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Solubility and Viscous Properties of Casein and Caseinates

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ABSTRACT

An integrated study was conducted on the effects of temperature, pH, salt type and concentration on the functionality of sodium caseinate, calcium caseinate and rennet casein. At $5.6 \leq pH \leq 6.2$, all proteins exhibited greatest solubility when sodium phosphate was added. Viscosities under these conditions were one to two orders of magnitude greater than solutions at pH < 4.6. Protein solutions and viscosities with added sodium phosphate were not temperature dependent.

INTRODUCTION

MILK PROTEINS are highly desirable as ingredients in foods. In addition to availability and high nutritive value, their utility is enhanced by the bland products they provide. Casein, the major protein component of cows' milk, and its caseinate derivatives, have physicochemical, functional and nutritive properties which make them useful worldwide (Southward, 1985). High concentrations of caseins have exceptional water binding capacity, fat emulsification properties, whipping ability, and are viscous and soluble in neutral or alkaline conditions (Jonas et al., 1976). These proteins are currently used in a variety of products, including coffee whiteners, cheese analogs and meat products.

The application of this, or any protein system, to a food product requires knowledge about its flow properties under various conditions. This information allows for the optimization of processing or transport operations such as mixing, pumping or spray-drying and for efficient investigations into the modification of functional properties to achieve the desired effects (Tung, 1978; Southward, 1985). The viscosity and solubility (two important properties for characterizing functionality) of casein and caseinates have been reported (Muller and Hayes, 1963; Hayes et al., 1968; Fox and Mullvihill, 1982; Hooker et al., 1982). The vast majority of this information, however, related only to effects of individual parameters such as temperature, pH, added salts and salt concentrations.

Generally, concentration is linear with and directly proportional to the logarithm of viscosity over a broad concentration range (Hermansson, 1975). Hayes et al. (1968) reported that relatively high concentrations (20%) of calcium caseinate and sodium caseinate showed an inverse relationship of viscosity with pH. The addition of calcium (0.05–1.5% w/w) to either of these protein solutions at pH 5.4 resulted in a decrease in viscosity in the range 30–55°C (gelation was observed above 55°C). Hermansson (1975) showed that the addition of sodium chloride (0.2–1.0M) to calcium or sodium caseinate (10–12% protein concentration, pH 6.5) considerably increased the viscosity. No salt effects were observed below 10% protein. By evaluating the viscosity at different shear rates, she noted an almost Newtonian caseinate flow behavior. Indications were that the increase in viscosity was more likely due to protein-water interactions than strong protein interaction forces. In studies with calcium caseinate, Roeper (1977) observed a rapid decrease in viscosity as the temperature was raised from 30 to 60°C. Hayes et al. (1968) reported a similar response with sodium caseinate at pH 6.6 to 7.2. Research has been reported on chemical and enzymatic modifications of the casein/casinates (Muller and Hayes, 1963; Salzberg and Simonds, 1965; Hooker et al., 1982) for viscosity and solubility adjustment; however, most of that work was aimed at industrial applications.

The objective of our study was to investigate, in an integrated fashion, the properties of various commercial caseins and caseinates. The parameters tested were temperature, pH, salt type and salt concentration. The effect of these parameters and their interactions on solubility and viscosity of the protein solutions were determined.

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MATERIALS & METHODS

Materials

Commercial (New Zealand Milk Products, Inc.) calcium caseinate (CaCA) Alanate* 310 (89.8% protein, 4.1% ash, 4.5% water, 1.1% fat and 0.1% lactose); sodium caseinate (NaCA) Alanate 130 (82.4% protein, 4.3% ash, 9.0% water, 1.1% fat and 0.1% lactose); and rennet casein (ReCN) Alanate 771 (80.6% protein, 7.8% ash, 11.0% water, 0.5% fat and 0.1% lactose) were used to prepare the solutions/dispersions. The proximate analyses are from manufacturer specifications.

