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Review article

A REVIEW ON THERMODYNAMICS AND FUNCTIONAL PROPERTIES OF COMPLEX COACERVATES

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ABSTRACT: Complex coacervation is defined as associative interactions between oppositely charged functional groups of proteins and polysaccharides, which on separation, form a phase rich in polymeric compounds in equilibrium with another aqueous phase. So coacervates are macro-ionic hydrated complexes of two charged neutralized bioploymers. Voorn and Overbeek developed the first model on complex coacervation by applying Flory-Huggins theory for random mixing of polyions. Alternatively, Veis and Aryani proposed that initially charged pair of symmetrical aggregates forms, followed by phase separation, for modeling diverse range of aggregates. Physicochemical properties such as pH, ionic strength, ratio of protein to polysaccharide, polysaccharide and protein charge, and molecular weight, mechanical properties (shear force) and temperature affect the formation and stability of coacervates. Improved structural, rheological, interfacial and delivery properties of these complexes than individual biopolymer can be exploited in numerous domains. This article intends to elucidate the salient features of coacervates which may contribute to better understanding of protein-polysaccharide systems, for their application in foods, cosmetics, pharmaceutical, and medicine.

Key Words: Complex coacervation, interactions, biopolymers, protein, polysaccharide, electrostatic complexes, functional properties.

INTRODUCTION

Two biopolymers in a solvent can make a homogenous solution only when resulting free energy of mixing is negative ($\Delta F_{Mix} < 0$) and the thermodynamic stability of the system depends on the combinatorial entropy of mixing, more precisely intermolecular interactions i.e. enthalpy of mixing and the free volume effect (Patterson, 1982). The combinatorial entropy of mixing represents all possible permutations with which the bio-molecules can mix in the solvent exploiting all sites of the system; it is a positional disorder of the system. In case of large molecules the stability of the system through entropy of mixing decreases (Olabisi et al., 1979). The intermolecular interactions depend on repulsive (steric hindrance) or attractive forces (electrostatic, hydrophobic, Van der Waals, London dispersion force) between atoms and molecules in the solution. The mixing of high molecular weight bio-molecules at low temperature results in decreased solution stability through increase in interaction parameter or the enthalpy of mixing (Schmitt et al., 1998). The free volume effect represents a change in volume in solution after mixing of the polymers i.e. difference in free volumes of solvent and of biopolymer. It brings the molecules of system together and has negative contribution to both entropy and enthalpy of mixing as the free volume of biopolymer is smaller than that of solvent (Schmitt et al., 1998). In high molecular weight bio-polymeric mixtures, the destabilizing factors overpower the stabilizing factors, which generally result in phase separation. Depending upon types of interactions between the polymers in the mixture, the phase separation is classified into two types. In case the interactions are repulsive then the polymers will separate in two opposite phases and is known as segregative type of phase separation (Zeman & Patterson, 1972). On the other hand, associative interactions usually occur because of electrostatic attraction between oppositely charged portions of proteins and polysaccharides and both the biopolymers will separate in the same phase leading to a phase rich in polymeric compounds in equilibrium with another aqueous phase (Piculell & Lindman, 1992).

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Hydrogen bridging and hydrophobic interactions also play an important role in the stabilization of the complexes (coacervates) formed (Doublier et al., 2000; Turgeon et al., 2007). This type of phase separation is known as associative type of phase separation or complex coacervation.

The word coacervate was first coined in 1623 (Onions., 1933) and may be defined as 'an aggregate of colloidal droplets (molecules of opposite charge) held together by electrostatic attractive forces. Latin word "acervus", which means aggregation (a heap), and the prefix "co", which means together and is used to describe the formation of a coacervate related to liquid-liquid phase separation. It has been applied to the separation of macromolecules from a homogeneous solution into a phase rich in the polyelectrolyte called the coacervate and the other phase is equilibrating solution (Bungenberg de Jong, 1949a). The coacervates are classified as simple and complex depending upon the number of macromolecules and process that leads to coacervation. In the former, only one type of polymer when placed in poly-ampholyte solution (contain both positive and negative charges), undergoes self-charge neutralization (normally in the presence of salt) and finally separate into a liquid coacervate phase, e.g. aqueous gelatin in the presence of alcohol or acetone (Mohanty et al., 2005). On the other hand, in complex coacervation two oppositely charged macromolecules associate to form a neutral complex and separate into polymer rich phase and aqueous equilibrium phase (Bungenberg de Jong, 1949).

Complex coacervation between oppositely charged proteins and polysaccharides was discovered by Tiebackx (1911) by mixing gelatin and gum arabic (GA) in an acetic acid solution. The electrostatic complex formed in complex coacervation may be either soluble or insoluble. Soluble complexes are formed when the electrostatic repulsion forces are minimized (Turgeon et al., 2007) at moderate ionic strength or when the pH of the solution is not close to isoelectric point (IP) of the protein and interactions occur between charged patches of the molecules (de Kruif et al., 2004; de Vries, 2004). Such complexes consist of a single polysaccharide chain bound to a few protein molecules. Under favorable pH and ionic strength, more number of proteins can be bound to polysaccharide until they achieve charge neutralization condition, which subsequently leads to formation of insoluble complexes by phase separation in the first step. In the second step, there is gain in entropy through random mixing of macromolecules in the dense phase and gain in entropy due to release of counter-ions (Bowman et al., 1997, Hattori et al., 2005, Antonov et al., 2010).

The physical properties of the macromolecule are controlled by complex interactions between them. Aggregation of the two biopolymers depends on the inter-particle interaction such as electrostatic interaction, van der Walls attractions, steric interaction, hydrophobic effects, hydrogen bonding and hydrophobic interactions. Non electrostatic (hydrogen and hydrophobic) forces stimulate the formation of junction zones between the biopolymers when they are in close proximity to each other (Tolstoguzov, 1993) These forces depend on the composition and structure of molecules present, for example amino acids and glycidic monomers sequence have compact or random coil structure (Xia & Dubin., 1993). The associative phase separation of protein and polysaccharides is the result of electrostatic interactions and are controlled by entropic and enthalpic factors (Girard et al., 2003b; Ou et al., 2006). In the complex coacervation, phase transition results in a gain in the entropy that arises from molecular rearrangement to form random aggregate phase (Burgess, 1990). Entropic factors, for example nature, structure and molecular weight of the macromolecule influence the complex formation. The enthalpic factors are associated to protein polysaccharide ratio, the interaction of proteins with soluble complexes to neutralize residual charges and to the energy gain resulting from the increased charge density in intermolecular soluble complexes (Burgess, 1990; Girard et al., 2003b). These entropic and enthalpic factors were predicted by Tainaka, (1979) and have also been found in computer simulation studies (Ou et al., 2006).

The aim of this paper is to illustrate how fundamental understanding of interactions between protein and polysaccharides and thermodynamic models, factors affecting formation of protein polysaccharide complex and coacervates are essential to efficient process design, development and functionality of novel products. Knowledge of thermodynamics of simple mixtures and effect of variables such as temperature, pH, ionic strength and polymer concentration provides a reference point to assess the potential behavior of the extremely complex multi-component system (a real food).

