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Phase behavior of ovalbumin and carboxymethylcellulose composite system

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ABSTRACT

The phase behavior, rheological properties and microstructure of ovalbumin and carboxymethylcellulose (OVA–CMC) conjugates were studied and the influence parameters were investigated. The results showed that the phase behavior of OVA–CMC conjugates was related to pH and concentration of CMC and NaCl. When pH was over 5.0, discrete phase separation occurred in the mixture system, which indicated that OVA and CMC were thermodynamic incompatible. The mixture system turned into uniform stable emulsion system when pH reduced below 5.0. The addition of NaCl can improve the stability of composite system against pH sensitivity. CLSM and particle size distribution and ultraviolet spectrum analysis results confirm that emptying interactions play a leading role in the separation system.

1. Introduction

Egg white proteins are widely used as a functional food material in food processing (Huang et al., 2012). Ovalbumin (OVA) is the major protein component of egg white and the most widely used model protein. It is an important food ingredient which possesses structural functionality including emulsifying property and foaming stability (Choi, Kim, Park, & Moon, 2005; Song et al.,2013). It is applied as foodstuffs because of not only their functional properties, but also high nutritive value. Its structure and properties predominantly affect the functional properties of egg white protein (Huang et al., 2012; Liu et al.,2014; Sun, Hayakawa, & Izumori, 2004).

The interaction between protein and polysaccharide was a hot scientific topic and the frontier in the field. The protein–hydrocolloid interactions in bulk solutions and at interfaces have an important influence on the stability properties of food dispersions (Doublier, Garnier, Renard, & Sanchez, 2000; Ercelebi & Ibanoğlu, 2007). The mixture of protein–hydrocolloid in an aqueous solution can exhibit one of the three different equilibrium situations: (a) miscibility, (b) thermodynamic incompatibility or (c) complex coacervation (or complexation) (De Kruif & Tuinier, 2001; Martinez, Baeza, Millan, & Pilosof, 2005). Both incompatibility and coacervation (or complexation) appear at high concentrations, which is depended on whether the protein–hydrocolloid interaction is a net repulsion or a net attraction, respectively (Dickinson, 2003). Phase-separated networks are formed by incompatible biopolymers where interactions between the different polymers are repulsive when the two types of polymers show different appetency toward the solvent (Doublier et al., 2000; Grinberg & Tolstoguzov, 1997; Van den Berg, van Vliet, van der Linden, van Boekel, & van de Velde, 2007). Phase-separated structures are most likely the outcome of gelation (Spotti, Santiago, Rubiolo, & Carrara, 2012; Tavares, Monteiro, Moreno, & Lopes da Silva, 2005).

Numerous studies about the OVA-saccharide conjugates have been carried out, focusing on the improvement of gelling property (Sánchez-Gimeno, Vercet, & López-Buesa, 2006) and foaming property, solubility, emulsifying property heat stability (Aoki et al., 1999; Kato, Minaki, & Kobayashi, 1993; Nakamura, Kato, & Kobayashi, 1992). Many researchers have investigated that the improvement of functionalities is due to using coexisting protein and polysaccharide (Babiker & Kato, 1998; Dickinson, 2003; Kim, Choi, Shin, & Moon, 2003). Scientific understanding of biological macromolecules hybrid system phase behavior can help to design and control the microstructure of the product. Phase behavior theory and its influence on product structure motivate many scientists study enthusiasm.

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Carbomethoxy cellulose (CMC), one of the most important derivatives of cellulose, is a typical anionic polysaccharide that has been widely utilized as a stabilizer in food (Toğrul & Arslan, 2004a, 2004b). CMC is one of the natural water-soluble cellulose derivatives that do not have harmful effects on human health (Su, Huang, Yuan, Wang, & Li, 2010). CMC is applied as a highly effective additive to improve the processing properties of products in fields of application varying from cosmetics, pharmaceuticals and foodstuffs (Schmitt, Sanchez, Desobry-Banon, & Hardy, 1998; Ye, 2008;Zhu et al.,2013)

In view of the poor research of interaction between OVA and the ionic polysaccharide, the relatively low price anionic polysaccharide sodium carboxymethylcellulose (CMC) was chosen to explore the influence that the physical blend and glycosylation modification of OVA and CMC on the structure and functional properties of products. So as to further reveal the mechanism of interaction between OVA with CMC. The study enriched the theoretical frame of interaction between protein and polysaccharide and provided fundamental resources for intensive study; at the same time, it also offered guidance on usage of mixture of food ingredients from egg source and polysaccharide.

2. Experimental

2.1. Materials

Ovalbumin (OVA) and nile blue chloride were purchased from Sigma Chemical Co. Ltd. (St. Louis, MO, USA). Sodium carboxymethylcellulose (CMC, viscosity 300–800 mPs, viscosity average molecular weight 9.72E+04), disodium hydrogen phosphate, sodium dihydrogen phosphate, sodium chloride, sodium hydroxide and hydrochloric acid were purchased from Sino harm Chemical Reagent Co., Ltd. (China). CMC were purified by alcohol precipitation before use and DS is 0.69.

