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Rheological Behavior of WPI Dispersion as a Function of pH and Protein Concentration

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Physical and flow properties of proteins can provide information necessary for the optimal design of unit processes and quality control of the manufacturing process and final products. Therefore, the purpose of this investigation was to characterize the rheological behavior of a whey protein isolate (WPI) (BiPRO) dispersion as a function of pH and protein concentration. A rotational viscometer was used to determine the apparent viscosity, shear rate, and shear stress of WPI dispersions. Both the consistency index (k) and the flow behavior index (n) were sensitive to changes in pH and protein concentration. Mathematical relations obtained from experimental values of k and n allowed the determination of a model for apparent viscosity (η) of WPI dispersions as a function of pH and protein concentration. At 5 and 10% BiPRO, whatever the pH, the rheological behavior appeared to be a newtonian fluid, while at 20% BiPRO, the rheological behavior appeared to be a nonnewtonian pseudoplastic fluid. Furthermore, at 20% Bipro, the apparent viscosity presented an increase in viscosity from 5.6 to 5.4, followed by a decrease from pH 5.4 to 5.0 at all shear rates. The highest viscosity was obtained at 20% pH 5.4, with an approximate value of 0.25 Pa.s, 10 times higher than the one obtained at 5 and 10% BiPRO.

KEYWORDS: Whey protein isolate; apparent viscosity; model; pH; protein concentration; rheological behavior

INTRODUCTION

Some proteins are known to form large aggregates, sometimes up to several microns in diameter, without phase separation at relatively high concentrations (*1*). Low concentrations, usually below 1%, are used in scientific works to avoid interference from aggregation. However, proteins are often used or treated in food industry at concentrations favoring aggregation (*2*). The rheological properties of protein solutions are governed by composition, molecular mass, size, shape, flexibility, degree of hydration, and intermolecular interactions (*3*). Most of these factors are in turn influenced by concentration, temperature, pH, ionic strength, and previous processing treatments (*4*, *5*). Intermolecular interactions between protein molecules may be especially important with respect to rheological properties: Proteins are charged particles, and it was shown that the presence of charges on particles increases the viscosity of dispersions (*6*).

Rheological properties of whey protein concentrate (WPC) solutions have been investigated by many workers (*7*−*11*), but a very small number of studies appears on whey protein isolate (WPI) dispersion. Furthermore, recently, Bazinet et al (*12*) demonstrated the feasability of bipolar membrane electroacidification (BMEA) for whey protein separation from a WPI solution and the influence of the initial protein concentration on the purity and yield of the separated fraction. At 5% WPI initial concentration, this technology allows the separation of 98% pure β-lactoglobulin (β-lg) fraction with a 44.0% recovery yield. However, with a 20% WPI solution, it was possible to reach pH 4.65 with conductivity control at 350 μS/cm, but protein precipitation was still low in comparison with 5% WPI (*13*). The changes in viscosity as pH decreases observed at 20% WPI would decrease the final precipitation rate of β-lg, since the viscosity of the 20% WPI dispersion was very different. Consequently, the design of the spacers actually used in BMEA for protein precipitation was not adapted. In fact, the change in protein conformation and aggregation of these proteins may lead to a fouling of the spacers, as already observed by Bazinet et al. (*14*).

In this context, flow properties of proteins can provide information necessary for the optimal design of BMEA unit process. Therefore, the purpose of this investigation was to characterize the rheological behavior of a WPI dispersion as a

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Table 1. Effect of Protein Concentration (5, 10, and 20%) and pH (from pH 6.0 to 4.0) on Consistency Index (k) and Flow Behavior Index (n) of WPI Solutions