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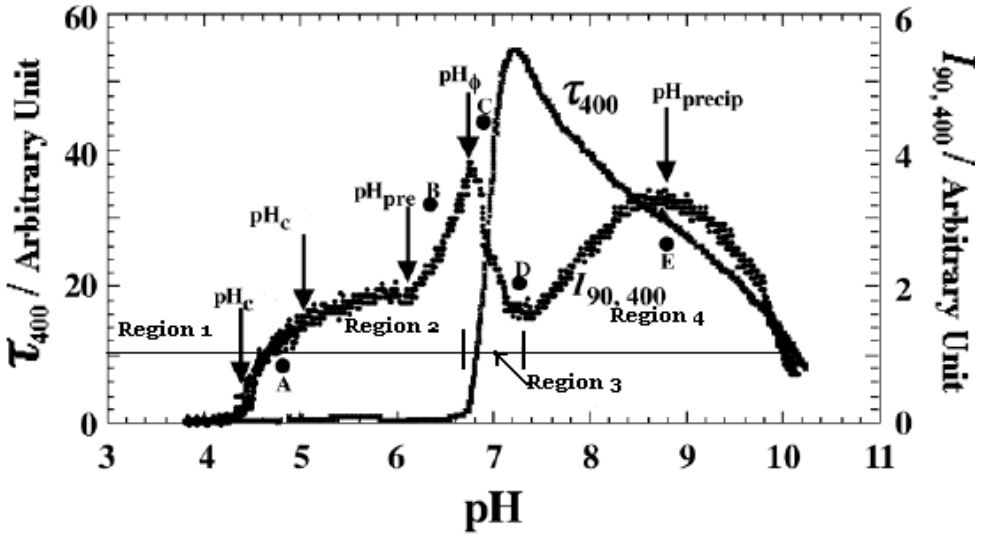

Figure 1: Coacervation process measured in terms of turbidity as a function of pH.(Adapted from Kaibara et al., 2000)

Table 1: Effect of pH on different protein/ polymer complex formation

Protein/ Polymer	pI of protein	pH_C	$pH_{\phi 1}$	$p_{\phi 2}$	References
Whey protein/ Gum arabic	α-lg (85%) = 5.2	5.3	4.8	4.75	Weinbreck et al., (2004a)
BSA/ (PDADMAC)	BSA = 4.9	5	6.2	8.8	Kaibara et al., (2000)
Gelatin A/ Gelatin B	Gelatin A = 9 Gelatin B = 5	5.5	6.3	6.5	Burgess & Carless, (1985)
Pea Protein Isolate (PPI) and GA	PPI = 4.4, GA = 1.8	4.2	3.7	2.5	(Liu et al., 2009)
Chitosan/ Gelatin (Total Biopolymer concentration = 3.15%w/w)	Chitosan = 8.7 Gelatin = 4.7	5.1	5.25	5.5	(Lopez & Bodmeier, 1996)

Theoretical Framework

We are often interested in the fate of mixtures of polymers in an aqueous solution. When proteins and polysaccharides are mixed together under aqueous conditions, it results in two main types of interactions: thermodynamic incompatibility or thermodynamic compatibility depending mainly on the electrical charges on both biopolymers. The solution will either be a homogenous mixture or will phase separate. Phase separation in mixed bio-polymer solutions is quite common and has important technological applications in foods and biotechnology. Thermodynamic compatibility of mixed polymers in solution depends on interactions between polymers, measured by the Flory-Huggins interaction parameter. The extrinsic or intrinsic factors affect phase separation and properties of coacervates. Extrinsic factors include the macromolecular mixing ratio, pH, ionic strength, temperature and rate of shear during acidification. Intrinsic factors are related to the nature and characteristics of the interacting molecules such as the molecular weight (Mw), net charge and flexibility of chains (Samant et al., 1993; Tolstoguzov, 1997). Proteins and polysaccharides are biopolymers that play important nutritional and functional roles in foods and are seldom found together as a bio-active compound (sensitive to environmental conditions such as high temperature and pH). Efforts have recently been made to produce either functional ingredients or for microencapsulation of foods. Rheological and textural properties as well as interfacial properties in emulsions and foams are the prerequisite for the successful use of protein and polysaccharide mixed systems. Novel microstructures can be generated using the wide spectrum of food biopolymers proteins and polysaccharides available by careful manipulation of mixing conditions and processing parameters.

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Theoretical modeling is necessary both for better understanding of the fundamental basis of the phase separation mechanisms and in prediction of the stability of any biopolymer system. Different models for complex coacervation have been developed on the basis of Flory-Huggins theory of the "liquid quasilattice" state for the entropy term in free energy of mixing and Debye-Huckel theory for electrical energy. The Flory-Higgins theory has been used to explain phenomena including phase separation and the kinetics of demixing, adsorption of polymers at interfaces. The quantitative models on thermodynamics of phase separation have been proposed by different researchers (Overbeek & Voorn., 1957; Veis-Aranyi, 1960-1970; Nakajima- Sato, 1972; Tainaka, 1979-1980). After considering possible interactions during phase separation, the following conclusions have been made: (i) the homogenous solution will remain stable till the free energy of the solute F_2 obeys the condition of thermodynamics, i.e. $\left(\frac{\partial^2 F_2}{\partial N_2^2}\right)_{N_1,T,P} > 0$, where N_1 & N_2 are molecules of solvent and solute at temperature (T) and pressure (P); (ii) phase separation is the result of charge neutralization of complex; (iii) there is release of water molecules and counter-ions from protein and polysaccharide during phase separation; (iv) precipitation of complex do not occur because of entropy gain due to molecular rearrangement to form random aggregate phase and gain in entropy due to release of counter ions from solute to the solvent. The details of assumptions, critical conditions and thermodynamics models are described in the following section.

In aqueous solution, complex coacervation takes place between two oppositely charged polymers owing to electrostatic attraction. For instance, complexation between proteins and anionic polysaccharides occurs below the protein iso-electric point and at low ionic strengths (Tolstoguzov, 1991). Factors that influence compatibility and complex formation are protein/ polysaccharide ratio, pH, ionic strength (Sanchez et al., 1997; Laneuville et al., 2000; Schmitt et al., 1999; Zaleska et al., 2000;), the nature of the polymers (molecular weight, net charge, ternary structure and flexibility of chains) (Walkenstrorn & Hermansson, 1998; Delben et al., 1998), and physical factors (temperature, shear, pressure and processing parameters) (Walkenstrorn & Hermansson, 1998). Pre-treatment of the polymer's solutions also enhances complex formation. High pressure (dynamic or hydrostatic) treatment and temperature have been reported to affect the stabilization of the new-formed complexes (Paquin, 1999; Bryant & McClements, 2000).

Properties of coacervates have been exploited in various sectors such as foods, cosmetics, pharmaceutical, and medicine (Schmitt et al., 1998). Protein-polysaccharide interactions play a significant role in the structure and stability of many processed foods. Thus, a basic understanding and control of the interactions and different non-equilibrium situations encountered in food systems has become important for improving product quality as well as for designing food materials (Sanchez et al., 2006). The potential application in food system include their use as thicken agent, stabilizers, and emulsifiers to improve the rheological properties of food gels and as safe delivery system for bioactive compounds which in turn improve the nutrition quality of food products (Schmitt et al., 2010). Novel products having better properties from that of pure colloid (protein and polysaccharide) could be prepared (Sanchez et al., 1997). Other prospective of electrostatic interaction in the food industry are: purification of macromolecules, microencapsulation of ingredients or cosmetics, fat substitutes, meat analogues, films and coatings. Considerable work has been reported on the associative interactions of various protein-polysaccharide binary systems but very fewer studies have focused on the changes of the complexes in real systems or during processing, when the critical conditions for the formation and stability of aggregates change over time. Only recently, a few researchers have focused on development of active components and their stability during processing (Flett et al., 2010), storage (Nigen et al., 2007; Dickinson, 2008) or digestion (Vandenberg et al., 2001).

Properties of coacervates have enabled the food scientist to develop novel products and in recent years many of such products have been developed and commercialized.

Thermodynamics of phase separation

Overbeek & Voorn (1957), Veis-Aranyi (1960-1970), Nakajima- Sato (1972), and Tainaka (1979-1980) have dealt with the development of quantitative models of complex coacervation. They have adopted a fundamental thermodynamic approach that the phase separation occurs when oppositely charged random chain polymers are mixed in the same solvent and studied by considering the free energy of mixing.

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They estimated the total free energy of the system as the sum of electrostatic free energy and free energy of mixing determined on the basis of Flory-Huggins solute-solvent interaction.

