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Complex coacervation of silk fibroin and hyaluronic acid

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Abstract

This study aimed to investigate the pH-induced complexation of silk fibroin (SF) and hyaluronic acid (HA). SF–HA complex coacervation was investigated by monitoring turbidity of the SF–HA system under slow acidification. Gravimetric analysis was performed to determine the yield of complex coacervation and viscosity of the system was measured to study the formation of the complexes at different pH values. The influences of total biopolymer concentration and biopolymer weight ratio on complex coacervation were examined during the analyses. Formation of the complexes was evidenced by the minimum viscosity and the maximum turbidity observed in the system. SF–HA complexes were formed within the pH–window of 2.5–3.5 regardless of the total biopolymer concentration or biopolymer ratio. Complex coacervation of SF–HA showed a reversible behavior and coacervation could be handled even in excess amounts of the biopolymers, which pointed out a non-stoichiometric complexation.

Keywords: Protein–polysaccharide interactions; Complex coacervation; Silk fibroin; Hyaluronic acid

1. Introduction

The study on interactions between biopolymers has been extensively subjected to many researches with respect to its promising applications in biotechnological and biomedical areas as well as its biological aspects. In biological systems, proteins and polysaccharides have an important role in the organization of the living cells and the interactions between these biopolymers lead to formation of macromolecular structures through association. On the other hand, their incompatibility may be the cause of a cellular event such as cell partition [1].

The mixtures of protein and polysaccharide undergo either of two kinetic processes: phase separation or complex coacervation. If the protein and the polysaccharide are incompatible, they repel each other and a thermodynamic phase separation, which is also called segregation or depletion interaction, occurs. On the other hand, if they attract each other through electrostatic interactions, the biopolymers associate excluding the solvent from their vicinity. This gives rise to the formation of protein/polysaccharide complexes [1,2]. Complex coacervation describes the phase separation of a liquid polymer rich-phase from a macromolecular solution, representing the separation of two liquid phases in a colloidal system. The phase more concentrated in colloid component is the coacervate and the other phase is the equilibrium solution [3]. In the mixture, electrostatically attracted and bound complexes can also be either soluble or insoluble. The coacervate layer occurs as the insoluble complexes concentrate in liquid coacervate droplets followed by further coalescence and phase separation [1–6].

Complex coacervation has been used in the fields of pharmaceuticals, medicine, foods, cosmetics, etc. Microencapsulation based on the ability of the coacervates to form a coating around sensitive materials (e.g. drug particle or an oil droplet containing flavors) has been investigated extensively and commercialized [7]. Gelatin/gum arabic (GA) coacervate system was extensively used for microencapsulation [8–10]. On the other hand, complexation has been studied in the purification and recovery of the macromolecules (e.g. protein) [11] and in the development of food ingredients, such as fat substitutes and meat replacers [12]. The studies on film formation properties of the coacervate complexes of sodium caseinate/wheat or starch corn have displayed that coacervate films are also promising in the fields of

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biopackaging or edible food packaging as they represent good mechanical and gas barrier properties [13]. Moreover, polyelectrolyte films produced by the chitosan/alginate coacervates have represented good biocompatibility and found promising in biomedical applications [14].

The trend nowadays is to replace the protein and polysaccharide components by other biopolymers to form complexes offering complexation mixtures that may result in materials with novel properties. To the best of our knowledge, no report concerning the use of silk fibroin and hyaluronic acid for complex coacervation has been published in the literature.

Silk has been commercially used as surgical sutures for decades and as a source of textile-grade fibers for centuries [15]. Silk synthesized by *Bombyx mori* consists of two kinds of proteins, sericin and fibroin. Fibroin is the structural fibrous protein and constitutes 70% of the intact silk, and sericin is the water-soluble glue-like protein that surrounds and binds the fibroin fibers. The silk fibroin molecule consists of heavy and light chain polypeptides of ~350 kDa and ~25 kDa, respectively [16,17]. Recently, interest has been concentrating and dramatically increasing on the use of several processed forms of the solubilized silk fibroin in biotechnological materials and biomedical applications. Reeled silk fibers easily redissolve in water with very concentrated neutral salt solutions (LiBr, LiSCN, $CaCl_2$) without inducing hydrolytic degradation [18]. Aqueous fibroin solution, which is also called regenerated silk fibroin (RSF), is environment friendly and it has been used in various applications such as in the development of biosensors [19], drug coating materials in the preparation of oral dosage forms [20], membranes/carriers for controlled drug delivery systems [21] and scaffolds for tissue engineering [22]. RSF is of interest since it represents a good starting material for the preparation of different forms of materials (e.g. gels, powder, foams, films, etc.) by the application of various processing techniques.