Methods

Fifteen percent casein(ate) solutions were prepared by mixing the protein powder with crushed ice and the appropriate amount of salt in a food processor. Salts were NaCl, $CaCl_2$ and NaH_2PO_4 at concentrations of 0.05, 0.15, 0.30, 0.45 or 0.55M. The mixing method (Strange and Konstance, 1990), for rapid and uniform dispersion, used a Cuisinart* food processor (750 mL bowl capacity) equipped with a stainless steel blade rotating at 1790 rpm. Statistical analysis of the moisture distribution and homogeneity of the moisture variance showed complete dispersion in less than eight minutes.

The pH was adjusted to 3.0, 3.6, 4.6, 5.2 or 6.0 on the fully dispersed solution using measured amounts of either 1N HCl or 1N NaOH. When the desired pH was reached, the appropriate amount of glass distilled water was added to bring the samples to 10% protein concentration. The samples were then incubated at 20, 28, 40, 52 or 60°C for 18 hr in water baths. Upon removal, samples were immediately centrifuged at room temperature at 1000 × *g* for 20 min. Supernatants were decanted for subsequent determination of viscosity and protein concentration.

Analysis

The viscosity of the supernatant protein solutions was determined, in triplicate, using a coaxial cylinder viscometer (Brookfield Model LVT*) at 25 ± 0.25°C and at shear rates of 3–40 s^{-1}. Over this range of shear rates the solutions were essentially Newtonian in behavior. A UL adapter was used to provide precise and accurate measurements of the low viscosity materials. The instrument was calibrated in the viscosity range of interest (1.0 to 101.4 centipoise).

Protein concentrations were determined using a Shimadzu Model UV240* spectrophotometer, by absorbance at 280 nm, assuming 0.86 absorbance units/mg casein/mL as calculated from data reported by Eigel et al. (1984).

Statistical design

A central composite design, with two qualitative variables (protein and salt type) and three quantitative variables (salt concentration, pH and temperature) was used to evaluate the effect of these design variables on the response variables: solubility and viscosity. Statistical analysis of the full model was accomplished using the General Linear Methods procedure, and step-wise regression analysis was used for the reduced model generation (SAS, 1985).