	5%			10%			20%		
	k[a]	n[a]	r^2	k	n	<r^2	k	n	r^2
6.0	0.069 ± 0.014	0.378 ± 0.011	0.69	0.038 ± 0.006	0.400 ± 0.036	0.92	0.062 ± 0.009	0.579 ± 0.035	0.93
5.8	0.094 ± 0.007	0.304 ± 0.015	0.85	0.030 ± 0.003	0.433 ± 0.049	0.91	0.039 ± 0.012	0.665 ± 0.049	0.95
5.6	0.081 ± 0.038	0.391 ± 0.007	0.73	0.042 ± 0.004	0.361 ± 0.006	0.92	0.038 ± 0.011	0.739 ± 0.077	0.96
5.4	0.053 ± 0.012	0.381 ± 0.111	0.85	0.040 ± 0.024	0.449 ± 0.143	0.83	0.676 ± 0.886	0.526 ± 0.271	0.21
5.2	0.060 ± 0.003	0.378 ± 0.039	0.83	0.046 ± 0.034	0.494 ± 0.141	0.56	0.383 ± 0.077	0.432 ± 0.043	0.97
5.0	0.070 ± 0.021	0.303 ± 0.071	0.83	0.038 ± 0.270	0.515 ± 0.182	0.74	0.127 ± 0.049	0.539 ± 0.084	0.94
4.8	0.056 ± 0.021	0.367 ± 0.051	0.64	0.023 ± 0.010	0.607 ± 0.089	0.88	0.081 ± 0.004	0.577 ± 0.013	0.98
4.6	0.048 ± 0.004	0.385 ± 0.030	0.97	0.030 ± 0.007	0.553 ± 0.039	0.96	0.093 ± 0.039	0.485 ± 0.104	0.89
4.4	0.037 ± 0.007	0.420 ± 0.040	0.95	0.023 ± 0.009	0.599 ± 0.071	0.95	0.088 ± 0.004	0.508 ± 0.028	0.88
4.2	0.041 ± 0.004	0.396 ± 0.018	0.93	0.023 ± 0.011	0.556 ± 0.027	0.86	0.103 ± 0.033	0.555 ± 0.084	0.89
4.0	0.040 ± 0.004	0.425 ± 0.026	0.98	0.022 ± 0.011	0.545 ± 0.067	0.93	0.130 ± 0.009	0.454 ± 0.042	0.84

[a] Mean ± standard deviation.

function of pH and protein concentration. This study has three objectives: (*1*) to study the effect of pH and protein concentration on the power law parameters (k and n) (*2*), to study the effect of pH and protein concentration on the apparent viscosity of WPI dispersion, and (*3*) to compare the model for apparent viscosity as a function of pH and protein concentration to the experimental data. The emphasis was to develop relationships that could be useful in engineering applications.

MATERIALS AND METHODS

Preparation of Dispersions. A commercially available WPI powder (BiPRO) was obtained from Davisco Foods International Inc. (Eden Prairie, MN). Bulk WPI solutions of 6.25, 12.5 and 25% (w/w) were prepared by placing a preweighed sample into a flask containing double-distilled water, mixing with a magnetic stirrer until dissolution was complete, and then storing overnight at 4 °C prior to testing to ensure that solution structure was in the fully recovered state. Samples for the rheological study were prepared as follows: 200 g of the bulk solution (initial pH ranging between 6.9 and 7.0) were acidified with 1.000 N HCl (VWR Scientific Products, West Chester, PA) to the required pH (from pH 6.0 to 4.0 by 0.2 pH unit). The final weight was adjusted to 250 g, such that final WPI concentrations were 5, 10, and 20% BiPRO (w/w). The experiment was carried out in triplicate.

Rheological Measurement. A rotational viscometer (Rheolyst, model AR 1000-N, TA Instruments Ltd., Leatherhead, England) equipped with a 60-mm 2° steel cone (truncation 46 μm, TA Instruments Ltd.) was used to determine the apparent viscosity, shear rate, and shear stress of the WPI dispersions laying on the rheometer plate, the temperature of which was automatically controlled at 22.2 °C. A computer controlled program (Rheology Advantage Instrument Control AR, version 1.0.71, TA Instruments Ltd.) in a rotational mode was used to shear samples at a linear rate from 5 to 100 s^{-1}. Shear stress (τ)−shear rate (γ) data were gathered as rheograms. Apparent viscosities were calculated at each shear rate for each combination of pH and protein concentration.

