Interactions of ε-Polylysine with Carboxymethyl Sweet Potato Starch with an Emphasis on Amino/Carboxyl Molar Ratio

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S *Supporting Information*

ABSTRACT: The interaction between ε-polylysine (ε-PL) and anionic polysaccharides has gained considerable attention recently because of its scientific impact on the stability and appearance of liquid food systems. The purpose of this study was to characterize the interactions between ε-PL and carboxymethyl sweet potato starch (CSS) using isothermal titration calorimetry (ITC), electrical charge, and turbidity measurements. The results showed that the interaction between ε-PL and CSS was electrostatic and mainly dependent on the molar ratio of amino groups in ε-PL to carboxyl groups in CSS. Additionally, the interaction between ε-PL and CSS was also associated with pH, degree of substitution (DS) of CSS, and ionic strength of the system. For the interaction of ε-PL with high DS (>0.235) CSS, three states of the ε-PL/CSS mixture were observed as transparent, turbid, and precipitated with a successive increase in amino/carboxyl molar ratio. Distinguishingly, a transparent mixture could be obtained for CSS with low DS (0.114) at a sufficiently high amino/carboxyl molar ratio. The present study provided basic guidance in designing liquid food systems containing both ε-PL and CSS.

KEYWORDS: *ε-polylysine, carboxymethyl starch, interaction, isothermal titration calorimetry, ζ-potential*

■ INTRODUCTION

ε-Polylysine (ε-PL) is a homopolymer consisting of 25−30 L-lysine subunits, as produced in the aerobic bacterial *Streptomyces albulus* fermentation.[1,2] It has been used as a preservative in the food industry for many years.[3−7] Studies on the absorption, distribution, metabolism, and excretion suggest that ε-PL is safe for human consumption.[8] Moreover, it has been approved as generally recognized as safe (GRAS in US) with a suggested maximum amount of intake of 8.4 mg per kilogram of bodyweight per day (mg/kg bw/d) at the 90th percentile.[9,10] Due to the presence of primary amine groups along its backbone, ε-PL is cationic at pH below its isoelectric point (≈9.0), which would be the case in most common foods (pH 2−7).[11] The antimicrobial ability of ε-PL is highly dependent on its cationic nature since it functions by adhering to negatively charged cell surfaces of microbes through electrostatic interactions.[12]

As common food components, negatively charged anionic polymers within the food matrix could interact with cationic ε-PL by electrostatic interactions, leading to the formation of electrostatic complexes. The status of the electrostatic complexes, soluble or precipitate, was related to the antimicrobial ability of ε-PL and decided by the ratio of ε-PL/polymer, pH, and charge density of the polymer, etc. The formation of undesirable precipitates may deteriorate the antimicrobial activity of ε-PL and present as food product cloudiness.[13] In this context, the electrostatic complexes between ε-PL and anionic polymers must be carefully formulated to maintain the antimicrobial activity of ε-PL and product appearance or stability.[13−15]

In recent years, the electrostatic interactions between ε-PL and natural anionic polysaccharides such as ι-carrageenan, pectin, and gum arabic (GA) had been systematically investigated. Girod et al. studied the polyelectrolyte complex formation between ι-carrageenan and ε-PL. They concluded that the polyelectrolyte complex formation process was proved to be conformation-directing.[16] Chang et al. investigated the interactions of pectin and ε-PL and the physicochemical properties and antimicrobial efficacy of the electrostatic complexes. Very recently, Chang et al. designed an antimicrobial delivery system based on electrostatic complexes of GA and ε-PL.[17] They found that the stability of ε-PL−GA complexes formed depended on the mass ratio of GA/ε-PL, and the antimicrobial efficacy of ε-PL decreased with this ratio. They also tested the stability of the complexes in apple juice. These works provided helpful information on how to control the functionality of ε-PL properly in foods containing anionic polysaccharides.

Theoretically, the interaction intensity between ε-PL and anionic polysaccharides is dependent on the number of cationic/anionic pairs. As a semisynthetic anionic polysaccharide, carboxymethyl starch (CMS) with controllable degree of substitution (DS) was capable of interpreting the interaction between ε-PL and anionic polysaccharide from a point of a cationic/anionic pair.[18,19] However, this is hard for natural anionic polysaccharides. Moreover, CMS was proved to be safe and has been used in the food industry for many years as a thickener in China and Japan in accordance with National Food Safety Standard of China (GB 2760−2011) and Japan's Specifications and Standards for Food Additives. The number of carboxylic acid groups, which have a pK_a value around 4.2,

was crucial to the physicochemical properties of CMS.[20] By using carboxymethyl sweet potato starch (CSS) as a model substrate, this study aims to understand the effect of amino/carboxyl molar ratio on interactions between ε-PL and anionic polysaccharide by isothermal titration calorimetry (ITC), electrical charge, turbidity, and visual appearance measurements. This work provides helpful information on how to use ε-PL properly in foods containing CMS, such as soft drinks and tea and fruit beverages.