Hyaluronic acid (HA) is a naturally occurring linear polysaccharide that is a copolymer of *N*-acetyl-D-glucosamine and D-glucuronic acid disaccharide units connected by regularly alternating β-(1 → 3) and β-(1 → 4) glucosidic bonds. This endogenic biopolymer was discovered first by Meyer and Palmer [23] and is present in the extracellular matrix of all higher animals as the only non-sulfated glucosaminoglycan [23–25]. Hyaluronic acid is an attractive building block for novel biocompatible and biodegradable biomaterials with potential applications in drug delivery [26] and tissue engineering [27], and with proposed applications in the production of artificial blood vessel and artificial skin [28].

The main objective of this study is to investigate the formation of complex coacervation between silk fibroin and hyaluronic acid. The influence of pH on the charge density of the biopolymers, therefore on complex coacervation and extent of complexation have been established by analyses on turbidity, viscosity and gravimetry of the system as well as by macroscopic observations. The effects of total biopolymer concentration (C_p) and the ratio of the two biopolymers (R = SF:HA weight ratio) on complexation have also been shown.

2. Experimental

2.1. Materials

Silk Fibroin (SF) was obtained in reeled form from Bursa Institute for Silkworm Research (Bursa, Turkey). Hyaluronic acid (HA) sodium salt (MW: 1600 kDa, from *Streptococcus equi*) was provided by Fluka-BioChemica (Buchs, Switzerland) in powder form. Ethanol (absolute GR for analysis) was from Merck (Darmstadt, Germany). Calcium chloride-2-hydrate was supplied from Riedel-de Haën (Seelze, Germany), sodium carbonate (99.5+%) was from Aldrich–Chemie (Steinheim, Germany). Dialysis tubing (MW Cut-off: 12–14 kDa) was obtained from Sigma (St. Louis, MO, USA). Sodium sulfide hydrate was provided by Fluka Chemie (Buchs, Switzerland). Deionized water was used during all experiments.

2.2. Preparation of biopolymer solutions

Silk fibroin solutions were prepared by subsequent processes of degumming and dissolution. During the degumming process, raw silk was kept in 50 times (v/w) of boiling aqueous 0.05% Na_2CO_3 for 30 min and this treatment was repeated three times. This was followed by washing several times with deionized water and the degummed silk was left drying at room temperature. To obtain aqueous SF solution, 1.2 g degummed silk was added to 20 times (v/w) of Ajisawa's reagent ($CaCl_2$/ethanol/water). The mixture was stirred for 2 h at 78 °C to form a clear solution. The resulting SF solution was then dialyzed against deionized water for at least 3 days at sub-ambient temperature to remove the neutral salts using cellulose tubing. Eventually, the dialysis was ended as the dialysate tested negative for chloride ion by performing silver chloride precipitation test using $AgNO_3$. The concentration of the SF solution was controlled using a rotary vacuum evaporator. Following these treatments, the average molecular weight of SF was determined by size exclusion chromatography (SEC). The system consisted of ZORBAX GF–250 column, HPLC (Agilent Tech. 1100) and a UV detector. 20 μl of sample dispersion (0.5 wt%) was injected into the column after filtration through 0.45 μm filters. Mobile phase was 200 mM sodium phosphate (pH 7.0) and flowrate through the column was kept at 2 ml/s.

HA was provided in powder form and it was soluble in water or any buffer solutions considered. However, HA particles were prone to coagulation during dissolution; therefore HA solution was stirred overnight to ensure complete solubilization.

