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Overcharging, Thermal, Viscoelastic and Hydration properties of DNA-Gelatin complex coacervates: Pharmaceutical and Food industries

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ABSTRACT

DNA (C_{DNA} = 5×10^{-3}% (w/v)) and Gelatin A (GA) ($C_{GA} \geq 0.1$% (w/v)) exhibit disproportionate binding and form variety of complex coacervates. Presence of charge neutral DNA-GA intermolecular soluble complexes were observed at C_{GA} = 0.1% (w/v) while for higher GA concentrations existence of polarity reversed and overcharged complexes were noticed. In this work, we observed an interesting behaviour of coacervates which showed varying mechanical properties of complex coacervates on overcharging while their thermal properties remains invariant. Rheological studies revealed neutral coacervates (formed of charge neutral complexes) possessed maximum storage modulii which decreased by a decade with overcharging as C_{GA} was increased to 0.25% (w/v). Raman data complemented rheological findings and suggested that in neutral coacervates structured water, 3200 cm^{-1} O-H band, was excluded from the coacervate matrix owing to dense packing of complexes which was regained in overcharged coacervates (formed of overcharged complexes) due to repulsion acting between positively charged complexes. Small angle neutron scattering data revealed that correlation length ξ increased from 1.27 to 1.90 nm as the complexes get overcharged and geometrical shape of the constituent complexes, determined from the low-q region showed presence of diffusion limited aggregates. Rheological characterization attributed anomalous gel-like features and Cole-Cole plot description implied presence of heterogeneity in these materials. The interesting phenomenology shown by this system has a good potential for gene therapy and modulating the physical properties of Gelatin based items in food industries.

Key words: DNA-protein, complex coacervates, overcharging, neutron scattering, rheology, Gene therapy, food industries.

1. Introduction

Associative interactions leading to the binding of DNA to proteins and oppositely charged spherical macroions has revealed interesting results pertaining to charge polarity reversal and overcharging of the intermolecular complexes. Nguyen and Shklovskii[1,2] examined the phase diagram of DNA and oppositely charged colloidal macroions and observed that at low colloid concentration, the DNA-colloid complexes are not fully neutralized and give negatively charged complex with DNA wrapping the colloids while at high colloid concentration the complexes showed charge reversal. Charge states of the complexes were decided by DNA-polycation charge ratio. The aforesaid two situations are separated by an intermediate phase where the complexes are fully charge neutralized; here DNA-colloid condensates exist. Thus, the concentration of colloidal macroions governed the condensation and re-entrant condensation in this system. In this study existence of overcharged intermolecular complexes was clearly proposed. Gurovitch and Sens[3] proposed an idealized model and applied self-consistent field theory to map the adsorption of weakly charged polyelectrolyte onto oppositely charged colloidal particles. They showed that the connectivity between the charges of the polymer leads to overcharging of the colloid.

Proteins can interact with DNA either specifically or non-specifically. In the case of non-specific interactions, the sequences of nucleotides do not matter, as far as the binding interactions are concerned. Histone (protein) - DNA interactions are an example of such interactions, and they occur between functional groups on the protein and the sugar-phosphate backbone of DNA. Specific DNA - protein interactions, however, depend upon the sequence of bases in the DNA and on the orientation of the bases that can vary with twisting and super-coiling. These DNA - protein interactions are strong, and are mediated by many interactions such as Hydrogen bonding mediated by water molecules, Ionic interactions like formation of salt bridges, protein side chains - DNA backbone interaction and van der Waals

and hydrophobic interactions. Such interactions can produce novel biomaterials and investigation of such systems aid to our general understanding of soft matter.

A clear experimental manifestation of the phenomenon of charge polarity reversal and overcharging was reported recently where binding between DNA and a protein (gelatin A, GA) was used.[4] The complexation mechanism could be broadly divided into two parts i.e. before and after charge neutralization point of complex. Prior to neutralization, initially size of complex decreased due to screening of electrostatic persistence length of DNA. Following this the sizes of the DNA-GA complex increased to further charge neutralize the complex. Beyond neutralization point, coacervation and interesting overcharging behaviour of the complex was noticed (disproportionate binding/ non uniform distribution of GA around DNA).[4] In spite of enhancing the coacervation after charge neutralization point, overcharging played an important role in suppressing the dynamics of coacervation. Overcharging phenomenon was modelled that showed the feasibility of binding of GA to pre-existing DNA-GA complexes leading to the overcharging post charge neutralization.

This raises a pertinent issue: how do the coacervates made up of charge neutralized DNA-protein complexes differ from those made up overcharged complexes as far as their thermal and viscoelastic properties are concerned. This constitutes the central objective of this work. This objective further throws light to harvest application in biomedical and food industries. Many literatures[5,6] has discussed Gelatin and different polyelectrolytes based complex coacervates for these kind of applications. Relevant use of our complex coacervates will be discussed in application section.

