International Food Research Journal 20(3): 1249-1254 (2013)
Journal homepage: *http://www.ifrj.upm.edu.my*

Complex formation between Tragacanth gum and beta-lactoglobulin in aqueous solution

*Nasirpour, A., Amir, M., Hajihashemi, Z. and Fazilati, M.

Department of Food Science and Technology, College of Agriculture, Isfahan University of Technology, Isfahan 84156, Iran

Article history

Received: 15 August 2012
Received in revised form: 10 November 2012
Accepted:15 November 2012

Keywords

Tragacanth gum
protein–polysaccharide interactions
associative phase separation
electrostatic interactions

Abstract

Proteins and polysaccharides are common ingredients in food products. In many food formulations, proteins and polysaccharides (two major biopolymers) are used simultaneously. Understanding their interactions can help controlling physical properties and stability of formulated foods. Interactions between β-lactoglobulin (BLG) and tragacanth gum (TG) were studied in aqueous solutions at pH ranging from 3.5 to 5.5 and at different biopolymers concentrations. BLG and TG solutions were prepared in distilled water under gentle stirring at 20°C. Different biopolymers concentrations were mixed and pH value were adjusted by HCl. Absorbance of the mixtures were measured at 650 nm using UV-Visible spectrophotometer. The measured absorbances were plotted against pH. In this study, pH_C, pH_φ, and pH_{opt} were identified and measured for different biopolymers concentrations. Phase separation observed during experiments is an associative phase separation. Complex sizes increased between pH_C and pH_φ because of soluble complexes formation and then a decrease in complex sizes was observed and this decrease was due to neutralization of initiate complexes and solubilization of biopolymers complexes. This wide range of complex formation helps product developer to use tragacanth gum in a large range of formulations.

Introduction

Proteins and polysaccharides are often used simultaneously in food industries. Interactions between proteins and polysaccharides play an important role in structure, stability and dietetic aspect of the formulated foods with complex structure (Cavallieril *et al*., 2011). Interactions between oppositely charged proteins and polysaccharides are of particular interest in food science, especially with respect to functional foods. One important type of complexation arises from electrostatic interactions between oppositely charged functional groups on proteins and polysaccharides. In the case of biopolymers with low charge density, these associative interactions lead to soluble complex formation in dilute solution, and to complex coacervation at higher concentrations. Insoluble complexes and precipitation may occur in systems containing biopolymers of high charge densities (De Kruif *et al*., 2004). β-lactoglobulin (BLG) is the major protein of milk serum proteins (40–50% in mass). It contributes significantly to milk serum proteins functional properties, i.e. thermal aggregation/gelation and surface properties (De Wit, 1998; Wong *et al.*, 1996; Kinsella, 1984). The isoelectrical pH (IEP) of BLG has been previously reported to be around 4.7–5.2 (Bromley *et al*., 2005; Sawyer *et al*., 1998). The association properties of BLG in aqueous solution depend strongly on pH (Gottschalk *et al.*, 2003). At pH 5–8, BLG exists as a dimer, at pH 3–5 the dimers are associated and form octomers, and at extreme pH values (< 2 or > 8) BLG exists mainly as monomers.

Tragacanth is one of the most acid-resistant gums and efficient natural emulsifiers for acidic oil-in-water emulsions (Weiping, 2000). Tragacanth gum consists of two major fractions: a water-soluble (Tragacanthic acid and small amount of an Arabinogalactan) and an insoluble but water-swellable fraction named Bassorin.Investigations on interactions between biopolymer mixtures, i.e., globular protein and anionic polysaccharide interactions continue in both industrial and academic sectors because of their large application in different industrial sectors. The effects of this character are important as they may result either in the phase separation of biopolymer through charge–charge repulsion or in association through charge-charge attraction (Piculell *et al*., 1994). Previous studies have shown that globular proteins can interact with anionic polysaccharides (e.g. gum arabic,

*Corresponding author.
Email: *ali.nasirpour@cc.iut.ac.ir*
Tel. (+98) (0311) 391 33 85; Fax: (+98) (0311) 391 33 80

carboxymethycellulose, pectin, and carrageenan) to form either soluble or insoluble complexes (Chang *et al*., 2000; Girard *et al.*, 2002; Weinbreck *et al*., 2003; De Kruif *et al*., 2004; Weinbreck *et al*., 2004). These complexes may be stabilized predominantly by electrostatic, ion-dipole or hydrophobic interactions. An improved understanding of the origin and nature of these interactions would lead to the design of foods with improved nutritional, physicochemical, and sensory properties. Therefore, in this study, the influence of pH, polymer concentration and protein to polysaccharide ratio on the interaction between bovine BLG and tragacanth gum in aqueous solutions was studied.