2.3. Electrophoretic mobility measurements

Measurements were performed with Zeta-Sizer (Malvern Ins.) apparatus. The biopolymer dispersions were injected using a plastic syringe into a quartz-measuring cell containing two electrodes. Three experiments (and three runs per experiment) were performed. 0.1% (w/v) HA and SF dispersions were prepared with 10^{-3} M KCl solution.

2.4. Turbidity measurement

As a simple method to evaluate biopolymer complexation between SF and HA, turbidity of the mixed solution was measured with respect to pH. Total biopolymer concentration (C_p) was varied from 0.1 to 2.5 wt% at constant biopolymer weight ratio (R) 32:1 and R was varied (32:1, 16:1) when C_p was set to 0.5%. The pH of the mixture was measured with WTW pH-meter (Inolab) equipped with a Sentix 41 pH electrode, which was calibrated with pH 7 and pH 4 buffers. Initial pH of the 30 ml biopolymer mixture was adjusted to pH 8.4 with 0.1 M NaOH. The turbidity of the solution was measured as a function of pH by titrating with 0.1 M NaCl with gentle magnetic stirring. Prior to each measurement, acid titrated mixture was stirred until the preset pH level was reached to a constant value. The turbidity of the SF–HA mixtures was then monitored by using HACH 2100AN turbidimeter. Final dilutions of SF and HA were approximately 5%.

2.5. Gravimetric analysis

The gravimetric yield studies were based on the study of Barbani et al. in 1999 [29]. Six sample mixtures were prepared by adding 3 ml (0.3 wt%) HA to each and by changing the added SF volume between 0.5–7 ml. The final volume in each tube was brought to 10 ml by addition of deionized water at the selected pH. pH of the solutions were adjusted with 0.1 M HCl while being stirred in a 25 ml beaker. Then the mixtures were dispensed into test tubes and centrifuged at 3000 rpm for 1 h. The same procedure was applied for pure fibroin and HA solution. The supernatants were analysed with a Shimadzu UV-1600 spectrophotometer with the absorbance measuring at 272 nm (specific wavelength for fibroin) and 229 nm (specific wavelength for hyaluronic acid) to search for the excess amount of each component. On the other hand, the phase-separated coacervates were washed twice with deionized water at the same pH of the experiment, dried and weighed. The amount of coacervate formation and separation was evaluated using Eq. (1) given below:

$$\text{coacervation yield (wt \%)} = \frac{\text{weight of complex recovered}}{\text{weight of SF} + \text{weight of HA}} \quad (1)$$

and it was represented as a function of SF/HA weight ratio.

2.6. Viscosity measurement

Intrinsic properties of biopolymer solutions were measured using a Brookfield DV-III rotational Rheometer interfaced to a personal computer and driven by a software package supplied by the manufacturers. The range of the rotational speed was 4–163 rpm. Shear rate was started from 5 1/s and increased up to 200 1/s with an increment of 5 1/s. The data was collected at 5 s intervals as an average of three simultaneous measurements. The viscosity measurements were carried out at room temperature (~25 °C) on 20 ml samples. Pure silk fibroin samples were measured at a concentration of 16 mg/ml and hyaluronic acid samples at a concentration of 0.5 mg/ml (due to its viscous nature). The viscosity of the SF–HA mixtures was measured for a total biopolymer concentration of 0.5% (w/v) at a SF:HA weight ratio of 32:1 and 16:1. The measurements were performed at three different pH values (7, 3.14 and 2.3) and repeated twice.

3. Results and discussion

Average molecular weight of SF was determined as 90 kDa by using the molecular weight distributions obtained by the HPLC–SEC analyses of the various silk fibroin solutions. Molecular weight of SF varied between 16 and 240 kDa. These analyses, together with the knowledge of the molecular mass of the native silk fibroin, denoted that the native fibroin molecule degraded into a mixture of polypeptides of various sizes during the preparation of the fibroin solution.