2. Materials and Method

Gelatin A (300 bloom) from porcine skin bought from Sigma Aldrich having molecular weight, $M_w = 1.3\times10^6$ Da and DNA purchased from Acros Organics having Mw = 50,000-100,000 Da were used without any further purification. Stock of GelatinA (GA) solution was prepared by dissolving known amount of protein in double distilled deionized water at 40 ^{0}C using a magnetic stirrer for almost an hour. DNA solution was prepared by dissolving DNA powder in double distilled deionized water at room temperature for an hour. Both solutions appeared transparent and optically clear after preparation. Finally solutions were prepared by mixing GA and DNA solution from their stock, appropriate dilution was made to achieve required mixing ratio. In all the final solutions concentration of DNA was maintained at 0.005% (w/v) and the GA concentration was varied from C_{GA} = 0.1 to 0.25% (w/v). The pH of all the final sample solutions measured was 6 ± 0.7. All preparations were made at room temperature, 20 ^{0}C, and the relative humidity in the room was less than 50%. Finally coacervates were extracted from these finally reacted solutions following standard procedure of repeated centrifugation and decantation of the supernatant[7-10], further details about the samples has been provided in Table S1 (Supplementary information). These extracted coacervates were then subjected to rheology and Raman studies. These final samples of coacervates were stored in airtight borosilicate glass bottles for further analysis which, in all instances, did not exceed more than 48 h after preparation. Heavy water from Sigma Aldrich was used instead of double distilled deionized water for SANS experiments. Protocol followed in making coacervates were the same.

Rheology measurements were performed on the coacervate samples using controlled stress AR 500 rheometer (TA Instruments, Surrey, England). In the frequency sweep experiments, coacervate samples of different GA concentrations were loaded onto the rheometer plate cooled to 20 ^{0}C. Coacervates measurements were carried out with a 20 mm cone plate

geometry using a constant oscillation stress of 6.3 Pa. Temperature sweep were carried with same geometry having oscillation stress fixed at 6.3 Pa and angular frequency fixed at 1 rad/s. GA and DNA measurements in solution phase were carried with 40 mm cone plate geometry with oscillation stress 0.06 Pa.

Raman spectroscopy was used to investigate the hydration of coacervates. Raman spectra for all coacervates were studied on a FT-RamanSpectrometer attached with a Microscope (Varian 7000 FT-Raman and Varian 600 UMA).

Small angle neutron scattering (SANS) requires a neutron source, i.e., a nuclear reactor or an accelerator based spallation source, and therefore, the experiments are performed at large scale facilities. The small angle neutron scattering experiments presented in this work have been performed at the SANS diffractometer at the Guide Tube Laboratory, Dhruva Reactor, Bhabha Atomic Research Centre, India. It makes use of polycrystalline block of beryllium oxide (BeO) filter as monochromator. The mean wavelength of the monochromator beam was 5.2 Å with a spread of $\Delta\lambda / \lambda \sim 15\%$. The angular distribution of neutrons scattered by the sample was recorded using a 1m long one-dimensional He position sensitive detector. The instrument covered a q-range of 0.015 – 0.35 $Å^{-1}$. Further details is available in ref.[11,12] Unless otherwise stated all experiments were carried out at room temperature.

3. Results and discussion

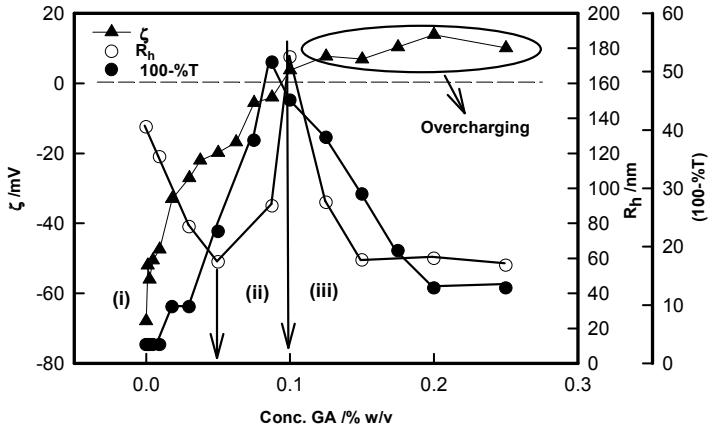


Fig. 1. Variation of hydrodynamic radii R_h, zeta potential of DNA-GA complex ζ, and solution turbidity 100-%T at different GA concentrations. The neutral and overcharged coacervates were extracted from the reacting solutions belonging to region (iii). Further detail about this plot can be obtained from ref.[4]. Solid lines are guide to eye.

The physical characteristics of biopolymers used have been discussed elsewhere.[4] It has been shown earlier[4] (Fig. 1) that maximum binding between DNA and GA occurred at charge neutralisation point of the DNA-GA complex which correspond to the concentration C_{GA} = 0.1% and $C_{DNA} = 5\times10^{-3}$% (w/v) respectively. At this concentration the solution turbidity rose sharply and charge neutralized soluble complexes were found in abundance. Increasing the concentration of GA in the range C_{GA} = 0.15 – 0.25% (w/v) while keeping DNA concentration fixed at 5×10^{-3}% (w/v) facilitated formation of overcharged soluble complexes in the dispersion (disproportionate binding). Overcharging of complexes increased considerably with increase in protein concentration. Herein, we refer to coacervates extracted from solutions containing charge neutral soluble complexes and those extracted from solutions having overcharged complexes as neutral and overcharged coacervates respectively. It is to be noted that coacervates can be extracted from the solution having $C_{GA} \geq 0.1$% (w/v) and fixed $C_{DNA} = 5\times10^{-3}$% (w/v) in the solution.[4] We discuss rheological, thermal and hydration properties of these coacervates as a function of the extent of their overcharge.

