Formation and Composition of Arachin/Carrageenan Complexes: Effects of Protein/Polysaccharide Ratio

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Abstract. The formation and composition of arachin/carrageenan complexes at various protein/polysaccharide ratios ($r$) have been studied by a combination of turbid metric titration and composition analysis. Our results suggest that an increase in $r$ favors the formation of complex coacervates. Unlike pectin/β-lactoglobulin, due to the strong polyelectrolyte nature of carrageenans, no pHφ2 was observed in the pH titration curves of arachin/carrageenan even at pH as low as 2.0. Our composition analysis results indicated that at higher $r$, the coacervate was more concentrated.

1. Introduction
Despite the widespread industrial applications and some fascinating biological implications, the complexation between polysaccharides and proteins is still an intriguing and important topic of investigation. The knowledge of their interactions is of importance not only in making cost-efficient use of functional ingredients, but also in designing novel food, controlling and improving food ingredients structures and textural properties of fabricated foods. Thus, systematic works are still needed to further disclose the relationship between physicochemical parameters, such as initial protein/polysaccharide ratio.

Because the dramatic increase of turbidity arises mainly from the change of mass and size of aggregates in the solution, the above changes in turbidity were supposed to be the result of the formation of protein/polysaccharide coacervates, similar to the other protein/polymer systems. Arachin and carrageenan are the bio macromolecules used in this study. Carrageenans, which are found in intercellular matrix materials of numerous species of red seaweed, are linear sulfated polysaccharides that are widely used as thickening, gelling, binding, and stabilizing agents in food, cosmetic, and pharmaceutical industries. The most important feature of carrageenans is that their linear charge densities ($ξ$) are well known. Linear charge density is normally defined as the number of sulfate group per disaccharide. The linear charge densities of four commercially available carrageenans, which include furcelleran, κ-carrageenan, ι-carrageenan, and λ-carrageenan, are 0.67, 0.92, 1.53, and 2.07, respectively. Stronger gels of protein/carrageenan were formed due to electrostatic interaction between the polymers. Studies have examined the complexation of whey proteins with carrageenan [1-3].

This work aims to study the complexes formed by four different linear charge densities carrageenans and arachin at various initial protein/polysaccharide weight ratios ($r$). The effect of $r$ on the formation...
of arachin/carrageenan complexes has been studied by turbidimetric titration, a typical way of monitoring complex formation, and the coacervate composition is also studied.

2. Materials and methods

2.1. Materials
Arachin powder was provided by Shandong Yuwang Industrial Co., Ltd (Shandong, CA). \(\kappa\)-, \(\iota\)-, \(\lambda\)-carrageenan and furcelleran were purchased from Shanghai Meryer Chemical Technology Co., Ltd (Shanghai CA). And used without further purification. Milli-Q water (18.3 \(\Omega\)) was used in all experiments.

2.2. Turbidimetric Titrations
The ratio of arachin to carrageenan varied from 1:5 to 2:1 and the concentration of the total carrageenan was fixed at 1 g/L in 0.1M NaCl solution. The coacervate samples of arachin/carrageenan mixtures were prepared by first adjusting the pH of the mixtures to 8.0 under magnetic stirring, followed by centrifuging at spin-speed of 8,000 RPM. The pH dependence of turbidity was measured using a TP309 turbid meter (Time power, Beijing). The colorimeter was calibrated to read 100% transmittance with Milli-Q water. The solutions were filtered with 0.45 mm Whatman filters before turbidimetric titration. A 0.5 M HCl solution was used to adjust the pH of the mixed solutions. After each small droplet of HCl was added, the turbidity value was collected, and the pH was monitored with a Thomas Scientific pH meter (Model 8025).

2.3. Composition Analysis
The compositions of arachin/carrageenan coacervates were analyzed using the method similar to the previously published procedures [1]. Water amount in the coacervates was determined at least twice by dry-weighting. To determine the concentrations of arachin and carrageenan in the coacervates, arachin/carrageenan coacervates were first dissolved in sodium phosphate buffer of pH 8.0. Next, the contents of arachin and carrageenan were determined using the size exclusion chromatography (SEC) system (DIONEX Ultimate 3000) connected with a ZORBAX GF-450 gel filtration column and a UV detector, with the absorbance measured at 280 and 214 nm, respectively. The contents of arachin and carrageenan in the coacervates were finally calculated according to the arachin and carrageenan concentration calibration curves.