Flory-Huggins theory provides information on existence of different phases in the blend as a function of temperature and molecular weight. The polymer solution is modeled as lattice, where each lattice site (a volume) is occupied by either solvent or polymer segment (the number of segment per molecule is x). The change in free energy of mixing (ΔF_{mix}) for a polymer solution is given by Flory-Huggins equation:

$$\Delta F_{mix} = F_{1+2} - (F_1 + F_2) = \Delta H - T\Delta S \quad (1)$$

ΔH and TΔS are enthalpic and entropic factors in the Gibbs free energy of mixing respectively (dependent on polymer concentration) and T is absolute temperature (K). There is gain in entropy due to higher mobility of polymers chain at low polymer concentration and to balance the effect enthalpy increases, to retain a homogenous system. At higher polymer concentration, low endothermic changes stimulate the phase separation.

$$\Delta F_{mix} \Big/ N = RT\left(\chi_{12}\varphi_1\varphi_2 + \frac{\varphi_1}{n_1}\ln\varphi_1 + \frac{\varphi_2}{n_2}\ln\varphi_2 \right) \quad (2)$$

$$\varphi_1 = n_1V_1 \Big/ \left(n_1V_1 + n_2V_2\right) \quad (3)$$

and, $\varphi_2 = 1 - \varphi_1$ (4)

where, R is universal gas constant, φ_1 and φ_2 are volume fraction of polymers 1 and 2, n_1 and n_2 are number of moles of polymers 1 and 2, V_1 and V_2 are partial volume of polymers 1 and 2 and N is the total number of lattice sites. First term in equation 2, represents enthalpic contribution or interaction energy between the solvent molecules and the polymer segments. The last two terms give the entropic contribution arising from different placements of polymers. The coefficient χ_{12} is called Flory-Huggins interaction parameter and is equal to

$$\chi_{12} = \Delta H_{mix} \Big/ \left(RTN_1\phi_2\right) \quad (5)$$

where, ΔH_{mix} is the energy involved in neighbor interaction. So, χ_{12} gives relation between energy involved in the interaction of neighboring molecules and thermal energy. Negative values of χ_{12} indicate miscibility or compatibility, while positive values indicate exclusion of one polymer from the neighbor of the other. That is, in thermodynamically incompatible mixtures, each polymer prefers to be surrounded by its own molecules rather than molecule of the other polymer.

At a given temperature the interaction parameter depends on degree of polymerization (x) of the dissolved polymer. Flory (1953) determined the limiting values of the interaction parameter for phase separation of a binary mixture (containing two polymers, having different degree of polymerization i.e. x_1, x_2) when $x_1 << x_2$, is given by

$$\chi_{12C} = \frac{1}{2} + \frac{1}{2x} + \frac{1}{\sqrt{x}} \quad (6)$$

For a large polymer ($x \to \infty$), χ_{12C} = 0.5, and upon gradual addition of a nonsolvent (with a large χ value), phase separation occurs in the order of decreasing x, i.e., when $\chi > \chi_C$. For a binary mixture of very long polymers, it has been proved that χ_{12C} approaches to zero and will almost always phase separate.

Overbeek & Voorn (1957) studied the associative phase separation by considering the free energy of mixing and developed the first model on complex coacervation in which they considered gelatin / GA (Gum Arabic) coacervation as a spontaneous process driven by gain in electrostatic free energy at the expense of a decrease in total entropy. The model is based on the assumptions that, the molecules have a random chain configuration; solvent–solute interactions are negligible; interactive forces are distributive in nature; and, the site-specific interactions are absent.

$F_{\text{total (T)}} = F_{\text{M (mixing)}} + F_{\text{e (electrostatic)}}$ (7)

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In the equation (7), F_M had been substituted by Flory-Huggins approximation. F_e was calculated by considering polyions are entirely distributed in the solution and are sum of single charges. Total electrical energy was then predicted by Debye-Huckel theory. The final equation deduced from equation (7), is:

$$F_T / N_x kT = \sum_i \frac{\phi_i}{x_i} \ln \phi_i - \alpha \left(\sum_i \sigma_i \phi_i \right)^{3/2} \quad (8)$$

where, N_x is the total number of sites in the system; k is Boltzman constant; x_i is number of sites occupied by particle I; σ_i is charge density of particle I; α is electrical interaction constant; ϕ_i is volume fraction of particle i.

$$\alpha = \frac{e^2}{3\varepsilon} \left(4\pi e^2 / \varepsilon kTv \right)^{1/2} \left(1/kT \right) \quad (9)$$

where, ε is solvent dielectric constant and e is elementary charge.

The first term in equation (8), is the Flory-Huggins entropy of the mixing components while the second term provides the electrostatic interaction contribution to the free energy of mixing, which increases with polyion concentration. The critical conditions for coacervation were determined from equation (8), for a binary mixture, in which the polyion is of same size and charge density and both having equal initial concentration. For the solvent $r_1 = 1$, $\sigma_1 = 0$, resulting expression can be given by equation:

$$\sigma^3 r = \frac{64}{9\alpha^2} \left(\frac{1}{(1-\phi)^2 (1+\phi)} \right) \quad (10)$$

Since the $\phi << 1$, coacervation would take place at normal temperatures in water when

$$\sigma^3 r \geq 0.53 \quad (11)$$

This model could be extended to three- or four-components systems; $\sigma^3 r$ is then increased to 1.06. Overbeek & Voorn explained that the suppression of coacervation by a salt excess was due to an increase of the solubility of the polyions, a decrease in the amount of polyions in the coacervate, and a decrease of charge density through charge screening by counterions. This model complies with the albumin/acacia complex coacervation under optimum conditions, that is, pH < pI and low ionic strength (Burgess et al., 1991).

Veis & Aranyi developed a theory at conditions where $\sigma^3 r < 0.53$, *i.e.* when the Voorn-Overbeek theory was not applicable (Veis and Aranyi, 1960; Veis, 1961, 1963; Veis *et al*., 1967). Veis modified the Voorn–Overbeek theory, including the Huggins interaction parameter, corresponding to the solvent–solute interaction. The Huggins interaction parameter increases significantly on temperature reduction. They considered coacervation as a two-step process rather than a spontaneous one as considered by Voorn-Overbeek (Burgess 1990). The gelatins spontaneously aggregate by electrostatic interaction to form neutral aggregates of low entropy (step I), and these aggregates then slowly rearrange themselves to form the coacervate phase (step II). The process is driven by the configurational entropy gain arising from rearrangement of aggregates into a randomly mixed coacervation phase.

The reactions of aggregate formation by the thermodynamic analysis were given by Veis & Aranyi (1960).

$$(PQ)_{aggregates,Cm} \longrightarrow \left[(PQ)_{aggregates,Ce} \right]^I + \left[(PQ)_{aggregates,Cc} \right]^{II} \quad (12)$$

where C_c is concentration of coacervate phase, C_e concentration of the dilute equilibrium liquid phase, C_m as functions of the initial mixing concentration. Veis found that the aggregates were present in equilibrium with both liquid and coacervate phase and given the name of symmetrical aggregate model. Equation (12) is concentration dependent since the equilibrium concentrations of the two phases are dependent on the initial mixture concentration. The reaction is rapid and irreversible at very low ionic strength because of the high activation energy required to separate the densely entangled oppositely charged polyion chains.

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In the symmetrical aggregate model, the main problem encountered was distribution of polyions within the aggregate of two oppositely charged polymers with a common domain. It was found that the distribution of polyions affects the free energy and distortion of distribution affects the entropy. The distortion factor by Wall, (1943) indicated that there is no difference in the entropy if the chains are crowded or stretched and second virial expansion coefficient by Flory could be used to estimate distribution of chain elements within the aggregate and electrostatic interaction parameter (α).

Gates (1968) & Veis (1970, 1983) devised a model for ΔF_M for the aggregates in both phases by calculating chemical potential of solvent in each phase based on aggregate model. Gibbs-Duhem equation was used to equate the chemical potential of solvent in each phase at equilibrium. The resultant equation is given as:

$$\ln(1-\phi_2^{II})+\phi_2^{II}-\frac{1}{N}(\phi_2^{II}-\phi_1^{II})+\chi[(\phi_2^{II})^2-(\phi_1^{I})^2]+[0.5-0.46\alpha\sigma^{3/2}(\frac{\alpha^3}{\theta_0})^{1/2}+ 0.5(\frac{\theta_0}{\sigma^3})](\phi_2^{I})^2+0.5\alpha(\sigma\phi_2^{II})^{3/2}=0 \quad (13)$$

where ϕ_2^{II} is volume fraction of polymer 2 in coacervate phase, ϕ_2^{I} volume fraction of polymer 2 in liquid phase ϕ_1^{II} volume fraction of polymer 1 in coacervate phase and ϕ_1^{I} volume fraction of polymer 1 in liquid phase.