(a) Rheological properties

Fig. 2 depicts the dispersion behaviour of viscoelastic parameters of the samples obtained from isothermal frequency sweep experiments carried out at room temperature. Frequency sweep studies have been done in oscillatory mode at constant oscillation stress which provided information about the microstructure in the same way as does an NMR. Rheology provides explicit information about the dispersive behaviour of storage modulus G'(ω) and its corresponding loss modulus G''(ω) thereby probing the material completely. A more comprehensive viscoelastic feature about the material can be obtained from the complex modulus G*(ω) = G'(ω)+*i*G''(ω).[13] The magnitude of the storage and loss modulus is often used to characterize the viscoelastic properties of a material. Fig. 2 shows the extremely low viscoelastic properties of pure GA (0.25% (w/v)) and pure DNA (0.005% (w/v)) in solution phase as compared to the viscoelastic properties of coacervates extracted after the complexation between DNA and GA. It is clear from this figure that value of G'(ω) and G''(ω) decreases with increase in protein concentration in the range C_{GA} = 0.10 – 0.25% (w/v). The frequency range chosen was from very low to very high (0.6283 – 100 rad/s). The explicit frequency dependence of storage modulus was determined by fitting the data to following power-law function

$$G' \sim \omega^n, \; G'' \sim \omega^n \qquad (1)$$

where ω is the angular frequency. The linear visco-elasticity model[14,15], for pre and post gel situations predicts that the stress-relaxation to follow the power-law frequency dependence behaviour given by Eq. (1) with 0 < n < 1. Stoichiometrically balanced and imbalanced crosslinked networks showed n = 1/2 (excess crosslinker) and n>1/2 (lack of crosslinker) respectively. However, this description strictly applies to chemically crosslinked gels, thus the observed smaller value of n, for the present system where no crosslinker is present *per se*

does not come as a surprise. Here, the very low values obtained for n indicate the elastic attribute of the coacervates material. The gel strength was found to be ≈ 10 KPa for neutral coacervates that got reduced by a decade for overcharged coacervates implying considerable softening of the material. This can be presumed to be arising from strong repulsive interactions prevailing inside the coacervate medium, an observation that indicated that this material was close to a pseudo-gel.

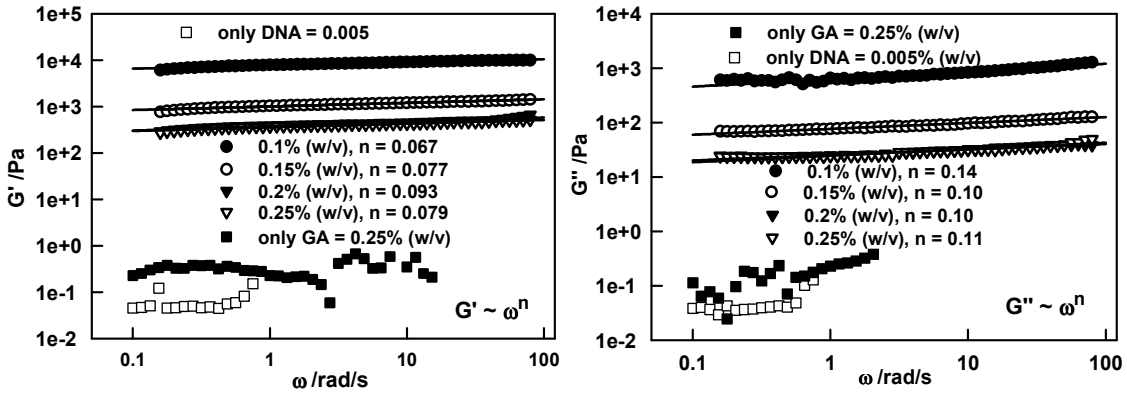


Fig. 2. Variation of storage modulus $G'(\omega)$ and loss modulus $G''(\omega)$ of DNA-GA coacervates at various GA concentrations. The measurements were performed at 20 ^{0}C. Solid lines represent fitting of data to Eq. (1). Data for only DNA and GA solutions are provided for comparison.

The modulus value reduced further with increase in protein concentration. It needs to be emphasized here that the extent of overcharge increased by about 200% which caused a drop in modulus by close to 70%. This is depicted clearly in Fig. 3 where the gel strength (G' at low frequency (0.1 rad/s), G_0) has been plotted as function of the overcharge.

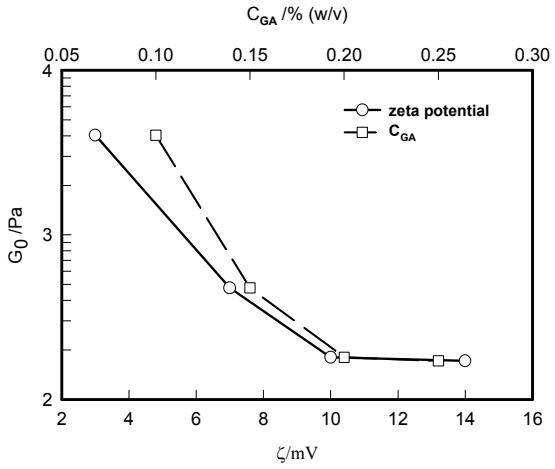


Fig. 3. Variation of coacervate rigidity modulus G_0 as function of overcharge carried by the DNA-GA soluble complex. The corresponding GA concentration is shown in the figure for comparison. Lines joining the data points are guide to the eye.

Having seen the elastic attribute of the coacervates, it was felt pertinent to resolve whether or not these had gel-like structures. In order to elucidate this, the loss tangent, tanδ = G''(ω)/G'(ω) was plotted as function of frequency which is illustrated in Fig. 4. It is well known that for gels the tanδ versus ω slope is positive, whereas at gelation it is zero while it is negative for melts.[15,16] Fig. 4 shows that the loss tangent as function of ω is associated with a small positive slope implying that the material was indeed gel-like.

