Chapter 2
Synthesis of Chitosan-Based Nanomaterials

2.1 Emulsion Cross-linking

Basic concept of this method is to convert the material into microscopic droplets through emulsion and further stabilize the particles into nano size by cross-linking. Water-in-oil (w/o) emulsion is prepared under mechanical stirring by mixing chitosan aqueous solution into oil. A suitable surfactant is used to achieve the stable emulsion. The stable emulsion is cross-linked by using an appropriate cross-linking agent such as glutaraldehyde, formaldehyde, genipin, glyoxal, sulfuric acid, poloxamer, etc. For efficient cross-linking, overnight stirring of the reaction should be performed. Stirring rate is based on the volume of the reaction and desired physical properties of nanoformulation. A translucent solution obtained from overnight stirring is then filtered and washed repeatedly with n-hexane followed by alcohol to remove oil. The surfactant can be removed by precipitation with appropriate salts like $CaCl_2$ followed by centrifugation. Resulted precipitates can then be lyophilized to get a fine dry nanomaterial (Akbuga and Durmaz 1994). Method is explained in Fig. 2.1.

By this technique, a narrow size distribution of nanoparticles can be obtained by varying the amount of cross linker and chitosan. Size of the nanoparticles also depends on the size of droplets formed in emulsion. Hence, before cross-linking, the size of the droplets can also be controlled by adjusting the oil, water and surfactant amount. Stirring speed during cross-linking also plays an important role in the size distribution and nature of distribution of particles. This method is tedious and time-consuming due to emulsion phase. Removal of surfactant and oil is also a wearisome step along with unkind shearing force and complexity in washing step. In addition to above-mentioned drawbacks, there are some benefits like high loading efficiency, controlled release with improved bioavailability and easy to control particle size (Agnihotri et al. 2004).

V. Saharan, A. Pal, *Chitosan Based Nanomaterials in Plant Growth and Protection*,
SpringerBriefs in Plant Science, DOI 10.1007/978-81-322-3601-6_2

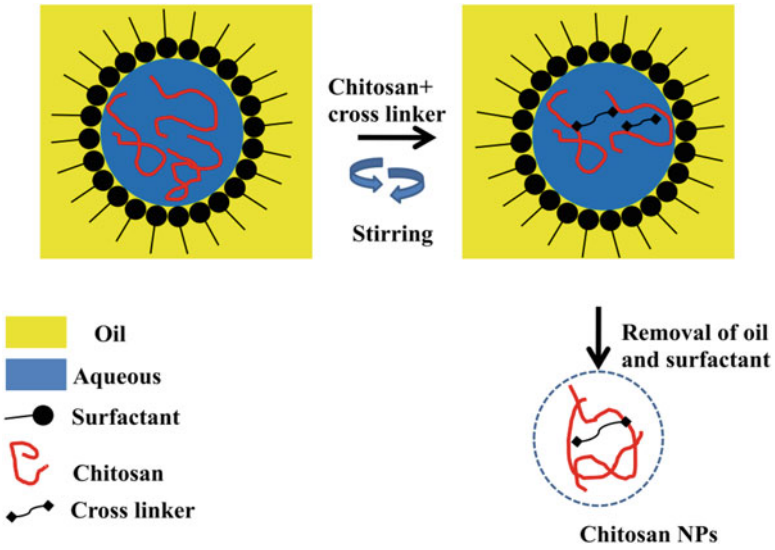

Fig. 2.1 Emulsion cross-linking

2.2 Emulsion-Droplet Coalescence Method

This method utilizes the principles of emulsion and precipitation without cross-linking (Tokumitsu et al. 1999; Anto and Annadurai 2012). First, a stable emulsion is established through aqueous solution of chitosan, oil and active ingredient. In the same way, a stable emulsion of chitosan aqueous solution with NaOH is also prepared. Both emulsions are mixed under high speed stirring to induce random collisions between droplets and subsequent precipitation of chitosan droplets gives small size particles (Fig. 2.2). This method also has same demerits as explained in emulsion cross-linking methods. However, this method does not use cross linker; hence, amino groups of chitosan are available to bind with active ingredients. Therefore, by using this method, it is possible to achieve higher loading efficiency and smaller particle size as compared to the emulsion cross-linking method.

