Chapter 8
Gelatin Nanoparticles via Coacervation

8.1 Introduction

Nanoscience has been a subject of considerable interest because of the special properties associated with nano-particles, such as large surface to volume ratio, increased surface reactivity as compared to bulk material and their porous or core shell structure. Nanoparticles have been around since Michael Faraday's time of 1857 when he first developed the gold colloidal particles. Its regime has broadened spanning three decades, and research on various methods of their preparation and applications is still in progress. In pharmaceutics, biodegradable and biocompatible polymeric nanoparticles have shown great potential as drug carriers. Compared to other colloidal carriers, like liposome, biopolymers represent better stability when in contact with biological fluids, and their polymeric nature allows one to obtain the desired controlled and sustained release of entrapped drug molecules [1-5]. Nanoparticles represent drug delivery systems suitable for most of the administration routes, even if a rapid recognition by the immune system limits their use as injectable carriers. These were initially devised as carriers for vaccines and anti-cancerous drugs [6]. Nano-particles made of gelatin (a denatured protein) biopolymers have the potential to be used as drug or gene delivery carriers. Gelatin is readily available, has a relatively low antigenicity and is in use in a number of parenteral formulations [7].

We have already discussed about coacervation, a liquid-liquid phase separation (see Chapter 4), giving rise to a polymer rich dense phase at bottom and transparent solution (supernatant) at above. These two liquid phases are incompatible, immiscible and are in equilibrium. The dilute liquid phase, the supernatant (rich in biodegradable nanoparticles) could be characterized by dynamic laser scattering light scattering (DLS), small angle neutron scattering (SANS), transmission electron microscopy (TEM) and electrophoresis measurements, which forms the objective of this chapter.
8.2 Results and Discussion

8.2.1 Analysis of DLS measurement

Table 8.1 shows the time dependent stability of gelatin nanoparticles observed through dynamic light scattering measurement. The procedure of sample preparations and theory of dynamic light scattering is well described in the section 3.6.2.1 (see Chapter 3). DLS measurement shows that the particles were quite stable at room temperature ~ 25 °C over a period of two months. The DLS data showed an average size of about ~ 45.9 nm (mean diameter by CONTIN analysis) and the average effective diameter (mean diameter by cumulant analysis) ~ 30.44 nm with an average polydispersity ~ 0.3. For DLS measurement we have used the solvent viscosity = 2.8 cP and refractive index = 1.342, which refers to ethanol concentration = 45 ± 2 (v/v %).

<table>
<thead>
<tr>
<th>Time/days</th>
<th>Diameter by CONTIN analysis/nm</th>
<th>Effective Diameter/nm</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>51 ± 5</td>
<td>30 ± 3</td>
<td>0.22</td>
</tr>
<tr>
<td>10</td>
<td>43 ± 5</td>
<td>30 ± 3</td>
<td>0.35</td>
</tr>
<tr>
<td>20</td>
<td>50 ± 5</td>
<td>34 ± 3</td>
<td>0.27</td>
</tr>
<tr>
<td>30</td>
<td>44 ± 5</td>
<td>31 ± 3</td>
<td>0.27</td>
</tr>
<tr>
<td>60</td>
<td>42 ± 5</td>
<td>27 ± 3</td>
<td>0.38</td>
</tr>
</tbody>
</table>

Table 8.1: Particle size determination by DLS through two techniques.

The cumulant analysis yields a very specific ratio of moments of the particle number distribution. If N(R) is the particle number distribution the cumulant analysis yields the ratio of the 6th to 5th moment of N(R) given by

$$R_{Cumulant} = \frac{\int N(R)R^6dR}{\int N(R)R^5dR}$$ (8.1)

The CONTIN algorithm calculates either volume distribution P (V(R)) = N(R) R^3 or an intensity distribution P (I(R)) = N(R) R^6. The average of the CONTIN result is defined as
\[ \frac{\int [P(V(R)) R \, dR]}{\int [P(V(R)) \, dR]} = \text{CONTIN} \]  
(8.2)

which is the ratio of the 4\textsuperscript{th} moment to the 3\textsuperscript{rd} moment of \( N(R) \)

or

\[ \frac{\int [P(I(R)) R \, dR]}{\int [P(I(R)) \, dR]} \]  
(8.3)

which is the ratio of 7\textsuperscript{th} to 6\textsuperscript{th} moment of \( N(R) \). In all cases the cumulant and the CONTIN analysis will give different values as long as the size distribution is broad alike in our case (polydispersity \( \sim 0.3 \)). The CONTIN method yields better results since it is insensitive to baseline fluctuations unlike the cumulant method that yields the effective diameter values [8]. These are compared in Table 8.1. It can be clearly seen that the cumulant method underestimates the size significantly. The large polydispersity can be ascribed to the polydispersity inherent in gelatin samples [9].

