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Effect of Conductivity Control on the Separation of Whey Proteins by Bipolar Membrane Electroacidification

LAURENT BAZINET,*,† DENIS IPPERSIEL,† AND BEHZAD MAHDAVI‡

Agriculture and Agri-Food Canada, Food Research and Development Centre, 3600 Casavant Boulevard West, St. Hyacinthe, Québec J2S 8E3, Canada, and Laboratoire des Technologies Électrochimiques et des Électrotechnologies d'Hydro-Québec, 600 Avenue de la Montagne, Shawinigan, Québec G9N 7N5, Canada

Since the limiting factor of the bipolar membrane electroacidification (BMEA) process at 20% WPI (whey protein isolate) was hypothesized to be the lack of mobile ion inherent to the protein solution at pH 5.0, the aim of the present work is to study the effect of the conductivity control on the precipitation behavior of whey protein. BMEA performances were evaluated by measuring electrodialytic parameters, protein kinetic precipitation, molecular profiles, and isolate chemical composition and purity. The highest protein precipitation with 10% WPI solution was obtained at pH 4.6 and at a conductivity level of 200 μS/cm maintained with many 0.4-mL additions of 1.0 M KCl (200 μS[+]), with a 46% precipitation of the total protein, β-lg composing the main part of the precipitated protein. With a 20% WPI solution, it was possible to reach pH 4.65 with conductivity control at 350 μS/cm. However, the 27% protein precipitation was still low. The changes in viscosity as pH decreases observed at 20% WPI would decreased the final precipitation rate of β-lg, since the viscosity of the 20% WPI dispersion was very different.

KEYWORDS: Electrochemical acidification; whey protein; fractionation; conductivity

INTRODUCTION

Fractionation of major whey proteins (α-la and β-lg) is performed by ion-exchange chromatography (*1*, *2*), metaphosphate complex precipitation (*3*), heat/acid separation (*4*−*7*), salting-out (*8*), and ion depletion at low pH (*9*, *10*). In their experiments, Amundson et al. (*9*) and Slack et al. (*10*) separated the two major whey protein fractions by demineralizing a concentrated whey solution and adjusting the pH chemically. With this method, they produced β-lactoglobulin-enriched fractions representing 33% of the original acid whey protein and 17% of the original sweet whey proteins.

Bipolar membrane electroacidification (BMEA) is a technology coupling the effects of demineralization and acidification by using bipolar membranes to split water at their interfaces and cation exchange membranes (CEM) to demineralize ionic species. This procedure, already used for soybean and milk casein protein, recently allowed the separation of a 98% pure β-lg fraction from a 5% whey protein isolate (WPI) solution (*11*). Furthermore, it appeared that the protein yield increased with an increase in initial protein concentration in the solution, and that electroacidification of a 20% WPI solution to pH 4.65 would allow a higher precipitation yield. Since the limiting factor of such a process at 20% WPI was hypothesized to be the lack of mobile ion of the protein solution at pH 5.0, Bazinet et al. (*11*) had suggested the addition of KCl to allow electroacidification to pH 4.65.

In this context, the aim of the present work is to study the effect of conductivity control on the precipitation behavior of whey protein to succeed in electroacidifying a 20% WPI solution to the optimum pH of 4.65 with a low salt concentration at this final pH. The performances of BMEA were evaluated by measuring electrodialytic parameters, protein kinetic precipitation, protein profiles, and isolate chemical composition and purity.

MATERIAL AND METHODS

Material. The starting material used in this study was a BiPRO whey protein isolate (Davisco Foods International Inc., MI).