Materials and Methods

Materials

BLG was purchased from Davisco (Davisco Foods International, Inc., Eden Prairie, MN). The powder composition was 93.2 ± 1% protein (N × 6.38), 5.1 ± 0.3% moisture and 2.3 ± 0.1 ash. Iranian tragacanth gum (*A. gossypinus*) used in this study was collected from Hindu Kush rangeland city Fereidan. Protein, ash and moisture content of whole gum were determined prior to preparation of stock solution using the standard methods of AOAC. The gum composition was 0.43 ± 0.1% protein (N × 6.38), 2.5 ± 0.1% ash, 9.8 ± 0.2% moisture content. All chemical reagents were of analytical grade.

Preparation of BLG and tragacanth gum stock dispersions

BLG and tragacanth gum solutions were prepared in deionized water under gentle stirring at 20°C. At low concentrations of biopolymer dispersions (i.e. ≤1), the dispersions were stirred for 2 h at room temperature and kept overnight at 5°C. At high concentrations of biopolymer dispersions (i.e. 5%), the mixtures were stirred 24 h at room temperature and kept overnight at 5°C in order to complete hydration of the biopolymers. BLG solutions and tragacanth gum were mixed at 50:1 to 1:2 weight ratios and total concentration from 0.1 to 3%. The pH (Jenway 3330, pH meter, Canada) of the dispersions was adjusted at 20 ± 1°C by 0.1N HCl.

BLG–Tragacanth gum complex formation

The effects of pH and protein to polysaccharide ratio were followed by mixing solutions of BLG and tragacanth gum at different pH values (2–6) and different total biopolymer concentrations (0.1–3 wt%). The absorbance of the mixtures was recorded at 650 nm using UV/Visible spectrometer (U.V-2100, Unico, New Jersy, USA). Complex formation points were determined by increasing optical density of the mixtures up to 20% at 650 nm (Schmitt *et al.*, 1999). Absorbances were plotted as a function of pH at specific protein to polysaccharide ratios and at concentrations of 0.1, 0.5, 1 & 2 wt%.

Dry matter measurement of phases after phase separation

After preparation of stock solutions of BLG (5 wt%) and tragacanth gum (0.1 wt%), the solutions were mixed at 2:1 ratio. The mixture pH was slowly decreased using HCl and then two phases were separated and poured in aluminum container and placed on a water bath. Then, it was dried at 105°C until constant weight was achieved.

BLG-Tragacanth aggregates size

Aggregates size was measured using laser light Scattering (Malvern Mastersizer S 2000) equipped with a 5 mW He/Ne (632.8 nm) laser beam. The BLG and tragacanth gum dispersions mixtures were prepared at different weight ratios of 1:1, 1:2, 1:20 and 2:1. Mixture pHs were then adjusted based on the beginning of complex formation. Optical properties of the mixtures were chosen according to Schmitt *et al*. (1999). All measurements were performed in triplicates.

Results and Discussion

Effect of pH on absorbance of biopolymers dispersions

The effect of pH on complex formation between BLG and tragacanth gum was studied by following the variation of the mixtures absorbances at 650 nm. In the first step, the contribution of each biopolymer in the absorbance values was determined at 650 nm as a function of pH. As can be seen in Figure 1, tragacanth gum dispersions at the mentioned concentration had a small contribution in the absorbance variation on the tested pH range. This shows that tragacanth gum solution is stable at this wide pH range. BLG dispersions displayed a different behavior which exhibited a sharp increase of the absorbance at pH values close to pH 5.0 (maximum at pH 4.8). Obviously, these results can be attributed to the presence of small and large BLG aggregates in the dispersions at these pHs. Therefore, the large increase in absorbance at 650 nm at pH values close to the Isoelectric Point (IEP) (pH = 5.2) is mainly due to the increase of the particles number and size. At other pH values (higher than 6 or lower than 3.5), the protein contains net (negative or positive) charges and no aggregates are formed due to electrostatic repulsion.