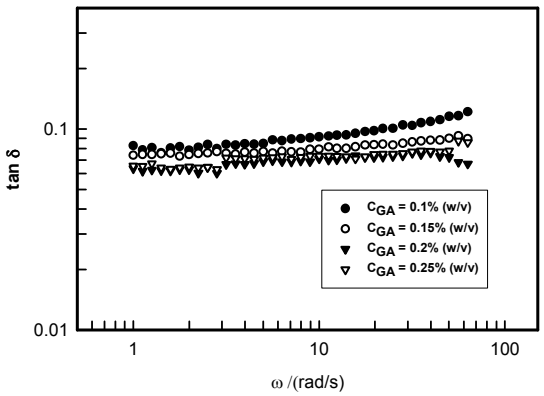


Fig. 4. Plot of loss tangent as function of frequency shown for various coacervate samples. Note the weak frequency dependence of the loss tangent, which implied that coacervates were in anomalous state, but closer to gels.

In a network of transiently connected chains, the shear modulus is proportional to the concentration of intermolecular bonds. The value of the length of elastically active strands, calculated from Eq. (2) is similar to the characteristic viscoelastic network size ζ_{el}, estimated from the low frequency shear modulus, G_0. This is a measure of elastic free energy stored per unit volume of a characteristic viscoelastic network of size, ζ_{el}.[17]

$$\zeta_{el}^3 \sim k_B T / G_0 \qquad (2)$$

The values for viscoelastic length obtained from Eq. (2) are plotted in Fig. 5 which suggests that this length increases with overcharging.

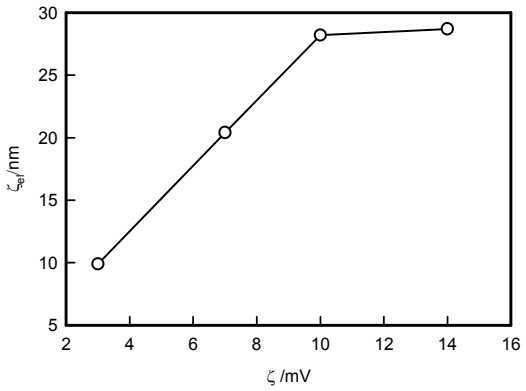


Fig. 5. Variation of viscoelastic length, ζ_{el} as function of overcharge carried by the DNA-GA soluble complexes. Data clearly reveals that overcharged coacervates are associated with stretched networks.

The phase heterogeneity is often deduced from Cole-Cole plot where the imaginary part of the complex viscosity (η'') is plotted as function of the real part (η'). Typically, in a polymer melt, at very low frequency, viscous behaviour is observed whereas at higher frequencies elastic properties dominate. In this formalism, the complex viscosity is represented as

$\eta^*(\omega)=\eta'(\omega) - i\eta''(\omega)$ and the low and high frequency viscosity values are given by η_0 and η_∞ respectively. The Cole-Cole empirical expression is written as[18]

$$\eta^* - \eta_\infty = \frac{(\eta_0 - \eta_\infty)}{\left[1 + (i\omega\tau_{cc})^{1-\alpha}\right]};\ 0< \alpha <1 \qquad (3)$$

The aforesaid expression is interpreted as arising from a superposition of several Debye relaxations.[18,19] The mean relaxation time is given by τ_{cc}. This plot has been used extensively to map homogeneity of polymer melts and composites. For a homogeneous phase, the Cole-Cole plot is a perfect semicircle (α=0) with a well defined relaxation time. Any deviation from this shape indicates inhomogeneous dispersion and phase segregation due to immiscibility. Such phases are associated with relaxation time distributions mentioned earlier.[20]

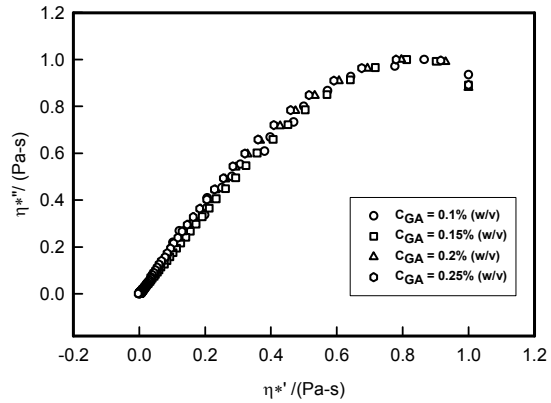


Fig. 6. Cole-Cole plot of coacervates for variation of normalized imaginary part of complex viscosity $\left(\eta^{*\prime} = {}^{\eta''}/_{\eta''_{max}}\right)$ as function of normalized real part $\left(\eta^{*\prime\prime} = {}^{\eta'}/_{\eta'_{max}}\right)$. Note that complex viscosity is measured at different angular frequency (rad/s) and τ_{cc} is taken from that angular frequency at which $\eta^{*\prime\prime}$ is maximum.

The Cole-Cole plot shown for our coacervate samples in Fig. 6 is a master plot which reveals two key features: (i) all the coacervates are heterogeneous to the same degree and (ii) the

mean relaxation time τ_{cc} = 0.13 s is invariant of protein concentration. Here, τ_{cc} was taken from the inverse of the angular frequency at which η'' shows maxima which refers to the mean viscoelastic relaxation time.

Thus, the overcharging though contributed to the softening of the coacervates did not affect the structural heterogeneity of the samples significantly. This was probed further by submitting these samples to isochronal temperature sweep experiments and the data is shown in Fig. 7. Interestingly, all the samples exhibited same melting temperature (40 ^{0}C) which is almost same as the melting temperature (~ 38 ^{0}C) for GA gels.[21] This behaviour is inclined to suggest that coacervate is mainly composed of physically cross-linked gelatin chains with DNA molecules playing the role of scaffolds, thus thermal property is dominated largely by GA-GA cross-links existing between neighbouring DNA-GA complexes.