Methods. *(a) Electroacidification Cell*. A MP-type electroacidification cell (100 cm^2 effective surface area) manufactured by ElectroCell Systems AB Co. (Täby, Sweden) was used with three Neosepta CMX cationic membranes and one Neosepta BP-1 bipolar membrane (Tokuyama Soda Ltd., Tokyo, Japan) according to the setup described by Bazinet et al. (*12*). This arrangement defines three closed loops containing the BiPRO whey protein isolate solution, a 2 g/L aqueous KCl solution, and a 20 g/L Na_2SO_4 solution. Each closed loop was connected to a separate external container, allowing continuous

* To whom correspondence should be addressed. Current address: Institute of Nutraceuticals and Functional Foods (INAF) and Centre de Recherche en Science et Technologies du Lait (STELA), Pavillon Paul Comtois, Université Laval, Department of Food Sciences and Nutrition, Sainte-Foy (QC G1K 7P4), Canada. Phone: (418) 656-2131, ext 7445. Fax: (418) 656-3353. E-mail: Laurent.bazinet@aln.ulaval.ca.

† Agriculture and Agri-Food Canada, Food Research and Development Centre.

‡ Laboratoires des Technologies Électrochimiques et des Électrotechnologies d'Hydro-Québec.

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recirculation. The electroacidification system was not equipped to maintain constant temperature (*12*).

(b) Protocol. Electroacidification was carried out in a batch process, using a current of 2.0 A, and after reaching 60 V, the voltage was maintained constant at 60 V. Electrolyte volumes of 1.5 L were used for the Na_2SO_4 and KCl solutions while a 1.0 L volume was used for the BiPRO solution. Initial pH varied between 6.8 and 7.0. The electroacidification was stopped at pH 4.4.

In the first part of this study, electroacidification of 10% (w/w) WPI solutions was carried-out in different conditions of conductivity control to evaluate the effect of the conductivity on the precipitation behavior of the whey protein. A 2 × 2 factorial array was set up; the conductivity of the solution was adjusted with a 1.0 M KCl solution at two different levels (200 and 250 μS/cm, abbreviated respectively in the text by 200 μS and 250 μS) by two different modes (many [+] 0.4-mL additions and one global addition [1]). Two replicates of each condition were performed in this experiment.

In the second part of this study, electroacidification of 20% (w/w) WPI solutions was carried out with conductivity control, following the results of the previous part: when pH 5.0 or 350 μS/cm was reached, conductivity was maintained constant at 350 μS/cm. The 350 μS/cm value was determined as the best combination of protein solubility and system resistance, according to a previous study (*11*); at this point the protein insolubility curve was close to its optimum and the system resistance began to increase. This value would allow a control of the system resistance at approximately 60 Ω at the end of the BMEA; this value represents 50% of the one obtained previously for BMEA of 20% Bipro Solution at pH 5.0 (*11*). Three replicates of the electroacidification were performed.

During acidification, 3.0-mL samples of the WPI solution were taken at different pH values: initial pH (around 6.8), pH 6.0, and then at every 0.2 pH unit decrease from pH 5.4 to 4.4. The time required to reach pH 4.4, the cell resistance, the conductivity, and the temperature were recorded as the acidification progressed. The concentration of soluble protein was determined on freshly acidified 3.0 mL samples.

(c) Analysis Methods. (1) System Resistance. The system resistance was calculated, using Ohm's law, from the voltage and the current intensity read directly from the indicators on the power supply.

(2) Conductivity. A YSI conductivity meter, Model 35, was used with a YSI immersion probe, Model 3418, cell constant $K = 1$ cm^{-1} (Yellow Springs Instrument Co.,Yellowsprings, OH), to measure the conductivity of the protein solutions. Since the electroacidification system was not equipped to maintain constant temperature, the conductivity of the WPI solutions was normalized at 25 °C according to Bazinet et al. (*11*).

(3) Protein Profile. The chromatographic analysis of the freeze-dried protein isolate and supernatant of centrifuged WPI solution samples was performed by reverse-phase HPLC according to Jaubert and Martin (*13*), in the conditions used by Bazinet et al. (*12*).