Interaction parameter (□1,agg) between the solvent molecules and the new component could be calculated by using experimental values ϕ_2^{II}, ϕ_2^{I}, ϕ_1^{II}, ϕ_1^{I} and M and □. A little change in electrostatic free energy was found on transfer of polyion form liquid phase to the coacervate phase and interaction parameter for aggregates was more than that required for coacervation, which supported the symmetrical aggregate model.

Veis found that when the total polymer concentration (C_T) is increased then the average number of chains participating in the initial aggregate formation becomes smaller. It is due to the fact that at higher concentration, the probability of intermolecular contact increased and original molecular clusters were smaller. At sufficiently high concentrations, the difference between the state of gelatin in randomly entangled dilute solutions and in more concentrated solutions becomes so slight, that coacervation drops abruptly as predicted by equation (14).

$$\varphi_{(r\to\infty)}^{II}\approx\alpha^2\sigma^3 \quad (14)$$

Veis – Aranyi (1960) considered that the molecules are not randomly distributed in both phases, but that ion-paired aggregates are present in the dilute phase. Moreover, the electrostatic term of the Voorn – Overbeek model was replaced by a term which is a function of concentration and charge density of the polymers (equation 14).

Nakajima & Sato (1972) studied an equivalent mixture of sulphated polyvinyl alcohol and aminoacetalyzed polyvinyl alcohol in microsalt aqueous solution. On experimentation Nakajima & Sato agreed to Voorn – Overbeek theory by including the Huggins parameter and changing the electrostatic term and also agreed to the finding of Overbeek and Voorn that the charges are uniformly distributed in both dilute and concentrated phases. Nakajima and Sato applied the theories of Vies (1970, 1983) to both the phases, and modeled the condition of phase separation, when the polymer concentration is very high.

Tainaka (1979) obtained the condition of phase separation when the polymer concentration is low. Gates (1968) & Veis (1970, 1983) obtained the virial coefficients upto second order when the symmetrical aggregates are neutral. Tainaka developed the interaction potential between symmetrical aggregates and calculates the virial coefficients upto fifth order.

Tainaka used Flory-Krigbaum potential for uncharged polymer as the basis to develop the interaction potential function U(R) to determine the potential acting at volume element i from symmetrical aggregate polymers at position l and k, separated at a distance R. Interaction potential function U(R) is given by:

$$U(R)=\sum_i\left[\Delta F\left(\varphi_{2k}^{i}+\varphi_{2l}^{i}\right)-\Delta F\left(\varphi_{2k}^{i}\right)-\Delta F\left(\varphi_{2l}^{i}\right)\right] \quad (15)$$

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where, U(R) is composed of two terms i.e $U_1(R)$ and $U_2(R)$. $U_1(R)$ is Flory-Krigbaum potential and $U_2(R)$ is due to electrostatic energy gain. The final equation after substituting the values of $U_1(R)$ and $U_2(R)$ is:

$$U(R)/kT = X_1 \exp[-0.75R^2/S^2] - X_2 \exp[-0.5625R^2/S^2] \quad (16)$$

where, S is radius of gyration, X_1 comprises of the size of polyion and the interaction parameter χ_{12}, and X_2 represents electrostatic interaction within the aggregate and charge density σ.

According to Tainaka (1978, 1979), the driving forces for phase separation are electrostatic and the attractive force between the aggregates, which become stronger when the molar mass and the charge density of the polymers increase. If the charge density or molar mass of the polymer becomes higher than the critical range, then a concentrated gel or a precipitate will be formed. On the other hand, for charge densities or molar mass below the critical range, short range repulsive forces will stabilize the dilute solution and coacervation will not occur. Tainaka developed the theory for long range electrostatic forces ($\chi_{12} < 0.5$) as he applied Debye Huckel theory to the volume elements of symmetrical aggregate polymer while Veis-Gates and Nakajima-Sato considered the short range electrostatic forces ($\chi_{12} > 0.5$). The Tainaka theory is more general than all the previous theories and is applicable to both high-charge and low-charge density systems.

3. Factors affecting Protein-polyelectrolyte coacervation

Phase separation in biopolymer mixtures is primarily influenced by factors that disrupt the electrostatic, attractive, or repulsive forces occurring between the protein and polysaccharides. The strength of complexation depends on the charge density of both biopolymers. Therefore, pH and ionic strength play fundamental role in the formation of complexes between polysaccharide and protein. Further, the total biopolymer concentration, temperature, pressure (shear force) and the ratio of the two biopolymers also influence complexation. The effect of each parameter is discussed below:

3.1 pH

pH plays a significant role in controlling the number of ionizable (or ionizing) reactive sites along the biopolymer's backbone (i.e., amino-groups and carboxylic-groups) and is particularly important in the case of proteins where the molecule can assume a positive charge at pH < pI and a negative charge at pH > pI. In protein and polysaccharide mixture, complex coacervation generally occurs between the pKa of the polysaccharide and the pI of the protein (Tolstoguzov, 1997; Ducel et al., 2004). As a consequence of change in pH, there is sequential formation of complexes and coacervates which are; (i) intrapolymeric soluble complexes (at the critical pH, pHc), (ii) interpolymer soluble and insoluble complexes; and, (iii) coacervates (at the pH of macroscopic/global phase separation, pH¢) (Weinbreck & Kurif, 2003). Effect of pH on complex coacervation have been documented for various other systems, for example Acacia gum–chitosan (Espinosa-Andrews et al., 2007), κ-carrageenan–gelatin (Fang et al., 2006), furcellaran–bovine serum albumin (BSA) (Laos et al., 2006) or silk fibroin–hyaluronic acid (Malay et al., 2007), gum arabic–BSA (Weinbreck et al., 2003), whey protein-carrageenan (Weinbreck et al., 2004), chitosan-gelatin (Lopezr, 1996).

Important studies on protein and polysaccharide (polyelectrolyte) associative interaction kinetics were conducted in the 1980s where results on pH-induced interaction demonstrated the existence of three clearly differentiated transitions at different critical pHs (Figure 1) (Burgess et al., 1984; Dubin et al., 1988; Park et al., 1992; Xia et al., 1993; Mattison et al., 1995, Mattison et al., 1998, Kaibara et al., 2000). In region 1, Coulombic repulsive forces between the positively charged protein and the positively charge polyelectrolyte prohibit the formation of complexes and the protein and polymer molecules coexist as separate entities within the solution. In region 2, formation of soluble primary complexes starts at "pHc" (point A) near pI of protein because the polymer protein complex formation involves a local domain and continued till point B at a constant pH. Then aggregation of primary complexes initiates with further increase in pH and resulted in macro/microcoacervate formation at $pH_¢$ (point C), which gradually increased in mass with the decrease in number of aggregates (Region 3), hence completion of coacervate formation (point D).

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Finally in region 4, the coacervate changed from liquid to solid precipitates at around pH 8.8 (point E). Primary complex formation, initiated at pHc is considered as a microscopic transition on the molecular scale, whereas coacervate droplet formation at $pH_{¢}$ is viewed as a global phase transition associated with a characteristic length scale of 10-100 nm (Kaibara et al. 2000). The pH of maximum coacervate yield is believed to correspond to the electrical equivalence pH (EEP), where both polymers carry equal but opposite charges (Burgess & Carless, 1984; Peters et al., 1992), which is followed by dissolution of the complexes at lower pH ($pH_{\varphi 2}$), due to protonation of reactive groups on the polysaccharide backbone (Liu et al., 2009). At the EEP, attracting forces between the charged components neutralize each other leading to a strong binding and the highest coacervate yield.

