for —OH group) and NMR (D_2O exchangeable signals) behavior.

Mass spectra of oxathiolanes (V, VII, IX) showed small molecular ion peaks. The ion peaks obtained due to the α-cleavages to the oxathiolane ring confirming the position and nature of the ring also were observed. A characteristic mass ion at m/z 115 observed in all the spectra, has been reported earlier as characteristic of oxathiolane grouping (12). The isomeric nature of IX was confirmed by mass fragmentation data shown in Scheme 4. Other prominent mass ions substantiating the structures have been elaborated in Schemes 2-4.

Here we observed that β-mercaptoethanol reacts with only one oxo group of methyl 9,10-oxooctadecanoate (III), whereas the ethane dithiol condenses with both the ketones of III as described earlier (8). This observation apparently is due to the difference in the reactivity of β-mercaptoethanol and ethanedithiol.

The formation of hemimercaptals (VI, VIII) from I, II respectively is explained in terms of lesser nucleophilicity of oxygen than of sulfur. Acetic acid was used in the reaction of III in order to dissolve the reactant, while I, II were found readily soluble in BF_3-etherate. There was no reaction of IV with β-mercaptoethanol in BF_3-etherate. The inductive effect caused by the chain and acid carbonyl group makes this oxo function less reactive. Steric hindrance may also play an important role in the nonreactivity of this acid.

ACKNOWLEDGMENT

The chairman of the Aligarh Muslim University Department of Chemistry provided necessary facilities, and the CSIR (New Delhi) provided financial support to two of us (SA and MK). The research was financed in part by a grant from the USDA under PL-480.

REFERENCES

1. Takeuchi, S., C. Higashikaze, A. Kawarada, S. Esumi, K. Sasaki, S. Kawabata, T. Saida, Y. Inoue, T. Yamamoto et al., *Chem. Abstr. 93:*210255h (1980).
2. Dimmoch, J.R., and L.M. Smith, *J. Pharm. Sci. 69:*575 (1980).
3. Robbe, Y., J.P. Farnandes, R. Dubief, J.P. Chapat, H. Sentenac-Roumanou, M. Fatome and J.D. Laval, *Eur. J. Med. Chem.-Chim. Ther. 17:*235 (1982).
4. Djerassi, C., and M. Gorman, *J. Am. Chem. Soc. 75:*3704 (1953).
5. Oliveto, E.P., T. Clayton and E.B. Hershberg, *J. Am. Chem. Soc. 75:*486 (1953).
6. Fieser, L.F., *J. Am. Chem. Soc. 76:*1945 (1954).
7. Larson, G.L., and A. Hernandes, *Synthetic Communications 4:*61 (1974).
8. Khan, M., M. Hashmi, F. Ahmad and S.M. Osman, *J. Amer. Oil Chem. Soc. 62:*525 (1985).
9. Husain, S.R., F. Ahmad and M. Ahmad, *Acta Chim. (Hung.) 120:*29 (1985).
10. Nasirullah, F. Ahmad, S.M. Osman and W. Pimlott, *J. Amer. Oil Chem. Soc. 59:*411 (1982).
11. Mustafa, J., M.S. Ahmad Jr., A. Rauf and S.M. Osman, *Ibid. 61:*941 (1984).
12. Fenselan, C., L. Milewich and C.H. Robinson, *J. Org. Chem. 34:*1374 (1969).

[Received August 19, 1985]

The Ionic Modification of the Surface Charge and Isoelectric Point of Soy Protein

W.S. Chen and W.G. Soucie*
Kraft Inc. R&D, 801 Waukegan Rd., Glenview, IL 60025

The effect of anionic and cationic binding on the surface charge of soy proteins was measured by electrokinetic analysis. All of the ions investigated suppressed the surface charge of the protein; however, certain multivalent ions such as Al (III), Fe (III), hexametaphosphate and tripolyphosphate also altered the isoelectric point of the protein. The results indicated the unpredictability of ionic effects on protein functionality, thus emphasizing the importance of making measurements of protein charge.