■ MATERIALS AND METHODS

Materials. ε-PL, with a purity of 99%, was provided by Zhengzhou Bainafo Bioengineering Co. Ltd. (Zhengzhou, China). Sweet potato starch was supplied by the Sweet Potato Research Center of Chongqing (Chongqing, China). Sodium hydroxide, hydrochloric acid, monochloroacetic acid, methanol, ethanol, and acetone were of analytical grade and from Kelong Reagent Chemical Co. (Chengdu, China). Carboxymethyl sweet potato starches (CSSs) with varied DS (0.114, 0.225, and 0.335) were prepared according to the reported method developed in our laboratory. [21] The as-prepared CSS product was first neutralized and then extensively washed with 85% ethanol to remove sodium hydroxide and sodium monochloroacetate so that it is safe and conforms to FDA regulations on Food starch-modified (section 172.892).

Solution Preparation. An aqueous ε-PL solution was prepared by dispersing powdered ε-PL into doubly distilled water to obtain a final concentration of 1.0% (w/v). Carboxymethyl sweet potato starch solution (0.3%, w/v) was prepared by dispersing powdered CSS into doubly distilled water, followed by heating until boiling. The pH of as-prepared solutions was adjusted to a desired value using either HCl or NaOH aqueous solution.

Determination of Enthalpies of Mixing. An isothermal titration calorimeter (ITC) (ITC200, GE Healthcare) was applied to measure enthalpies of mixing at 25 °C. Twenty 2 μL aliquots of ε-PL solution (1.0% w/v, pH 4.2) were injected sequentially into a 200 μL titration cell initially containing 0.3% w/v CSS (pH 4.2). Each injection lasted 5 s, and there was an interval of 180 s between successive injections. The solution in the titration cell was stirred at a constant speed of 1000 rpm throughout the experiments.

Electrical Charge and Turbidity Measurements. The preparation of ε-PL/CSS complexes for the measurement of electrical charge and turbidity was mimicked as in ITC experiments with a similar total ε-PL concentration range and ε-PL/CSS molar ratio. In detail, aliquots of ε-PL solution (0–600 μL, 1.0% w/v, pH 4.2) were injected into glass tubes initially containing 6.0 mL of 0.3% w/v CSS solution (pH 4.2). The resulting solutions were then vortexed thoroughly and stored overnight at room temperature prior to analysis.

The electrical charge (ζ-potential) of ε-PL/CSS complexes in the solutions were measured in folded capillary cells with a Nano-ZS90 Zetasizer (Malvern Instrumernts, UK). The optical turbidity expressed as the absorbance at 600 nm was measured against distilled water using a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan) with 1 cm path length cells.

Statistical Analysis. Measurements of enthalpy were carried out in duplicate, and electrical charge and turbidity were conducted in triplicate. The results were expressed as mean ± standard deviation. Statistical analysis was conducted using SPSS16.0.

■ RESULTS AND DISCUSSION

Enthalpy Changes Associated with ε-PL/CSS Interactions. ITC was widely applied to investigate the interaction between oppositely charged biopolymers.[22−24] The curves of ITC at pH 4.2 were obtained by injecting ε-PL solution (1.0% w/v) into either water or CSS (DS 0.335, 0.3% w/v) (see Supporting Information). The enthalpy change per injection in the reaction cell was calculated by integration of the heat flow versus time profiles. The dependence of enthalpy change on ε-

PL concentrations was shown in Figure 1. In the absence of CSS, a slightly exothermic enthalpy change was observed and
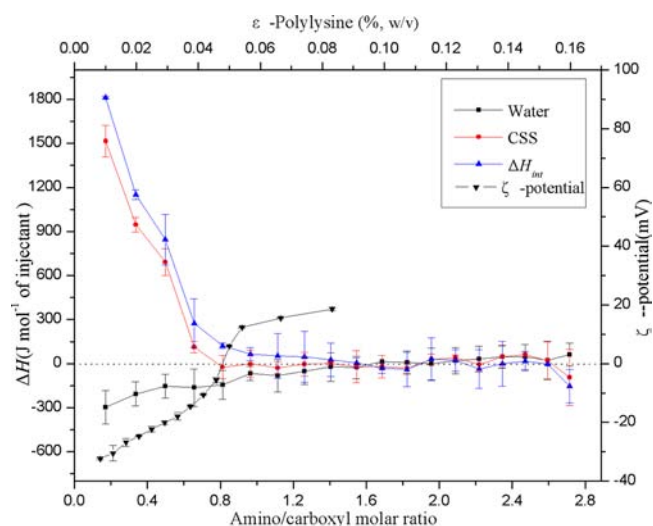