These results were consistent with observations of absorbance of BLG solutions by Schmitt *et al.* (1999).

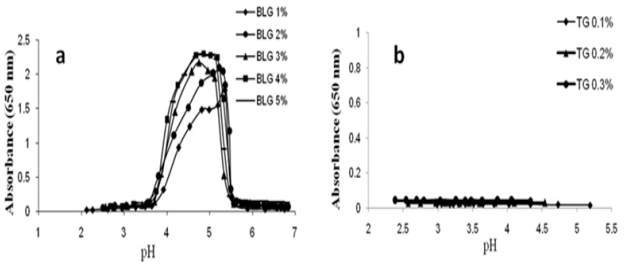

Figure1. Absorbance of BLG (a) and tragacanth gum (b) solutions (at 650 nm) as function of pH at different total biopolymer concentrations

By decreasing pH of mixture solutions of BLG and tragacanth gum at different combined ratios and total concentration, firstly, soluble complexes were formed and the solution turbidity was increased. Further decrease of the pH caused formation of insoluble complexes. In this condition, the mixture became completely opaque and by that time, BLG-tragacanth complexes were sedimented and two separated phases were observed. As pH was further decreased, the amount of formed complexes was reduced due to protonation of tragacanth gum and as a result, solution color became transparent. The increase of absorbance of the biopolymers mixture reveals three phases which are presented in Figure 2.

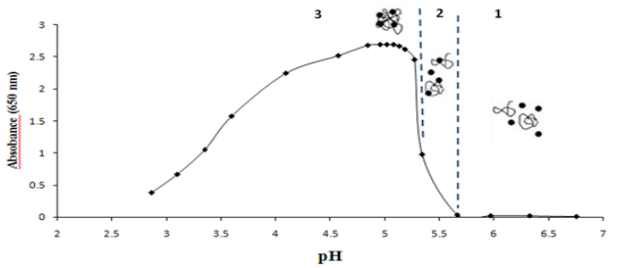

Figure 2. Absorbances of BLG-tragacanth mixture solutions at 50:1 ratio at different pH measured at 650 nm. pH_c, $pH_{\varphi 1}$, pH_{opt} and $pH_{\varphi 2}$ are 5.7, 5.3, 5 and 3.5, respectively

The absorbance of BLG/ tragacanth mixture is almost constant and low at a pH above a critical pH value (pH_c) (region 1). The second phase is between pH_c and a pH of phase separation (called $pH_{\varphi 1}$) where the absorbance increased slightly with decreasing pH (region 2). Then, at $pH_{\varphi 1}$ (and below), the absorbance increased rapidly. Later, it decreased because of sedimentation of BLG-tragacanth complexes. Initial interactions between BLG and tragacanth were started at a pH around 5.7 (pH_c) and then at pH= 5.3 ($pH_{\varphi 1}$) particle numbers and sizes were increased and insoluble complexes between BLG-tragacanth were formed. Optical density was increased to its maximum at pH = 5 (pH_{opt}). These regions were previously found by some researchers working on other protein-polysaccharide systems (Semenova *et al*., 2009; Weinbreck *et al*., 2004; Zaleska *et al*., 2001). This pH is the best as it ensures the maximum of interactions between the biopolymers. At pH lower than pHo_{pt}, formed complexes were started solubilizing due to protonation of tragacanth gum. This phenomenon was observed at pH = 3.5 ($pH_{\varphi 2}$) and at this pH and lower values, the biopolymers can remain co-soluble. pH_c and $pH_{\varphi 1}$ depend on many parameters such as isoelectric pH of protein and protein to polysaccharide ratio. For example, by increasing protein concentration in the mixtures, $pH_{\varphi 1}$ is increased to higher values.