Much effort has been given to understanding of the functional properties of proteins. Functional properties of proteins are dependent on their physicochemical characteristics. For example, effects of ion binding to polyelectrolytes have been determined by emulsion rheology studies (1). The effect of pH and ionic strength on protein solubility has been determined (2) and is well known. In the food industry, the emphasis has been on studying the functional properties of the proteins rather than on measurements of the underlying physicochemical properties that actually control protein functionality. Because binding of either hydrogen ions or salts affects protein functionality by modifying the electrical properties of the protein, a method was used to measure these electrical properties. The method chosen was electrokinetic analysis (3,4), and the results of an electrokinetic study on the effect of pH and ionic modification of soy protein isolate are reported herein.

*To whom correspondence should be addressed.

MATERIALS AND METHODS

Soy isolate was from Kraft, Inc., Glenview, Illinois. Other chemicals were of reagent grade. Electrokinetic analysis was performed either by a System 3000 Electrokinetic Analyzer (PenKem, Inc., Bedford Hills, New York) or by a Zeta Meter, (Zeta Meter, Inc., New York, New York). The methods described in the instruction manuals (3,4) for the electrokinetic equipment were followed to make mobility determinations. The ionic strength of solutions was measured as specific conductance (micromhos/cm) using either the System 3000 Electrokinetic Analyzer or the Zeta Meter.

Soy protein isolate concentration was 0.28 mg/ml (solids basis) in distilled water. About 50-100 ml of