2.2. Preparation of OVA/CMC conjugates

The bulk OVA and CMC solutions were prepared by dissolving a certain amount of OVA and CMC powder into 50 mM phosphate buffer at room temperature. The OVA–CMC mixture systems were obtained by adding CMC solution into OVA solution. The concentration of OVA was fixed at 1 wt% and CMC concentration was varied concentration from 0 to 0.5 wt%. Each sample was kept stirring for 1.5 h at room temperature after mixing.

2.3. Phase diagram

The phase diagram was set up by transforming pH, NaCl and CMC concentration. pH of all the solutions were adjusted from 2.5 to 7 with 1 M HCl and NaOH. 0–500 mM NaCl were added into the mixture system. The OVA–CMC mixture systems were stored overnight at the room temperature before measurement. The phase diagram was draw out through the macroscopic observation.

2.4. Confocal laser scanning microscope (CLSM)

The microstructure of OVA–CMC conjugates was observed by CLSM (LSM 510 META, Zeiss, Germany) equipped with a He/Ne mixed gas laser. Nile blue chloride was used to dye OVA. A drop of nile blue chloride was added into the fresh OVA solutions or OVA–CMC mixture and then mixed well by vortex mixer. An aliquot of sample was then placed on a microscope slide, covered by a cover slip. A 40× objective lens was used to capture confocal images. Digital image files were acquired in 1024 × 1024 pixel resolution.

2.5. Rheological properties

Rheological properties were evaluated on a stress-controlled AR2000ex rheometer (The United States TA instrument Co., New Castle DE, USA) with parallel plates (d = 40 mm) at 25 °C. The sample was dumped on the bottom of frustum of a cone, and lowered the fixture. Determination was carried out with steady shear experiment and selected the flow pattern and determination of shear rate range was 1–300 s^{-1}.

2.6. Determination of average particle size

The droplet size distribution of the OVA–CMC conjugates systems was measured using a laser light scattering instrument (Malvern Instruments, Nano-ZS, Worcestershire, UK). Each sample was diluted under the same ion strength and pH of phosphate buffer before the test on the volume ratio of 1:10. Each sample was analyzed in triplicate.

2.7. UV–Vis analysis of OVA–CMC conjugates

The UV–Vis analysis of OVA–CMC conjugates was performed using a spectrophotometer (UV-1700, Japan). The conjugate was centrifuged for 20 min at 15,000 r/min. Model reaction conjugates with thirty-fold dilution by phosphate buffer was placed in quartz cuvette, and the sample was scanned from 220 to 400 nm.

2.8. Transmission electron microscopy (TEM)

For the analysis of the OVA–CMC conjugates by JEM-2010FEF (NEC, Japan), a droplet of a dilute suspension of OVA–CMC composite solution was deposited on a carbon-coated grid and allowed to dry (Dolphen & Thiravetyan, 2011). The accelerating voltage was 200 kv.

3. Results and discussion

3.1. Influence of pH, CMC and ion concentration on the phase behavior of OVA–CMC composite system

The phase behavior of OVA–CMC mixture system was studied by varying pH, NaCl and CMC concentration. The resulting phase diagram and visual images were shown in Fig. 1. (Overall, three kinds of phenomenon were observed, which were precipitation, phase separation with lower turbidity and relatively transparent upper phase and milky white uniform phase.)

Fig. 1a displayed the influence of pH on the phase behavior of OVA–CMC mixture systems. When pH was over 5, discrete phase separation obviously occurred by forming the epinephelos lower layer and relatively transparent upper layer. This is because OVA and CMC have the same charge and they may occur depletion interaction. It could also be observed that the phase separation was independent on the concentration of CMC. The height of lower phase increased with higher CMC concentration. The results were consistent with the previous study by Blonk et al. (Blonk, van Eendenburg, Koning, Weisenborn, & Winkel, 1995). Protein macromolecules are easy to form relatively compact lower phase at low concentration, presenting the formation of network structure. Part of polysaccharides embed in the network structure. Along with the increase of the concentration of polysaccharide, height of the lower phase increases.

When pH was less than 5, there might be electrostatic interactions between CMC and OVA molecules. Because OVA is positively charged when pH is lower than the isoelectric point of OVA (pI 4.6), which prompted the intermolecular electrostatic interaction between OVA and anionic polysaccharide CMC.