Data Analysis. Rheograms were evaluated using the power law rheological model. The consistency index (k) and the flow behavior index (n) were evaluated using a modified Turian approach (*15*) through a regression analysis of log(shear stress) versus log(shear rate), where the consistency index (k) and flow behavior index (n) represent the intercept on the shear stress axis and the slope of the linear regression, respectively. The effect of the pH and protein concentration on the consistency index (k) and flow behavior index (n) were evaluated by use of TableCurve 3D (Automated surface fitting and equation discovery, Version 3.0 for Windows 95 and NT, SPSS Inc., Chicago, IL). For k and n index, the more realistic equation fitting for both indexes was determined on the basis of the best coefficient of determination (r^2) and higher F-value of the analysis of variance performed by TableCurve 3D. For the apparent viscosity, the difference between the predicted data and the experimental data was evaluated using the determination coefficient, calculated from the square of the Pearson's coefficient.

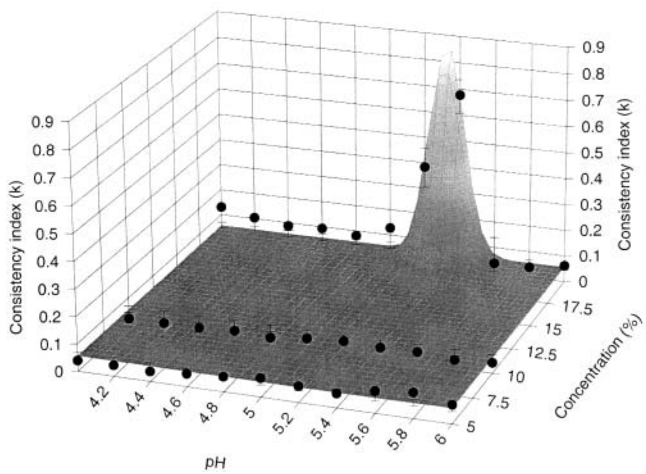

Figure 1. Evolution of the consistency index (k) as a function of pH and protein concentration during chemical acidification.

RESULTS AND DISCUSSION

Rheological Models and Effect of pH and Protein Concentration on Flow Parameters. The power law model was applied to characterize the flow behavior of the WPI dispersion in the different conditions of pH and protein concentration. **Table 1** shows the means and standard deviations of the power law parameters. Coefficient of determination (r^2) was calculated and for most cases was over 0.8 and indicated a good fit for both models. Except for 20% BiPRO and pH 5.4, the r^2 was under 0.5, while for 5% pH 6.0, 5.6, and 4.8 and 10% pH 5.2 and 5.0, the r^2 was lower than 0.8 but over 0.56. The pH values of 4.8, 5.2, and 5.4 seem to be transition pH for the rheological behavior of the WPI dispersion at 5, 10, and 20% BiPRO, respectively. Aggregates appeared in the soluble phase of the dispersion.

Consistency Index (k). The consistency index was modeled using TableCurve 3D as the following equation (**Figure 1**):

$$k_{(\mathrm{pH},[\mathrm{Prot}])} = 0.0564 + 0.8111\exp\left\{-0.5\left[\left(\frac{\mathrm{Ln}\frac{\mathrm{pH}}{5.33}}{-0.0179}\right)^2 + \left(\frac{[\mathrm{Prot}]-20}{-1.022}\right)^2\right]\right\} \quad (1)$$

Table 2 shows the results of the analysis of variance. It appeared that the fit of the data by a Log-Normal(pH) and

Table 2. Numeric Summary of the Analysis of Variance Performed by Table Curve 3D on the Consistency Index (k) Model Equation

$$k_{(\mathrm{pH,[Prot]})} = a + b\exp\left\{-0.5\left[\left(\frac{\mathrm{Ln}\left(\frac{\mathrm{pH}}{c}\right)}{d}\right)^2 + \left(\frac{[\mathrm{Prot}] - e}{f}\right)^2\right]\right\}$$

r^2 coef det	DF Adj r^2	fit std err	F-value
0.9435	0.9304	0.0320	90.2139

source	sum of squares	DF	mean square	F statistic	$P > F$
regression	0.46207	5	0.09241	90.2139	0.00001
error	0.02765	27	0.00102		
total	0.48972	32			