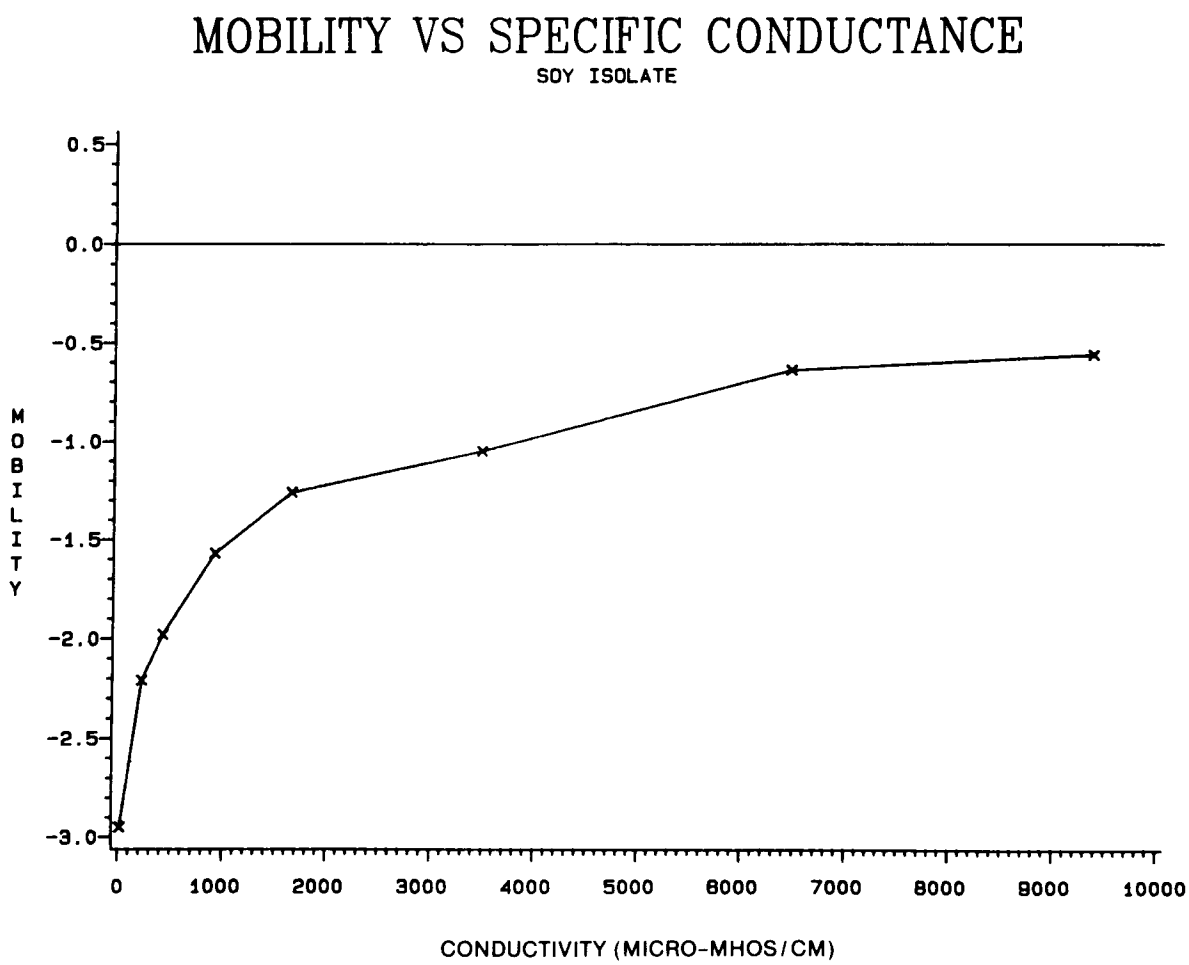

FIG. 1. Mobility units are 10^{-8} m/sec/volt/m. pH = 7.0. Protein concentration was 0.28 mg/ml. The pI is at zero mobility. Conductivity was adjusted using sodium chloride. 1500 micromhos/cm is about 11 mM sodium chloride.

solution was sufficient to generate a single mobility vs pH profile. The pH of the soy solution was adjusted using 0.1 M HCl or 0.1 M NaOH. Mobilities were determined at pH increments of from .1 to 1.0 pH unit, depending on the response of mobility to the pH adjustment. Once the sample was loaded into the instrument, the specific conductance was determined followed by measurement of the electrokinetic mobility. The mobility data were then plotted against pH in order to generate the electrokinetic mobility vs. pH profiles. One mobility unit is defined as 1.0×10^{-8} m/sec/volt/m. Mobility vs pH profiles were taken where the specific conductance was constant. For comparative purposes, zeta potentials, in millivolts, can be estimated by multiplying mobility values by the factor 1.3×10^{9}. For example, a mobility of -1.0×10^{-8} m/sec/volt/m is approximately -13 mV in zeta potential. The isoelectric point of the protein (pI) is the pH where the net electrokinetic mobility (or zeta potential) is exactly zero.

RESULTS AND DISCUSSION

The charge on a protein molecule is due to the ionization of amino acid functional groups. Ionic strength of the solvent therefore affects the extent of dissociation of these functional groups, altering protein charge and consequently electrokinetic mobility. Figure 1 shows the effect of ionic strength on the mobility of soy isolate (specific conductance was used to measure ionic strength) at pH 7.0. The ionic strength of the protein solution was adjusted by adding sodium chloride. Notice in Figure 1 that the mobility was reduced biphasically. At low ionic strength the mobility was very sensitive to concentration whereas at specific conductances greater than 1500 micromhos/cm (~11mM NaCl) the decrease in mobility was less rapid. Visual observation of soy proteins indicated aggregation at zeta potential values less negative than -1.5 mobility units (-20 mV) which is somewhat more electronegative than the -1.1 mobility units (-14 mV) stated by Riddick (5) as the point of aggregation for colloids in general. Soy isolate has a mobility of -1.5 units at the steeper part of the curve in Figure 1. Thus, small increases in salt concentration above 1000 micromhos/cm (<8 mM NaCl) may cause soy proteins to aggregate because the soy mobility reaches the critical value of -1.5 mobility units. At values greater than 1500 micromhos/cm soy protein was aggregated and susceptible to precipitation. The explanation for the decrease in mobility as ionic strength increases is that more ions are packed closer to the protein surface, resulting in the reduction of the electrical double layer thickness (9). Reduction in the