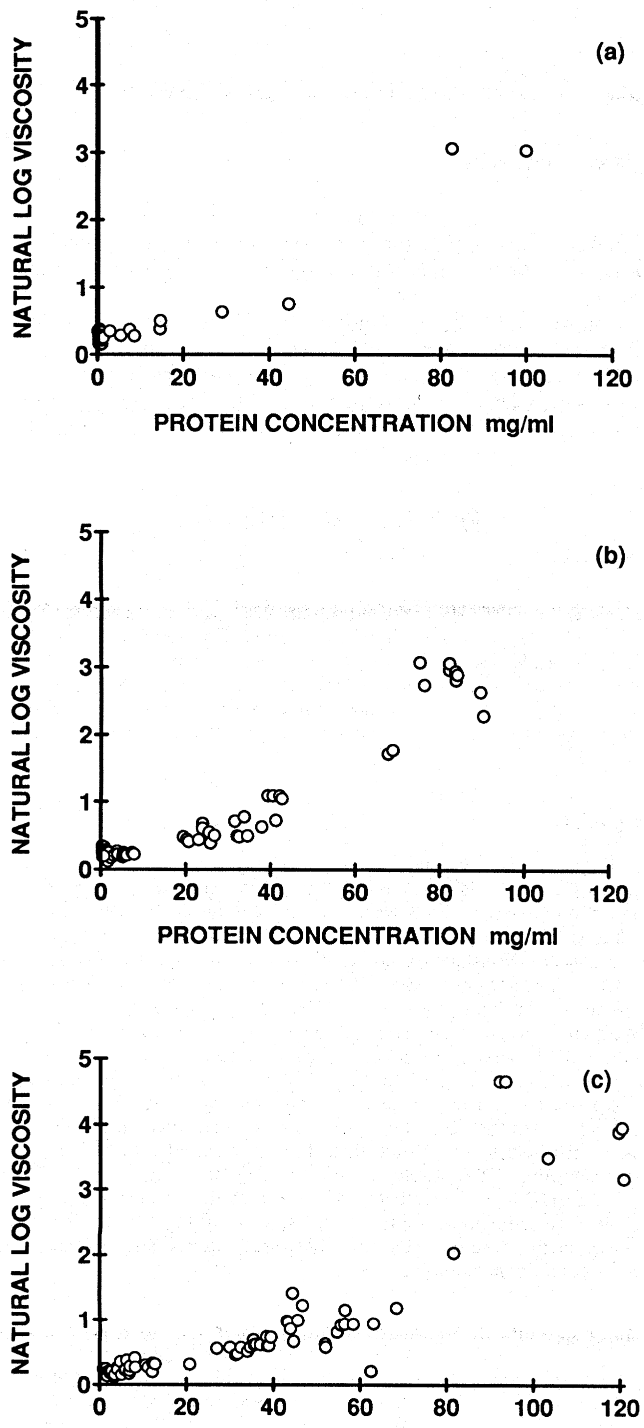

Fig. 1—Effect of protein concentration on natural log of viscosity. Effects of casein(ate) type. (a) Rennet casein (ReCN); (b) sodium caseinate (NaCA); (c) calcium caseinate (CaCA).

RESULTS & DISCUSSION

Viscosity vs concentration

Rennett casein (ReCN)(Fig. 1a) remained insoluble under most conditions studied. However, with certain specific treatments, the ReCN solubilized and some solutions exhibited unusually high viscosities. The ReCN supernates, which had protein concentrations greater than zero with viscosities appropriate to the protein concentration, had either $CaCl_2$ or NaCl added at pH > 5.6 and were incubated at temperatures < 28°C. The ReCN which showed both greatly increased solubility and enhanced viscosity had NaH_2PO_4 added instead of $CaCl_2$ or NaCl. Humansson (1975) reported that ReCN required treatment with alkali (to pH 9.0) or addition of complex phosphates at a pH of about 7.5 to effectively disperse. We have shown that ReCN would disperse (dissolve) with addition of a simple phosphate (NaH_2PO_4) at a much lower pH (pH = 6.2).

Sodium caseinate (NaCA)(Fig. 1b) also showed a linear reponse for ln viscosity versus protein concentration when the concentrations were ≤ 70 mg/mL. Significant increases in solubility, as measured by protein concentration in the supernatant, only occurred at pH ≥ 4.6. Deviation from the linear relationship of ln viscosity vs. concentration at pH ≥ 5.6 occurred when either NaCl or NaH_2PO_4 was added. Sodium caseinate was the most soluble of the proteins studied (Morr, 1979; Southward, 1985). As shown in Fig. 1c, calcium caseinate (CaCA) behaved similarly to NaCA, except the deviation from the expected linear relationship occurred only when NaH_2PO_4 at pH ≥ 5.6 was included.

Viscosity of the three casein(ate)s studied showed a strong dependency on protein concentration. Behavior of these proteins, at their resultant concentrations, was essentially Newtonian, exhibiting little or no change in viscosity as a function of shear rate. Hermansson (1975) also showed almost Newtonian behavior for caseinates at concentrations of less than 12%. The relationship of concentration with the natural logarithm (ln) of the viscosity of the three proteins was essentially linear when the concentration was less than 70 mg/mL (7%). The data shown represent the individual casein(ate)s without regard for types or concentration of salt, pH or temperature.