2.3 Ionic Gelation Method

This method is most accepted method for the synthesis of stable, non-toxic and organic solvent free chitosan nanoparticles. The synthesis of chitosan NPs is based on electrostatic interactions between positively charged amino groups ($-NH_3^+$) of chitosan and negatively charged cross linkers (Saharan et al. 2013; Saharan et al. 2015). In this procedure, chitosan is dissolved in week acidic aqueous solution and mix systematically into aqueous solution of negatively charged cross linkers. In this

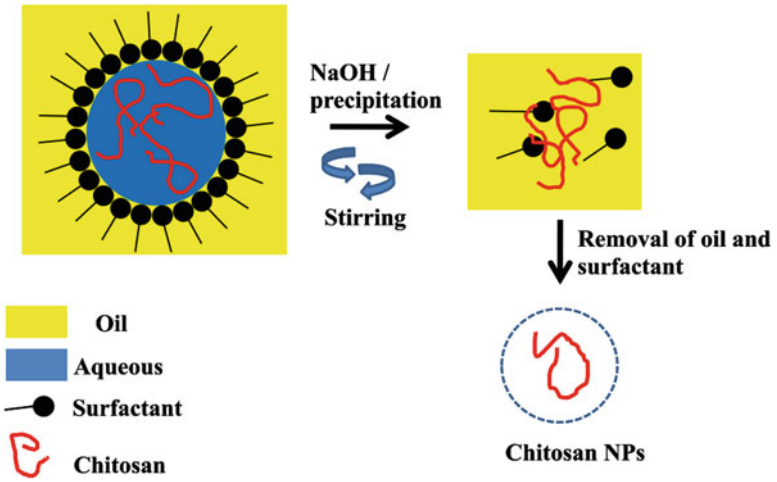

Fig. 2.2 Emulsion-droplet coalescence method

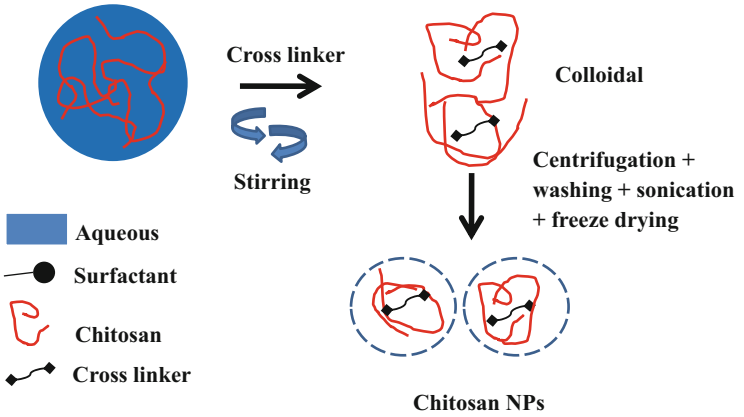

Fig. 2.3 Ionic gelation method

method chemical cross-linking is avoided and a reversible physical cross-linking by electrostatic interaction is performed. Tripolyphosphate (TPP), a polyanion, has become very popular because of its non-toxic property. TPP is added drop wise under steady stirring to acidic chitosan solution. Chitosan undergoes ionic gelation and precipitates to form particles (Fig. 2.3). It is important to understand the volume and concentration ratio of chitosan and TPP as it affect the size and surface charge of particles. In addition, the deacetylation of chitosan is also crucial for particles formation, more is the percentage of deacetylated chitosan, more is the electrostatic interaction. Stirring rate during gelation is also important as it provides equal probability of interaction between opposite charges. This method demonstrates some striking features like easygoing procedure, no chemical cross-

linking, minimum toxic effects of chemicals, no additional steps for removal of oil and surfactant, etc. which make it more reliable and accurate (Jaiswal et al. 2012).