Figure 1. Dependence of enthalpy change (ΔH) and ζ-potential on amino/carboxyl molar ratio. To determine ΔH per injection, 2 μL of 1.0% w/v ε-PL was titrated into a 200 μL reaction cell containing 0% or 0.3% w/v carboxymethyl sweet potato starch (CSS) (DS 0.335, pH 4.2, 25 °C). The interaction enthalpy change (ΔH_{int}) was calculated as the difference between titration into CSS solution and titration into water ($\Delta H_{\text{int}} = \Delta H_{\text{CSS}} - \Delta H_{\text{water}}$).

progressively weakened with increasing ε-PL concentrations and finally became stable around zero, suggesting that the dilution of ε-PL solution was exothermic in nature. In the presence of CSS, a highly endothermic enthalpy change (1515.37 J/mol) was observed at the beginning of ε-PL titration into CSS solution. Then, the enthalpy change rapidly fell to zero when the mixture attained 0.0478% w/v ε-PL and an amino/carboxyl molar ratio of 0.687. No significant enthalpy change was observed with the further titration of ε-PL solution into the mixture.

To highlight the enthalpy changes associated with the ε-PL/CSS interactions (ΔH_{int}), the difference in enthalpy changes in the presence (ΔH_{CSS}) and absence (ΔH_{water}) of CSS was calculated as $\Delta H_{\text{int}} = \Delta H_{\text{CSS}} - \Delta H_{\text{water}}$ and was plotted against ε-PL concentrations (Figure 1). The relatively high values in ΔH_{int} at the initial part of titration, with ε-PL concentration ranging from 0.00200% w/v to 0.0478% w/v, indicated a strong endothermic interaction between cationic ε-PL and anionic CSS.[23] The ΔH_{int} values sharply decreased with an increase in ε-PL concentration, suggesting a significant decline in the interaction efficiency between ε-PL and CSS. As expected, the ΔH_{int} values decreased slowly to zero with the ε-PL concentration increased from 0.0478% w/v to 0.0910% w/v and remained stable at higher ε-PL concentrations. Moreover, all CSS molecules in the reaction cell were complexed utmost with titrated ε-PL at an amino/carboxyl molar ratio of 1.41, which suggested that excessive amounts of ε-PL were essential to completely complex CSS molecules in the reaction cell. It is therefore pointed out that not all of the amino groups on ε-PL chains are fully interacted with carboxyl groups of CSS during complexation, possibly due to the steric hindrance of the ε-PL backbone.[25] Theoretically, a similar situation like ε-PL chains would happen with carboxyl groups in CSS.[26]

Electrical Characteristics of ε-PL/CSS Complexes.
Although ITC was very informative in determining the molecular interaction involved in different molecules in solution, it only provided information on the very initial stage of intended molecular interaction and failed for the successive interaction that occurred within the long-term stability of the resulting mixture. The measurement of ζ-potential is useful to overcome this limitation as it is an indicator for the long-term stability of particulate systems. If the particles in a suspension have an absolute large ζ-potential value, they will repel each other and do not aggregate, which is reflected by the stability of the suspension. However, if the particles have an absolute low ζ-potential value (close to zero), they are apt to aggregate, leading to the destabilization of the suspension. Approximately, an absolute value of ζ-potential beyond 20 mV is necessary to obtain a physically stable suspension.[27] The plots of ζ-potential versus amino/carboxyl molar ratio were shown in Figure 1 using CSS (DS 0.335) as substrate. Pure CSS solution presented a highly negative ζ-potential ($\zeta \approx -36.9$ mV), which could be ascribed to the partly deprotonated carboxylic groups ($-COO^-$) along the CSS backbone at pH 4.2. Similar results were reported for pectin and GA.[16,17] The introduction of ε-PL increased the ζ-potential of the resulting mixture with a dose dependence manner. The particles of ε-PL/CSS complexes were negatively charged at low amino/carboxyl molar ratios but positively charged at high amino/carboxyl molar ratios. As estimated from Figure 1, charge neutralization occurred at an approximate amino/carboxyl molar ratio around 0.80, which implied that ε-PL/CSS complexes contained more carboxyl groups than amino groups. This was due to the higher protonation degree of amino groups ($pK_a \approx 9.0$) on ε-PL chains than the deprotonation degree of carboxyl groups ($pK_a \approx 4.2$) of CSS at pH 4.2.[5,27] The relatively high absolute values in ζ-potential (>20.0 mV) with amino/carboxyl molar ratio below 0.40 indicated the resulting ε-PL/CSS particles were stable enough without aggregation. Practically, the introduction of ε-PL into liquid foods containing CSS in this situation was acceptable and would not deteriorate the stability of foods.