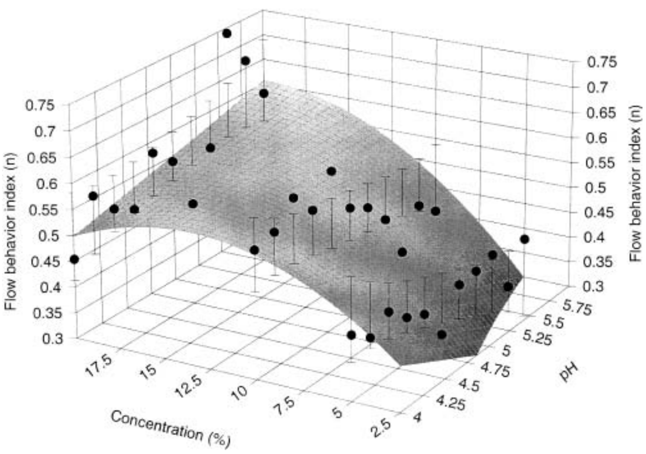

Figure 2. Evolution of the flow behavior index (n) as a function of pH and protein concentration during chemical acidification.

Gaussian (protein concentration) was very good, with an r^2 value of 0.94 and an F-stat value of 90.21. Furthermore, according to the residuals of the consistency index, the difference between the predicted data and the experimental data were realistic, and we were able to conclude that there was a good adequation between the model and the experimental data. From these results, it appeared that the consistency index was stable at an average value of 0.045 ± 0.019 Pa.s^n between 5 and 10% BiPRO, whatever the pH, while at 20% BiPRO, k reached maximum value between pH 5.4 and 5.2, 0.676 ± 0.886 Pa.s^n and 0.383 ± 0.077 Pa.s^n, respectively (**Table 1**). The standard deviation observed at 20% pH 5.4 was important and characterized a complete destabilization of the solution at this pH, reflected by an increase in the k index: This explained the low r^2 obtained at this combination of factors.

Flow Behavior Index (n). The flow behavior index (n) was modeled as the following equation (**Figure 2**):

$$n_{(\mathrm{pH,[Prot]})} = 0.8386 - 0.1416\mathrm{pH} + 0.0014[\mathrm{Prot}] + 0.0020\mathrm{pH}^2 - 0.0013[\mathrm{Prot}]^2 + 0.0087\mathrm{pH}\cdot[\mathrm{Prot}] \quad (2)$$

Table 3 shows the results of the analysis of variance. The data fitted by the model equation was good, with an r^2 value of 0.65 and an F-stat value of 10.27. Furthermore, according to the residuals of the flow behavior index, the difference between the predicted data and the experimental data were realistic for a biological model. These results indicate that the flow behavior index increased mainly with an increase in protein concentration; All pH values averaged in the flow behavior index values are 0.375 ± 0.039, 0.501 ± 0.081, and 0.550 ± 0.089 at 5, 10, and 20% BiPRO respectively (**Table 1**). Furthermore, the maximum n values were obtained at high pH and high protein concentration values.

Table 3. Numeric Summary of the Analysis of Variance Performed by Table Curve 3D on the Flow Behavior Index (n) Model Equation: $n_{(\mathrm{pH,[Prot]})} = a - b\cdot\mathrm{pH} + c\cdot[\mathrm{Prot}] + d\cdot\mathrm{pH}^2 - e\cdot[\mathrm{Prot}]^2 + f\cdot\mathrm{pH}\cdot[\mathrm{Prot}]$

r^2 coef det	DF Adj r^2	fit std err	F-value
0.6555	0.5760	0.0660	10.2747

source	sum of squares	DF	mean square	F statistic	$P > F$
regression	0.22408	5	0.04482	10.2748	0.00001
error	0.11777	27	0.00436		
total	0.34185	32			

Since the contribution of [Prot] and pH^2 was extremely low on the variation of the flow behavior index, as shown in **Figure 3**, eq 2 was simplified as follows:

$$n_{(\mathrm{pH,[Prot]})} = 0.8386 - 0.1416\mathrm{pH} - 0.0013[\mathrm{Prot}]^2 + 0.0087\mathrm{pH}\cdot[\mathrm{Prot}] \quad (3)$$