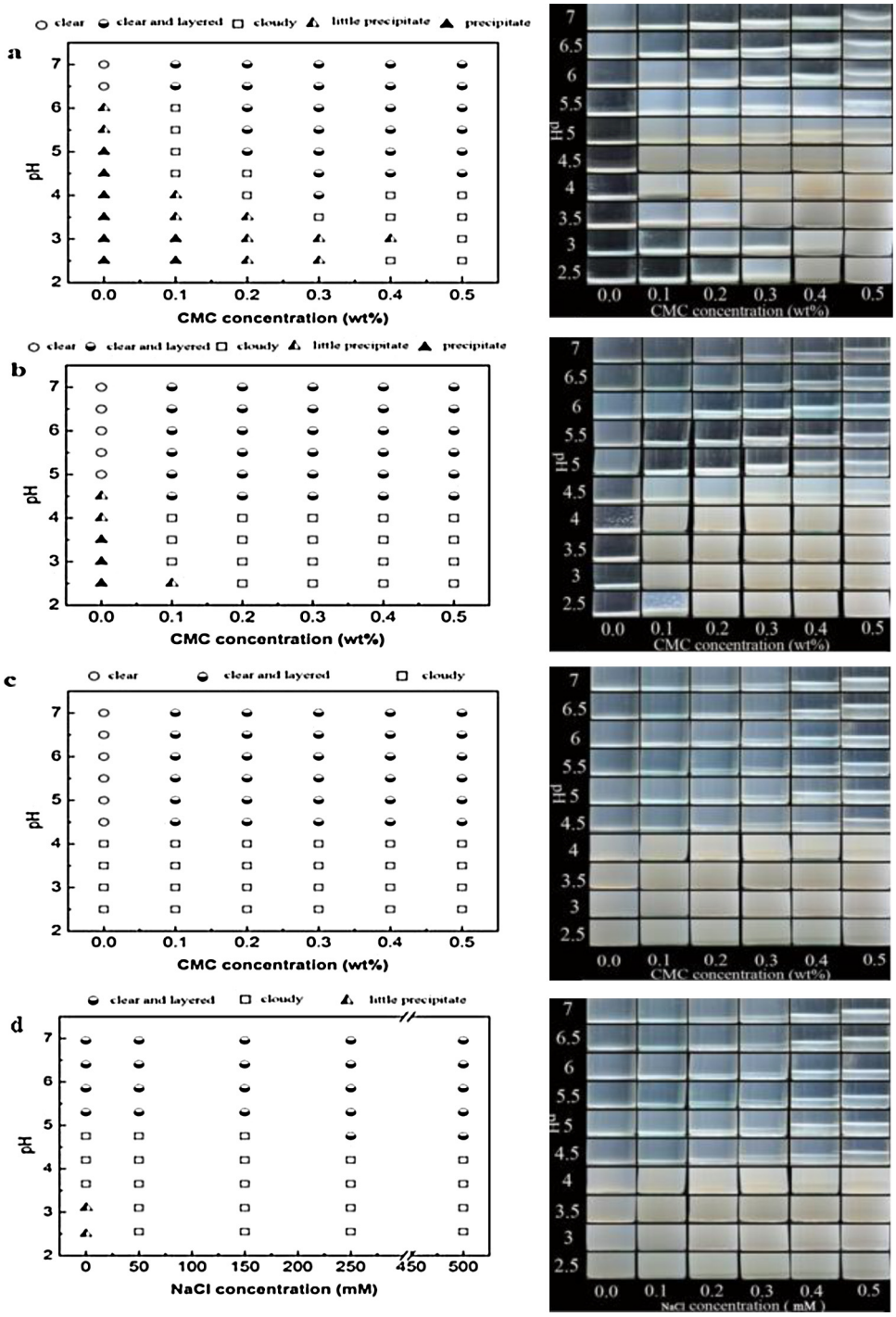

Fig. 1. Phase diagrams and picture of OVA–CMC systems. (a) 0 mM NaCl, (b) 150 mM NaCl, (c) 500 mM NaCl at different pH values and different CMC concentrations respectively. (d) 1 wt%OVA + 0.4 wt%CMC at different pH and NaCl concentration.

They arise non-positioning of the adsorption and form the milky white uniform solution. When CMC concentration is less than 0.4%, phase separation occurs easily. It is because there is electrostatic adsorption between CMC and OVA at low pH condition. At low CMC concentration, the positive area of OVA is relatively large and CMC molecules can interact with multiple proteins, forming larger particles. Due to the flocculation between OVA and CMC, the mixture system is separated into two layers. The upper layer is clear transparent water phase and the lower layer is the compound precipitated phase rich in CMC and OVA complex. With higher CMC concentration, a certain thickness of the adsorbed layer is formed on the protein surface and the system is stable. As pH is lowering, the higher CMC concentration is required to stabilize OVA, due to the higher positive charge of protein.

The influence of NaCl concentration on the OVA–CMC system is shown in Fig. 1b and c. At lower NaCl concentration (Fig. 1b), the plug color tube phase separates significantly at pH > 4.5, compared with Fig. 1a. However, when pH is lower than 4.5, most of them exhibit a milky white uniform stable solution without visible separation. The forming of floc area decreased. At higher NaCl concentration (Fig. 1c), phase separation area is more obvious (pH > 4.5). But the formed solution is uniform ivory at pH < 4.5.