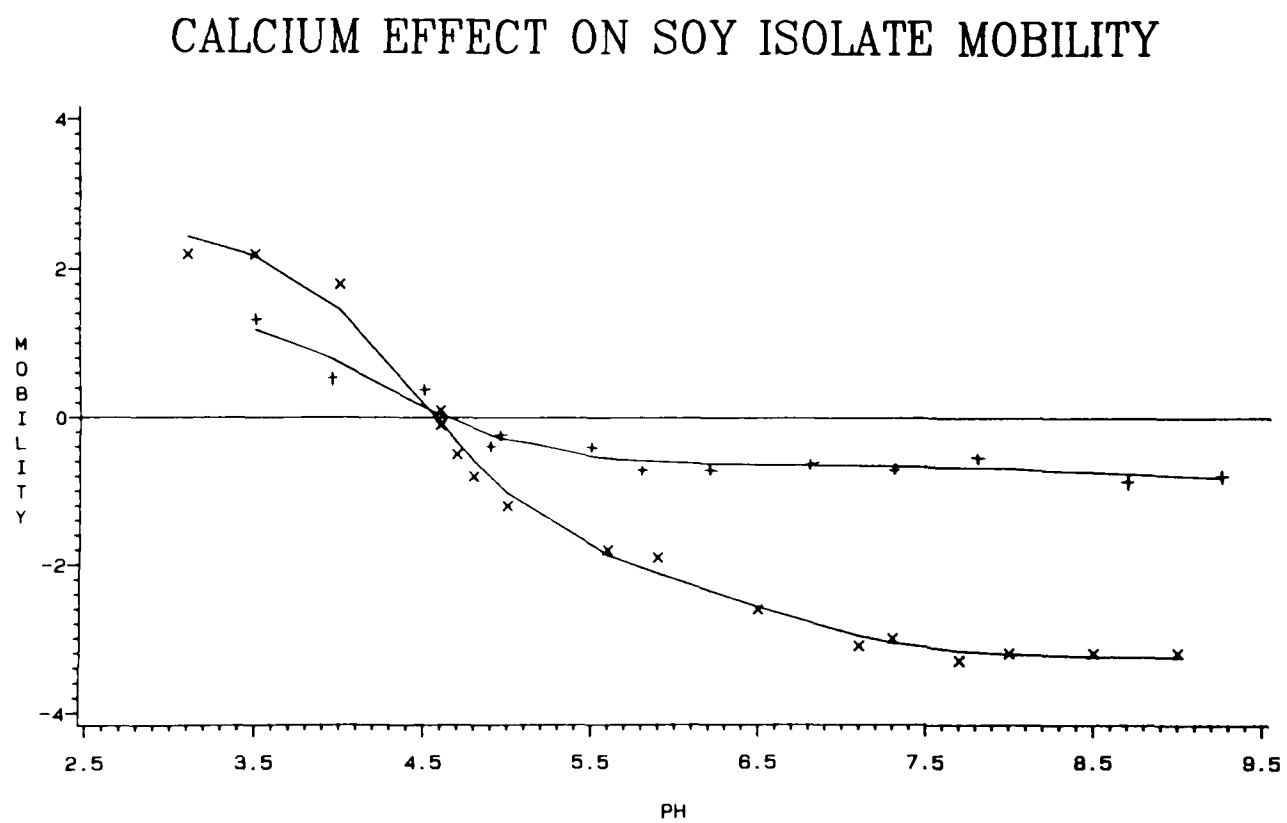

FIG. 2. Mobility units are 10^{-8} m/sec/volt/m. The pI is at zero mobility. (x), no calcium added. (+), 1 mM calcium chloride added.