Prior to complexation experiments, electrophoretic mobilities (μ_e) of the two biopolymers were investigated in order to predict the most appropriate region for the formation of the electrostatic complexes (Fig. 1). The μ_e of the hyaluronic acid (HA) was negative during all analyses and decreased with increasing pH, i.e. −3 e.m.u. at pH 3.08 versus −4.5 e.m.u. at pH 5.01. The pK_a value of hyaluronic acid was determined as 2.5, which was compatible with the pK_a value of the carboxyl groups being 2.5, but lower than the reported pK_a value of HA, which was given as 2.9 [26].

μ_e of silk fibroin decreased by increasing the pH (5 e.m.u. at pH 2.97 to −0.1 e.m.u. at pH 3.93). The variation of μ_e was dependent on the charge balance between the amino and carboxyl groups carried by fibroin. A zero value was obtained around pH 3.9, which indicated the isoelectric point (IEP) of the prepared SF sample. In literature, it has been given within the range of 3.8–4.2.

Electrophoretic mobility studies showed that silk fibroin is in the protonated form, positively charged, below pH 3.9, whereas above the pH 2.5 hyaluronic acid is in the deprotonated form, and hence negatively charged. For this reason, complex coacervation was expected between these pH values.

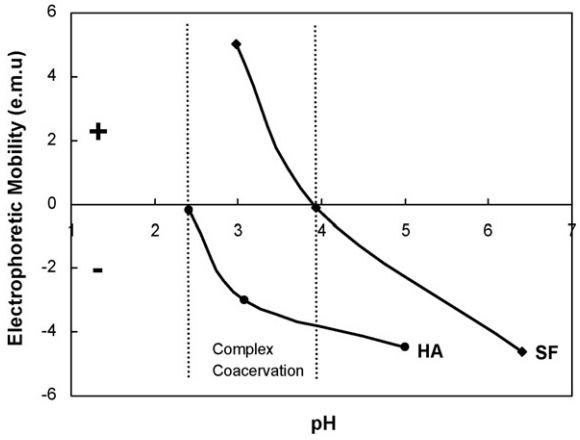

Fig. 1. Electrophoretic mobility of 0.1 wt% biopolymer dispersions at 20 °C.

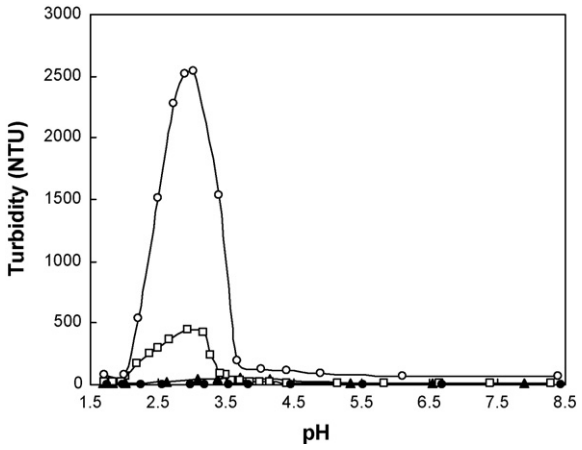

Fig. 2. Turbidity of pure SF (▲) and HA (●) solutions; SF–HA system as a function of pH for C_p = 0.5% (□) and C_p = 2.5% (○) with SF:HA ratio of 32:1.

3.1. Turbidimetric titration under acidification

Turbidity is proportional to both the molecular weight and the concentration of the particles in a system. Therefore, turbidimetric analysis presents a powerful technique in monitoring the complex coacervation. Turbidimetric titrations were performed to obtain qualitative information about the interaction of SF and HA.

The mixtures between SF and HA showed good compatibility above pH 5 and consequently, transparent and homogeneous mixture was obtained with no precipitate formation when these two biopolymers were mixed above this pH. Turbidimetric curves of the acid titrated SF–HA mixtures were shown in Figs. 2 and 3 in terms of NTU (Nephelometric Turbidity Unit) versus pH. These figures revealed that complexation between SF and HA were in the favor of the proposed pH-window (2.5–3.9).