Apparent Viscosity Model and Effect of pH and Protein Concentration. Since the apparent viscosity is a function of the shear stress (τ) and the shear rate (γ), as shown in eq 4

$$\eta = \frac{\tau}{\gamma} \quad (4)$$

and according to power law equation (*16*)

$$\tau = k\cdot\gamma^n \quad (5)$$

by replacing the value of τ in eq 4 as calculated in eq 5, it can be deduced that

$$\eta = \frac{k\cdot\gamma^n}{\gamma} \quad (6)$$

or by simplification of eq 6

$$\eta = k\cdot\gamma^{(n-1)} \quad (7)$$

Finally, when the modeled values in eq 7 of k (eq 1) and n (eq 3) are replaced, eq 7 becomes

$$\eta_{(\mathrm{pH,\,[Prot]})} = k_{(\mathrm{pH,\,[Prot]})}\cdot\gamma^{(n_{(\mathrm{pH,\,[Prot]})}-1)} \quad (8)$$

or more precisely

$$\eta_{(\mathrm{pH,[Prot]})} = \left\{0.0564 + 0.0811\exp\left\{-0.5\left[\left(\frac{\mathrm{Ln}\left(\frac{\mathrm{pH}}{5.33}\right)}{\text{-0.0179}}\right)^2 + \left(\frac{[\mathrm{Prot}] - 20}{-1.022}\right)^2\right]\right\}\right\}\cdot\gamma^{(-0.1614-0.1416\mathrm{pH}-0.0013[\mathrm{Prot}]^2+0.0087\mathrm{pH}\cdot[\mathrm{prot}])} \quad (9)$$

Equation 9 gives a model of the apparent viscosity (in Pa.s) as a function of pH and protein concentration. Explanation of the experimental data by the model was adequate, as confirmed by the r^2 calculated for each protein concentration; $r^2 = 0.66$ at 5% BiPRO (**Figure 4**), $r^2 = 0.80$ at 10% BiPRO (**Figure 5**) and $r^2 = 0.85$ at 20% BiPRO (**Figure 6**). Since the main