Figure 3 shows absorbance of BLG-tragacanth mixtures at different ratios and total concentration as a function of pH. For 0.1 wt% biopolymers concentration (Figure 3 (a)), no absorbance variation was observed at pH values above 4. In this case, maximum absorbance value was obtained below pH 3.3. By increasing Ps: Pr ratio in the mixtures, pH variation had no effect on the absorbance and stayed near zero (biopolymer total concentration 0.1 wt%). Low biopolymer total concentration limited the frequency of collision of the particles. Therefore, at this biopolymer concentration, electrical charge of the particles had an important effect on complex formation. Consequently, in this case, at lower pH the complexes between the biopolymers were observed. When the total biopolymer concentration increased to 0.5 wt %, maximum absorbance was achieved at a higher pH. At 1 and 2 wt% biopolymer concentrations, maximum absorbance was identified around pH 5. At these concentrations, the absorbance varied at all pH values and ratios and only at pH values higher than 5.8, absorbance variation was not observed. These findings showed both the effect of pH and biopolymer concentration. Our results also show that at high total biopolymers concentrations, i.e. 1 and 2 wt%, a considerable change was not observed in $pH_{\varphi 1}$ as compared with lower total biopolymers concentrations, i.e. 0.1 and 0.5 wt%. This is because the soluble complex formed between polysaccharides and proteins is not completely dependent on biopolymers concentrations.

Effects of protein to polysaccharide (pr:ps) ratio on complex formation

The Pr:Ps ratio is a meaningful parameter in mixed biopolymer systems. It controls the balance of macromolecules charges and accordingly, the

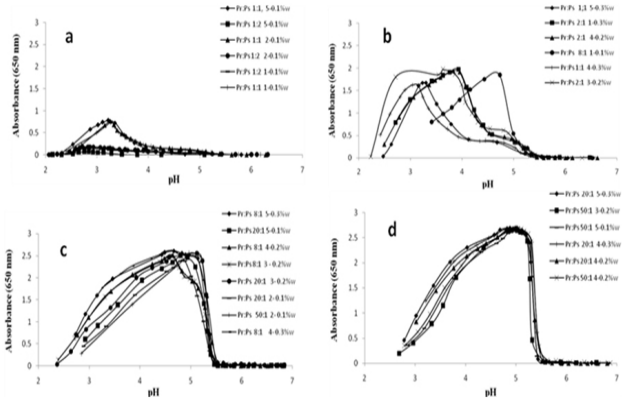

Figure 3. Absorbance of BLG–tragacanth gum mixtures (at 650 nm) as function of pH at different total biopolymer concentrations and Pr:Ps ratios: (a) 0.1 wt% total biopolymer concentration; (b) 0.5 wt% total biopolymer concentration; (c) 1.0 wt% total biopolymer concentration; (d) 2.0 wt% total biopolymer concentration. (protein:polysaccharide ratio, BLG-tragacanth concentration)

intensity of the electrostatic interactions. Regarding the absorbance at 0.1 wt%, variation only occurred at pH values below 4 and protein to polysaccharide ratios less than 2. At 0.5 wt% total biopolymer concentration, absorbance changes occurred at higher ratios and at pH close to protein isoelectric pH. For 1.0 wt% total biopolymer concentration, the absorbance of samples began rising for the 1:2 ratio at pH 3.3. Moreover, for the 2 wt% total biopolymer concentration, at pH 4-5, increase in absorbance was shown at higher ratios than 8:1. All these data confirmed the effect of the total biopolymer concentration on the coacervation formation between BLG and the tragacanth gum. Coacervation arises at low protein to polysaccharide ratios for pH values far from the IEP of the BLG. For low biopolymer concentrations (0.1 wt%), the phase separation only occurs when the protein is in excess. Otherwise, the number of protein molecules is not sufficient to neutralize all the tragacanth gum ones. The resulting complexes still carry a net negative charge and become dispersed. In the same vein, for an excess of protein, the complexes are positively charged. When the biopolymer concentration increases, phase separation appears even if the ratio is in favor of the tragacanth gum because of the large number of protein molecules able to interact. Studying the effect of such a parameter on BLG–tragacanth gum system indicated how much protein portion increases at protein to polysaccharide ratio and diagrams move to side of pHs near protein isoelectric pH and maximum absorbance considerably. This phenomenon is due to electrical charge balance between BLG and tragacanth gum that control phase separation and aggregation.

Particle size distribution

Mean particle size and turbidity are used for exploring of knowledge in relation to soluble and insoluble complexes formation. In BLG and tragacanth gum mixtures, at pH 3.6, the absorbance of mixed solutions for the mixture containing 5% BLG and 0.3% tragacanth gum (ratios of 20:1) was decreased. Moreover, at this concentration, mean particle size decreased and was about 230 μm. At pH 4.9, the absorbance of mixed solutions and mean particle size for the mentioned concentrations increased. Maximum absorbance value of biopolymer mixed solutions and maximum mean particle size were observed at pH 5.8. Figure 4 presents particle size distribution of the biopolymers mixtures.