(4) Solubility Profile. A 0.2 N hydrochloric acid solution was added gradually to 250 mL of 2% (w/v) isolate protein solution. Aliquots of 1.5 mL were taken at pH 6.6, 5.8, 5.4, 5.2, 5.0, 4.8, and 4.6 and centrifuged at 500 g for 10 min at 4 °C. Soluble protein concentration was determined in the supernatant by using Bradford's method (*14*). Nonlinear regression equations were calculated according to Bazinet et al. (*15*).

(5) Ash Content. In accordance with method 930-30 (*16*) approximately 1.5 g of lyophilized sample was added to preweighted crucibles. The sample was then ashed at 550 °C for 16 h and weighed again after cooling to room temperature.

(6) Apparent Viscosity. The apparent viscosity was measured on a Brookfield digital rheometer (Model DV-III, Stoughton, MA) with RV spinddles (Model RV I and II). The shear rate was increased from 10 to 200 s^{-1}. During the measurement, the temperature of the dispersion (250 mL) was constant at 23.5 ± 0.5 °C.

(7) Soluble Protein and Total Protein Determination. The soluble and total protein determinations were done on an FP-428 LECO apparatus (LECO Corporation, Saint Joseph, MI) according to the method of Bazinet et al. (*12*) in the following conditions: loop select = high range; and flow constant = high, 10 s; high, 30 s; high, end.

(8) Statistical Analyses. The system resistance, the conductivity, and the percent soluble protein as a function of pH were subjected to a multivariate analysis of variance with SAS software (*17*). The duration of the electroacidification data was subjected to an analysis of variance. Regression equations and curve fitting were calculated for the system resistance, conductivity and percent soluble proteins as a function of pH, using SigmaPlot (version 3.0 for Windows, Jandel Scientific, Corte Madera, CA). The ash and total protein contents were analyzed by analysis of variance and examined by Duncan tests to determine the significance of differences between the different samples. The percents of BSA, α-lactalbumin, and β-lactoglobulin in the supernatant during BMEA were subjected to a split-block analysis of variance, while the percentage of each protein fraction measured in the isolate was subjected to an analysis of variance.

RESULTS AND DISCUSSION

10% BiPRO Solution Electroacidification. *(a) BMEA Parameters: Conductivity and System Resistance.* The change in conductivity as the electroacidification proceeded was influenced by the level at which the conductivity was controlled ($P < 0.0295$). In addition, the evolution of the system resistance during BMEA was different according to the level ($P < 0.0001$) and the mode ($P < 0.0001$) of conductivity control.

Since the conductivity was not adjusted from pH 6.8 to 5.0 during BMEA of Bipro WPI, the evolution of the 25 °C normalized conductivity was the same with a decrease from 899 ± 71 to 220 ± 34 μS/cm. As expected at pH 4.8, one addition of 1 M KCl for 250 μS (250 μS[1]) increased the conductivity to 401 μS/cm in comparison with 232, 206, and 197 μS/cm at repectively 250 μS[+], 200 μS[1], and 200 μS-[+]. At pH 4.6, the conductivity for 250 μS[1] was still high at 340 μS/cm. At this pH value, one addition of KCl for 200 μS-[1] increased the conductivity of the WPI solution to 262 μS/cm in comparison with values of 192 and 221 μS/cm respectively for 200 μS[+] and 250 μS[+]. Subsequent additions of 1 M KCl as BMEA ran allowed a conductivity control at the desired value, whatever the level of conductivity to be maintained. The final conductivity whatever the mode of conductivity adjustment was similar at 186 μS/cm for 200 μS and 221 μS/cm for 250 μS.

As the pH decreased from pH 6.8 to 5.0, the system resistance evolution was the same with an increase in resistance from 18.5 ± 1.4 to 43.5 ± 6.9 Ω. After pH 5.0 was reached, the conductivity of the system was controlled. Consequently, the resistance decreased with an increase in the level and the mode of conductivity adjustment. In the case of 250 μS[1], the system resistance was decreased back to the initial resistance value. With one addition of KCl, the system resistance dropped by 16.7 and 14 Ω respectively for 250 and 200 μS. With many additions of KCl, the system resistance was maintained constant and did not reach as high a value such as 120 Ω observed in a previous work (*11*). In this previous work, no addition of KCl was done, which explained the increase in the system resistance due to the demineralization of the solution treated. In the present work, during BMEA at a control conductivity value, the demineralization of the intrinsic ionic species was compensated by addition of KCl and consequently no real increase in system resistance was observed.

