and J. M. S. Cabral. 1992. Purification of lipases. *J. Biotechnol.* 26:111–142.
- Wong, N. P., R. Jenness, M. Keeney, and E. H. Marth. 1983. Fundamentals of dairy chemistry. 3rd Ed., p. 142. Van Nostrand Reinhold, New York.
- Wong, C. W., and D. L. Watson. 1995. Immunomodulatory effects of dietary whey proteins in mice. *J. Dairy Res.* 62:359–368.