Aggregation Characteristics of ε-PL/CSS Complexes.
To provide further evidence on long-term stability of ε-PL/CSS complexes, the turbidity of complexes was determined after 24 h storage at room temperature. Plots of turbidity changes at the tested CSS concentrations (0.10%, 0.20%, and 0.30% w/v) against amino/carboxyl molar ratio were shown in Figure 2. It is shown that the solution was transparent (turbidity <0.16) at lower amino/carboxyl molar ratios (<0.40), suggesting the formation of soluble ε-PL/CSS complexes in this range. The particles of ε-PL/CSS complexes were relatively highly charged, and they repelled with each other, preventing aggregation or precipitation. However, the solution turned turbid when the amino/carboxyl molar ratios ranged from 0.40 to 0.80 (Figure 2), indicating ε-PL/CSS complexes with much larger average size. The result was in agreement with the change in ζ-potential of ε-PL/CSS complexes. In addition, the maximal turbidity was associated with CSS concentration; i.e., a higher value in turbidity maximum occurred at higher CSS concentration. This could be ascribed to the formation of increasing number of ε-PL/CSS particles at higher CSS concentration, which made light scattering stronger. White sediments occurred in the solutions at an amino/carboxyl molar ratio around 0.80, indicating the formation of insoluble ε-PL/CSS complexes. The result implied that the ε-PL/CSS complexes were less
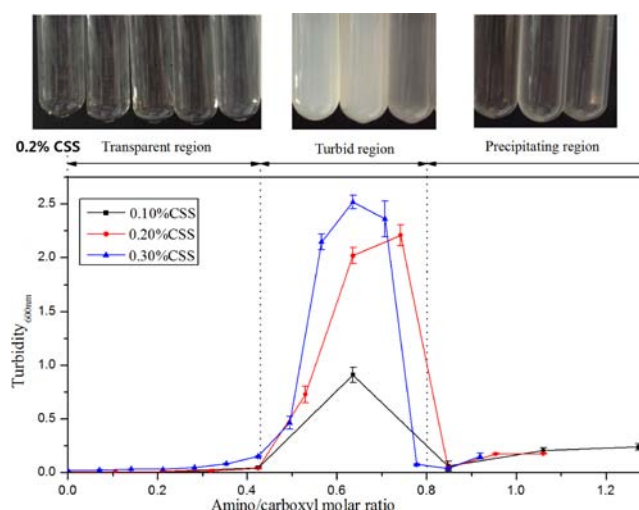
Figure 2. Dependence of amino/carboxyl molar ratio on the turbidity (at 600 nm) of ε-PL/CSS solutions. ε-PL solution (1.0% w/v) was titrated into 0.1%, 0.2%, and 0.3% w/v CSS (DS 0.335) at pH 4.2. Appearance images corresponded to 0.2% w/v CSS.

positively charged at an amino/carboxyl molar ratio around 0.80 and tended to aggregate, resulting in precipitation.

According to the visual appearance of ε-PL/CSS suspension in the present study (pH 4.2, 0.3% w/v CSS, DS 0.335), the interaction between ε-PL and CSS (DS 0.335) can be divided into three amino/carboxyl molar ratio regions.

Transparent Region I: 0−0.40 Amino/Carboxyl Molar Ratio. With an increase in amino/carboxyl molar ratio, (i) the enthalpy change varied from highly endothermic to less endothermic; (ii) ε-PL/CSS complexes were highly and negatively charged with an absolute ζ-potential beyond 20 mV; and (iii) the ε-PL/CSS suspension was transparent with its turbidity at 600 nm below 0.1.

Turbid Region II: 0.40−0.80 Amino/Carboxyl Molar Ratio. With an increase in amino/carboxyl molar ratio, (i) the enthalpy changed close to zero from minor endothermic; (ii) ζ-potential of ε-PL/CSS complexes went from highly negative to close to zero; and (iii) the turbidity of ε-PL/CSS suspension at 600 nm first increased at a very high speed and even reached 2.5 and then dramatically decreased with the formation of ε-PL/CSS precipitate.