Fig. 2 represented the titration curves of SF and HA mixtures for the C_p's of 0.5 wt% and 2.5 wt% at constant SF:HA weight ratio (R) of 32:1. At pH > IEP_{SF}, both SF and HA are negatively charged and hence repulsive Coulombic forces prevented the complexation. This showed that biopolymers were soluble in the mixture and the system exhibited a blend of two biopolymers miscible in each other. In this region, the turbidity was constant and the level of turbidity was as low as that of the pure biopolymer solutions. Further decrease of the pH led to the increase in turbidity. All curves illustrated that turbidity represented a gradual increase as the pH of the mixture approached to the isoelectric point of fibroin (IEP_{SF} = 3.9). The very first small variations in turbidimetric signals in a polyelectrolyte mixture were considered as the pH_c (pH critical) as the formation of primary soluble intrapolymeric complexes [3,6]. This variation was seen within the pH range of 4.5–6. The possibility of such variations above IEP of the protein, on the wrong side of the IEP, was attributed to the existence of a local protein domain forming a charge patch with an effective charge opposite in sign to net protein charge [30]. Around pH 3.5 a strong increase in system turbidity was observed and this point was generally symbolized as pH_ϕ, coacervate formation pH [1–6]. This sharp rise in turbidity was hypothesized as the aggregation of very large number of inter- and intrapolymeric complexes in a cooperative manner giving rise to coacervation, but not the beginning of phase separation [1,31].

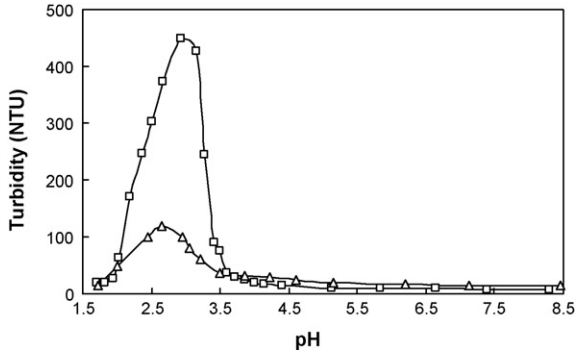

Fig. 3. Turbidity of SF–HA system as a function of pH for SF:HA ratio of 32:1 (□) and 16:1 (△) for C_p = 0.5%.

These observations were in the favor of the formation of electrostatic complexes between the two biopolymers and can be confirmed by the abrupt increase of the turbidity values of the mixtures as the protonated amino groups of the protein associated with the deprotonated carboxyl group of the polysaccharide.

The curves overlapped differing only by their intensities by the existence of small pH shifts and they showed a peak around pH 3.0. This revealed that formation pH of the complexes was independent of total biopolymer concentration. Total biopolymer concentration only influenced the amount and probably the size of the complexes formed. Since the coacervates may not be fully charge-neutralized, they can attract other coacervates, SF molecules and/or HA molecules in the mixture and hence, grow in size. Therefore, increase in total biopolymer concentration may increase the size of the complexes.

On the other hand, during turbidimetric titration analysis it was recognized that there was no coacervate formation below C_p of 0.1 wt% (results not shown here), which simulated the co-soluble highly diluted case for this system. It was also observed that the turbidity of the system increased abruptly as the total biopolymer concentration was increased from 0.5% to 2.5%.

The curves were symmetrical and initial turbidity values were obtained as the pH approached to 2.5. Below pH 3.0, turbidity decreased as the dissociation of the carboxyl groups was suppressed, and hence the interactions between the biopolymers were weakened. The elevated turbidity values were seen due to complex coacervation pH values even lower than 2.9 (reported pK_a of HA). This was attributed to lower pKa value (2.5) of the carboxylic groups (COO^-). Eventually, it was considered that all coacervates dispersed spontaneously into soluble complexes. The final turbidity values were similar to that of the soluble complexes formed prior to coacervation indicating that complexation was reversible.

On the other hand, as shown in Fig. 3, at constant total biopolymer concentration, as SF:HA ratio was decreased, maximum turbidity value decreased, which showed that SF was the limiting biopolymer for the coacervation.