A regression analysis on the combined data, from each of the proteins in their linear range (ReCN ≤ 20mg/mL, NaCA and CaCA ≤ 70 mg/ml), resulted in an R^2 of 0.80 ($P \leq 0.001$, df = 74). When each of the proteins was analyzed separately, the slopes indicated a difference in the relationship between concentration and ln viscosity. ReCN exhibited the lowest viscostiy and NaCA showed the highest. The deviation from linearity for CaCA occurred at a lower concentration (7–8%) and resulted in a higher viscosity than that found by Hermansson (1975). Investigations of the flow properties of NaCA (Hermansson, 1975) indicated the increase in viscosity (deviation from initial linearity) was more likely due to hydration than protein-protein interactions. Swelling, which is related to the hydration of the proteins, increased dramatically with pH (Hermansson, 1972).

Effect of salts

Figure 2 illustrates the effect of salt type on concentration vs. ln viscosity relationship. The data used in Fig. 1 were rearranged to show the effect of individual salts without regard for protein type, pH, temperature or salt concentrations. The addition of calcium chloride (Fig. 2a) resulted in limited solubilization of the proteins. Under the conditions studied, a maximum of 60 mg/mL protein concentration in the supernatant was observed. The viscosity of these protein solutions was low and exhibited no significant deviation from linearity over the concentration range. The effects of sodium chloride (Fig. 2b) on the concentration-viscosity relationship at protein concentrations < 60 mg/mL were similar to those observed with calcium chloride. When the protein concentration of the NaCl solution exceeded 60 mg/mL, the ln viscosities showed a tenfold increase over those expected from a simple linear correlation between concentration and ln viscosity. When NaH_2PO_4 (Fig. 2c) was added, the viscosity at protein concentrations ≥ 70 mg/mL increased 50 fold over the expected linear response.

A regression analysis on the linear portion of each of the concentration-viscosity relationships in Fig. 2 confirmed the similarity of results for $CaCl_2$ and NaCl addition and indicated a greater tendency for solubilization and viscosity development when sodium phosphate was added. Ca^{+2} ion addition usually results in quantitative precipitation of caseins (Whitney, 1988)