The addition of K^+ allows the H^+ to be used for the acidification and not be lost for the process. During the process, as the pH decreased by electrogeneration of the H^+ on the cationic side of the bipolar membrane, the K^+ migrated through the cation exchange membrane. The K^+ added to the whey protein was used by the process to keep the solution electrically neutral (*15*).

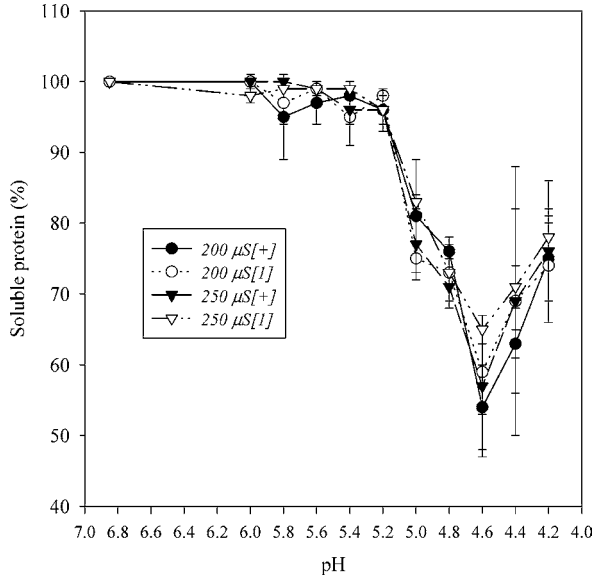

Figure 1. Effect of the conductivity control on the evolution of the soluble protein during BMEA of 10% BiPRO.

(b) Soluble Protein. The split block analysis of variance of the data showed that the percent soluble protein as the pH decreases by BMEA was similar whatever the mode ($P > 0.3167$) and the level of conductivity control ($P > 0.3123$).

The pH had the main effect on the evolution of the percent soluble protein ($P < 0.0001$) (**Figure 1**). From initial pH to pH 5.2, the percent soluble protein remained constant at 100%. Afterward, the percent soluble protein dropped to a minimum averaged value of 58.7% at pH 4.6, and increased back to 75.7% at pH 4.0. However, although the split-block analysis of variance showed no difference, the analysis of variance carried out at the different values of pH showed a possible difference at pH 4.6 ($P > 0.0872$). Since the probability level was closed to the acceptance level of 5%, the mode and level of conductivity adjustment would have an impact on the percentage of soluble protein at pH 4.6. The averaged percent soluble protein increased from 206.5 to 301 μS/cm with an increase in the mode of conductivity adjustment (one [1] versus many [+] additions of KCl) and from 227 to 280 μS/cm with an increase in the level of conductivity (200 μS versus 250 μS).

The percent soluble protein was very sensitive to the conductivity and pH values. Protein precipitation was maximum at pH 4.6 and 200 μS[+], with a 46% precipitation of the total protein. One global [1] addition of 1 M KCl decreased the protein precipitation yield by resolubilizing protein. As previously observed by Amundson et al. (*9*) the optimum pH to precipitate whey protein was close to pH 4.6. The pH 4.65 value has been reported as the optimal pH for maximum formation of β-lg A octamer (*18*). The increase in percent soluble protein, after pH 4.6 was reached, was due to a decrease in the association degree of the β-lg. After pH 4.65 and below, β-lg dimerizes and is more soluble (*19*).