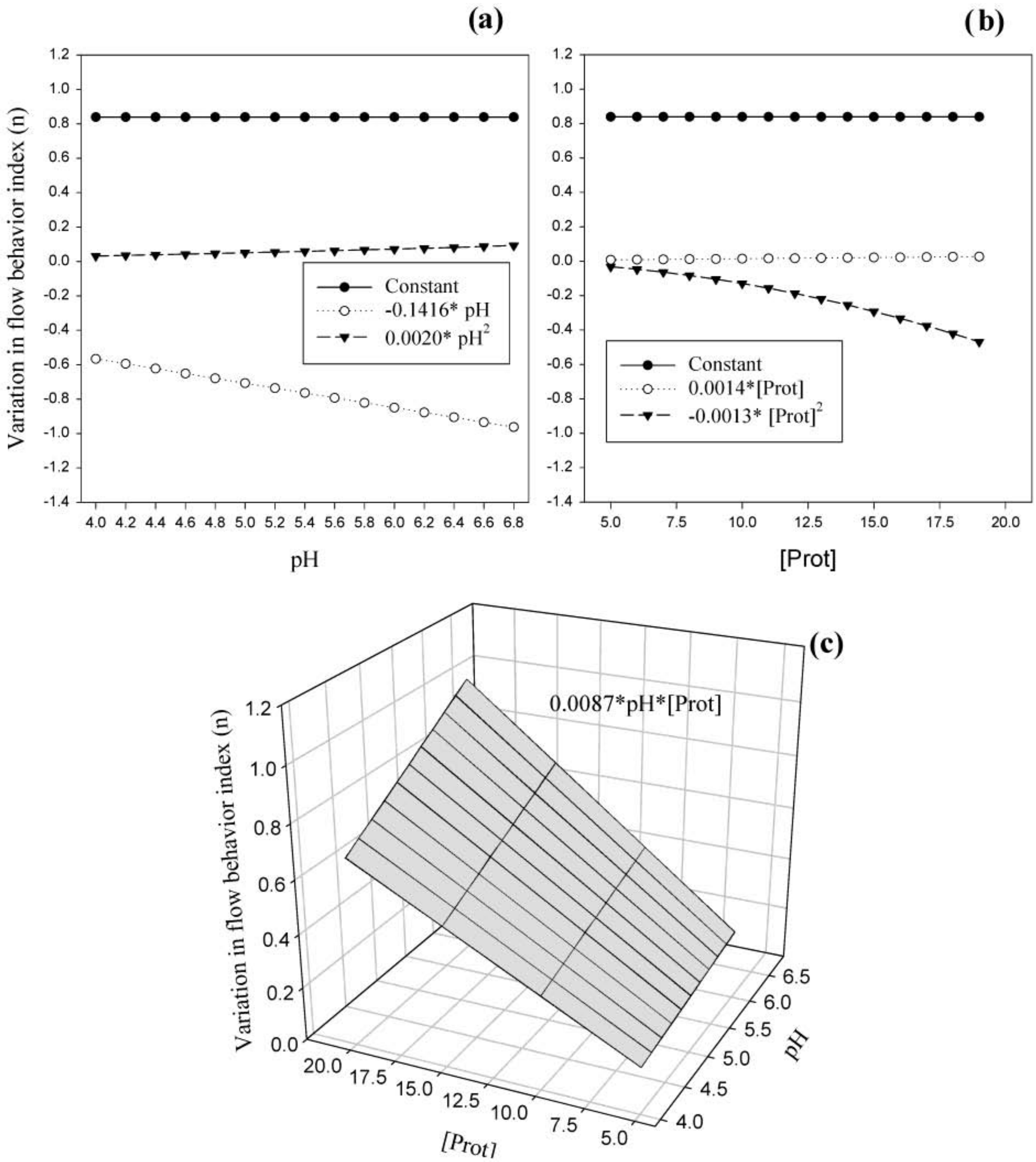

Figure 3. Relative contribution of (**a**) pH, pH^2; (**b**) [Prot], [Prot]^2; and (**c**) pH × [Prot] variables on the flow behavior index (*n*).

variations in viscosity appeared at 20% BiPRO, the model explained in a better way the experimental data obtained at higher concentrations than at lower concentrations. It appeared that the rheological behavior of the WPI dispersion depends mainly on the protein concentration. At 5 and 10% BiPRO in a shear rate range from 5 to 100 s^{-1} whatever the pH, the evolution of the apparent viscosity was relatively similar, with highest viscosity values of about 0.025 Pa.s. The rheological behavior was apparently a Newtonian fluid, which means that the apparent viscosity was independent of shear rate. At 20% BiPRO, the evolution of the apparent viscosity was completely different from lower protein concentration. The rheological behavior was apparenting a non-Newtonian pseudoplastic fluid, which means either that the apparent viscosity decreases when the shear rate is increased or that the fluid is shear thinning in nature. The highest viscosity was obtained at pH 5.4 with an approximate value of 0.25 Pa.s, 10 times higher than the one obtained at 5 and 10% BiPRO. Furthermore, the increase in apparent viscosity observed from 5.6 to 5.4 and the decrease from pH 5.4 to 5.0 was present at all shear rates, while it was not observed at lower concentrations.

These results agreed with those of Tang et al. (*9*) concerning the effect of protein concentration on the rheological behavior. They observed, with WPC at pH 7.0 in a shear rate range from 10 to 240 s^{-1}, that at protein concentrations $\leq$10%, solutions were Newtonian. At concentrations of 15−30%, apparent viscosity decreased slightly with shear rate at low shear rates, indicating slight shear thinning. However, Tang et al. (*9*) demonstrated that flow curves for 10, 20, and 30% solutions could all be fitted by power equations as eq 4, and that deviation from Newtonian behavior >10% increased slightly (lower *n*) as concentration increased. The value of *k*, which is a measure