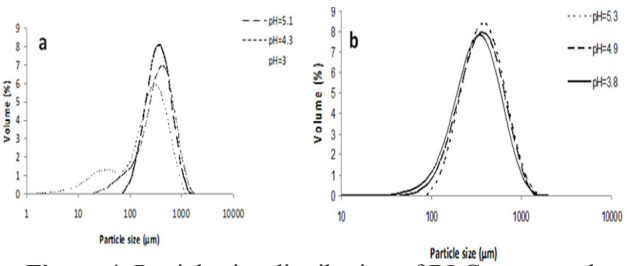

Figure 4. Particle size distribution of BLG-tragacanth mixture at 20°C and different ratio: a- BLG-tragacanth ratio 1:1, b- BLG-tragacanth ratio 20:1

These results illustrate that the increase in turbidity, being dependent on pH at mixed biopolymer, is due to electrostatic interaction between BLG and tragacanth gum as Weinbreck *et al.* (2004) for whey proteins and carrageenan and Schmitt et al (1999) reported for BLG and acacia gum. At pH > pH_c, proteins and polysaccharides are both negatively charged and therefore, repulsive electrostatic forces prevent the complexation. This means that biopolymers are soluble in the aqueous solvent. At a pH between pH_c and $pH_{\varphi 1}$, mean particle size increased. This can be explained by the formation of soluble complexes of BLG and tragacanth gum. At pH < $pH_{\varphi 1}$, the primary complexes and phase separation took place and mean particle size decreased. The decrease of the particle size could be due to the shrinkage of the molecule and reduced intramolecular repulsion. The results of particle size distribution show that protein to polysaccharide ratio had an important effect on complexes size. It was observed that at protein to polysaccharide ratio of 1:1, a small difference was observed in particle size at pHs studied (the difference was due to the shrinkage of complexes). At ratios of 1:2, 2:1 and 20:1, by increasing protein to polysaccharide ratio, mean particle size was slightly increased.

Considering particle size distribution, BLG/tragacanth mixtures exhibited a slight increase of the mean diameter when the biopolymer concentration was increased. This is in accordance with the effect of the total biopolymer concentration. The presence of protein aggregates in the initial BLG dispersion has been found to have a marked effect on the size of the obtained complexes. The large complexes at high Pr:PS ratios are possibly the result of protein aggregation and/or the formation of complexes covered with a protein multilayer. As explained by Sanchez and Paquin (1997) at a pH value close to the protein's IEP, the attraction between proteins is maximal and large aggregates tend to form. The quantity of tragacanth in the solution may also have, to a certain extent, controlled the final size of the complexes since the presence of the polysaccharide would have an important role in preventing the over aggregation of proteins.

Effect of temperature on complex formation

Absorbances of the biopolymers mixtures were studied at 25 and 5°C. Generally, hydrophobic interactions are less strong and the number of hydrogen bonding is higher at a lower temperature. By mixing the biopolymers solutions at 5°C, pH_c tended to rise to higher values. The same result was found for carrageenan-whey proteins (Weinbreck *et al.*, 2004). Also, complex formation was more rapid as compared with the results found at 25°C (results are not presented).

Conclusion

Tragacanth gum contains two fractions; soluble (tragacanthin) and insoluble (tragacanthic acid). This hydrocolloid has an important position in food and pharmaceutical industries. Interactions of tragacanth with BLG were investigated in this study. The results support the hypothesis of complex coacervation phenomenon between BLG and tragacanth gum in aqueous medium. Building of phase diagrams gives a general overview of the pH-dependence of the areas of the two phases. The effects of pH and protein to polysaccharide ratio were demonstrated by following the absorbance of the mixtures at 650 nm. Complexes formation between the two biopolymers strongly depended on the solution pH. By decreasing pH, the biopolymers concentration in which the instability occurred, decreased. Complexes formation had an important effect on physical properties of the mixture such as turbidity and viscosity. Complexes formation in this case also depends on biopolymers concentrations. At higher concentrations of biopolymers in the solution, the pH of complex formation was higher. Further researches should be done to study the effect of soluble fraction of tragacanth gum and its contribution on complexation with BLG. Moreover, Isothermal titration calorimetry can be used to determine the type and magnitude of the energies involved in the complexation process of tragacanth gum and BLG.