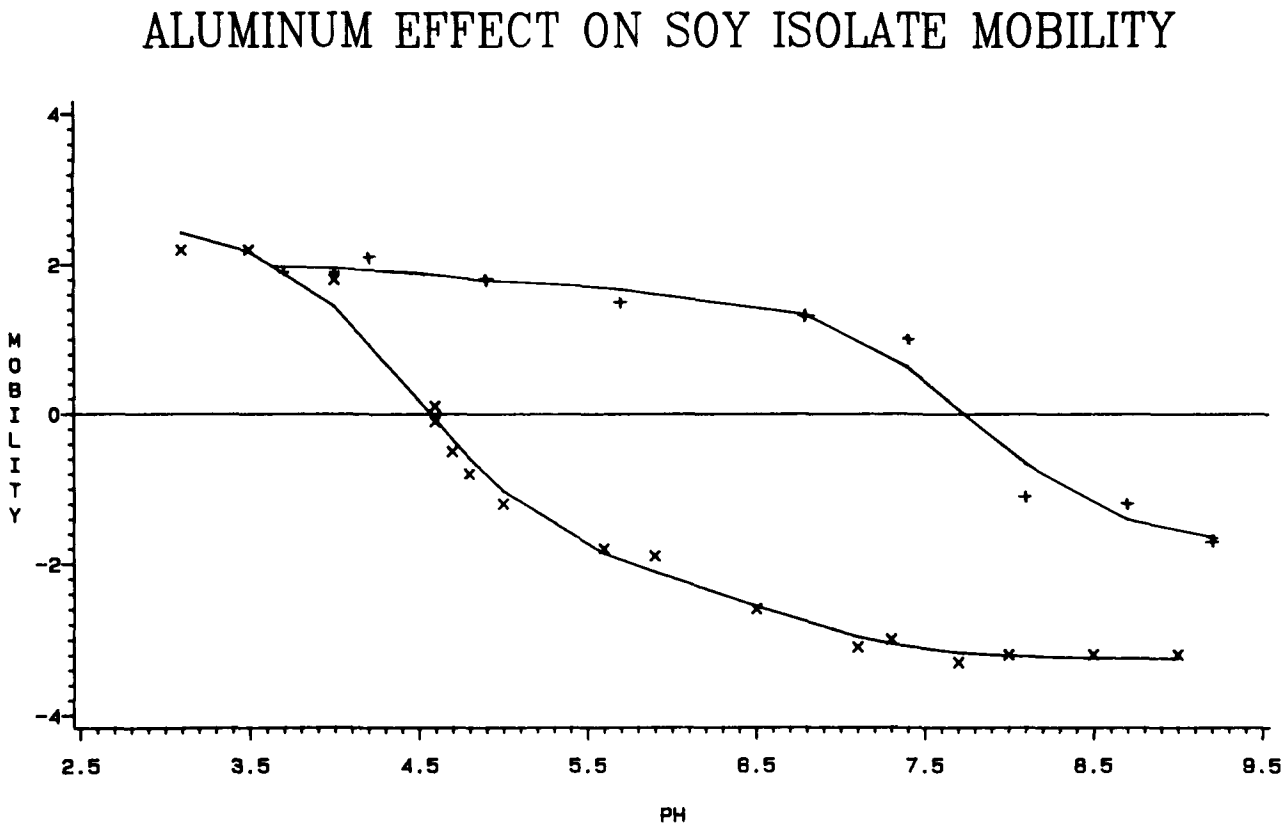

FIG. 3. Mobility units are 10^{-8} m/sec/volt/m. The pI is at zero mobility. (x), no added aluminum. (+), 1 mM added aluminum chloride.

double layer thickness results in a decreased mobility (zeta potential). Electrostatic repulsions are reduced sufficiently that the particles can approach each other closely enough for van der Waals forces to predominate (9). Equations relating the effect of ionic strength on the electrical double layer can be found in several texts (7,10). Although electrostatic effects are very important for protein stability in aqueous solutions, it should be noted that hydrophobic effects are also very important (11).

In Figure 2 the mobility of soy isolate in 1 mM calcium chloride is compared to a control that contained no added calcium. The specific conductance of the 1 mM calcium chloride solution was about 200 micromhos/cm, and at pH 7.0 the soy mobility was about −0.6 mobility units. Note in Figure 1 that at 200 micromhos/cm the mobility was about −2.5 mobility units. Therefore, in the absence of other contributing factors the mobility of soy in 1 mM calcium should have been −2.5 mobility units also. Obviously the soy mobility in calcium was much less than −2.5 units; therefore, electrostatic binding in calcium chloride solutions was much greater than in sodium chloride solutions. Taylor and Bosmann (12) have also observed this effect for calcium binding to reovirus (a class of animal viruses) coat proteins. At pH values above the isoelectric point multivalent cations are much more effective at penetrating the hydrodynamic boundary of a negatively charged surface than are monovalent cations (12). The result is a decrease in zeta potential due to multivalent cation binding. The calcium binding was very effective in reducing the negative charge on the protein at pH values near neutrality (Fig. 2); however, calcium ion binding was not strong enough at pH 4.5 to compete with the hydrogen ions, and the pI (pH where net mobility is zero) of soy protein was not shifted in the presence of calcium ion (Fig. 2). The specific binding of ions to proteins is well known (13), and Klotz (14) described the binding of calcium to casein by application of the law of mass action to ion binding by proteins. Coagulation of milk casein micelles has been attributed to electrostatic interactions between the negatively charged casein and various cationic species (15-18). Since the mobility of the protein was well below −1.5 units at all pH values above the pI (Fig. 2), electrostatic repulsion was not sufficient to prevent aggregation and the proteins precipitated.