8.2.2 Analysis of SANS measurement

The small angle neutron scattering (SANS) experiments were performed at the Swiss Spallation Neutron Source SINQ, Paul Scherrer Institute, Switzerland. The details of theory and collections of data are discussed in section 3.6.2.2 (see Chapter 3). Dilute systems are ideally suited for studying the shapes and sizes of the particles. In these systems, particles concentration is very low. The intensity of scattered neutrons \( I(q) \) for this system (see Eq. 3.20, Chapter 3) is given by

\[ I(q) = n (\rho_p - \rho_m)^2 V^2 \exp\left(-\frac{q^2 R_g^2}{3}\right) \]  
(8.4)

where, \( (\rho_p - \rho_m)^2 \) is referred to as the contrast factor, \( n \) is the number of particles per unit volume of the sample; \( V \) is the average volume of the single particle. Plot of \( I(q) \) vs. \( q \) in Figure 8.1 shows the scattering in supernatant samples at temperature 25 \( ^\circ \)C. A plot of logarithm of scattering intensity \( I(q) \) versus \( q^2 \) will be a straight line fitting to Eq. 8.4 in the small \( q \) region (\( q < 3R_g^{-1} \)) and the slope gives the radius of gyration, \( R_g \sim 20.5 \text{ nm} \) (see Figure 8.2). By assuming the shape of particle to be a sphere, we have converted the radius of gyration \( R_g \) into a particle radius, \( R \) through

\[ R = \sqrt[5]{\frac{5}{3}} R_g \]  
(8.5)
was estimated that the radius of the particle is \( \sim 26 \text{ nm} \) and the particle size (diameter) \( 52\pm2 \text{ nm} \), comparable to the size measured by DLS.

**Figure 8.1:** SANS data for supernatant performed at 25 °C, Plot of scattering intensity, \( I(q) \) versus wide range of \( \log(q) \), \( (0.03 \text{ nm}^{-1} = q = 3.5 \text{ nm}^{-1}) \).

**Figure 8.2:** SANS data for supernatant, Guinier logarithmic plot of scattering intensity \( a.u \) versus \( q^2 \) (\( q = 0.07 \text{nm}^{-1} \)).
8.2.3 Analysis of TEM measurement

TEM data (see Figure 8.3) shows that gelatin particles have diameters in the range of 45nm to 90nm. Larger particles (~ 90 nm) seen in the TEM picture could arise due to the aggregation on the substrate surface. Though the TEM picture shows the large variation of particle sizes, the dynamic light scattering and Small angle neutron scattering measurements showed only the average particles range in the order of 46 ± 2 nm and 51 ± 2. Secondly, TEM sample preparation involved drying of the supernatant on the grid at ambient temperature. There is a lot of discussion usually of what the drying effects are on the fluctuational organic nanoparticles as they are in a state of non-equilibrium during drying and as a result alter their shape and size considerably. Mostly, freeze fracture or cryo-TEM is usually performed to establish such structures.

![Figure 8.3: TEM picture of a supernatant sample at concentration 45±2% (v/v), pH = 5 at T = 25 °C after staining. Particles of sizes 48nm, 65nm and 90 nm are clearly seen in the picture.](image)

8.2.4 Analysis of Electrophoresis measurement

At the time of electrophoresis measurement, the particles (moving in Brownian motion in bulk) were viewed in a microscope (x 500) fitted with a reticule through optical dispersion of a laser beam. A voltage of 50V was applied during the measurement and the particle migration was noted to be stable. The result of average zeta potential value and
the corresponding electrophoretic mobility was \(-23.9\) mV and \(0.422\) um/sec.cm/V. The surface charge distribution was found to be negative as the zeta potential was negative. We have only shown the representative data on frequency (no. of particles) vs. zeta potential of gelatin nanoparticles in the supernatant medium (see Figure 8.4).