3. Result and Discussion
Strong polyelectrolytes, \(\kappa\)-, \(\iota\)-, \(\lambda\)-carrageenan, and furcelleran, which have similar chemical structure but different sulfate contents (linear charge density), were used to study the effect of ionic strength on the phase separation behaviors with arachin. For turbidimetric titration, turbidity increased due to the aggregation of polymer. So coacervates formed were detected by dramatically increased turbidity. By adding HCl to a mixture of arachin and carrageenan, complexation of biopolymers could be studied in the pH range 8.0-2.0. The initial pH of the mixture was set at 8.0 and, by adding 0.5 M HCl, it decreased slowly to pH 2.0 in approximately 2h. Carrageenan is a negatively-charged sulphated polyelectrolyte, while arachin is either positively or negatively charged at above or below the isoelectric point (pI). There are three regions observed in this pH titration curve: (1) at pH>7.0, there is no change in solution turbidity; (2) at 5.5<pH<7.0, the solution turbidity change slightly, which is identified as the incipient of the soluble complex (pHc); and (3) at pH<5.5, the turbidity rapidly increases and reaches a maximum, which corresponds to the global phase separation point (pHφ). It should be pointed out that soluble complex occurs at pH>pI, suggesting that even though the overall charge characteristics of arachin is negative, locally there are positively charged patches on the protein surface, which can bind to the negatively-charged carrageenan. On the other hand, global phase separation only occurs at pH<pI where the positive charges dominate the protein surface.
Figure 1 shows the titration curves of turbidity (100-transmittance T) % from turbid metric titration versus pH for mixtures of arachin/carrageenan as a function of various $r$ values. From the figure 1, we can easily see that the more coacervate formed with increasing $r$ value from 1:5 to 2:1. The interaction between protein molecules and polysaccharide chains expected to be enhanced in higher $r$. higher $r$ means that more protein molecules are available for binding onto polysaccharide chains. It has been reported that protein/poly saccharide coacervates have higher amount of protein molecules in the coacervates phase [1]. More proteins molecules bound in polysaccharide chains may cause higher electro-neutrality of polysaccharide chains. Consequently, higher $r$ will cause protein/poly saccharide complexes to aggregate more tightly to form protein/poly saccharide coacervates.

![Figure 1. The plots of turbidity versus pH for the mixture of arachin/carrageenans with different $r$: (a) furcelleran, (b) $\kappa$-, (c) $\iota$-, and (d) $\lambda$-carrageenan.](image)

Both the initial complexation and phase separation are characterized by well-defined pH values, i.e., pH$_c$ and pH$_{\phi}$, which are directly related to the protein net charges. Two characteristic pH values, pH$_c$ and pH$_{\phi}$, were observed in the pH titration curves. The variation of pH$_c$ and pH$_{\phi}$ values as functions of $r$ and $C_{NaCl}$ is given in Figure 2. The well-defined point of initial turbidity increase is designated as pH$_c$, beyond which the turbidity increases gradually with increase of turbidity, while the sharp changes in $d(100\%-T)/dpH$ is correspond to pH$_{\phi}$. The initial $r$ is an important factor in protein/poly saccharide concentration. The soluble complexes originate from the electrostatic interaction between protein and polysaccharide, and the phase separation is induced by the aggregation of the electrostatically neutralized soluble complexes, so the values of pH$_c$ were reported to be independent of the initial $r$, whereas pH$_{\phi}$ values first increased and then stabilized with increasing initial $r$ for the concentration between protein and anionic polysaccharide [1]. But our result of the general trend is that by increasing $r$ the pH$_c$ and pH$_{\phi}$ shift to higher values is not very coincident with it, probably due to the polysaccharide we used. Usually, pH$_{\phi}$ values may be used as the starting point of protein/poly saccharide concentration. The higher the pH$_{\phi}$ value, where arachin carries less positive charge for interacting with negatively charged carrageenan, the stronger the tendency for arachin and carrageenan to form coacervates.
However, no $\text{pH}_{\text{φ}2}$ is observed in the pH titration curve of arachin/carrageenan, which was seen in $\beta$-lacto globulin/pectin [4], even at pH as low as 2.0, suggesting that $\text{pH}_{\text{φ}2}$ is mainly affected by the polysaccharides used, because carrageenan is strong polyelectrolytes. For negatively charged weak acid (e.g., carboxylic acid)-based polysaccharides like pectin, with the decrease of pH below its pKa, due to the low charges of polysaccharide chains as well as the repulsion between the positively charged proteins, protein (e.g., $\beta$-lacto globulin) /polysaccharide (e.g., pectin) coacervates may dissociate into soluble complexes, or even uninteracted protein molecules and polysaccharide chains.