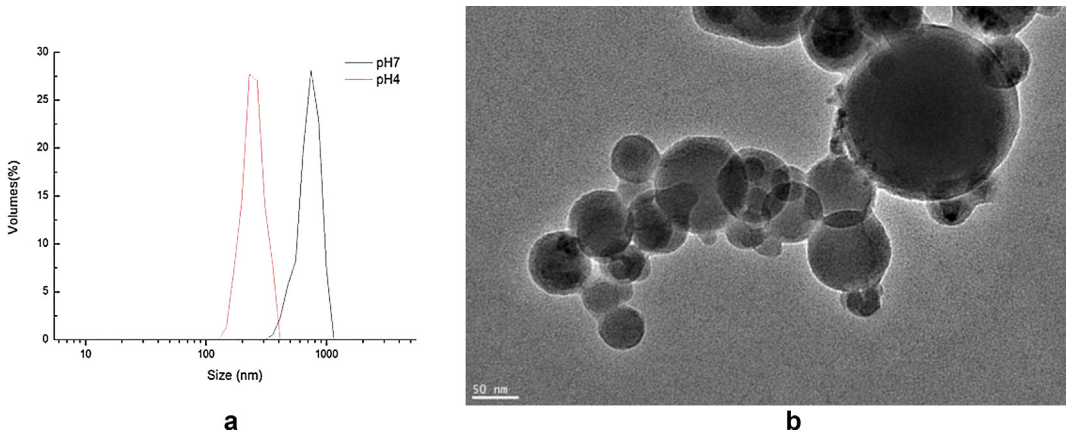

Fig. 2. (a) Particle size distribution of OVA–CMC mixtures (1 wt%OVA + 0.4 wt%CMC) with different pH values. (b) TEM image of OVA–CMC mixtures (1 wt%OVA + 0.4 wt%CMC, pH 4.5, NaCl 500 mM).

Interestingly, precipitated phase does not appear even under low polysaccharide concentration.

The results show that the phase behavior of OVA and CMC depends on environmental pH, CMC and salt concentration. It has been reported that ionic strength can induce and accelerate the phase separation in the protein–polysaccharide system. Fig. 1d shows that phase separation becomes more obvious and extended with increasing NaCl concentration. This is because that on one hand salt ion concentration promotes the phase separation between proteins and polysaccharides; on the other hand, it affects the aggregation of protein. Salt ion shields the charge of protein molecules and reduces the intermolecular repulsive force, which promotes protein crosslinking, thus forming the larger particles aggregate. Phase diagram results show that larger aggregate particles, formed by protein, are more likely to be separated with CMC under a certain ionic strength. It further proves that the emptying interaction in OVA and CMC phase separation plays an important role.

3.2. Particle size distribution of OVA–CMC composite

Fig. 2 reveals the particle size distribution of OVA–CMC (1 wt% OVA + 0.4 wt% CMC) composite solution under pH 4.0 and 7.0. The particle size under pH 7.0 is larger than that under pH 4.0, which is changed from 229 nm to 741 nm. This may be because that the phase separation obviously occurred in the composite system under pH 7.0. Aggregate increases and results in the increase of particle size. However, under pH 4.0, it forms the uniformity stable emulsion. The aggregate is few, and particle size is small. This is consistent with the result of the CLSM. As there is micro blue light in the OVA–CMC composite system when NaCl concentration is 500 mM and pH value is over 4.5, it is supposed that nano gel is formed in the system, which is further verified by TEM observation. The round particles are observed under TEM.

3.3. Microstructure of OVA–CMC composite system

The microstructure of OVA–CMC composite system under different pH conditions is shown in Fig. 3. The black areas of the diagram is CMC enrichment region and water phase. The green luminous area is protein-rich area dyed by nile blue. For pure OVA system, protein distributes uniformly in the water phase at pH 6.0 and 7.0. Under pH 4.0 and 5.0, there is some flocculation, which is more obvious at pH 4.0. This is because the OVA dehydrates and condenses at the isoelectric point. For OVA–CMC composite system, the aggregates are apparent under pH above 5.0, which proves that phase separation occurs. When the pH value is 4.0, particle dispersion is relatively uniform in the OVA–CMC system, and there is no obvious phase separation aggregate.

In order to further prove that the phase separation of OVA and CMC composite system is due to the depletion interaction. Shaking plug color tube of phase separation, it can be found that lower phase is easier dispersed through the dilution. The phase separation process is reversible. The separated phase can be become the initial system after mixing the lower and upper phase. This is consistent with the depletion interaction mechanism (Hemar, Tamehana, Munro, & Singh, 2001).

3.4. Rheological properties of OVA–CMC composite system

Fig. 4 shows that the viscosity of CMC and OVA–CMC composite decreases with the increase of shear rate under different pH conditions, which belongs to non-Newtonian fluid. When the shear rate increases to a certain degree, viscosity is stable. In the medium concentration range, the CMC is a typical shear-thinning fluid. Clasen had a systematic study of the CMC rheological properties and its influence factors (Clasen & Kulicke, 2001). It is due to the polymer intermolecular clew structure is destroyed with the increase of shear rate, leading to stronger molecular directional property and decreased intermolecular forces. At the same time, it is also observed that, the reduction of pH value has produced significant influence to the compound solution and pure CMC solution.

The change of shear stress with shear rate directly reflects the touch degeneration of pseudoplastic fluid, which conform to the power-lower model $\tau = K\gamma^n$ of power law where τ is shear stress; γ is shear rate and K is the consistency coefficient. Bigger K belongs to more viscous liquid. Through which the solution sticky degree

Table 1
K and n values for OVA–CMC (1 wt%OVA + 0.4 wt%CMC) mixtures.

pH	7	6.5	6	5.5	5	4.5	4	3.5	3
K	0.0324	0.0395	0.0783	0.1605	0.2063	0.2139	0.2429	0.3563	0.4287
n	0.7541	0.7315	0.6440	0.5126	0.4909	0.4329	0.3539	0.2854	0.2739
R^2	0.9983	0.9995	0.9919	0.9873	0.9981	0.9918	0.9975	0.9735	0.9902