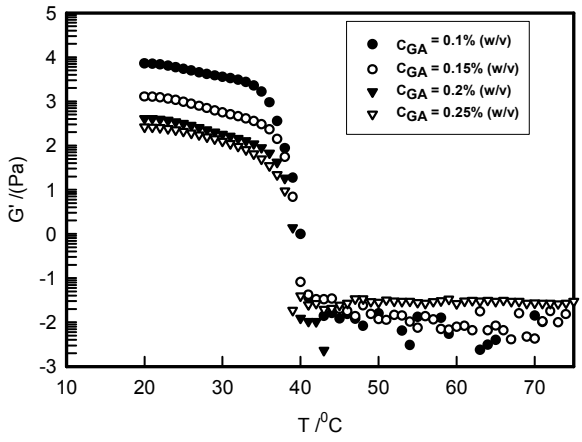


Fig. 7. Variation of elastic modulus, G'(ω) shown as function of temperature of different protein concentrations in coacervates. Melting temperature for all the coacervates are 40 ^{0}C. Temperature ramp = 1 ^{0}C/min was applied to the samples kept under constant oscillation frequency of 1 rad/s and constant strain maintained at 6.3 Pa.

The rheology data in hand implied that the overcharge had no discernible impact on the thermal property (melting) of the coacervates whereas the viscoelastic attributes were considerably influenced. In particular, the shear storage modulii changed significantly with overcharging. Hydration governs the chain conformation and solution phase stability of biopolymer molecules. Thus, it was felt necessary to probe the structure of water inside the coacervate samples through Raman spectroscopy.

(b) Hydration behaviour

We have adopted Raman Spectroscopy to investigate structure of water in all the systems because vibrational spectra are very sensitive to the local molecular environment. Slightest change that is incorporated into the coacervates due to overcharging can alter the vibrational spectra of water. Thus, direct investigation of vibrational modes of water provides the hydration state of the system concerned. In order to make these studies comprehensive, the dispersions containing the soluble complexes were examined too.

Water is associated with three distinct Raman bands located at 3200, 3300 and 3460 cm^{-1} which are identified as follows: (i) peak around 3200 cm^{-1} arises from in phase vibration of, OH stretching mode, structurally arranged water, which is sometimes referred to as ice-like structures[22], (ii) the origin of the Raman peak around 3300 cm^{-1} in the water spectra appears intriguing. It has been argued by Ratajska-Gadomska and Gadomski[23,24] that the peak around 3300 cm^{-1} owes its origin to H-bonds linking ethanol molecules; they recorded no such peak in water spectra. Our data is at variance with their results. We observed a strong peak around 3300 cm^{-1} in water samples and its peak area or height was found to be independent of gelatin concentration. Note that this O-H band can overlap with weak N-H band (from gelatin molecule). We did not observe any distinctive signature of this in our data. Thus, it can not be arising from H-bond formation of water with amine group of gelatin. It can be attributed to

the OH stretching of partially structured water molecules (liquid-like water[16]) and (iii) the peak around 3460 cm^{-1} arises from poorly H-bonded water molecules (amorphous water[16]). The water spectra could not be fitted to more than three Gaussian-Lorentzian functions unlike what was reported by Gadomska and Gadomski earlier.[23,24]

A set of representative Raman spectra is shown in Fig. 8 corresponding to C_{GA} = 0.15% (w/v). Raman spectra were deconvoluted to three component Lorentzian function using Origin 6.1 software. Fig. 9 illustrates the fractional peak area pertaining to different O-H vibrational modes for solution and coacervate samples. Fractional area of 3200 cm^{-1} band dominated the spectrum, thus all the analysis related to hydration was focussed on the attributes of this particular band. It is to be noticed that as the protein concentration was increased one observed reduction in the structured water component (fractional area of 3200 cm^{-1} band) with the minima occurring at C_{GA} = 0.1% (w/v) (complete charge neutralization point). Interestingly, as disproportionate binding of protein to DNA-GA complexes ensued with the concomitant occurrence of overcharging the aforesaid component began to rise and it regained its original value. This typical behaviour can be attributed to the following explanation: close packing of charge neutralized complexes inside the coacervate is possible only at C_{GA} = 0.1% (w/v) as a result there is a paucity of structured water inside this matrix. However, in the overcharged coacervates the electrostatic repulsion occurring between similarly charged complexes allowed voids to be created that facilitated entry of structured water into the core of the coacervate.

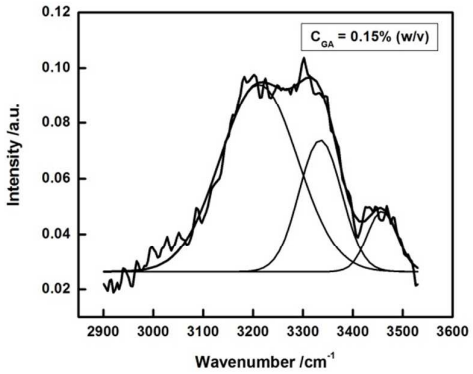


Fig. 8. Representative Raman spectra of a coacervate samples was deconvoluted to yield three distinct peaks located at frequency 3200, 3300 and 3460 cm^{-1} representing vibrational modes of structured, partially structured and amorphous water respectively.