![Figure 2. The variation of $\text{pHc}$ and $\text{pHφ1}$ as a function of $r$](image)

The contents of carrageenan, arachin, H$_2$O, as well as the actual $r$ in arachin/carrageenan coacervates prepared at various initial $r$ and $C_{\text{NaCl}}$ have been determined by a combination of UV, size exclusion chromatography, and dry-weighting methods. Actual arachin and carrageenan contents in arachin/carrageenan coacervates exhibit an increasing variation when $r$ increases from 1:5 to 2:1, indicating that the higher $r$ will provide protein molecules and carrageenan chains more chances to interact and form protein/polysaccharide coacervates, and cause a larger amount of arachin and carrageenan to settle in the coacervate phase. The arachin content in arachin/carrageenan coacervates is seen to become bigger with increasing $r$, which is also consistent with whey protein/gum Arabic coacervates [1]. Moreover, our results also agree with the report that protein/polysaccharide coacervates have higher amount of protein molecules in the coacervates phase [1]. And, here, the higher the initial $r$ results the higher actual $r$ in the coacervate phase. This result was already found by Schmitt et al. for a system of pure $\beta$-lacto globulin and gum Arabic [1, 5]. Depending on the $r$ ratio, the internal structure of coacervate droplets appeared vesicular to sponge-like exhibiting numerous inclusions of water [5]. The occurrence of vacuoles was explained by the presence, at the interface of oacervate droplets, of GA that was not electrostatically neutralizing $\beta$-lacto globulin, so that water was entrapped [6]. In time, neutralization proceeded leading to rearrangements within the coacervates, and the vacuoles disappeared. Thus, it seemed that the coacervate is a very flexible system that adapts to external parameters. Recent work also showed that the coacervate phase was a very dynamic system which rearranged continuously and turned to more transparent and homogeneous in time [1].
Table 1. Compositions of arachin/carrageenan coacervates for various initial $r$ at 0.1M NaCl solution

| carrageenan species | $r$ | carrageenan % | arachin % | H$_2$O % | actual $r$ |
|---------------------|-----|--------------|----------|---------|-----------|
| furcelleran         | 1:5 | 0.66±0.01    | 0.62±0.03| 98.72±0.31| 0.94      |
|                     | 1:2 | 1.13±0.04    | 1.30±0.08| 97.57±0.19| 1.15      |
|                     | 1:1 | 1.23±0.05    | 2.18±0.15| 96.60±0.54| 1.77      |
|                     | 2:1 | 2.06±0.11    | 4.57±0.16| 93.37±0.84| 2.22      |
| $\kappa$-carrageenan| 1:5 | 0.84±0.02    | 0.61±0.03| 98.56±0.25| 0.73      |
|                     | 1:2 | 1.08±0.03    | 2.01±0.15| 96.91±0.74| 1.86      |
|                     | 1:1 | 1.20±0.03    | 3.19±0.16| 95.61±0.23| 2.66      |
|                     | 2:1 | 1.59±0.09    | 4.97±0.21| 93.44±0.46| 3.13      |
| $\iota$-carrageenan | 1:5 | 0.79±0.03    | 0.36±0.01| 98.85±0.28| 0.45      |
|                     | 1:2 | 1.32±0.09    | 2.36±0.16| 96.33±0.52| 1.79      |
|                     | 1:1 | 1.42±0.07    | 3.13±0.15| 95.45±0.36| 2.20      |
|                     | 2:1 | 1.49±0.06    | 4.54±0.20| 93.97±0.68| 3.06      |
| $\lambda$-carrageenan | 1:5 | 0.69±0.06    | 0.62±0.04| 98.69±0.35| 0.89      |
|                     | 1:2 | 1.40±0.08    | 2.13±0.15| 96.47±0.81| 1.52      |
|                     | 1:1 | 1.79±0.11    | 3.60±0.21| 94.60±0.73| 2.01      |
|                     | 2:1 | 1.90±0.12    | 5.14±0.23| 92.96±0.27| 2.71      |

4. Conclusion
In summary, physicochemical parameters such as protein/polysaccharide ratio significantly influence the formation of arachin/carrageenan coacervates. In general, an increase of $r$ promotes the arachin/carrageenan concentration. The diffusion properties and the barrier properties will be very dependent on this structure, which can be very useful for using the coacervates as barriers in encapsulation applications for instance.

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