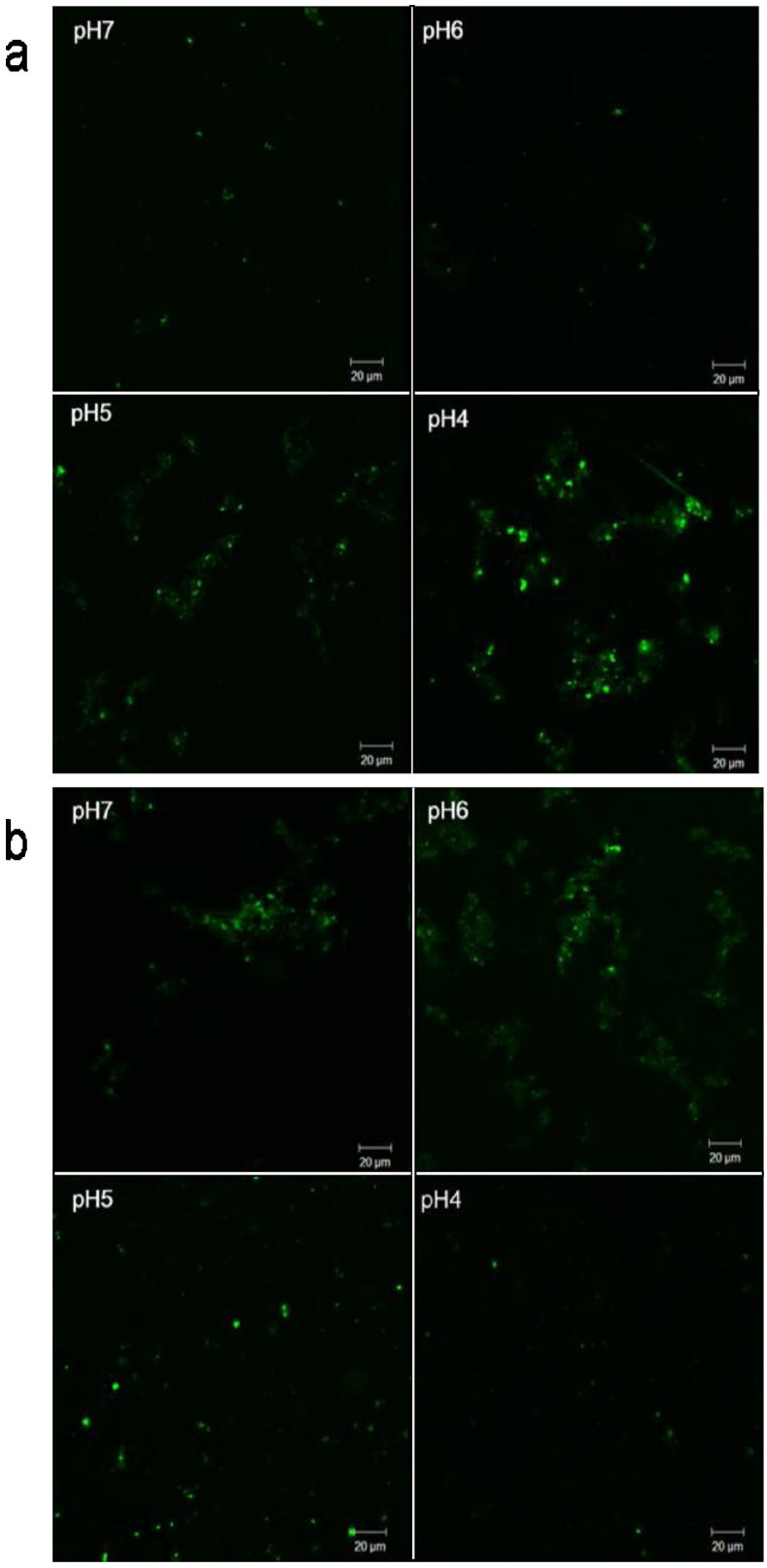

Fig. 3. CLSM images of (a) 1 wt%OVA and (b) 1 wt%OVA + 0.4 wt%CMC mixtures (150 mM NaCl).

can be measured. *N* value is a fluid type index. When *n* value is less than 1, it is the shear thinning pseudoplastic fluid. The more *n* value deviate from 1 the more likely to dilute and it also means that it has the greater the degree of pseudoplastic. Fig. 5 displays the change of shear stress of OVA–CMC composite solution under different pH conditions. The corresponding calculated *K* and *n* values are shown in Table 1. For the composite system, *K* value increases with the decrease of pH, and the *n* value constantly decreases, which demonstrates that the pseudoplastic of OVA–CMC composite system is pH-dependence.

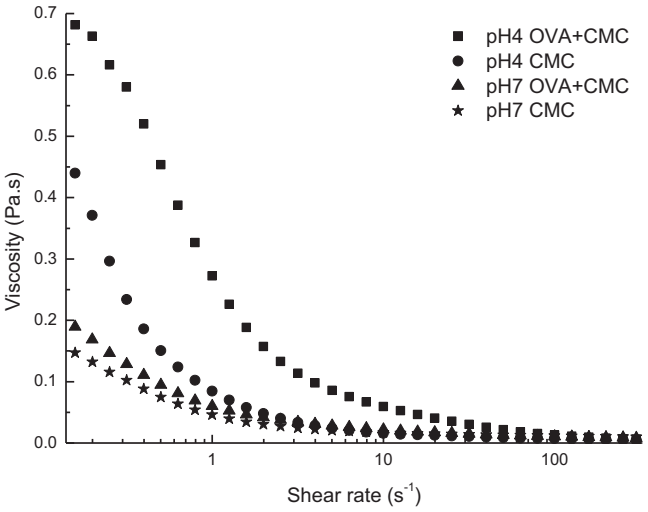

Fig. 4. Viscosity as a function of the shear rate for 0.4 wt%CMC and 1 wt%OVA–0.4 wt%CMC mixtures.