As expected, trivalent cations were found to have an even greater effect on soy protein mobility than divalent ions. In Figure 3, the effect of adding 1 mM aluminum chloride is compared to the untreated control. Again, the ionic strength effect is not enough to explain the results because at pH 7 the conductivity of the aluminum chloride solution was approximately 400 micromhos/cm, which would give a mobility of approximately −2.0 mobility units (Fig. 1). Instead, the mobility at pH 7 was approximately +1.0 unit. Not only was the mobility reduced, but the net charge on the protein was reversed from negative to positive in the presence of 1 mM aluminum. The results are consistent with the Schulze-Hardy rule which states that the electronegative colloids

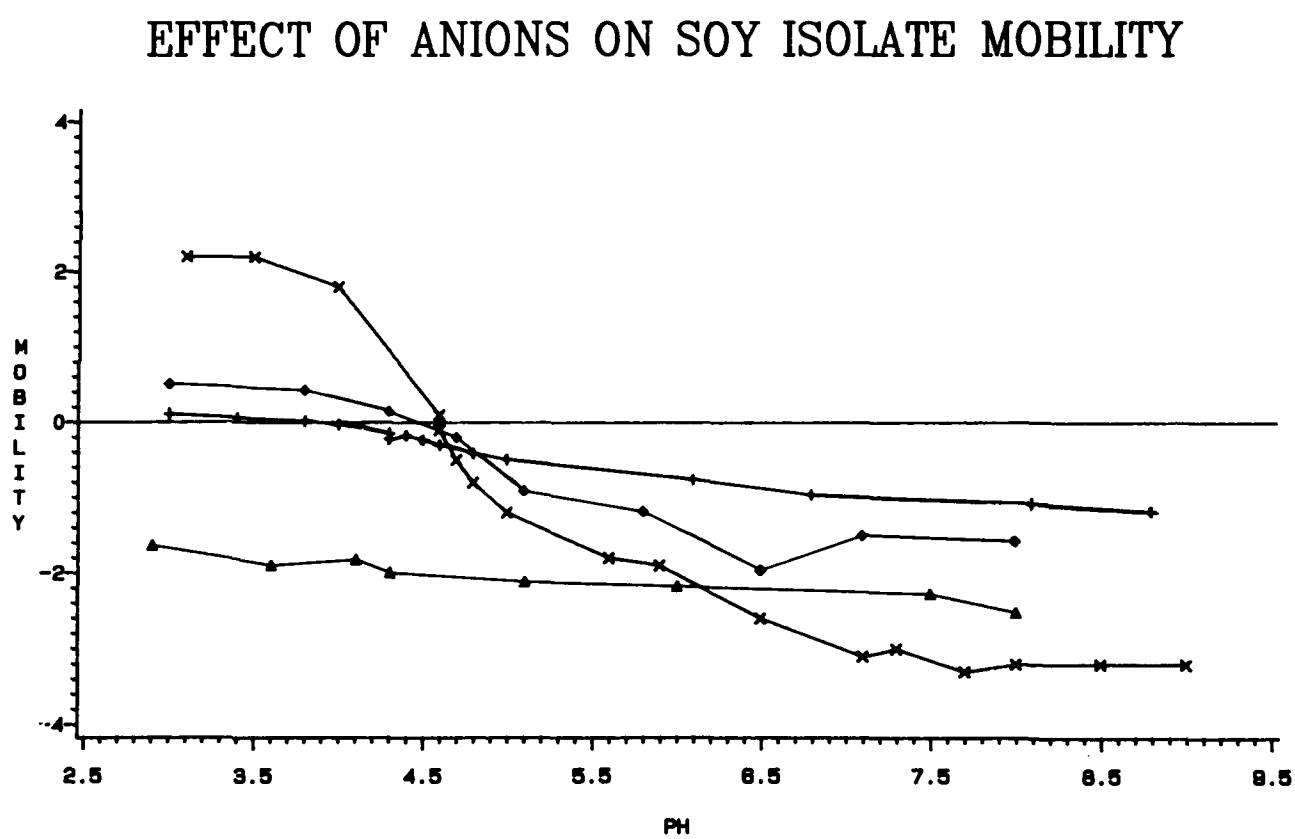

FIG. 4. Mobility units are 10^{-8} m/sec/volt/m/. The pI is at zero mobility. (x), no added ions; (+), 10 mM sodium tripolyphosphate; (◆), 10 mM sodium sulfate; (▲), 1 mM sodium hexametaphosphate.

are flocculated by ions according to the following order: trivalent > divalent > monovalent (6,9). The reverse of the protein surface charge is most likely explained by preferential counter-ion binding of aluminum which, according to Shaw, could cause a reversal of charge in the ionic double layer (8). Figure 3 also shows a significant shift in the pI of the protein from about a pI of 4.5 to a pI of 7.7. This shift in pI is indicative of specific ion interaction with the protein (12), and it is reasonable to assume that the interaction would be between the amino acid carboxyl groups and the aluminum ions. Binding of molecules to proteins may be due to nonspecific van der Waals interactions (19); however, the decrease in mobility in Figure 3 is evidence that electrostatic ion binding to specific sites is also significant.