(c) Protein Profiles. The split-block analysis of variance dealing with the results of the different whey protein fractions showed that pH has a highly significant effect on the percentage of α-la ($P < 0.0001$) and β-lg ($P < 0.0001$) in the supernatant. Duncan tests were carried out on each fraction percentage in the supernatant from pH 5.2 to 4.4 to identify significant differences between the mode and the level of conductivity adjustment at these specific pH values.

The fractions showed different trends (**Table 1**). Between pH 6.8 and 4.8, the α-la was stable, accounting for 10.7 ± 0.3% of the proteins in the supernatant. Since Duncan Tests carried out at pH 4.6 and 4.4 showed no difference between the mode and the level of conductivity adjustment ($P > 0.4916$ and $P > 0.6393$, respectively) the concentration of α-la in the supernatant during BMEA of 10% Bipro solution decreased slightly to 9.9 ± 0.4% at pH 4.6, and was fairly stable afterward at 10.0 ± 0.3% at pH 4.4. Although the analysis of variance showed a difference ($P < 0.0003$) for the BSA data, there are no real difference in the percentage of BSA in the supernatant whatever the pH and the conductivity adjustment, since the standard deviations were relatively high at 200 μS[1] and 250 μS[1]. The β-lg concentration was stable between pH 6.8 and 5.2 accounting for 85.2 ± 1.8% of the total protein in the supernatant. According to the Duncan tests performed at pH 5.2, 5.0, 4.8, and 4.6, no differences were observed whatever the mode and the level of conductivity (respectively $P > 0.9731$, $P > 0.1525$, $P > 0.5538$, and $P > 0.2604$, respectively): between pH 5.2 and 4.6 the β-lg concentration in the supernatant dropped from 85.2% to its minimum averaged value of 50.4 ± 5.5%, and increased afterward to 58.2 ± 2.4% at pH 4.4. However, the maximum precipitation of β-lg appeared at pH 4.6 and 200 μS[+].

β-lg composed the main part of the protein-precipitated fraction. The BSA did not seem to be affected during BMEA of 10% Bipro solution, while a small amount of α-la was precipitated at pH 4.6 and afterward. These results agreed with the literature (*9*, *18*) and previous results obtained for the soluble protein on the optimum precipitation pH of the whey protein at pH 4.65. In our previous work (*11*) a 40.5 ± 1.9% percentage of β-lg at pH 4.6 was obtained with a 25 °C normalized conductivity value of 107 μS/cm. The difference in β-lg precipitation observed between this work and the previous may be explained by the different final conductivity levels reached:

Table 1. Evolution of the Percent Total Peak Area of β-lg, α-la, and Bovine Serum Albumin in the Supernatant during 10% WPI Bipolar Membrane Electroacidification Ran at Different Modes and Levels of Conductivity Adjustment.