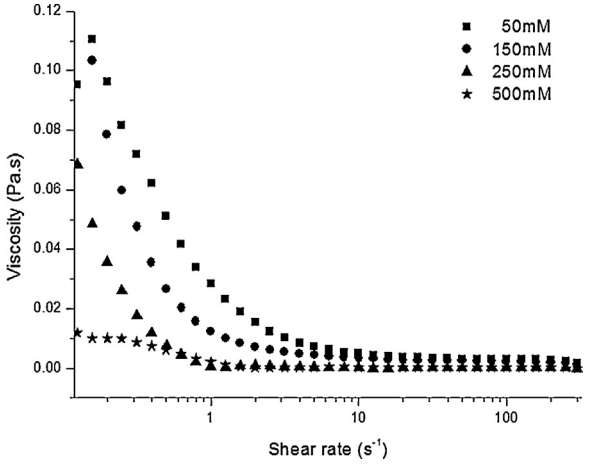

Fig. 6. Viscosity as a function of the shear rate for OVA–CMC mixtures (1 wt%OVA + 0.4 wt%CMC) mixture with different NaCl concentration (pH 4.0).

CMC aqueous solution general is pseudoplastic fluid. Its zero shear viscosity increases with the rise of CMC molecular weight and degree of substitution. However the degree of substitution of solution has less effect to the rheology qualitative. As shown in Fig. 6, with the increase of NaCl concentration, viscosity of the OVA–CMC composite solution declines, which may be related to the nature of the CMC. The viscosity of CMC rises with the increase of concentration. A gel system can be formed when CMC concentration is high enough. CMC solution has a typical polyelectrolyte effect. The viscosity of CMC solution reduces with the increasing concentration of sodium ion (when CMC solution adds sodium ion, viscosity reduces with the increase of salt ion concentration) which may because that sodium ion changes the conformation of the CMC in the solution, and molecular chain area tends to crimp. Internal friction between

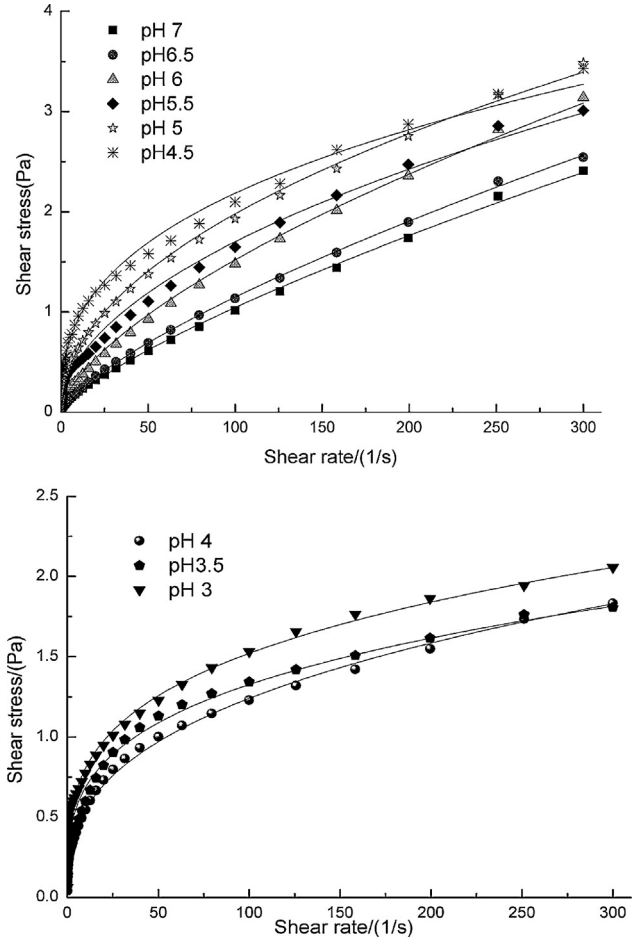

Fig. 5. Shear stress of OVA–CMC (1 wt%OVA + 0.4 wt%CMC) mixtures under different shear rate with different pH values.