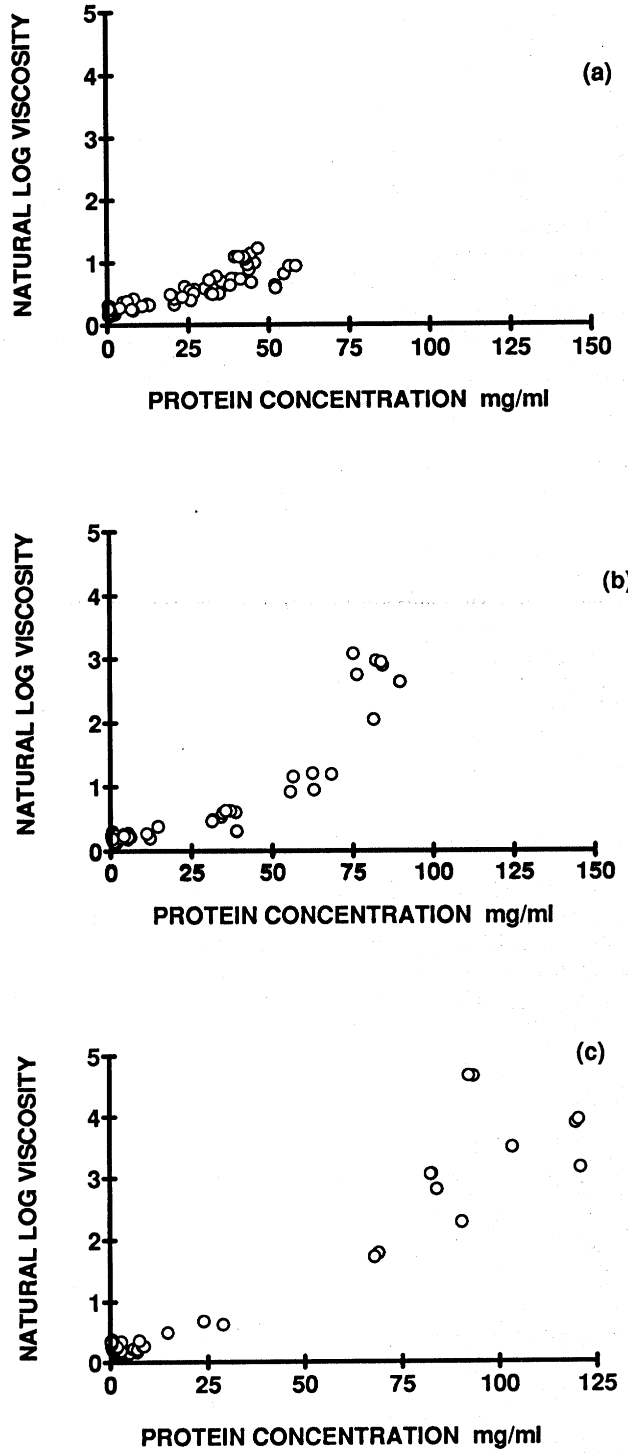

Fig. 2—Effect of added salts on protein concentration vs. natural log of viscosity. Effects of salt type. (a) $CaCl_2$; (b) NaCl; (c) NaH_2PO_4.

at 0.07M $CaCl_2$, pH = 6.6 and 3°C. Farrell et al. 1988, however, demonstrated that high concentrations of Ca^{+2} (> 0.05M) result in a salting-in effect on certain caseins. Our results demonstrated that this salting-in effect occurred with commercial-type caseinates over a varied temperature and pH range. Hayes et al. (1968) also found calcium addition to be a viscosity limiting factor. Hermansson (1975) reported an increase in viscosity with addition of NaCl in 12% dispersions of CaCA. This increase was reported as highly salt concentration-dependent, with little or no effect at dispersions of less than 10%. Sodium phosphate addition had the greatest impact on solubility and viscosity, with solubilization observed at low salt concentrations at pH 6.2.

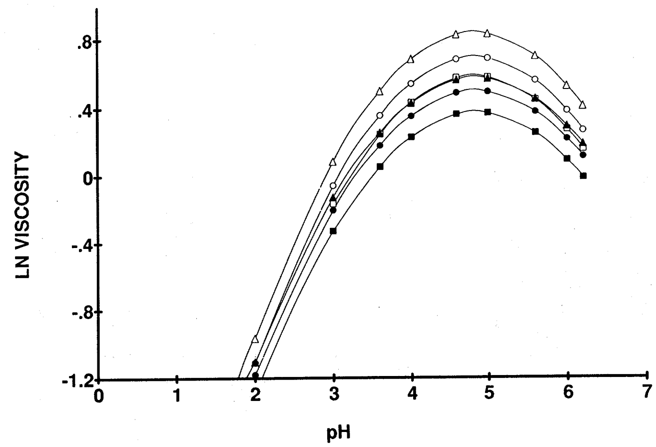

Fig. 3—Modeled data for added $CaCl_2$. Calcium caseinate: ○ 20C, 0.15M; △ 20C, 0.45M; □ 60C, 0.45M. Sodium caseinate: ● 20C, 0.15M; ◆ 20C, 0.45M; ■ 60C, 0.45M.

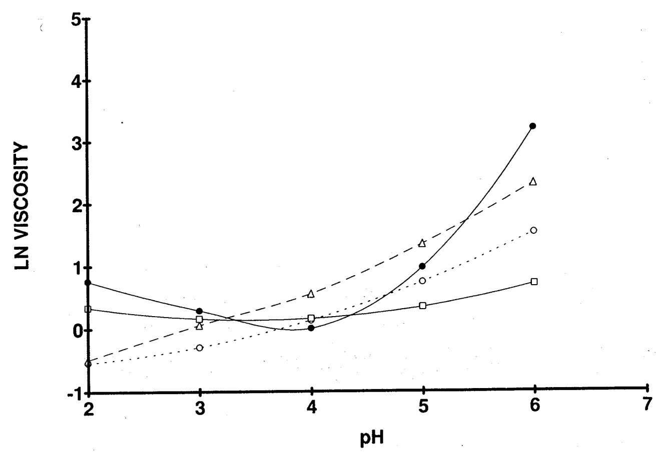

Fig. 4—Modeled data for added NaCl. Calcium caseinate: ○ 20C, 0.15M, △ 20C, 0.45M; □ 60C, 0.45M. Sodium caseinate: ●.