Moreover, the pH value where the maximum turbidity was obtained shifted to lower pH values, since fewer SF molecules were available per HA chain and a more acidic pH was

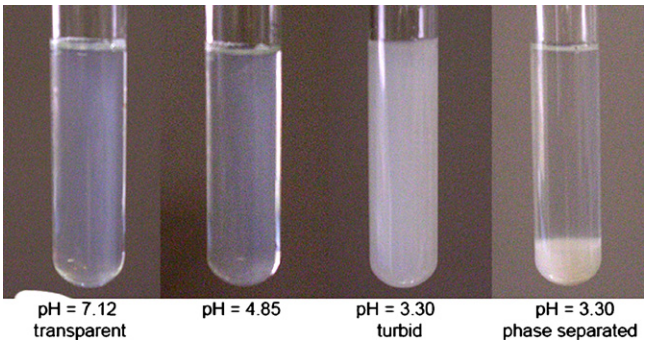

Fig. 4. Formation of soluble and insoluble complexes with respect to pH.

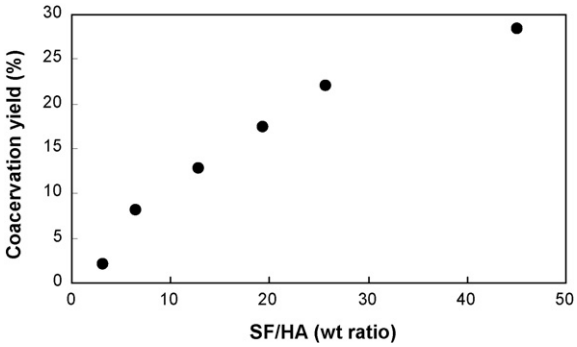

Fig. 5. Coacervation yield (%) vs. SF/HA (wt ratio) in the complex mixture.

necessary to provide more positive charges on SF. Therefore, charge compensation and stabilization and hence aggregation of the coacervates could be established by further protonation of the amino groups and resultant increase in the electrophoretic mobility of the protein at lower pH levels for lower SF:HA ratio. Consequently, the pH, at which the maximum complexation was obtained, was lower when the SF/HA ratio was decreased.

All the curves represented for SF:HA ratio of 32:1 (Fig. 2) exhibited similar trends. Maximum turbidity level, which was reported as the charge neutralization of the coacervates, was around pH 3.0. Consequently, by turbidimetric titration, the complex coacervation between SF and HA was observed around the pH range of 2.5–3.5 as expected. The plots given in Fig. 2 and 3 exhibited a pH-dependent two-step increase and symmetrical decrease in turbidity for the biopolymer mixtures. This can be considered as the indication of pH–induced complex coacervation between SF and HA, and this should be dominantly due to an electrostatic interaction between the SF and HA as shown in Eq. (2) below.

$$[\text{HA-COO}^-] + [\text{SF-NH}_3^+] \leftrightarrows [\text{HA-COO}^-][\text{NH}_3^+\text{-SF}] \quad (2)$$

During the whole pH range turbidimetric analysis was performed, HA solution did not exhibit any change in turbidity. However, there was a slight increase in the turbidity of the SF solution around the isoelectric point. This was accepted as a typical behavior of proteins since they aggregate around their isoelectric point as a result of charge neutralization.

Complex coacervation can also be evidenced by naked-eye observations by the whitening of the biopolymer mixture as shown in Fig. 4. At pH 7.12, the mixture was transparent. Change in the color of biopolymer mixture was observed as the pH was decreased and finally at pH 3.3 it was translucent with a white color. The centrifuged sample, which had a pH of 3.3, presented the complete phase-separation. Both phases were homogeneous.

Centrifugation was applied since settling of the coacervates was very slow. It was indicated by the study of Weinbreck et al. [5] that coacervate droplets settle down faster if they are fully charge-balanced than if some residual charges are present. The prepared SF:HA mixture at pH 3.3 was in excess of HA, therefore SF was in insufficient quantity and it could not totally compensate the negative charges of the HA. For this reason, a surface layer of HA stabilized the coacervates as the case reported for β-lactoglobulin and acacia-gum [32]. It was considered that the stabilization of the coacervates inhibited the interactions between coacervate droplets, and rearrangement of the coacervates was needed that resulted in longer time to settle down.