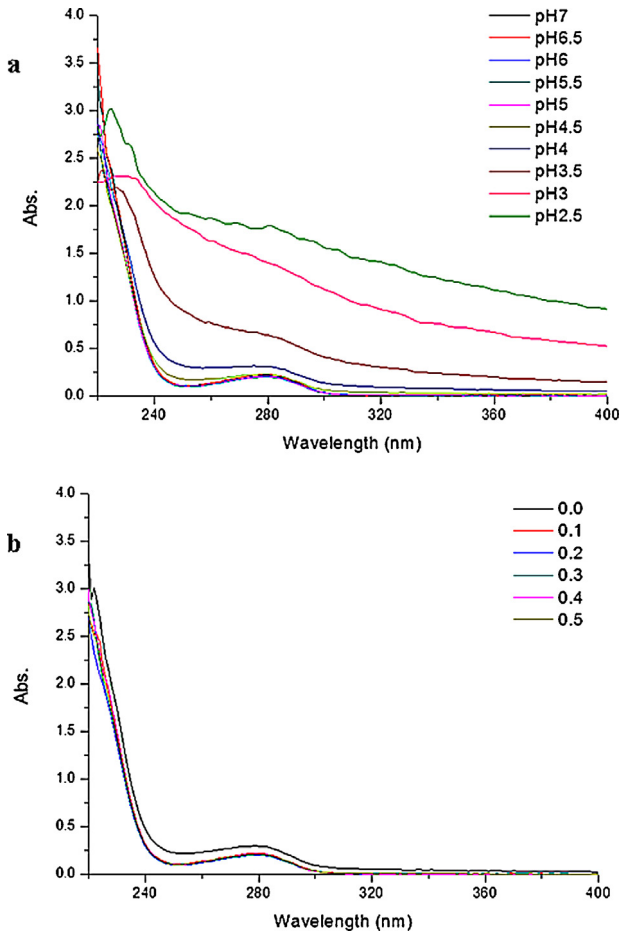

Fig. 7. Ultraviolet spectrum of OVA–CMC mixtures. (a) 1 wt%OVA + 0.4 wt%CMC with different pH values and (b) mixtures with different CMC concentrations

the molecules reduces, and the viscosity of the solution declines so that it is more conducive to flow.

3.5. UV–Vis spectrum analysis of OVA–CMC composite system

Generally speaking, ultraviolet spectrum refers to the near ultraviolet area which is from 200 to 400 nm, and the characteristic absorption of protein is 280 nm. As shown in Fig. 7a, it is the ultraviolet spectrum of OVA–CMC composite solution under different pH values. From pH 7.0 to pH 4.5, the protein contained in the sample supernatant fluid has similar absorption intensity at 280 nm, and its intensity is lower than that under pH less than 4.5. This may be due to the phase separation which occurred seriously at the pH value over 4.5, and protein mainly distributed in the lower layer. However, under the condition of the pH value less than 4.5, the solution is uniformity stable emulsion, which is consistent with the result of CLSM. Fig. 7b shows the ultraviolet spectrum of the supernatant fluid proteins which is obtained by the different polysaccharide concentration under pH 4.5. It can be found that the different concentration of the polysaccharide does not cause significant influence to the protein absorption. But compared with the purified protein without polysaccharides, protein showed lower absorbance.

4. Conclusion

The phase behavior of OVA–CMC composite system depends on pH and concentration of polysaccharide and NaCl. Under neutral condition, discrete phase separation occurs in the complex system. When pH is lower than the isoelectric point of OVA, the system is a milky homogenous solution. With the absence of NaCl, OVA–CMC system is apt to phase separation when CMC concentration is less than 0.4%. Rheology analysis shows that the CMC and OVA–CMC composite solution are pseudoplastic fluid, which is more obvious with lower pH. The presence of NaCl can shift the occurrence of phase separation into low pH value and improve the pH stability of OVA–CMC composite system. The viscosity of composite system decreases with higher NaCl concentration. (CLSM confirms that emptying interactions play a leading role in the separation system. Particle size distribution results and ultraviolet spectrum analysis results are consistent with the results of CLSM.) Nano gel is formed in the uniform OVA–CMC system.

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References

Aoki, T., Hiidome, Y., Kitahata, K., Sugimoto, Y., Ibrahim, H. R., & Kato, Y. (1999). Improvement of heat stability and emulsifying activity of ovalbumin by conjugation with glucuronic acid through the Maillard reaction. *Food Research International*, *32*(2), 129–133.

Babiker, E., & Kato, A. (1998). Improvement of the functional properties of sorghum protein by protein–polysaccharide and protein–protein complexes. *Food/Nahrung*, *42*(05), 286–289.

Blonk, J. C. G., van Eendenburg, J., Koning, M. M. G., Weisenborn, P. C. M., & Winkel, C. (1995). A new CSLM-based method for determination of the phase behaviour of aqueous mixtures of biopolymers. *Carbohydrate Polymers*, *28*(4), 287–295.

Choi, S., Kim, H., Park, K., & Moon, T. (2005). Molecular characteristics of ovalbumin-dextran conjugates formed through the Maillard reaction. *Food Chemistry*, *92*(1), 93–99.

Clasen, C., & Kulicke, W.-M. (2001). Determination of viscoelastic and rheo-optical material functions of water-soluble cellulose derivatives. *Progress in Polymer Science*, *26*(9), 1839–1919.

De Kruif, C., & Tuinier, R. (2001). Polysaccharide protein interactions. *Food Hydrocolloids*, *15*(4), 555–563.

Dickinson, E. (2003). Hydrocolloids at interfaces and the influence on the properties of dispersed systems. *Food Hydrocolloids*, *17*(1), 25–39.

Dolphen, R., & Thiravetyan, P. (2011). Adsorption of melanoidins by chitin nanofibers. *Chemical Engineering Journal*, *166*(3), 890–895.

Doublier, J.-L., Garnier, C., Renard, D., & Sanchez, C. (2000). Protein–polysaccharide interactions. *Current Opinion in Colloid & Interface Science*, *5*(3), 202–214.