Precipitating Region III: >0.80 Amino/Carboxyl Molar Ratio. With an increase in amino/carboxyl molar ratio, (i) the enthalpy change remained close to zero; (ii) ζ-potential of ε-PL/CSS complexes varied from zero to slightly positive; and (iii) the ε-PL/CSS complexes rapidly precipitated with a turbidity at 600 nm below 0.2.

Influence of DS on ε-PL/CSS Interactions. DS is one of the most important structural parameters of CSS.[28,29] Regarding the structure−activity relationship, DS was expected to influence the formation and physicochemical properties of ε-PL/CSS complexes. Practically, the investigation addressing this topic was valuable in selecting optimal modification degree for CSS to prepare ε-PL/CSS complexes. As shown in Figure 3, the change in ΔH_{int} increased by the first aliquot of ε-PL solution (2 μL) and decreased with the increasing DS of CSS, and this was attributed to a stronger interaction between ε-PL and CSS molecules with higher charge densities. At relatively high amino/carboxyl molar ratio, the interaction enthalpy of three DSs was close to zero. Higher DS of CSS was easier to get
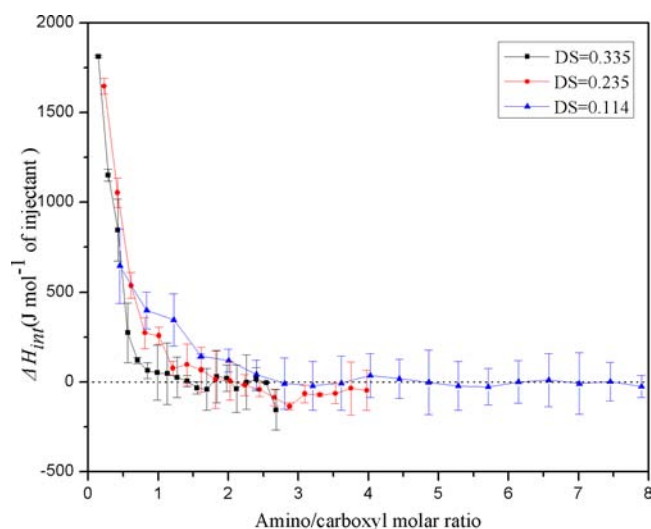
Figure 3. Effect of DS on amino/carboxyl molar ratio dependence of interaction enthalpy change (ΔH_{int}). The ε-PL solution (1.0% w/v, pH 4.2) was injected into a reaction cell containing 0.3% w/v CSS with varied DS (0.114, 0.235, and 0.335) (pH 4.2, 25 °C).

zero, indicating that higher complexing efficiency occurred with a higher DS of CSS. This could be explained by the fact that more carboxyl groups were available for complexation in solution of CSS as its DS increased. Correspondingly, the lower the DS of CSS, the slower the rate of ΔH_{int} decreased with increasing amino/carboxyl molar ratio. As assumed, a value of ΔH_{int} close to zero indicated the end of complexation. Figure 3 illustrated that CSSs with a lower DS were more efficient in complexing with ε-PL than those CSSs with a higher DS. The increase in number of carboxyl groups on the CSS backbone impaired the accessibility of carboxyl groups to complex with ε-PL due to steric hindrance.

Figure 4 showed the effects of the DS of CSS on ζ-potential of ε-PL/CSS complexes. In the absence of ε-PL, the absolute ζ-potential value of CSS solution increased with its DS, which could be ascribed to the difference in the amount of carboxyl
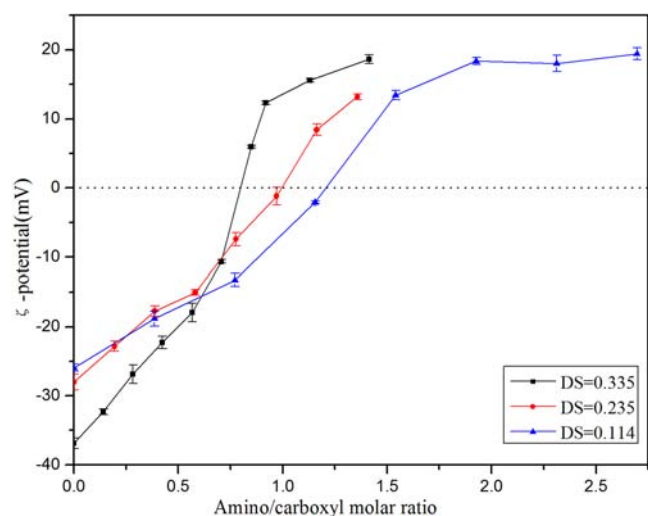