Figures 1 and 2 illustrate the effects of the qualitative variables (casein type and salt type) on viscosity and solubility. To establish effects of quantitative variables (temperature, pH and salt concentration) in an integrated fashion, a full model regression (SAS, 1985) analysis with second-order interactions was evaluated. The response variable used in the regression analysis was viscosity. Solubility was not evaluated because solubility and ln viscosity are highly significantly related.

Modeled data

Table 1 shows the results of the regression analysis for each protein/salt combination. The correlation coefficients (r) for these data indicated a good fit to the model and were highly significant ($P < 0.001$, df = 25)(Volk, 1958). The lowest set of correlations was observed for the ReCN which had relatively small responses to treatment. The data were further analyzed using a backward-stepwise regression analysis (SAS, 1985) to evaluate and eliminate those parameters which showed nonsignificant ($P > 0.05$) contribution to the response variable. These nonsignificant parameters, annotated in Table 1, indicated similar viscosity dependency within salt groupings, with exception of the NaCA/NaCl combination which, like the NaH_2PO_4 grouping, was dependent on pH alone.

Figures 3, 4, and 5 illustrate the viscosity response of Na and Ca caseinates to the quantitative variables using the reduced model. The data were plotted as ln viscosity vs pH

Table 1—Regression coefficients for full model regression analysis
[a](Ln Viscosity = $A_0 + A_1T + A_2P + A_3C + A_4T^2 + A_5P^2 + A_6C^2 + A_7TP + A_8TC + A_9PC$)

Protein/ Salt[c]	r	A_0	A_1	A_2	A_3	A_4	A_5	A_6	A_7	A_8	A_9
RECA	0.807	0.38	−4.2E-3	−0.03	−0.52	8.0E-5	3.5E-3	1.03	−4.9E-4[b]	−2.3E-3[b]	0.04[b]
CACA	0.949	−6.18	0.04	2.27	6.21	5.5E-4	−0.23	−7.87	−2.3E-4[b]	2.7E-3[b]	−0.29[b]
NACA	0.882	−6.71	0.06	2.20	8.51	−7.4E-4	−0.21	−8.68	−4.0E-5[b]	−3.8E-3[b]	−0.63[b]
RENA	0.802	−0.30	0.02	0.05[b]	0.41[b]	−1.0E-5[b]	4.6E-3	0.53	−2.8E-3	−8.6E-3[b]	0.09
CANA	0.929	−1.83	0.08	−0.07[b]	0.44[b]	−1.1E-4[b]	0.09	−0.68[b]	−0.01	−0.06	0.63
NANA	0.949	10.91	−0.50[b]	−5.08	−3.32[b]	8.3E-4[b]	0.69	4.83[b]	−4.3E-3[b]	6.0E-3[b]	0.08[b]
REPO	0.861	4.84	0.07[b]	−3.29	3.72[b]	−6.4E-4[b]	0.44	−5.28[b]	−5.2E-3[b]	1.9E-2[b]	−0.21[b]
CAPO	0.932	18.50	−0.10[b]	−7.78	−14.53[b]	1.3E-3[b]	0.96	8.96[b]	−3.9E-3[b]	0.02[b]	2.05[b]
NAPO	0.983	9.44	−0.10[b]	−4.48	4.66[b]	2.1E-4[b]	0.55	1.37[b]	0.02[b]	0.04[b]	−1.57[b]

[a]A_n = regression coefficients; T = Temperature (°C); P = pH; C = salt concentration (M).
[b]Not significant in reduced model after backward-stepwise regression.
[c]Protein Salt Combination: RECA—Rennet Casein with Calcium Chloride; CACA—Calcium caseinate with Calcium Chloride; NACA—Sodium Caseinate with Sodium Chloride; RENA—Rennet Casein with Sodium Chloride; CANA—Calcium Caseinate with Sodium Chloride; NANA—Sodium Caseinate with Sodium Chloride; REPO—Rennet Casein with Sodium Phosphate; CAPO—Calcium Caseinate with Sodium Phosphate; NAPO—Sodium Caseinate with Sodium Phosphate.