Potentiometric titration with hydrochloric acid showed that the titratable equivalents of carboxyl groups were reduced when soy protein was in the presence of aluminum ions (Ford, L.D., Kraft Inc., personal communication, 1984), i.e., some of the aluminum ions were not displaced by protons during the titration. This decrease in titratable anions resulted in the protein charge being more positive, thereby shifting the pI in the basic direction.

Binding with aluminum shows how significantly the charge profile on a protein may be altered by low concentrations of trivalent ions. Between pH 4.5-7.0 soy protein normally would be negatively charged; however, in the presence of aluminum the protein was positively charged.

Not only did di- and trivalent cation binding alter protein charge but anion binding also influenced protein mobility. Figure 4 shows the effects of sodium hexametaphosphate (1 mM), sodium sulfate (10 mM) and sodium tripolyphosphate (10 mM) on soy protein mobility. The sulfate and tripolyphosphate salts decreased the mobility at pH 3.5 from about +2.4 units for the control to less than +0.6 units. The tripolyphosphate also had an effect on the pI, decreasing it from 4.6 to about 4.0. Hexametaphosphate had a large effect on the pI of the protein. Even at a pH of 3.1 the protein was still very negatively charged (−1.8 mobility units compared to +2.2 mobility units for the control). At pH 4.5, where soy protein is usually insoluble, the hexametaphosphate-bound protein was still quite soluble due to the negative charge repulsion imparted to the protein by the metaphosphate anion. The change in the mobility of the soy protein above pH 4.5 in Figure 4 can be explained by the ionic strength effect already demonstrated in Figure 1.

Ionic salts were used to modify and control the protein charge. Electrokinetic analysis is a useful method for determining the effect of ionic modification of proteins because the charge on the protein surface alters mobility in an electric field. Unpredictable changes in protein functionality now may be partly explained through determination of protein charge by electrokinetic analysis.

REFERENCES

1. Ralston, J., T. vanVliet and J. Lyklema, *J. Coll. Inter. Sci. 82*:53 (1981).
2. vanMegen, W.H., *J. Agric. Food Chem. 22*:126 (1974).
3. *The PenKem 3000 Electrokinetic Analyzer Reference Manual*, PenKem, Inc., Bedford Hills, NY (1980).
4. Zeta-Meter Operating Manual zm-75, Zeta-meter, Inc., New York, N.Y. (1968).
5. Riddick, T.M., in *Control of Colloid Stability Through Zeta Potential*, Creative Press, New York, 1968, p. 2.
6. Riddick, T.M., *Ibid.*, p. 21.
7. Shaw, D.J., in *Introduction to Colloid and Surface Chemistry*, 2nd ed., Butterworths, Boston, 1970, p. 139.
8. Shaw, D.J., *Ibid.*, p. 141.
9. Shaw, D.J., *Ibid.*, p. 168.
10. Hunter, R.J., *Zeta Potential in Colloid Science, Principles and Applications*, Academic Press, New York, 1981, p. 27.
11. Melander, W., and C. Horvath, *Arch. Biochem. Biophys. 183*:200 (1977).
12. Taylor, D.H., and H.B. Bosmann, *J. Coll. Inter. Sci. 83*:153 (1981).
13. Arakawa, T., and S.N. Timasheff, *Biochemistry 21*:6545 (1982).
14. Klotz, I.M., *Arch. Biochem. 9*:109 (1946).
15. Green, J.L., *Netherlands Milk Dairy J. 27*:278 (1973).
16. Green, M.L., and R.J. Marshall, *J. Dairy Res. 46*:365 (1979).
17. Pearce, K.N., *Ibid. 43*:27 (1976).
18. DiGregorio, F., and R. Sisto, *Ibid. 48*:267 (1981).
19. Klotz, I.M., H. Triwush and F.M. Walker, *J. Amer. Chem. Soc. 70*:2935 (1948).

[Received November 18, 1984]