Conversely, Liu et al. (2009) reported that in pea protein isolate and gum arabic system, both biopolymers carried similar charges at both above and below pH_c and $pH_{\varphi 2}$ and resulting in electrostatic repulsion inhibited complex coacervation. However, in case of protein isolate and carrageenan, associative phase separation reported to occur when the overall net charge on both biopolymers is negative (Weinbreck et al., 2004a). Similar results haves been reported for pectin and WPI (Zaleska et al., 2000), and bovine serum albumin and polydimethyldiallylammonium chloride (Wen & Dubin, 1997), and due to the interactions with localized positively charged patches on the protein's surface (Schmitt et al., 1998; Doublier et al., 2000; de Kruif et al., 2004; Weinbreck et al., 2004a).

With progressive change in pH (increase in the case of polycations and decrease for polyanions), the charge on protein becomes opposite to that of the polyion, so that the protein-polymer charge may approach electro neutrality (phase separation), which favors the higher order association and, eventually, phase separation. Prior to pH_c, protein charge density (σ_{pr}) has been reported to be too small to initiate complexation. At pH > pH_c, however, σ_{pr}, has increased substantially for the mass action effects to be observed. Thus, pH_c, has been described as initial phase of molecular binding of proteins to polyions and at $pH_{¢}$ these complexes undergo extensive higher order aggregation. It has been reported that self-association of such species to form larger particles and eventually coacervate would sometimes be suppressed by the presence of a substantial net charge (Mattison et al., 1998).

Mattison et al. (1995) observed that production of primary complexes (at $pH_{¢}$) is directly dependent on protein concentration and higher molecular weight complex was observed when excess protein was present. Consequently, charge per protein molecule required to achieve electro-neutrality also decreases. It had been concluded that proteins are not uniformly distributed among the polymer, and there is no possibility of cooperative binding (Kabanov & Mustafaev, 1981, Li et al., 1996). Further, at constant protein concentration (*r*), the total solute concentration did not effect on both pH_c and pH¢. Strege et al. (1990) had reported that the pH is also independent of *r* and in case the complexation resulted from charge neutralization, the pH is inversely related to ***r*** given by equation (17)

$$Z_T = Z_P + \bar{n} Z_{pr} \qquad (17)$$

and at the point of phase separation

$$Z_T = Z_P + \bar{n}_{\phi} Z_{\phi} = 0 \qquad (18)$$

where, $\mathbf{Z_P}$ is the net polymer charge (independent of pH), *n* is the mean number of bound proteins per polymer chain, $\mathbf{Z_T}$ is the charge of the primary complex, and Z_{pr} is the pH-dependent protein charge, defined as Z¢, at the point of phase separation and taken to be opposite in sign to Zp and $n_{¢}$ is mean number of bound protein per polymer chain at the point of phase separation (i.e at $pH_{¢}$) (Strege et al., 1990).

Ionic Strength

Coacervation involves electrostatic interactions and is highly affected by factors that change the electrical charge density on the interacting molecules such as the pH and the ionic strength (Burgess & Carless, 1984; Mattison et al., 1995; Wang et al., 1999; Weinbreck et al., 2003b). The charge neutralization of biopolymer by microions can hinder the electrostatic interaction, which in turn can suppress the complex coacervation. At low ionic strength, the microion concentration helps to promote electrostatic interactions between biopolymers, as ions associate with protein structure alter its conformation to expose additional charged groups. Depending on the biopolymers used, the amount of salt that must be added to promote complex coacervation varies. However, at high salt concentrations, the net charge carried by the biopolymer is reduced due to screening (charge neutralization) of charges by the presence of microions, resulting in lesser electrostatic attractive forces between macromolecules (Weinbreck et al., 2003a).

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The coacervation improved on addition of salt only up to a certain concentrations. The reasons for this might be that the proteins are amphoteric, contain both positive and negative charges at the same time. Therefore there is simultaneous existence of both electrostatic attraction and repulsion between protein and polysaccharide. Dubin model (Seyrek et al., 2003) has defined Debye length (Rd) as the distance over which significant charge separation can occur, outside of which charges are screened, (R^+) as average distance between the protein's positive sites and the polysaccharide's negative sites and (R^-) as the average distance between the protein's negative sites and the polysaccharide's negative sites. Debye length (R_d) decreased by increasing salt concentration and the relation was given by

$$R_d \approx 0.3/[NaCl^{1/2}] \quad (19)$$

For β-lactoglobulin (β-lg)/pectin coacervation, at low salt concentrations, there may be $R^+ < R_d < R^-$, and increased salt concentration mainly screen the electrostatic repulsion without affecting electrostatic attraction. Net attraction will be enhanced on increasing salt concentration up to 0.1M. On the other hand, when C_{NaCl} is above 0.1 M, $R_d < R^+ < R^-$ could make both electrostatic attraction and repulsion be screened significantly because of the higher amount of salt.

Weinbreck et al. (2003a) reported that a concentration of <50 mM NaCl improved complex coacervation between WPI and gum Arabic, whereas at levels >50 mM NaCl complexation was inhibited due to the screening effect. Liu et al. (2010a) reported that 100 mM NaCl suppressed complex formation between pea protein and gum Arabic mixtures. Lopez & Bodmeier (1996) for gelatin-chitosan system has reported that a concentration 130mM of NaCl is required for complete suppression of coacervation due to strong interactions between gelatin-chitosan. Wang et al. (2007) studied β-lactoglobulin and pectin mixtures and have reported that there is an increase in the values of $pH_{¢1}$ and $pH_{¢2}$ on addition of salt up to the concentration 0.1M and further increase results in again decrease in the pH. Thus, the C_{Nacl} upto 0.1M will help to improve the coacervate yield. For whey protein (WP)/ carageenan (CG) system, [NaCl] < 45 mM, pH¢ also reported to increase the pH values and maximum turbidity which means increase in number and/or size of the biopolymer complexes. The charge density of CG will be so high that electro-neutrality of the complex is not fully achieved by the WP only. Therefore, if microions are present in the solution, they will screen the residual negative groups of CG and thus effectively reduce repulsion between complexes and allow an effective phase separation. When the concentration of NaCl > 45 mM, the pH¢ shifted to lower values, and for [NaCl] > 1 M, no phase separation occurred (Weinbreck et al., 2004).

Biopolymer Mixing Ratio

Protein to biopolymer ratio also has a significant effect on phase separation. Due to the constant charge density of proteins and polysaccharides at a given pH, by changing the biopolymer mixing ratio, the net charges available for coacervate formation can be modified (Liu et al., 2009). For ratios where one of the biopolymers is in excess, soluble complexes are obtained because of the presence of non-neutralized charges. Several studies have demonstrated that the protein and polysaccharide mixing rario has a important effect on the characteristics of the resulting complexes, namely, the complexes size, composition, and viscosity in solution (Wang et al., 2007c; Turgeon et al., 2007; Laneuville et al., 2005b; Weinbreck et al., 2004d). At low mixing ratio (r) only intra-polymeric complexes are formed, whereas at higher r the coacervate or inter-polymeric complexes concentration is increased (Wang et al., 2000). The structure of complexes or coacervates was found to be greatly influenced by the mixing ratio (r), at low r, smaller coacervates form and coalesce rapidly into very large coacervates, whereas at higher r the coacervates did not coalesce as readily (Schmitt et al., 2001b). At high protein content, very large complexes containing an excess amount of protein form (Laneuville et al., 2005b). Wang et al. (2007b) reports that increasing the proportion of proteins in a β-lactoglobulin and pectin system results in the formation of coacervates with a higher elastic character.

Liu et al. (2009) reported pHc to be dependent on biopolymer mixing ratios of below 4:1 after which pHc became stable at higher biopolymer mixing ratios upto 10:1, because gum arabic interacts with more than one protein molecules. Similar dependence of pHc for gelatin–agar system has been reported by Singh et al. (2007). On the other hand, some researchers observed that the pHc was independent of mixing ratios and this stability was thought due to the interaction of a simple polysaccharide chain with given amount to protein (Weinbreck et al., 2004a, Weinbreck et al., 2003b, Mattisson et al., 1995).