	pH	200 μS[1]	200 μS[+]	250 μS[1]	250 μS[+]
BSA	6.8	2.5 ± 1.0	2.9 ± 0.3	2.2 ± 1.1	3.3 ± 0.1
	6.0	2.3 ± 1.0	2.8 ± 0.1	2.1 ± 1.0	3.4 ± 0.2
	5.8	2.4 ± 1.2	3.0 ± 0.4	2.1 ± 1.1	3.3 ± 0.3
	5.6	2.4 ± 1.0	3.0 ± 0.1	2.2 ± 1.1	3.2 ± 0.1
	5.4	2.4 ± 1.0	2.9 ± 0.3	2.1 ± 1.0	3.2 ± 0.0
	5.2	2.2 ± 0.9	3.0 ± 0.3	2.0 ± 1.0	3.2 ± 0.2
	5.0	2.4 ± 1.1	3.1 ± 0.6	2.2 ± 1.0	3.2 ± 0.1
	4.8	2.5 ± 0.8	3.1 ± 0.4	2.2 ± 1.1	3.2 ± 0.2
	4.6	2.3 ± 1.0	2.9 ± 0.4	2.1 ± 0.9	3.4 ± 0.2
	4.4	2.5 ± 1.0	3.1 ± 0.3	2.0 ± 0.8	3.2 ± 0.1
α-la	6.8	11.0 ± 0.5	10.7 ± 0.0	11.3 ± 0.6	10.7 ± 0.2
	6.0	10.7 ± 0.2	10.4 ± 0.6	10.6 ± 1.1	10.8 ± 0.5
	5.8	10.9 ± 0.0	10.6 ± 0.4	10.8 ± 1.4	10.7 ± 0.6
	5.6	11.1 ± 0.7	10.9 ± 0.1	10.7 ± 1.1	10.6 ± 0.6
	5.4	10.9 ± 0.3	10.6 ± 0.2	10.7 ± 0.6	11.0 ± 0.4
	5.2	10.4 ± 0.4	10.4 ± 0.0	10.3 ± 1.0	10.6 ± 0.7
	5.0	10.8 ± 0.1	10.1 ± 0.5	10.4 ± 0.6	10.7 ± 0.0
	4.8	10.4 ± 0.7	10.5 ± 0.4	10.4 ± 0.8	10.4 ± 0.4
	4.6	10.3 ± 0.3	9.3 ± 0.4	10.0 ± 0.8	10.1 ± 0.2
	4.4	10.4 ± 0.1	9.7 ± 0.3	9.9 ± 0.6	10.0 ± 0.4
β-lg	6.8	86.5 ± 0.5	86.4 ± 0.3	86.5 ± 0.4	86.0 ± 0.1
	6.0	84.9 ± 3.4	84.0 ± 4.5	84.7 ± 10.1	87.5 ± 4.0
	5.8	86.0 ± 2.6	85.2 ± 3.8	85.3 ± 14.3	85.1 ± 3.2
	5.6	87.0 ± 4.6	87.4 ± 1.2	85.4 ± 10.6	85.8 ± 4.7
	5.4	86.0 ± 0.0	83.0 ± 2.3	84.9 ± 6.3	88.1 ± 3.5
	5.2	81.0 ± 1.0	83.2 ± 0.6	81.3 ± 9.3	83.2 ± 4.3
	5.0	69.0 ± 1.9	68.2 ± 2.4	68.9 ± 3.3	76.8 ± 9.1
	4.8	61.6 ± 0.1	65.4 ± 2.5	64.6 ± 6.7	65.7 ± 0.5
	4.6	52.2 ± 2.0	43.0 ± 1.7	56.1 ± 7.3	50.6 ± 4.7
	4.4	60.8 ± 3.2	56.9 ± 0.1	59.7 ± 7.0	55.6 ± 1.0

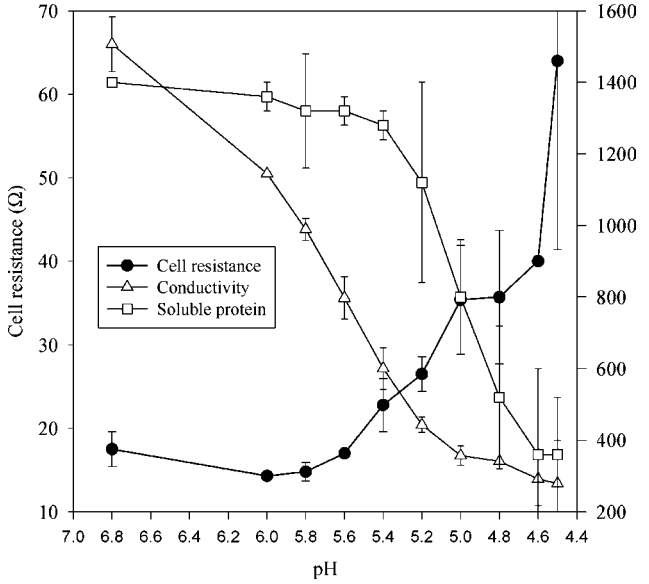

Figure 2. Evolution of the cell resistance, conductivity, and soluble protein during BMEA of 20% BiPRO.