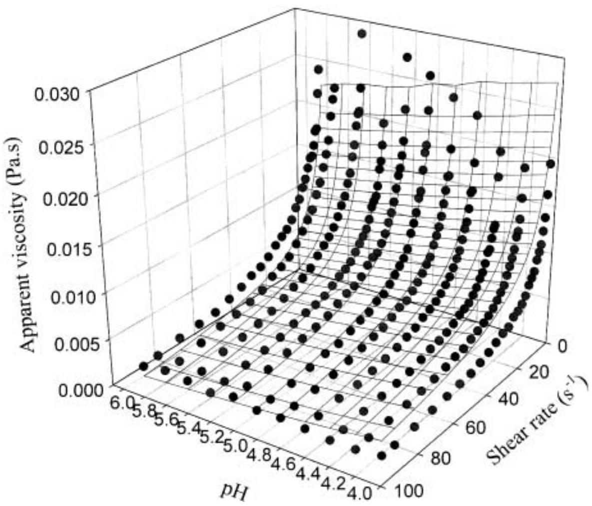

Figure 4. Evolution of the apparent viscosity (η) as a function of pH and shear rate during chemical acidification of a 5% WPI concentration.

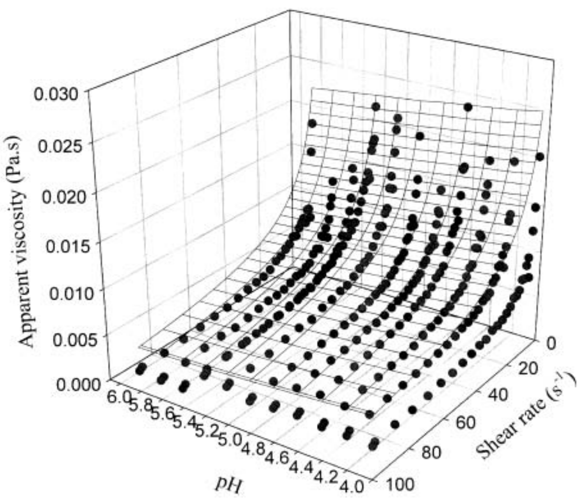

Figure 5. Evolution of the apparent viscosity (η) as a function of pH and shear rate during chemical acidification of a 10% WPI concentration.

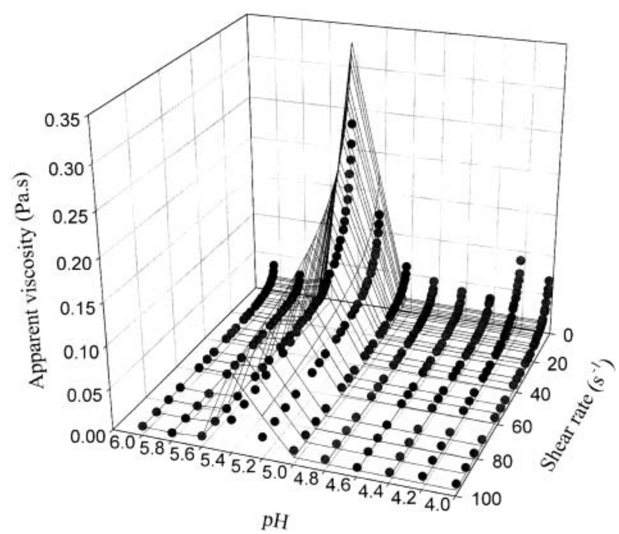

Figure 6. Evolution of the apparent viscosity (η) as a function of pH and shear rate during chemical acidification of a 20% WPI concentration.

of viscosity, increased markedly with concentration (*9*). These characteristics are typical of pseudoplastic liquids. According to Pradipasena and Rha (*17*), the strong dependence of apparent viscosity on shear rate at high protein concentration may be explained by the disaggregation of particles, due to shearing, occurring at a rate higher than the normal formation of aggregates as a result of Brownian motion. The shear thinning exhibited at concentrations >10% is usually attributed to two phenomena for protein solutions: (1) progressive orientation of protein molecules in the direction of flow with deformation or removal of the protein hydration sphere, and (2) rupture of weak bonds such as hydrogen and ionic bonds resulting in dissociation of protein aggregates or networks (*4*, *18*). Both phenomena could be caused by hydrodynamic interaction. According to Tang et al. (*9*), the second phenomena was probably dominant for WPC solutions since shear thinning were more marked at high protein concentrations.