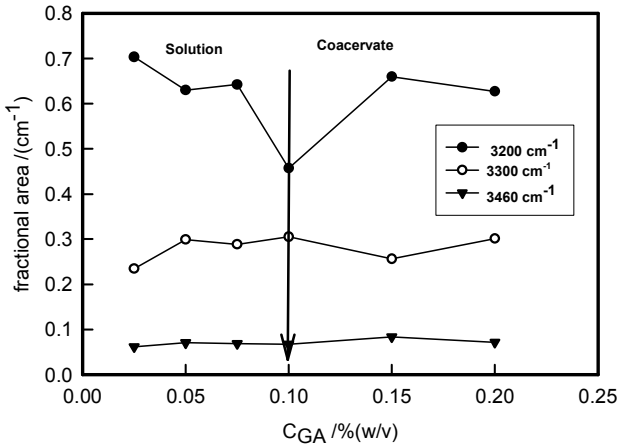


Fig. 9. Fractional ar s corresponding to structured, partially nown for complex coacervate and solution samples. Note the loss of structured water sharply at C_{GA} = 0.1% (w/v), where neutral coacervate formed. Structured water again enters in coacervate phase beyond C_{GA} = 0.15% (w/v) due to overcharging.

(c) Structure of coacervates

Raman and rheology data suggested repulsion between overcharged DNA-GA complexes inside the coacervate matrix at C_{GA} = 0.15% (w/v) where the coacervates begin to regain the structured water content. In order to gain further insight into the microstructural arrangement of the biopolymers inside the samples, these were subjected to SANS studies where samples were probed over a length scale of several nanometres. For this, we have used SANS data

pertaining to coacervates having protein concentration C_{GA} = 0.1 and 0.15% (w/v).The static structure factor data obtained from these experiments is depicted in Fig. 10. For comparison SANS of pure GA in solution phase having concentration 0.1% (w/v) were also plotted.

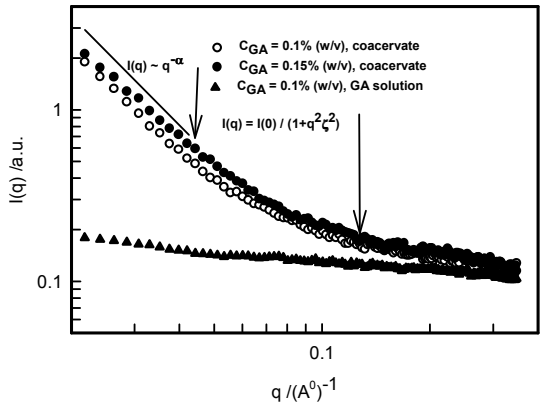


Fig. 10. Plot of static structure factor I(q) measured by SANS on two DNA-GA coacervate samples and only GA sample in solution phase at 25 ^{0}C. Arrows indicate the distinctive q-regions where power-law and Ornstein-Zernike regimes prevailed. See text for details.

The scattering cross section for pure GA (0.1% (w/v)) in solution phase is too low and scattering profile for the same could not be fitted to any function. However, the scattering profile depicted in Fig. 10 shows SANS data of DNA protein complex coacervates could be spliced into two distinct regions: (i) for 0.0173 < q < 0.05 Å^{-1}, data showed power-law dependence given by[25]

$$I(q) \sim q^{-\alpha} \quad (4)$$

and (ii) for 0.05 < q < 0.15Å^{-1}, data was fitted to function to Ornstein-Zernike function[26]

$$I(q) = I(0) / (1+q^2\xi^2) \quad (5)$$

Region with q > 0.15 Å^{-1} was too noisy and comprised of incoherent scattering. The two q-regions were separated manually by examining the I(q) versus q and I(q) versus q^2 plots. A clear slope change was discernible at q* = 0.05 Å^{-1}. For q < q*, the data was fitted to power-

law and for q > q* to the Ornstein-Zernike function, which yielded reproducible and robust data fitting with chi-squared values > 0.95. The exponent defined by Eq. (4) owes its origin to the geometry of the scattering moiety present in the coacervate. The analysis of SANS data in region (i) yielded $\alpha = 1.8 \pm 0.1$ for coacervates having protein concentration C_{GA} = 0.1 and 0.15% (w/v). This value conforms to the geometry of clusters formed via diffusion limited aggregation (DLA) mechanism in a given system.[27] Note that in this q-region I(q) remained invariant of overcharge.

The intermediate q-region was analyzed using Ornstein-Zernike function arises from density fluctuations prevailing inside the condensed phase. The correlation length of these fluctuations is given by ξ.[26] In gel and network theory this length is referred to as mesh size. However, correlation length obtained from the experimental data using Eq. (5) gave ξ = 1.27 nm for C_{GA} = 0.1% (w/v) and ξ = 1.90 nm for C_{GA} = 0.15% (w/v). The increase in the correlation length which was close to 50% is attributed to repulsion prevailing between overcharged complexes inside the coacervate matrix. It should be noted here that same behaviour was manifested in the Raman data (dense neutral coacervate versus rarefied overcharged coacervate).

Coacervate with protein concentration C_{GA} = 0.1% (w/v) when subjected to heating at different temperatures showed same neutron scattering profiles shown in Fig. 11. Thus, it is believed that correlation length at these temperatures do not change as coacervates are mainly composed of protein chains implying these contain networks of GA-GA chains in propensity with DNA providing the scaffold (Scheme 1). Such a structure will not allow any change in the mesh size upon heating. However, high-q resolution SANS data could give further insight about the temperature dependent characteristics of these coacervates which was not accessible due to instrumental limit.