References

AOAC. 1984. Official Methods of Analysis. Association of Official Analytical Chemists, Washington, DC.

Bromley, E.H.C., Krebs, M.R.H. and Donald, A.M. 2005. Aggregation Across The Length-Scales in Beta-Lactoglobulin. Faraday Discussions 128: 13-27.

Cavallieril, A.L.F., Fialho, N.A.V. and Cunha, R.L. 2011. Sodium Caseinate and κ-Carrageenan Interactions in Acid Gels: Effect of Polysaccharide Dissolution Tempreture and Sucrose Addition. International Journal of Food Properties 14(2): 251–263.

Chang, H.M., Lu, T.C., Chen, C.C., Tu, Y.Y. and Hwang, J.Y. 2000. Isolation of Immunoglobulin from Egg yolk by Anionic Polysaccharides. Journal of Agricultural and Food Chemistry 48(4): 995-999.

De Kruif, C.G., Weinbreck, F. and De Vries, R. 2004. Complex Coacervation of Protein and Anionic Polysaccharides. Current Opinion in Colloid and Interface Science 9(5): 340-349.

De Wit, J.N. 1998. Nutritional and Functional Characteristics of Whey Proteins in Food Products. Journal of Dairy Science 81(3): 597-608.

Girard, M., Turgeon, S.L. and Gauthier, S.F. 2002. Interbiopolymer Complexing Between β-Lactoglobulin and Low- and High-Methylated Pectin Measured by Potentiometric Titration and Ultrafiltration. Food Hydrocolloids 16(6): 585-591.

Gottschalk, M., Venu, K. and Halle, B. 2003. Protein Self-association in Solution: The Bovine Pancreatic Trypsin Inhibitor Decamer. Biophysical Journal 84(6): 3941-3958.

Kinsella, J.E. 1984. Milk Proteins, Physicochemical and Functional Properties. Critical Reviews in Food Science and Nutrition 21(3): 197-261.

Piculell, L., Iliopoulos, I., Linse, P., Nilsson, S., Turquois, T., Viebke, C. and Zhang, W. 1994. Association and Segregation in Ternary Polymer Solutions and Gels. In Phillips, G.O., Williams, P.A., Wedlock, D.J. (Eds). Gums and stabilisers for the food industry 7, p. 309-322. Oxford: Oxford University Press.

Sanchez, C. and Paquin, P. 1997. Protein and Protein-Polysaccharide Microparticles. In Damodaran, S., Paraf, A. (Eds). Food proteins and their applications in foods, p. 503-552. New York: Marcel Dekker.

Sawyer, L., Brownlow, S., Polikarpov, I. and Wu, S.Y. 1998. β-Lactoglobulin, Structural Studies, Biological Clues. International Dairy Journal 8(2): 65-72.

Schmitt, C., Sanchez, C., Thomas, F. and Hardy, J. 1999. Complex Coacervation Between β-Lactoglobulin and

Acacia Gum in Aqueous Medium. Food Hydrocolloids 13(6): 483-496.

Semenova, M.G., Belyakova, L E., Polikarpov, Y.N., Antipova, A.S. and Dickinson, E. 2009. Light Scattering Study of Sodium Caseinate+Dextran Sulfate in Aqueous Solution: Relationship to Emulsion Stability. Food Hydrocolloids 23(3): 629-639.

Weinbreck, F., Nieuwenhuijse, H., Robijn, W.G. and De Kruif, C.G. 2004.Complexation of Whey Proteins with Carrageenan. Journal of Agricultural and Food Chemistry 52(11): 3550-3555.

Weinbreck, F., Vries, F., Schrooyen, P. and De Kruif, C.G. 2003.Complex Coacervation of Whey Proteins and Gum Arabic. Biomacromolecules 4(2): 293-303.

Weiping, W. 2000. Tragacanth and Karaya. In Phillips, G.O., Williams, P.A. (Eds). Handbook of hydrocolloids, p. 231-245. Cambridge: Woodhead Publishing Limited.

Wong, D.W.S., Camirand, W.M. and Pavlath, A.E. 1996. Structure and Functionalities of Milk Proteins. Critical Reviews in Food Science and Nutrition 36(8): 804-844.

Zaleska, H., Ring, S. and Tomasik, P. 2001. Electrosynthesis of Potato Starch-Whey protein Isolate Complexes. Carbohydrate Polymers 45(1): 89-94.