200 or 250 μS/cm versus 107 μS/cm, respectively. The conductivity appears to influence strongly the extent of protein precipitation.

20% BiPRO Solution Electroacidification. Since the conductivity control of a 10% WPI solution allowed it to reach a lower pH than 4.6, a 20% (w/w) Bipro solution was electroacidified in the same way.

With conductivity control at 350 μS/cm, it was possible to reach pH 4.65 (**Figure 2**). From pH 6.8 to 5.6, the system resistance was stable at 18 Ω and increased in a linear fashion afterward from 18 Ω at pH 5.6 to 40 Ω at pH 4.6. As expected, with conductivity control, the system resistance was under 60 Ω at the end of the run at pH 4.6. After pH 4.6 was reached, the system resistance increased over 60 Ω since at pH 4.6 the conductivity was not controlled any more. Furthermore, a 27% protein precipitation was observed at pH 4.6. This value was higher than the 21% precipitated protein obtained previously for the same solution at pH 5.0 and at a normalized conductivity value of 161 μS/cm (*11*). However, this percentage was still low in comparison with the 46% protein recovery obtained with 10% Bipro solution at 200 μS[+].

The apparent viscosity of 10% and 20% Bipro solution was determined during chemical acidification with 1 N HCl. The apparent viscosity was measured at different shear rates: 10 and from 20 to 200 s^{-1} every 20 shear rate unit and at initial pH of Bipro solution (approximately pH 6.9) and from pH 6.0 to 4.4 every 0.2 pH unit. It appeared that the 10% Bipro solution should be modeled as a linear surface response regression (R^2 = 0.795, **Figure 3**) while the 20% Bipro solution should not (R^2 = 0.275, **Figure 4**). For a 10% Bipro solution, the apparent viscosity was quite stable at an averaged value of 17.5 ± 6.8 mPa·s (all shear rates and pH values combined). For a 20% Bipro solution, the apparent viscosity showed a drastic increase between pH 5.2 and 4.8 with a maximum at pH 5.0, whatever the shear rate (**Figure 4**).

Since the viscosity of the 20% BiPRO dispersion was very different in comparison with 10% BiPRO solution, the viscosity of the protein dispersion should have a real impact on the final precipitation yield. Some factors have an influence on the viscosity: particle size, polydispersity of diameters, and the electroviscous effect in the case of charged particles. Large monodispersed particles tend to give lower relative viscosities

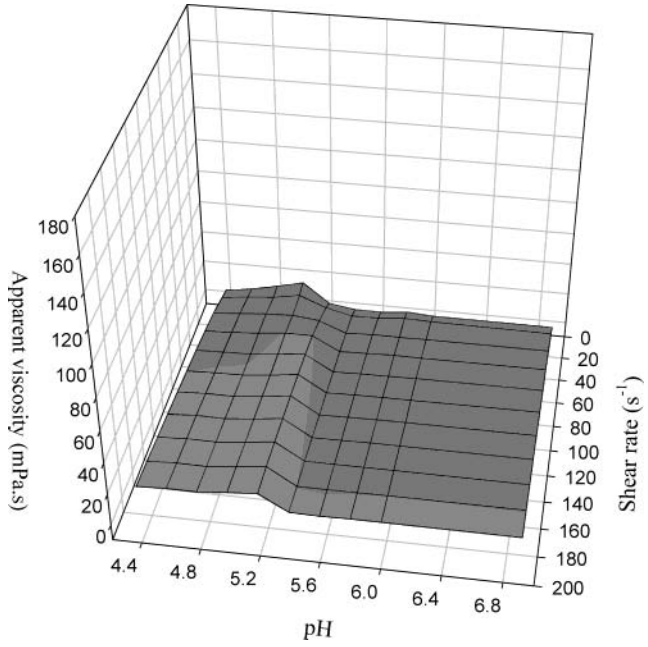

Figure 3. Evolution of the apparent viscosity of a 10% BiPRO solution as a function of pH and shear rate.