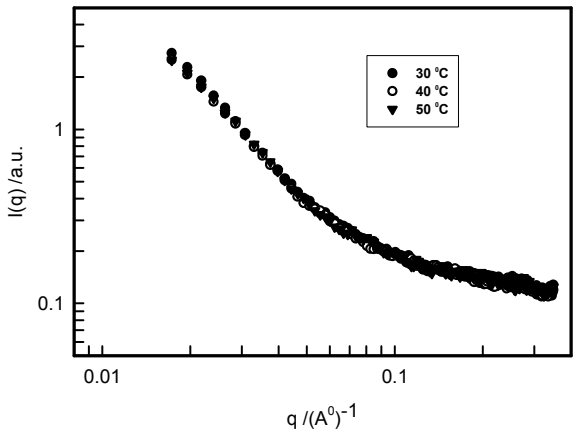
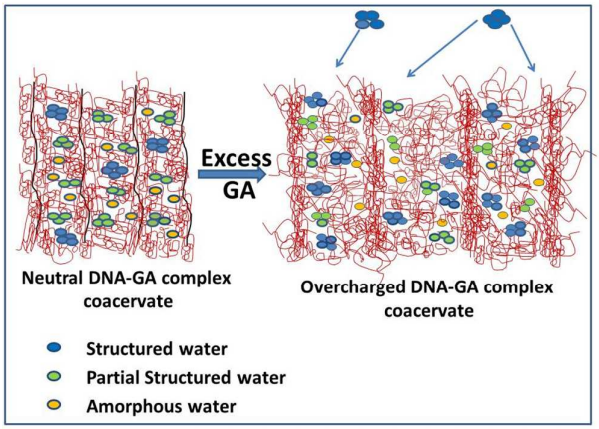


Fig. 11. Logarithmic plot of DNA-GA complex coacervate at C_{GA} = 0.1% (w/v) at different temperature. Note the marginal dependency of the scattering profile on temperature.

(d) Gel – like behaviour

Binding between the hydrated complexes inside the coacervate matrix at C_{GA} = 0.1% (w/v) resulted in the observation of maximum storage modulus. Such high storage modulus was facilitated due to dense packing of the charge neutral DNA-GA complexes. As far as the thermal studies of the coacervates by SANS and rheology (temperature sweep) experiments are concerned these implied that structurally the matrix was dominated by the presence of dense GA-GA crosslinks. Moreover, increasing the concentration of protein above C_{GA} = 0.1% (w/v) led to the overcharging of the complexes and presence of such mutually repulsive complexes inside the coacervate matrix allowed increase in their mesh size and facilitated the

structured water fraction to rise inside the matrix. This hydration of coacervates led to the decrease in the storage modulus observed. A schematic depiction of this model is provided in Scheme 1 where the protein network supported by the DNA scaffold swells for coacervates containing overcharged complexes and allows for the presence of more structured water.



Scheme 1. Schematic showing the coacervate matrix due to overcharge and the consequent inclusion of structured water inside the matrix and weakening of its mechanical strength.

Physical properties of neutral and overcharged complex coacervates have been summarized in Table 1 which shows the occurrence of softening in overcharged complexes.

Table 1

Summary of physical property of two selected coacervate samples differentiated as function of overcharge.

SI No.	Parameter	Neutral C_{GA}=0.1% (w/v)	Overcharged C_{GA} = 0.15% (w/v)	Effect of Overcharging
1	Charge on Complex	0 mV	15 mV	
2	Melting Temp.	40 ^{0}C	40 ^{0}C	None
3	Storage Modulus	5820 Pa (0.1 rads^{-1})	500 Pa (0.1 rads^{-1})	Softening
4	Viscoelastic Length	10 nm	28 nm	Softening
5	Correlation Length	1.27 nm	1.9 nm	Softening
6	Complex Assembly	DLA type	DLA type	None
7	Hydration	Poor	Substantial	Softening
8	Heterogeneity	Heterogeneous	Heterogeneous	None
9	Micro Structure	Dense gel like	Open gel like	Softening

4. Possible biomedical and food industries application

Gene therapy is the huge progress in molecular biology. In this technique a vector is needed to transport DNA through the cell membrane to restore or correct or inhibit the expression of pathogenic genes.[28] In general drug has to pass many potential barriers[29] in order to reach their target. Drug in gene therapy should meet few basic conditions for its usage like its non toxicity[30,32] and its transportation through blood stream or the extracellular fluid which is related to water solubility properties of drugs. Moreover, active substance (DNA) must be encapsulated in a proper vector, Gelatin in our case, for protection and effective transportation. DNA- Gelatin complexes as gene therapy in our case has an extra advantage of overcharging. Since cell membrane is negative in charge, we can tune the charge of DNA-Gelatin complex from neutral to positive in charge (overcharging) so as to drive it electrostatically towards the cell membrane.

In another aspect of application, chemical treatment or modification of proteins is almost inevitable in food industries to enhance their properties.[32-34] Nonetheless, our overcharged complexes have a good application in food industry. For the simple fact that most food processing technology depends on the thermo-mechanical properties of food. Tonnes of Gelatins are used in food industries to tune the gelation and adhesive properties of the food items. So, DNA-Gelatin complexes has a potential to change the mechanical properties owing to overcharging as discussed in rheology section. Invariance of thermal properties is as good as we are not thermally stabilising our food product and so that we can get melting and bursting taste of our food in our mouth (body temperature).