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For the β-lg and low methoxy (LM) pectin, the ratio of 8:1 was found to be optimum, which means that 0.05% β-lg in solution is required to saturate the LM-pectin at 0.00625%. For high methoxy (HM)-pectin, the ratio of 4:1 was optimum. The difference between LM- and HM-pectin is due to the dependency of pHc values on the polysaccharide charge density (Girard, 2002, Park et al., 1992). This is explained by the lower content of carboxylic groups in HM-pectin, which limits the possibility of electrostatic binding with β-lg (Girard et al., 2002). Thus HM-pectin becomes saturated with proteins faster than the LM-pectin. The LM-pectin holds more H^+ than β-lg/HM pectin which shows that LM-pectin is strongly complexed to β-lg than HM-pectin.

At low ionic strength, the complexation can occur over a wide range of biopolymer concentration (Tolstoguzov, 1986). However, at too high concentrations, self-suppression of the interaction occurs, because intrapolymer complex changes interpolymer complex which is precursor of coacervation, and at this point there is no entropy gain which is a driving force to phase separation.

Biopolymer Molecular Weight (MW)

The number of proteins bound to polyelectrolyte mainly depends on the polymer length, chain flexibility, and protein dimensions (Dubin & Murrell, 1988). Theoretically, increase in the molecular weight of the biopolymers is expected to lower the biopolymer compatibility in solution by lowering the combinatorial entropy of mixing. Voorn & Overbeek (1960) predicted coacervation as a function of molecular weight of the biopolymers.

Wang et al. (1996) suggested that larger MW polymer form large coacervates of lower solubility. Therefore, a low MW polymer has less affinity to bind with proteins due to lesser binding sites available on low MW polymer. When MW of polymer is sufficiently large, the solubility of the protein-polymer complexes becomes less and less dependent on the polymer chain length. Studies on the effect of the MW of the complex characteristics established that biopolymers with higher MW leads to formation of larger primary complexes which aggregated more readily into inter-polymer complexes (Li et al., 1994; Laneuville et al., 2005b; Wang et al., 2000), presumably since the polyelectrolyte acts as the backbone for the formation of primary complexes (Park et al., 1992; Laneuville et al., 2005b) and possibly also due to higher entropy gain (Tainaka, 1979). It has been observed that coacervation with soy globulin increased with molecular weight of dextran, due to higher space filled by the polysaccharide in solution which makes it more accessible for the protein (Semenova, 1996). Wang et al. (2000) studied effect of molecular weight on polyelectrolyte-micelle coacervation and demonstrated that a critical molecular weight for coacervate formation was required, below that coacervation was not possible and by adjusting the size of the polyelectrolyte or the micelles, the size of coacervates can be controlled. Moreover, the internal structure of complexes can be controlled by varying the polyelectrolytes MW, lower MW results in denser complexes, thus allowing additional control of their properties (Laneuville et al., 2005b).

However, simulations studies showed that increased chain flexibility and charge mobility resulted in stronger binding, this effect apparently being more important for micelles than for single proteins (Grymonpre et al., 2001; Kayitmazer et al., 2003; Cooper et al., 2006). Mattison et al. (1998) suggested that the length of the binding segment was controlled by the intrinsic stiffness of the polymer chain and that this parameter may be stronger than the effect of the polyelectrolyte linear charge density. Shieh & Glatz (1994) also suggested that little effect of MW is observed for protein-polyelectrolyte pairs exhibiting strong electrostatic interactions, as in the case of Polyacrylic acid (PAA)-lysozyme and PAA-ovalbumin complexes

Charge Density of Biopolymers

The charge density of the biopolymers can be defined as the number of charges present on the biopolymer per unit length. Charge density of biopolymers has important contribution in the formation of coacervates. There is a critical charge density value below which no coacervates are formed because phase separation is charge neutralized state of aggregates. If the charge density is below a critical range, biopolymer will bind to lesser number of molecules of other biopolymer for neutralization; consequently the size of the aggregate will be very small which will remain soluble in solution. It has also been suggested that a more compact conformation of the polyelectrolyte results in increased charge density and therefore higher polarizing effect on the protein, which promotes a stronger attraction (Bowman et al., 1997).

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The charge distribution of biopolymer can affect the interaction affinities (Kayitmazer et al., 2003). Takahashi et al. (2000) studied interactions of ribonuclease and lysozyme (both have same molecular weight and basic groups) with same polyelectrolyte and demonstrated that the two protein interacted differently to the polyelectrolyte due to different distribution of their basic residues. Lysozyme has a random distribution of basic amino acids whereas in ribonucleases these are in close proximity to each other. As a result, large and more coacervates were formed with lysozyme due to better polarizability. Girard et al. (2002a) compared highly methoxylated pectin (HM pectin) and low methoxylated pectin (LM pectin) for electrostatic interactions with β-lactoglobulin. The results showed that HM pectin has less affinity to bind to the protein and also it binds to lesser number of proteins as it has lesser charged carboxylic groups than LM pectin. LM pectin interacted strongly with the β-lactoglobulin. Another comparison of i-carrageenan and k-carrageenan for binding with casein revealed that i-carrageenan formed a stronger complex with protein as it has higher sulphate groups (Burova et al., 2007).

Temperature

In general change of temperature influences the biopolymer/biopolymer interactions by changing the Flory- Huggins interaction energy. Enthalpic interactions other than Coulombic interactions (hydrogen bond and hydrophobic interactions) are also influenced with change in temperature. Low temperatures favor hydrogen bond formation, while hydrophobic interactions are enhanced by rise in temperature.

If hydrophobic interactions contribute, it should be possible to observe temperature dependence of phase behavior, since hydrophobic interactions are known to exhibit strong temperature dependence (Tanford, 1980). The coacervate yield decreased at higher temperatures due to increased solubility of the polymers in solution and lesser probability of complex formation. Further, the complex formation of β-lg with LM-pectin and HM-pectin decreased with increase in temperature from 4 to 40°C. The destabilization effect of increasing temperature has shown that hydrophobic interactions are not significant at low temperature. Hydrophobic interactions are possible between aliphatic and aromatic amino acids of β-lg and ester group of pectin. Heat treatment was necessary to expose hidden hydrophobic groups of β-lg (Giarard et al., 2002a). Similar results were reported by Lopez & Bodmeier (1996) for temperature equilibration studies on chitosan-gelatin coacervates. The correlation between the presence of hydrophobic domains and the tendency for coacervation also appears to be quantitative. The temperature at which aggregational transition occurs is generally inversely dependent on the mean hydrophobicity of the polypeptide (Luan et al., 1990, Urry et al., 1991) and direct proportionality between the heat of transition and mean hydrophobicity as revealed by differential scanning calorimetry studies (Urry et al., 1991, 2004).

On the basis of temperature, coacervation systems can be classified into two types: one which involves hydrophobic interactions are temperature dependent systems, for example polydimethyldially1ammonium chloride (PDADMAC) and anionic micelles showed a temperature-induced phase separation (Dubin et al., 1990). Other include electrostatic interactions are temperature independent systems for example in whey protein (WP) and a nongelling carrageenan (CG) (Weinbreck et al., 2004) and BSA-PDADMAC systems (Kaibara et al., 2000), the temperature had a little effect form 5-50°C on the complex formation.

Shear force

Shear force can have an impact on the properties of complexes and coacervates and is an important parameter to control for industrial scale productions; however few studies have dealt with these issues in detail. It has been found that when shear force is applied during complexation, restructuring processes of interpolymeric complexes takes place by a competition between attractive electrostatic forces and rupture forces caused by shear (Laneuville et al., 2005b). In systems forming coacervates, finding the right conditions of temperature and shear allowed stabilization of the system against flocculation (Sanchez et al., 2001). Also, by adjusting the polysaccharide aggregation degree or by changing its MW, e.g. by mechanical degradation prior to the complexation process, can allow the formation of particulate complexes instead of fibrous ones (Laneuville et al., 2005b). Galazka et al. (1999) found that by applying a pressure treatment to the complexes led, upon pressure release, to the strengthening of the interaction, due in part to a partial denaturation of the protein, which exposed more charged groups. Similarly, BSA and anionic polysaccharide interactions were stronger following heat denaturation due to the increased molecular flexibility in the denatured state, which permitted configurationally adjustments that allowed interactions to be maximized, yielding more stable complexes than those formed with the native proteins (Samant et al., 1993).