![Graph](image)

**Figure 8.4:** Electrophoresis measurement of gelatin nanoparticle in supernatant medium at pH = 5 at 25 °C. Plot of zeta potential vs. frequency is shown.

Smoluchowski’s formula [10] was used to calculate the zeta potential of gelatin nanoparticles. The equation is described as:

\[
\zeta = \frac{4\pi\eta}{\varepsilon} \cdot \mu \times 9 \times 10^7
\]  

(8.6)

where \(\zeta\) = Zeta potential (mV), \(\eta\) = Viscosity of solution (0.028 poise), \(\varepsilon\) = Dielectric constant (56.4) and \(\mu\) = Electrophoretic mobility. The value of viscosity and dielectric constant were taken at ethanol concentration 45 ± 2% (v/v).

For a neutral sample, for which the ensemble average charge asymmetry is equal to zero, concentrating the samples leads to the formation of a supernatant containing counterions and almost neutral globules while the precipitate at the bottom remains neutral [11].
For samples with asymmetric charge distributions, the supernatant contains highly charged globules while the precipitates are neutral [11]. In our experimental data, the particles in supernatant carried negative charge which confirms that the solution initially had asymmetric charge distributions. We have argued earlier that the possibility of particle contraction due to ethanol-induced dehydration results in contraction of random coil particles into hard nano-spheres [12].

### 8.3 Conclusions

Though gelatin nanoparticles were previously prepared by desolvation method [13], the simple coacervation [14] process is a new method by which we could get very stable nanoparticles in the range of $\approx 45$ nm which was confirmed through DLS, SANS and TEM experiments (see Table 8.2).

<table>
<thead>
<tr>
<th>Techniques</th>
<th>Diameter of nanoparticle/nm</th>
</tr>
</thead>
<tbody>
<tr>
<td>DLS</td>
<td>$\sim 46 \pm 2$</td>
</tr>
<tr>
<td>SANS</td>
<td>$\sim 51 \pm 2$</td>
</tr>
<tr>
<td>TEM</td>
<td>$\sim 48 \pm 2$</td>
</tr>
</tbody>
</table>

**Table 8.2:** Average particle size of gelatin particles.

This is very small compared to the same reported earlier for this polypeptide ($\sim 220$nm) [15]. In our simple coacervation studies, the high molecular weight fraction of gelatin (aggregates of size $\sim 200$ nm) goes to coacervate phase. Low molecular weight species formed the nanoparticles of diameter $\approx 50$ nm in the supernatant [14]. The objective of specifying different of molecular weight is needed to account for the effect of polydispersity inherent in gelatin samples though the weight average molecular weight was $\approx 90 \pm 10$ kDa. So the removal of the high molecular weight gelatin fraction by coacervation process enabled the enhancement of stability of these particles.
The physical mechanism of aggregation can be visualized as follows. Gelatin is not soluble in alcohols whereas water is a good solvent. As ethanol is added to water, the water molecules will preferentially bind to the alcohol molecules through hydrogen bonding and the resultant binary mixture becomes a marginal solvent for gelatin molecules [9]. Furthermore it has been shown that the binary liquid mixture of ethanol and water exhibit maximum hydrogen bonding at ethanol concentration = 45±2% (v/v) which is supported by thixotropic and viscosity data [9]. The prepared gelatin nanoparticles showed high stability in water and ethanol binary mixture (at concentration ~ 45±2% (v/v)), which forms the dispersion medium. Particle stability is also enhanced by presence of ethanol in the supernatant phase because contamination due to bacteria is avoided significantly. It was evident from DLS, SANS and TEM studies that the particles showed neither sedimentation nor flocculation and the particle size remained constant during the investigation time of two months.

Electrophoresis measurement confirms the particles are negatively charged. However, we believe that this not only provides us with new information about nanoparticle but also has consequences that reach beyond nanosciences. Previously, the concepts of polymer physics has had an enormous impact on the understanding of structural and dynamic properties of coacervation, but we are now in a position to return this favor and use supernatant as ideal model system for “equilibrium polyelectrolytes”. It must be realized that the experimental studies with highly hydrogenous polyelectrolyte systems using SANS has suffered weak scattering power, which makes it impossible to produce data with a sufficient accuracy over a required wave vector range from diluted supernatant solutions.
8.4 References