5. Conclusions

We have systematically studied the mechanical and thermal properties of DNA-GA complex coacervates as function of overcharge. The mechanical property of the coacervates decreased

significantly with increasing overcharging of the complexes comprising the coacervate matrix while the thermal properties remained invariant. Variation in the mechanical property is attributed to overcharging due to disproportionate binding and causing differential hydration of coacervates having neutral and overcharged DNA-GA complexes. The same has been verified by SANS data which showed increase in mesh size and considerable swelling of the coacervates containing overcharged complexes. However, invariant thermal property has been observed from the melting curves in rheology experiment. SANS data revealed no distinct structure factor as function of temperature. Indistinguishable thermal property of a coacervate for a given protein concentration and the same observed for varying protein concentration was attributed to the presence of physically cross-linked gelatin chains where the DNA molecules provided the necessary scaffold. However, it is possible that present SANS experiments could not sense the change in structure factor on temperature variation, similarly the Porod region remained unexplored due to lack of quality data in high-q region. In summary, it was clearly observed that gel-like networks of the protein chains supported by DNA scaffold existed inside the coacervate matrix in propensity and the network density was much reduced in overcharged coacervates with the concomitant change in their hydration properties. The manifestation of anomalous thermal and viscoelastic properties of these materials owe their origin to the aforesaid differential hydration behaviour. Moreover, we have also discussed the potent application of DNA-Gelatin complex coacervates in biomedical and food industries due to overcharging phenomenology of our coacervates.

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References

[1] T.T. Nguyen, B.I. Shklovskii, J. Chem. Phys. 115 (2001) 7298-7308.

[2] R. Zhang, B.I. Shklovskii, Physica A 352 (2005) 216-238.

[3] E. Gurovitch, P. Sens, Phys. Rev. Lett. 82 (1999) 339-342.

[4] N. Arfin, H.B. Bohidar, J. Phys. Chem. B 116 (2012) 13192-13199.

[5] F. Bordi, S. Sennato, D. Truzzolillo, J. Phys. Condens. Matter 21 (2009) 203102.

[6] K.B. Djagny, Z. Wang, S. Xu, Critical Reviews in Food Science and Nutrition 41 (2001) 481-492.

[7] Y. Wang, J.Y. Gao, P.L. Dubin, Biotechnol. Prog. 12 (1996) 356-362.

[8] P.L. Dubin, S.S. The, D.W. McQuigg, C.H. Chew, L.M. Gans, Langmuir 5 (1989) 89-95.

[9] A.B. Kayitmazer, H.B. Bohidar, K.M. Mattison, A. Bose, J. Sarkar, A. Hashimoto, P.S. Russo, W. Jaeger, P.L. Dubin, Soft Matter 3 (2007) 1064-1076.

[10] K. Kaibara, T. Okazaki, H.B. Bohidar, P.L. Dubin, Biomacromolecules 1 (2000) 100-107.

[11] P.S. Goyal, V.K. Aswal, J.V. Joshi, Current Science 79 (2000) 947-953.

[12] P. Thiyagarajan, J.E. Epperson, R.K. Crawford, J.M. Carpenter, T.E. Klippert, D.G. Wozniak, J ApplCrystllogr. 30 (1997) 280-293.

[13] J.D. Ferry, Viscoelastic Properties of Polymers, John Wiley, New York, 1961.

[14] H.H. Winter, F.J. Chambon, Rheology 30 (1986) 367-382.

[15] H.A. Barnes, Handbook of Elementary Rheology, University of Wales, Wales, 2000.

[16] N. Arfin, H.B. Bohidar, Int. J. Biol. Macromol. 50 (2012) 759-767.

[17] A. Ajji, L. Choplin, Macromolecules 24 (1991) 5221-5223.

[18] K. S. Cole, R.H. Cole, J. Chem. Phys. 9 (1941) 341-351.

[19] T.C. Warren, J.L. Schrag, J.D. Ferry, Biopolymers 12 (1973) 1905-1912.

[20] D.W. Davidson, R.H. Cole, J. Chem. Phys. 19 (1951) 1484-1490.

[21] A. Veis, The Macromolecular Chemistry of Gelatin, Academic Press, New York, 1964.

[22] D.M. Carey, G.M. Korenowski, J. Chem. Phys. 108 (1998) 2669-2675.

[23] S. Boral, H.B. Bohidar, J. Phys. Chem. B 116 (2012) 7113-7121.

[24] B. Ratajska-Gadomska, W. Gadomski, J. Chem. Phys. 133 (2010) 234505, 1-7.

[25] J.L. Burns, Y. Yan, G.J. Jameson, S. Biggs, Langmuir 13 (1997) 6413-6420.

[26] P.G. de Gennes, Scaling Concept in Polymer Physics 2nd ed., Cornell University Press, Ithaca, New York, 1985.

[27] F. Gaboriaud, A. Nonat, D. Chaumont, J. Phys. Chem. B 103 (1999) 5775-5781.

[28] E. Rayburn, H. Wang, J. He, R. Zhang, Lett. Drug Design Discov. 2 (2005) 1-18.

[29] O.G. Mouritsen, Life - as a matter of fat, Springer, Berlin, 2005.

[30] A. El-Aneed, J. Control. Release 94 (2004) 1-14.

[31] S. Zhang, Y. Xu, B. Wang, W. Qiao, D. Liu, Z. Li, J. Control. Release 100 (2004), 165-180.

[32] L.R. Hass, Biochemistry 3 (1964) 535.

[33] E.F. Mellon, A.H. Korn, S.R. Hoover, J. Amer. Chem. Soc. 69 (1947) 827.

[34] J.C. Monti, R. Jost, J. Dairy Sci. 61 (1978) 1233-1237.

Overcharging, Thermal, Viscoelastic and Hydration properties of DNA-Gelatin complex coacervates: Pharmaceutical and Food industries

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TOC Abstract