Table 4. Comparison of BiPRO Composition with Those of Whey Protein Concentrates (WPC) Used by Tang et al. (1993) and Rattray and Jelen (1995) for Their Rheological Studies

	BiPRO	Tang et al. (1993)	Rattray and Jelen (1995)		
	WPI	WPC	WPC 132	WPC 312	WPC 472
protein (g/100 g)	93.1	79.8	81.5	78.7	79.6
moisture (g/100 g)	4.9	4.4	4.2	4.2	3.7
lactose (g/100 g)	< 0.1	4.6	6.2	10.8	8.0
fat (g/100 g)	0.3	7.1	4.9	3.3	6.1
ash (g/100 g)	1.8	1.5	2.5	3.9	3.6

Concerning the effect of pH on the apparent viscosity, Tang et al. (*9*) observed that the apparent viscosity of 10% WPC solutions in the pH range 4–8 was only slightly dependent on solution age up to 168.5 h after preparation. These results confirmed our results at low protein concentration. However, they observed that the apparent viscosity of 20% solutions under similar conditions to the above was also independent of solution age in the same pH range up to 4.5 h after preparation. Moreover, Rattray and Jelen (*10*) concluded that from pH 6.8 to 4.0 at 20 °C, WPC dispersions containing 11 or 20% (w/v) protein prepared from three different WPC powders displayed low viscosity with negligible variations (averaged values of 0.017, 0.013, and 0.022 Pa.s for WPC 132, 312, and 472, respectively). This was in contradiction with our results. This difference in rheological behavior should be due to the composition difference of the protein source (**Table 4**). In our experiment, a WPI was used, while in the experiments of Tang et al. (*8*, *9*), a WPC was used. The difference in purity, mainly the difference in salts, could explain this difference. In their colloid chemical approach of the effects of ionic strength on the solubility of whey protein products, de Wit and Van Kessel (*3*) demonstrated that at pH 4.6, significant differences appeared in the pH of minimum solubility between WPCs and WPIs. WPI showed minimum solubility near the isoionic point of β-lactoglobulin A, the major protein component, (pH 5.2) which differed sufficiently from denaturation and salting-in effects at pH 4.6. WPC having $\geq$80% protein on total solids showed minimal solubility in the pH range 4.6–5.0, which coincided with insolubility induced by whey protein denaturation. Furthermore, these features seemed to be related to specific adsorption of multivalent anions on the acid side of the β-lg isoionic point already mentioned by Taylor (*19*).

Conclusion. We can conclude from the data presented in this study that the rheological behavior of WPI dispersions is dependent on protein concentration. At 5 and 10% BiPRO, whatever the pH, the rheological behavior appeared to be a newtonian fluid, while at 20% BiPRO, the rheological behavior appeared to be a nonnewtonian pseudoplastic fluid. The highest viscosity was obtained at 20% pH 5.4, with an approximate value of 0.25 Pa.s, 10 times higher than the one obtained at 5 and 10% BiPRO. Furthermore, at 20% BiPRO, the apparent viscosity presented an increase in viscosity from 5.6 to 5.4, followed by a decrease from pH 5.4 to 5.0 at all shear rates, which was not observed at 5 and 10% BiPRO.

The different mathematical relations obtained from experimental values of k and n allowed the determination of a model for η of WPI dispersions as a function of pH and protein concentration. The pH and protein concentration dependency of k and n was modeled using a modified Turian approach. Both k and n were sensitive to changes in pH and protein concentration. Moreover, the comparison of predicted and experimental data for apparent viscosity as a function of pH and protein concentration, by obtaining high r^2 for each protein concentration, has confirmed the validity of the apparent viscosity model.

These data on physical and flow properties of whey protein isolates will provide information necessary for the optimal design of BMEA spacers and more generally unit processes, quality control of the manufacturing process, and final products, for BMEA protein precipitation and on suitable fields of application for a new protein product and of some limited use in providing information about changes in the molecular structure.

ABBREVIATIONS USED

k, consistency index (Pa.s^n); τ, shear stress (Pa); γ, shear rate (s^{-1}); η, apparent viscosity (Pa.s); n, flow behavior index; [Prot], protein concentration (%); r^2, coefficient of determination; F, test of Fisher value; WPI, whey protein isolate; WPC, whey protein concentrate; BMEA, bipolar membrane electroacidification; β-lg, β-lactoglobulin

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