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The effect of temperature (25 or 50°C) and shear (1000 s^{-1} or complex shear) was studied for the β-lactoglobulin/acacia gum system. Combinations of both parameters could either lead to a stable system (low temperature/complex shear) or to a very unstable one (high temperature/ complex shear), with a marked phase separation due to the flocculation and coalescence of coacervates (Sanchez et al., 2001).

Applications of protein–polysaccharide complexes

Formation of non-covalent electrostatic complexes between proteins and polysaccharides enables designing of novel microstructures. Owing to the biological significance of protein–polysaccharide electrostatic interactions and the huge industrial potential of complexes and coacervates, increasing number of research groups have focused on the study of these systems during the last decade. The industrial applications include micro- and nano-encapsulation processes (Xing et al., 2004, 2005; Champagne et al., 2007; Daniel, 2007), design of multi-layers structures (Noel et al., 2007), formation and stabilization of food emulsions (Dickson et al., 2006), formation of new food gels (Haug et al., 2004), and recovery of proteins from industrial byproducts (Vikelouda et al., 2004, Damianou et al., 2006, Montilla et al., 2007). The microstructures can be formed in the coacervate phase depending on the total biopolymer concentration, having novel rheological properties. Interfacial properties can be changed by introducing organic phase in the aqueous complex system, due to protein–polysaccharide complexation synergistic effects (McClements, 2006). Various properties (thickening agents, whipping and emulsifying properties, stable delivery system) of coacervates presented in the preceding section provide a foundation not only for basic understanding but also for their practical applications for the development of novel food products with higher nutritive value and minimum loss of bioactivity during processing.

As Thickening agents and Stabilizers

Each individual biopolymer (polysaccharide) exhibits different rheological behavior on association with other biopolymer (protein). After association, molecular weight of the complex and coacervate increases due to complex formation. Consequently, the viscosity increases due to formation of hydrated polymeric complex and is dependent on the nature of polysaccharide. Rheological properties have been studied for soluble complexes, coacervates and inter-polymeric insoluble complexes and applied to various food systems. Viscosity of a mixture of biopolymers is affected by molecular weight, total biopolymer concentration, hydration capacity, pH, ionic strength, and presence of other functional groups. The spherical structure of acacia gum is responsible for viscosity of acacia gum/ protein coacervates (Weinbreck et al., 2004) whereas pectin/ protein coacervate resulted in gel, when same protein has been used in both systems (Wang et al., 2010). For whey protein and gum arabic coacervates, the highest viscosity was obtained at pH 4.0, the pH at which electrostatic interactions were the strongest (Weinbreck et al., 2004a) i.e. in charge neutralization conditions (Weinbreck et al., 2004a, Schmitt et al., 2005b, Espinosa et al., 2010). Viscosity is also affected by the ionic strength of solution as optimum salt concentration favors the electrostatic interactions thus stronger tendency for coacervate formation. Improved gel strength has been reported for β-lactoglobulin/pectin coacervates at 0.21M NaCl (Wang et al., 2010). Higher concentration of NaCl supported protein–protein interactions and coacervates contained more water and protein-rich domains. The parameters for coacervate formation strongly affect the solubility and viscosity of gel, and are to be carefully controlled (Schmitt et al 2010). For example, if strong intermolecular attractive forces will be applied, the system will have less hydration capacity and consequently a lower viscosity (Delben, 1997, Laneuville et al., 2000).

Highly charged soluble complexes are formed at initial phase of associative interaction between biopolymers and electrostatic repulsion stabilizes the system against aggregation. For example, carrageenans-casein complex is formed at pH > EEP of protein (Teramoto et al., 1999) and thus improved rheological properties.

Another example, where pectin is used to stabilize positively charged casein, thus preventing over aggregation by electrostatic repulsion (Tromp et al., 2004). Negatively charged exo-polysaccharide produced by lactic acid bacteria have strong ability to interact with milk protein and are used to replace synthetic stabilizers in yogurt (Ruas-Madiedo et al., 2002). Using negatively charged exo-polysaccharides (sulphated) gels can be formed at higher pH and in lesser gelation time in comparison to neutral polysaccharides and resulted in firm and brittle gel (Ding et al., 2002; Girard et al., 2008). Several other proteins (β-lactoglobulin, BSA, ovalbumin) and polysaccharides (gellan gum, carrageenan, xanthan) exhibit similar behaviour. Use of exo-polysaccharide in fermented dairy products has allowed improving the viscosity, texture and the overall sensory and water-retention properties of the product.

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As a texturing agent, protein-polysaccharide complexes have been used to produce meat analogs and fat replacers (Chen and Soucie, 1986; Chen et al., 1989). Tolstoguzov et al. (1974) first patented the process for production of polysaccharide gels entrapping proteins as minced meat mimetics under conditions such as protein to polysaccharide ratios varying from 1:1 to 30:1, pH was near IEP of protein and heat treatment at 40 to 90□C for 20 to 120 min. Soucie and Chen. (1986) patented the process of microencapsulation for production of protein-polysaccharide complexes exhibiting fibrous structure and used in the formulation of low-fat meat products. Microparticulation technique was patented by Chen et al. (1989) for production of fibrous complex (made of milk and egg protein with xanthan gum) to be used as fat replacers. Thermal treatment (50 to 90□C, 3 to 5 min) was applied to improve their stability, causing denaturation of proteins and preventing any dissociation of the complexes through protein hydrophobic interactions. The resulting coacervates, after fragmentation can be used as fat substitutes directly, were of spherical shape with a mean diameter 2- 10□m. Another example, where core-shell microparticles having a gelified polysaccharide core and a protein shell had been utilized as fat replacers (Perrau et al., 1989; Bishay et al., 1996).

Protein-polysaccharide complexes thus can be exploited as a tool to manipulate rheological properties of several food products. A limiting factor in the coacervate structure is functional properties of proteins changes with pH. However, understanding the role of process parameters (like pH) on the structure of coacevate can help to get the desired functional properties of complexes at particular ratio of biopolymers.

Whipping ability and emulsification

Protein–polysaccharide complexes have combined properties of individual biomolecule especially hydrophilic and hydrophobic nature which can be used to modify the adsorption kinetics of proteins at the air/water interface, but also to modify the microstructure of the adsorbed layer and to control its stability (Ganzevles et al., 2006b). Here, two models of adsorption have been proposed depending on the initial protein to polysaccharide mixing ratio: (i) a thermodynamically limited protein adsorption (electrostatic barrier) at the interface, when the complexes are charged because of an excess of polysaccharide, and (ii) a diffusion limited adsorption of complexes, when the protein to polysaccharide ratio is close to charge neutralization.

The interfacial properties of β-lactoglobulin/acacia gum complexes obtained at pH 4.2 and a mixing ratio of 2:1 to stabilize air/water interface have been studied (Schmitt et al., 2005b). Protein/polysaccharide complexes formed much stronger viscoelastic interfacial films with a thickness of about 250 Å when compared to pure protein (Liz et al., 2006). As a result, there was a decrease in gas permeability of a thin-film stabilized by the complexes (0.021 cm/s) in comparison to pure β-lactoglobulin (0.521 cm/s). This property was successfully used to improve air bubble stability of strawberry ice cream and lemon sherbet, which increased perception of creaminess. Whey protein isolate/acacia gum complexes have been similarly used to obtain dairy gels and replaced the synthetic gelatin by whey protein isolate/acacia gum complexes (Schmitt et al., 2010). Similar air/water surface activity had been reported for complexes based on β-lactoglobulin/pectins (Ganzevles, 2006b), ovalbumin/pectin (Kudryashova et al., 2007), or β-lactoglobulin/carboxylated pullulan (Ganzevles et al., 2007c).