Ercelebi, E. A., & Ibanoğlu, E. (2007). Influence of hydrocolloids on phase separation and emulsion properties of whey protein isolate. *Journal of Food Engineering*, *80*(2), 454–459.

Grinberg, V. Y., & Tolstoguzov, V. (1997). Thermodynamic incompatibility of proteins and polysaccharides in solutions. *Food Hydrocolloids*, *11*(2), 145–158.

Hemar, Y., Tamehana, M., Munro, P. A., & Singh, H. (2001). Viscosity, microstructure and phase behavior of aqueous mixtures of commercial milk protein products and xanthan gum. *Food Hydrocolloids*, *15*(4–6), 565–574.

Huang, X., Tu, Z., Xiao, H., Wang, H., Zhang, L., Hu, Y., et al. (2012). Characteristics and antioxidant activities of ovalbumin glycated with different saccharides under heat moisture treatment. *Food Research International*, *48*(2), 866–872.

Liu, X., Wang, X., Zhang, J., Wang, X., Lu, Y., Tu, H., Deng, H., & Jiang, L. (2014). Protein–polymer co-induced exfoliated layered silicate structure based nanofibrous mats and their cytotoxicity. *RSC Advances*, *4*(17), 8867–8873.

Kato, A., Minaki, K., & Kobayashi, K. (1993). Improvement of emulsifying properties of egg white proteins by the attachment of polysaccharide through Maillard reaction in a dry state. *Journal of Agricultural and Food Chemistry*, *41*(4), 540–543.

Kim, H. J., Choi, S. J., Shin, W.-S., & Moon, T. W. (2003). Emulsifying properties of bovine serum albumin–galactomannan conjugates. *Journal of Agricultural and Food Chemistry*, *51*(4), 1049–1056.

Martinez, K., Baeza, R., Millan, F., & Pilosof, A. (2005). Effect of limited hydrolysis of sunflower protein on the interactions with polysaccharides in foams. *Food Hydrocolloids*, *19*(3), 361–369.

Nakamura, S., Kato, A., & Kobayashi, K. (1992). Enhanced antioxidative effect of ovalbumin due to covalent binding of polysaccharides. *Journal of Agricultural and Food Chemistry*, *40*(11), 2033–2037.

Sánchez-Gimeno, A. C., Vercet, A., & López-Buesa, P. (2006). Studies of ovalbumin gelation in the presence of carrageenans and after manothermosonication treatments. *Innovative Food Science & Emerging Technologies*, *7*(4), 270–274.

Schmitt, C., Sanchez, C., Desobry-Banon, S., & Hardy, J. (1998). Structure and technofunctional properties of protein–polysaccharide complexes: A review. *Critical Reviews in Food Science and Nutrition*, *38*(8), 689–753.

Song, R., Yan, J., Xu, S., Wang, Y., Ye, T., Chang, J., Deng, H., & Li, B. (2013). Silver ions/ovalbumin films layer-by-layer self-assembled polyacrylonitrile nanofibrous mats and their antibacterial activity. *Colloids Surf B Biointerfaces*, *108*, 322–328.

Spotti, M. J., Santiago, L. G., Rubiolo, A. C., & Carrara, C. R. (2012). Mechanical and microstructural properties of milk whey protein/espina corona gum mixed gels. *LWT-Food Science and Technology*.

Su, J.-F., Huang, Z., Yuan, X.-Y., Wang, X.-Y., & Li, M. (2010). Structure and properties of carboxymethyl cellulose/soy protein isolate blend edible films crosslinked by Maillard reactions. *Carbohydrate Polymers*, *79*(1), 145–153.

Sun, Y., Hayakawa, S., & Izumori, K. (2004). Modification of ovalbumin with a rare ketohexose through the Maillard reaction: Effect on protein structure and gel properties. *Journal of Agricultural and Food Chemistry*, *52*(5), 1293–1299.

Tavares, C., Monteiro, S. R., Moreno, N., & Lopes da Silva, J. (2005). Does the branching degree of galactomannans influence their effect on whey protein gelation? *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, *270*, 213–219.

Toğrul, H., & Arslan, N. (2004a). Carboxymethyl cellulose from sugar beet pulp cellulose as a hydrophilic polymer in coating of mandarin. *Journal of Food Engineering*, *62*(3), 271–279.

Toğrul, H., & Arslan, N. (2004b). Extending shelf-life of peach and pear by using CMC from sugar beet pulp cellulose as a hydrophilic polymer in emulsions. *Food Hydrocolloids*, *18*(2), 215–226.

Van den Berg, L., van Vliet, T., van der Linden, E., van Boekel, M., & van de Velde, F. (2007). Serum release: The hidden quality in fracturing composites. *Food Hydrocolloids*, *21*(3), 420–432.

Ye, A. (2008). Complexation between milk proteins and polysaccharides via electrostatic interaction: Principles and applications – A review. *International Journal of Food Science & Technology*, *43*(3), 406–415.

Zhu, K., Ye, T., Liu, J., Peng, Z., Xu, S., Lei, J., Deng, H., & Li, B. (2013). Nanogels fabricated by lysozyme and sodium carboxymethyl cellulose for 5-fluorouracil controlled release. *Int J Pharm*, *441*(1–2), 721–727.