Figure 4. Effect of DS on amino/carboxyl molar ratio dependence of ζ-potential of ε-PL/CSS solutions. ε-PL solution (1.0% w/v, pH 4.2) was titrated into aqueous CSS solution (0.3% w/v, pH 4.2) with varied DS (0.114, 0.235, and 0.335).

groups on CSS molecules. In the presence of ε-PL, a sharp increase in ζ-potential of ε-PL/CSS complexes was inevitably accompanied with the increase in amino/carboxyl molar ratio, especially in the early stage of complexation. Crucially, the changing rate of ζ-potential versus amino/carboxyl molar ratio was tightly related to the DS of CSS. The higher the DS, the steeper the changing rate was. This finding reinforced the results obtained for enthalpy changes.

The impact of DS of CSS on aggregation characteristics of ε-PL/CSS complexes was determined in terms of turbidity at 600 nm, and the results were shown in Figure 5. With an increase in DS of CSS, the critical amino/carboxyl molar ratios, where the ε-PL/CSS suspensions turned turbid or highly precipitated, decreased.
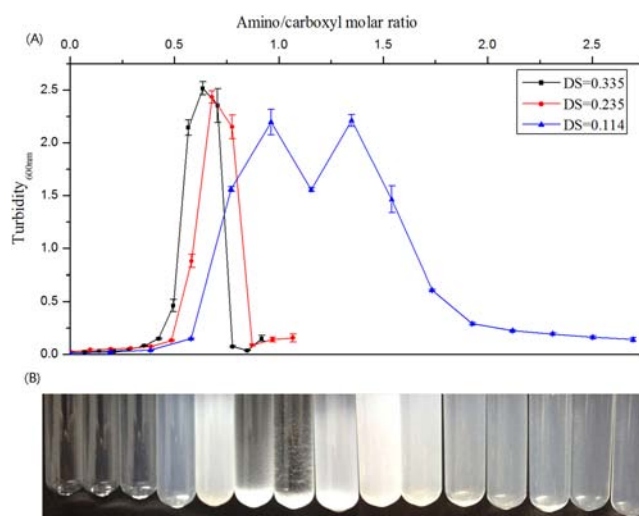

Figure 5. (A) Effect of DS on amino/carboxyl molar ratio dependence of the turbidity (at 600 nm) of ε-PL/CSS solutions. ε-PL solution (1.0% w/v, pH 4.2) was titrated into CSS solution (0.3% w/v, pH 4.2) with varied DS (0.114, 0.235, and 0.335). (B) Appearance images corresponded to DS of 0.114.

By combining the results from Figure 4 and Figure 5, it is revealed that, as the amino/carboxyl molar ratio increased, ε-PL/CSS suspensions with higher increasing rate of ζ-potential were more apt to become turbid than those with lower increasing rate of ζ-potential. Importantly, the changing tendency of turbidity and visual appearance of ε-PL complexes with lower DS (0.114) CSS were significantly different from that with higher DS (0.235 and 0.335) CSS. As suggested by the images of ε-PL/CSS (DS 0.114) suspension, its visual appearance was transparent with amino/carboxyl molar ratio below 0.40, and it became turbid with further increase in amino/carboxyl molar ratio and slightly precipitated with an amino/carboxyl molar ratio of 1.16. Interestingly, unlike the behavior of ε-PL/CSS suspensions with high DS (0.235 and 0.335) CSS, highly precipitated suspensions were not observed for ε-PL/CSS suspensions with low DS (0.114) CSS, instead of a progressively clarified suspension. This was possibly due to the difference in average size of ε-PL/CSS complexes formed. For CSS with higher DS (0.235 and 0.335), the higher increasing rate of ζ-potential caused more rapid aggregation of ε-PL/CSS complexes and resulted in larger particles, which sunk at a higher speed. When the DS of CSS reached its critical gelling point, all particles of ε-PL/CSS complexes were embedded in this gel network and could not be separated

from each other. Oppositely, for CSS with a low DS (0.114), a smaller degree of aggregation occurred, which resulted in smaller particles. These particles slowly sunk and thereof were difficult to form CSS gel. It led to the conclusion that CSS intrinsic flexibility/structure was imposing the precipitate rheological properties.[30] In this situation, the increased ζ-potential of ε-PL/CSS complexes with amino/carboxyl molar ratio generated a strong electrostatic repulsion between these particles. Consequently, the loosely packed aggregates of ε-PL/CSS complexes would dissociate at sufficiently high amino/carboxyl molar ratio.