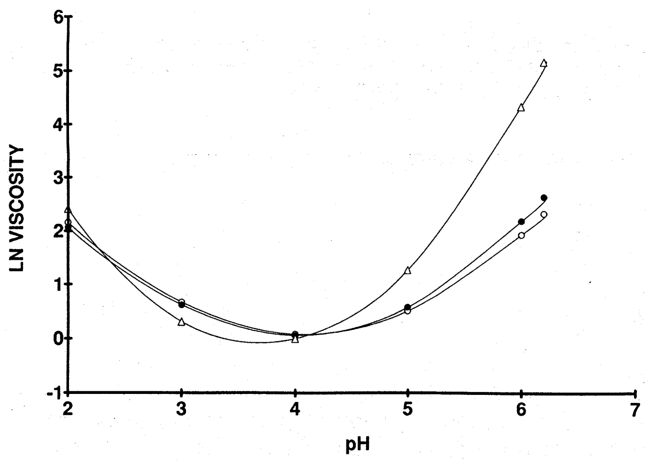

Fig. 5—Modeled data for added NaH_2PO_4. Rennet casein ○, sodium caseinate ●, calcium caseinate, △.

because of the common dependency on pH with constant temperature and increasing salt concentration or with constant salt concentration and increasing temperature. Figure 3 shows the effects of $CaCl_2$ concentration and temperature on the relationship between ln viscosity and pH. Both Na and Ca caseinate showed remarkably similar behavior with the effects of pH on viscosity being slightly greater for Ca caseinate. However, the differences in viscosity between Ca and Na caseinate (1.0 to 1.5 cp), while significant, were of relatively small magnitude. Maxima at pH 4.6 were observed for these curves and the viscosities increased with increased $CaCl_2$ concentration and decreased temperature. The maxima at pH 4.6 may be explained by solubility increases due to lack of available charges for the binding of calcium thereby decreasing sensitivity of caseins to Ca^{+2}. The relationship between viscosity and pH was completely inverted from what we would expect if pH were the controlling factor in casein solubility.

With the addition of sodium chloride (Fig. 4), the viscosity of calcium caseinate increased with salt concentration and decreased with temperature (temperature being the overriding factor). This response was similar to that observed with addition of $CaCl_2$ but viscosity changes were considerably greater. Viscosities of the sodium caseinate/sodium chloride solutions were dependent on pH alone and were at a maximum at pH 6.2, the highest pH we used. Added sodium phosphate (Fig. 5) had the greatest impact on viscosity and, like the NaCA/NaCl combination it was dependent only on pH. The viscosities of CaCA/NaH_2PO_4 were on the order of 100 cp, the highest observed. These high viscosities may have been due to increased water uptake by the casein. Ruegg and Moor (1984) suggested that the increase in water uptake, and therefore swelling, was determined primarily by the amount and type of cation. They found that the addition of Na^+ caused a greater tendency toward water uptake. We observed, however, that anion dependence also played a significant role. The addition of phosphate ion impacted on the viscosity, due to its high ionic strength compared to the chloride ion.

CONCLUSIONS

THE INTEGRATED STUDY provided valuable information for formulation of foods based on casein(ate) functionality. Viscosity and solubility of casein(ate)s could be altered by addition of salts and temperature and pH control. Casein(ate)s were solubilized at the isoelectric point with the use of $CaCl_2$ at appropriate concentration and temperature. The addition of NaH_2PO_4 provided limited solubility of the normally insoluble rennet casein and was effective in viscosity enhancement of the proteins studied.

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