Two techniques have been widely used for stabilization of emulsions, either layer by layer or use of coacervates leading to mixed emulsions (Jourdain et al., 2008). In layer by layer technique, a primary emulsion is stabilized by a protein and thereafter dropped in a polysaccharide dispersion to encourage interfacial complex formation (McClements, 2006, Jourdain et al., 2008). It was found through interfacial shear rheology experiments on sodium caseinate/dextran sulfate that mixed emulsions were much more viscoelastic than bilayer emulsions (McClements, 2006). Interestingly, the emulsions have been tested for stability under acidic conditions where the mixed emulsion was shown to be much more stable to reduce flocculation compared to the bilayer ones. Similar pH stability was obtained when dextran sulfate was replaced by the sulfated carrageenan polysaccharide (Jourdain et al., 2008). Higher oil/water surface activity for the pea globulin or α-gliadin complexes or coacervates with acacia gum have been reported (Ducel et al., 2005). Coacervate films were characterized by a very long relaxation time and a high surface elasticity. Several other protein/polysaccharide pairs (β-lactoglobulin complexed with alginate, carrageenan or acacia gum) were used to produce acid stable bilayer emulsions and might find interesting application in the beverage industry (Guzey & McClements, 2006, Harnsilawat et al., 2006). The use of the cationic chitosan enables production of stable emulsions based on whey protein isolate closer to the neutral pH range, i.e. pH 6.0 (Laplante et al., 2005a) at an optimum protein to polysaccharide mixing ratio. Interestingly, the use of complexes enables production of heat stable emulsions, which was not the case for pure protein systems (Girard et al., 2002a).

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Plant proteins (alpha-gliadin and pea globulin) had been studied by Ducel et al. (2004) to complex with gum arabic and carboxy methyl cellulose. Another example producing coacervates, gelatin and a modified guar gum (sodium carboxymethyl guar) was utilized to encapsulate clove oil (Thimma et al., 2003). The addition of surfactants was reported to enhance encapsulation yield (Mayya et al., 2003), decrease droplet diameter and accelerate coacervation in a gelatin and arabic gum system (Tan et al., 2008). WPI and LM-pectin inter-polymeric complexes have been used to evaluate their functionality as an entrapment matrix for thiamine by Bedie et al. (2008). Silletti et al. (2007) reported that the in-mouth behaviour of food emulsions could be stimulated by the charge of the interfacial layer in order to promote interaction with salivary glycoproteins such as mucins. Hence, mucins have been shown to strongly interact with polysaccharides, like chitosan (Dedinaite et al., 2005).

Stable delivery system for release of bioactive compounds

The encapsulation covers all aspects of protection of bioactive molecules against degradation through physicochemical, enzymatic, or mechanical damages (oxidation or acidification in the presence of O_2 or CO_2,) and processing (temperature, pH and shear.). It also enables controlled release over the time for delivery of the encapsulated material and to specific gastrointestinal targets. Encapsulation conditions should be gentle for active compounds and the release can be controlled by mechanical process, pH variations or enzymatic action.

Complex coacervation is suitable for food applications as this technique neither uses organic solvents nor requires drastic temperatures (Schmitt et al., 1998). On the other hand, protein and polysaccharides also exhibit nutritional and functional properties. Coacervates have also been applied in flavor industry for release (Yeo et al., 2005; Prata et al., 2008) and nutrient protection (Junyaprasert et al., 2001; Lamprecht et al., 2001; Pierucci et al., 2007). Gelatin and gum arabic encapsulation has been done to release a flavour (oil) during cooking in baked goods which was released at temperature $\geq 100^{o}C$ (Yeo et al., 2005). Weinbreck et al. (2004a) used whey protein and arabic gum coacervates to encapsulate lemon and orange flavors.

Partitioning of flavor compounds in the oil phase is main challenge in using coacervates for flavor encapsulation. Partitioning rate depends on hydrophobicity, which determines the release of flavoring compound (Taylor, 2002). In solution, the properties of coacervates could be fine-tuned to balance the limitation induced by the partitioning of the chosen flavor compound which can be achieved by controlling the environmental conditions during coacervation and the appropriate choice of the coacervating macromolecules (Taylor, 2002; Madene et al., 2006).

Cross-linking agents can further contribute to harden the coacervate layer after formation of the microcapsules (Leclercq et al., 2009). Transglutaminase introduces covalent links between carboxyl group of a glutamine and amino group of lysine in protein molecules. Soeda et al. (2003) patented a simple coacervation technique for encapsulation of flavors and oil-soluble cores including omega-3 fatty acids, using transglutaminase as the crosslinking agent for hardening the capsule wall formed by salting out. The resulting product has been shown to be suitable for a wide range of food applications. Food grade alternatives have been recently studied as stable delivery system, e.g. tannic acid (Xing et al., 2005) and glycerine (Huang et al., 2007). Sagis et al. (2008) added HM-pectin and whey proteins (pre-heated to denature the proteins) in alternate on emulsion droplets and observed increased freeze-drying stability. (β-lg + sodium caseinate) or (β-lg + arabic gum) coacervates had been used for encapsulation of sensitive lipophillic compounds such as vitamins or oils and also to reduce the bad odour of some fatty acids (Mellema, 2005).

Pre-complex coacervate formation between therapeutic proteins and oppositely charged macromolecules are able to enhance the stability and control the release and absorption rates of proteins. An optimized novel delivery system containing dual advantages of complex coacervation and temperature responsiveness has been introduced and the potential as an efficient protein drug delivery system has been demonstrated (Kwang et al., 2007).

Protein-polysaccharide complexes had also been targeted for microencapsulation and controlled release of pharmaceutical products because the protein is not a foreign protein to the living system and can be in contact for temporary or definitive periods. Encapsulation of micro-oil droplets containing lipophilic drugs into gelatin-acacia gum microcapsules to preserve their activity had been proposed by Jizotomo et al (1993). Easton and Gorham, (1986) patented a process for production of protein polysaccharide complexes (collagen, xanthan gum, alginate, and CMC) that can be used in the medical and pharmaceutical industries.

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Covalent oxyhemoglobin-carboxylic dextran complex have been used as blood replacer during surgical operations, having similar capacity for binding and delivery of oxygen as that of hemoglobin. Similarly, chitosan and ovalbumin mixtures for production of nanogels has been studied and resulted in nanogels (size ranging from 120 to 500 nm) with a stable size distribution even after long storage periods and under wide pH conditions of 2.0 to 10.5 which could be used to load drugs.

Thermal stability of globular proteins can be improved by considering biopolymer pairs. An increased thermal stability of proteins was observed in flaxseed gum-meat protein (Chen et al., 2007) and carboxymethylcellulose-α-lactalbumin or β-lactoglobulin systems (Capitani et al., 2006). In contrast, thermal aggregation of proteins significantly increased between β-lactoglobulin and chitosan by electrostatic complex formation (Mounsey et al., 2008). β-lactoglobulin aggregation was prevented in presence of chitosan at pH 4 (no interaction is supposed to occur at this pH). Heparin also reduced heat stability of lysozyme, presumably due to presence of multiple binding sites (van de Weert et al., 2004). Since soluble complexes are charged, electrostatic repulsion between complexes could prevent heat-induced protein–protein interactions and large scale aggregation. Importantly, heating may induce a stabilization of protein–polysaccharide complexes against subsequent pH changes (Hong & McClements., 2007). It was assumed that stabilization could be due to the formation of a network of aggregated proteins with the polysaccharides.

CONCLUSION

The here-described work based on past literature is mainly attending the thermodynamics of phase separation, difference between various thermodynamic models, the intrinsic and extrinsic factors controlling complex formation and the most important functional properties of protein-polysaccharide complexes. The interesting functional properties of these entities can used in diverse range of food formulations such as fat replacer, stabilizers, texturing agent, purification of proteins and microencapsulation of bioactive compounds. This is undoubtedly an area of future intensive research which can contribute to food functionality, better control of food quality and formulation of novel food and processes without thermal treatment and chemicals to preserve the natural characteristic with reasonable production cost. Even if a number of protein-polysaccharide systems had been already studied, numerous binary combinations of proteins with biopolymers will still wait to be tested, as well as protein interaction with a mixture of polysaccharides with combination of low- and high-molecular-weight polyelectrolytes.

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