Influence of pH on ε-PL/CSS Interactions. Regarding the facts that the charge magnitudes of CSS and ε-PL varied with pH and the formation of ε-PL/CSS was driven by the electrostatic interactions among its components, pH was assumed as a crucial environmental parameter for ε-PL/CSS interactions.[30−32] Consequently, the impact of pH on ζ-potential of ε-PL/CSS complexes and turbidity of the resulting suspension were checked. The effects of pH on ζ-potential of ε-PL/CSS complexes were shown in Figure 6. The changes in ζ-
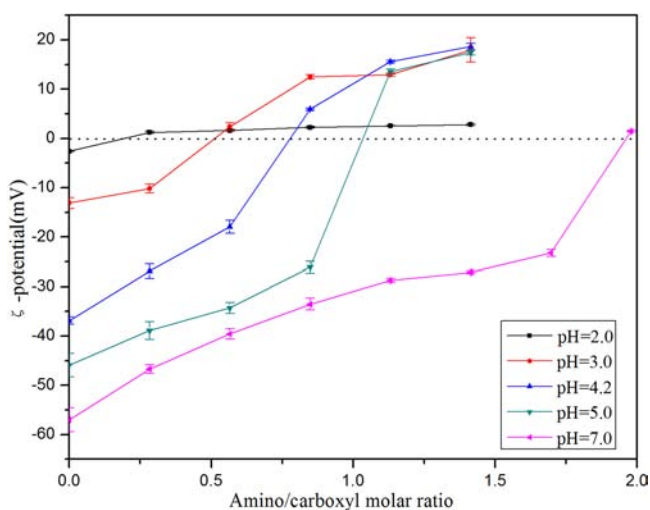

Figure 6. Effect of pH on amino/carboxyl molar ratio dependence of the ζ-potential of aqueous ε-PL/CSS solutions. The ε-PL solution (1.0% w/v) was titrated into 0.3% w/v CSS (DS 0.335) solutions at pH of 2.0, 3.0, 4.2, 5.0, and 7.0.

potential versus ε-PL/CSS molar ratio were highly dependent on pH. At pH 2, only a minor increase in ζ-potential was observed as the amino/carboxyl molar ratio increased from zero to 0.707. This was due to the fact that nearly all carboxyl groups on CSS molecules presented in the protonated form (−COOH) at this pH, and they cannot interact with highly protonated amino groups (−NH$_3$$^+$) in ε-PL counterparts. Across the tested range of pH (2.0−7.0), ε-PL molecules were nearly protonated (99∼100%), but the ionization degree of CSS changed significantly. Assuming CSS as a simple acid, the ionization degrees of its carboxyl groups were 5.9%, 50%, 86.3%, and ≈100% at pH of 3.0, 4.2, 5.0, and 7.0, respectively, according to the equation of pH = pK_a + lg([COO$^-$]/[COOH]). This was also evidenced by the fact that pure CSS molecules were more negatively charged at higher pH (see data at an amino/carboxyl ratio of zero in Figure 6). In this context, the electrostatic interactions between ε-PL and CSS were mainly dependent on the ionization degrees of the carboxyl groups on CSS molecules. Evidently, the increasing rate in ζ-

potential of ε-PL/CSS complexes gradually decreased with pH. A higher ε-PL loading capacity, defined as the highest amino/carboxyl molar ratio in ε-PL/CSS suspension with a turbidity below 0.1, could be obtained for CSS at higher pH values (Figure 7). For instance, the loading capacities at pH 3.0 and 7.0 were 0.141 and 1.131, respectively.
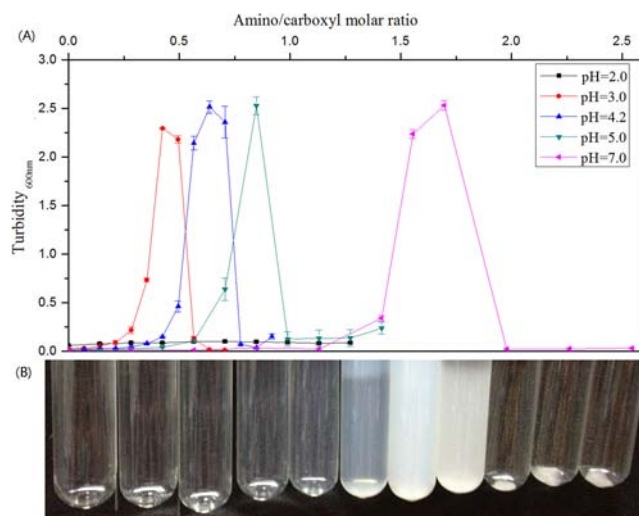