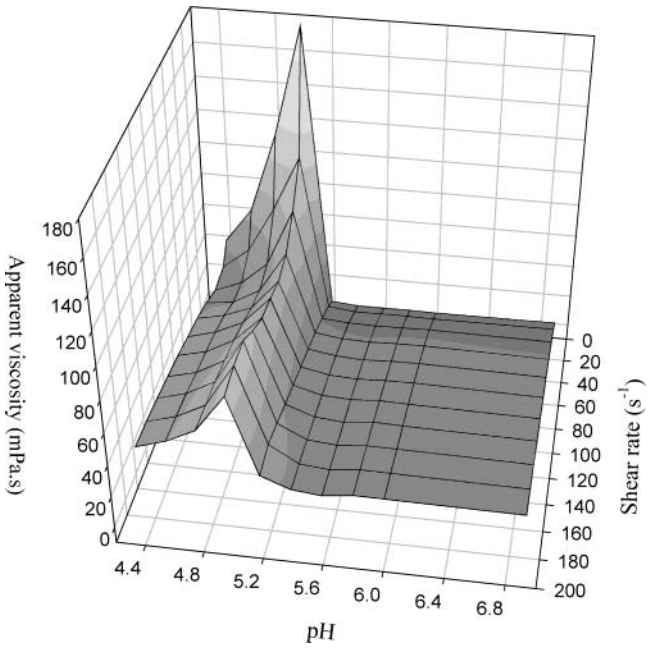

Figure 4. Evolution of the apparent viscosity of a 20% BiPRO solution as a function of pH and shear rate.

than smaller particles at equivalent volume fractions but the differences between them decrease as the mean diameter (*20*, *21*). This change in viscosity by the mean of one or a combination of the three factors would decrease the precipitation yield of β-lg. An increase in the voluminosity of the particles related by an increase in viscosity would slow the migration or diffusion of the β-lg and consequently its aggregation with another monomer or dimer.

It appeared from these results that the extent of precipitation for a 20% BiPRO solution was influenced not only by the conductivity level at pH 4.6, but probably also by the viscosity of the protein solution at this pH. In fact, whey proteins give Newtonian dispersions in medium range concentrations up to 12% (*22*). The Newtonian protein dispersion may be considered as a suspension of spherical noninteracting, nondeformable

particles. Then the viscosity of these dispersions may be treated as such on the basis of the simple hydrodynamic model presented in the literature (*23*). However, for protein dispersion over 12% this model does not fit the reality. For protein concentration higher than 12%, the viscosity of the protein dispersion should influence the precipitation behavior of the proteins. Moreover, since proteins are charged particles, the presence of charges on these particles would increase the viscosity of their dispersions (*24*).

CONCLUSION

The percent soluble protein was very sensitive to conductivity and pH values. The highest protein precipitation was obtained at pH 4.6 and 200 μS[+], with a 46% precipitation of the total protein. One global [1] addition of 1 M KCl decreased the protein precipitation rate by resolubilizing protein. β-lg composed the main part of the protein precipitated fraction. The difference in β-lg precipitation observed in this work may be explained by the different final conductivity levels reached.

With conductivity control at 350 μS/cm, it was possible to reach pH 4.65, the optimium pH for the whey protein precipitation. However, the 27% protein precipitation was still low in comparison with the 46% protein recovery obtained with 10% Bipro solution at 200 μS[+]. Since the viscosity of the 20% whey protein dispersion was very different and presented a non-Newtonian profile, the change in viscosity as pH decreases observed at 20% WPI would decrease the precipitation rate of β-lg. Moreover, the increase of viscosity should have a direct consequence on the intern hydrodynamic design of the BMEA cell.

A study is currently under way on the changes in particle size and viscosity during chemical and electrochemical acidification to understand the precipitation behavior of high protein concentration solutions during BMEA and to improve the hydrodynamics of the cell.

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