3.2. Gravimetric analysis

The coacervation yield of the SF–HA system was investigated by gravimetric analyses and it was evaluated using Eq. (1). Fig. 5 represents the coacervation yield with respect to change in SF/HA weight ratio in the complex mixture at pH 3.0. As the initial SF amount was decreased (for constant HA amount), the coacervation yield decreased. The maximum amount of coacervate that could be isolated by the gravimetric analyses was close to 30%. The trials for the contribution of higher amounts of SF into the biopolymer mixture did not permit the phase separation of the coacervates to end up with higher coacervation yield. It was reported that when biopolymer concentration exceeds a critical value, biopolymers become limitedly co-soluble due to the large size and the rigidity of biopolymer molecules [2]. Therefore, the entropy of mixing of biopolymers was several orders of magnitude smaller than that of the monomers. Biopolymer incompatibility may occur even the corresponding monomers were miscible in all proportions. Biopolymer incompatibility was observed between SF and HA biopolymer mixture when the concentration of SF exceeded 3 wt%. In literature, this value changes within the range of 2–12 wt% biopolymer concentration with respect to the charge density and the structure of the protein whether it is globular or fibrous [2].

UV analysis of the supernatant solutions showed that there was high excess of both polymers that not incorporated in coacervate formation. This may be attributed to presence of small peptide chains of SF and the soluble complexes formed by the small peptide chains of SF with the excess of HA in the system. On the other hand, the repeated gravimetric analyses showed that coacervate formation was also affected by the stirring time of the coacervate mixture.

3.3. Viscosity measurement

The viscosity measurements aimed to investigate the compatibility of the polymers in the mixture and the association between

Table 1
Calculated vs. measured viscosity

pH	SF/HA weight ratio, R	Calculated viscocity[a] (cP)	Measured viscosity[b] (cP)
2.3	32	0.83	1.66
2.3	16	1.20	1.87
3.2	32	1.72	1.69
3.2	16	2.82	1.63
7.5	32	2.17	2.30
7.5	16	3.82	4.53

[a] Calculated by Eq. (3).
[b] Measured at room temperature.

the interpolymer complexes in solution. If the viscosity, concentration, and weight fractions of the polymers are known, the following Eq. (3) constituting the additivity rule of the biopolymers can be established [33]:

$$\left(\frac{\eta}{c}\right)_{\mathrm{m}} = w_1\left(\frac{\eta_1}{c_1}\right) + w_2\left(\frac{\eta_2}{c_2}\right) \quad (3)$$

where subscripts (1) and (2) refer to SF and HA, respectively; c is the total biopolymer concentration; w_1 and w_2 are the weight ratios of the corresponding two biopolymers in the mixture; η_1/c_1 is the dynamic viscosity of SF at concentration of c_1; η_2/c_2 is the dynamic viscosity of HA at concentration of c_2.

Measured and calculated viscosity values of SF–HA mixtures at varying weight ratio (R) and pH are given in Table 1. The results indicated that at pH 3.2 (within the proposed pH window of 2.5–3.5), viscosity values of the mixtures were lower than the calculated viscosity values evaluated by Eq. (3). This was attributed to formation of compact interpolymeric complexes. Higher viscosity values occurred outside this range was attributed to good compatibility and gel-like association between biopolymers [33,34].

The viscosity of the SF/HA mixture, regardless of the total biopolymer concentration and the ratio of the biopolymers in the mixture, decreases at the pH of complex formation. It was demonstrated by Bungenberg de Jung in 1929 that the decrease in viscosity before and during the actual complexation was induced by the reduction of the amount of liquid occluded inside the complexes [35]. The decrease in viscosity of the polyelectrolyte systems and low viscosity close to the point of complexation was reported to be consistent with intrapolymer condensation.

The effect of pH on the viscosity of the complexation mixture was shown in Fig. 6. The viscosity measured at three pH levels (above, within and below the proposed pH window, 2.5–3.5, for complexation). The decrease in viscosity of the biopolymer mixture may be attributed to coacervate formation between the oppositely charged SF and HA around pH 3.0.