Figure 7. (A) Effect of pH on amino/carboxyl molar ratio dependence of the turbidity (at 600 nm) of ε-PL/CSS solutions. The ε-PL solution (1.0% w/v) was titrated into 0.3% w/v CSS (DS 0.335) solutions at pH of 2.0, 3.0, 4.2, 5.0, and 7.0. (B) Appearance images corresponded to pH 7.0.

Influence of Ionic Strength on ε-PL/CSS Interactions. The effects of ionic strength (50 and 100 mM NaCl) on the interaction between ε-PL and CSS were shown in Figure 8. The tested NaCl concentrations were selected according to the common addition of NaCl in beverages and soups. With the addition of NaCl, the onset of turbidity of solution shifted to a higher amino/carboxyl molar ratio. This implied that the presence of NaCl hindered the formation of ε-PL/CSS
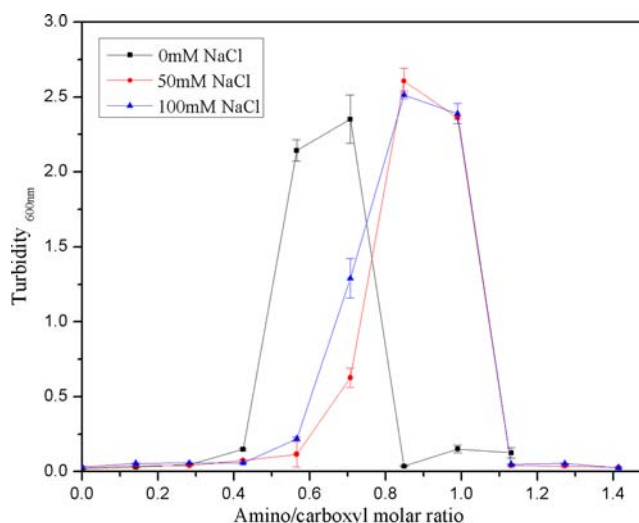

Figure 8. Effect of ionic strength (0−100 mM NaCl) on amino/carboxyl molar ratio dependence of the turbidity (at 600 nm) of ε-PL/CSS solutions. The ε-PL solution (1.0% w/v) was titrated into 0.3% w/v CSS (DS 0.335) solutions, containing 0−100 mM NaCl.

complexes, presumably because the electrostatic screening effects of Na$^+$/Cl$^-$ weakened the attractive interactions between ε-PL and CSS. As expected, the electrostatic screening effect of Na$^+$/Cl$^-$ with a specific NaCl was more significant for a system with lower amino/carboxyl molar ratio.[13] Therefore, even with NaCl, the attractive interactions between ε-PL and CSS were strong enough to form ε-PL/CSS complexes, which thereof resulted in turbid solution.

The interactions between ε-PL and carboxymethyl starch were investigated in this study. The results indicated that ε-PL interacted with oppositely charged CSS in aqueous solutions. These interactions caused the formation of ε-PL/CSS complexes and made ε-PL/CSS solutions turbid and even lead to precipitate. These changes were dependent on CSS concentration, pH, and DS of CSS and highly related to the amino/carboxyl molar ratios. Practically, the results of the present study are appreciable in designing liquid foods containing both ε-PL and CSS. By selecting applicable CSS concentration, pH, DS of CSS, amino/carboxyl molar ratio, and ionic strength, a turbid but homogeneous or transparent appearance would be endowed for liquid foods containing both ε-PL and CSS. Additionally, previous reports suggested that the formation of complexes between ε-PL and pectin or between ε-PL and GA deteriorated the antimicrobial capacity of ε-PL. Further research should be conducted on this topic to estimate the effect of ε-PL/CSS complexation on antimicrobial capacity of ε-PL.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Supplement 1. Heat flow versus time profiles resulting from injection of 2 μL aliquots of 1.0%, w/v ε-PL into a 200 μL titration cell containing water (A, B) at pH 4.2 (25 °C).

Supplement 2. Heat flow versus time profiles resulting from injection of 2 μL aliquots of 1.0%, w/v ε-PL into a 200 μL titration cell containing 0.3%, w/v CSS (A, B) at pH 4.2 (25 °C). This material is available free of charge via the Internet at http://pubs.acs.org.

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