The viscosity of a dilute polymer mixture was reported to be directly related to the size of the particles. Viscosity decreases by a drastic reduction of the hydrodynamic radius of two polyelectrolytes through complexation and formation of the compact complexes [34]. Therefore, it can be concluded that higher amounts of more compact and larger coacervates were formed at

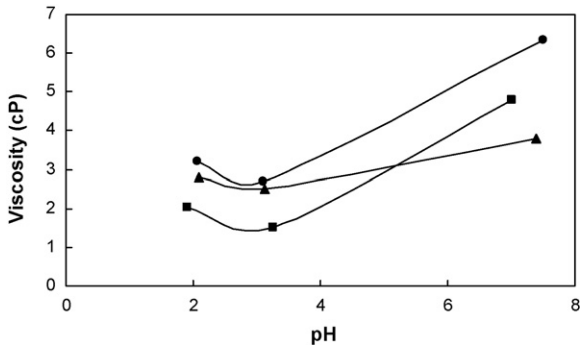

Fig. 6. Viscosity vs. pH for $C_p = 1.5\%$ and SF:HA = 32 (■); $C_p = 0.5\%$ and SF:HA = 32 (▲); $C_p = 0.5\%$ and SF:HA = 16 (●).

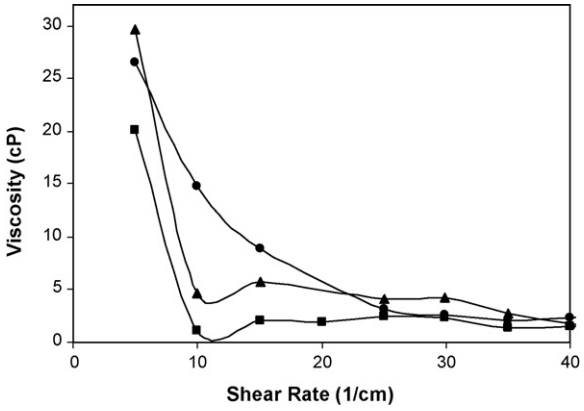

Fig. 7. Viscosity vs. shear rate plots of SF–HA complex mixtures at pH = 3.0. The curves represent $C_p = 1.5\%$ and SF:HA = 32 (■);$C_p = 0.5\%$ and SF:HA = 32 (▲); $C_p = 0.5\%$ and SF:HA = 16 (●).

the pH level where minimum viscosity and maximum turbidity were simultaneously observed.

The viscosity versus shear rate plot for $C_p = 1.5\%$ and SF:HA = 32 represents lowest viscosity values below the shear rate of approximately 20 1/cm among other systems represented (Fig. 7). On the contrary, highest turbidity values were obtained for this system. In case of $C_p = 0.5\%$ and SF:HA = 16, the higher values for viscosity were obtained due to higher excess amount of HA. This was attributed to much higher viscosity of HA as compared to SF solution and also to selective binding of HA to the poorly flexible chains of SF. Moreover, the complexation systems showed a limited shear thinning behavior below the shear rate of 10 1/cm as shown in Fig. 7.

4. Conclusions

In this study, SF–HA complexation was introduced as a novel system for complex coacervation. SF–HA complex coacervation was investigated and it was shown that the complexation was dominantly induced by pH that determined the charge states of the biopolymers. Insoluble complex coacervate formation was observed within the narrow pH range of 2.5–3.5. It was shown that the complexes were formed due to electrostatic interactions between SF and HA in this pH range, where these two biopolymers were oppositely charged.

It was revealed by turbidimetric analysis that the formation of insoluble complexes was reversible and the pH value at which the complexes occurred was independent of total biopolymer concentration. Gravimetric analysis performed at constant pH showed that total biopolymer concentration and ratio of the biopolymers in the complex mixture influenced the amount and probably the size of the complexes formed. Formation of the insoluble complex was confirmed and detailed by viscosimetric analysis. The complexation was shown by the decrease of the mixture viscosity due to formation of a dispersion composed of aggregated coacervates. Consequently, coacervate formation was evidenced by the minimum viscosity and the maximum turbidity observed in the system. The properties of SF–HA coacervation were affected by pH, biopolymer ratio and total biopolymer concentration. Therefore, SF–HA complex coacervation could be promising to develop new biomaterials with novel properties by the optimization of the effective parameters.

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