2.4 Reverse Micellar Method

Basic principle of this method is to first establish thermodynamically stable, monodisperse, isotropic reverse micelles. To initiate reverse micellar method, a suitable surfactant and organic solvent is mixed to prepare translucent, fine microscopic micelles droplets solution. For nanoparticles' synthesis, acidic aqueous solutions of chitosan along with active ingredient to be encapsulated are added to isotropic reverse micelles with constant vortexing to avoid any turbidity. In the next step, a cross-linking agent is added to this ultra-fine transparent solution with constant stirring. Subsequent cross-linking is achieved by overnight stirring which leads to the formation of nanoscale size chitosan particles. Dry material is obtained by evaporating organic solvent and surfactant is further removed through suitable salt precipitation. The mixture is then subjected to centrifugation and nanoparticles containing supernatant is further used (Fig. 2.4). Monodisperse, thermodynamically stable, narrow size particles are the most vital aspects of reverse miceller method. However, this method is very comprehensive in making nanoparticles in the range of 50–100 nm as compared to other methods where size range exceeded 200 nm.

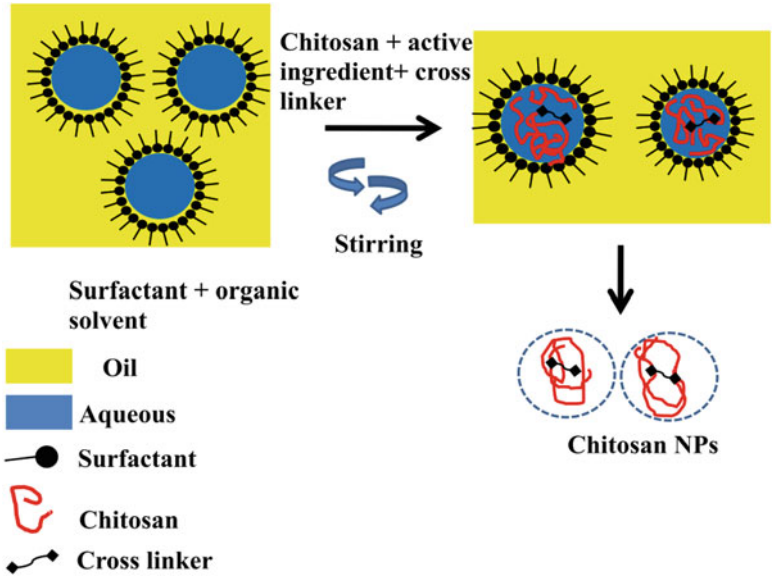

Fig. 2.4 Reverse micellar method

This method has tedious and laborious steps and needs an accuracy in performing the reaction (Brunel et al. 2008).

2.5 Sieving Method

It is still a challenge to produce stable and mono-dispersed chitosan nanoparticles for achieving desirable results. Methods invented till date are not accurate for the synthesis of desirable size of nanoparticles. Owing to this obstacle, filtering of nanomaterials through desirable mesh size was invented (Agnihotri and Aminabhavi 2004). Here, the synthesized chitosan materials are passed through the sieve having fixed size. This leads to the accumulation of filtered material with higher percentages of mono-dispersed nanoparticles (Fig. 2.5).

2.6 Spray Drying

Spray drying method is used for the synthesis of dry powder, granules and pellets of chitosan nano/microparticles. This is an atomized procedure for rapid and mass synthesis of chitosan nanomaterials. In this method, acidic aqueous chitosan solution along with cross linker and active ingredient is passed through a nozzle with hot air stream. Compressed air induces the cross-linking and size reduction of

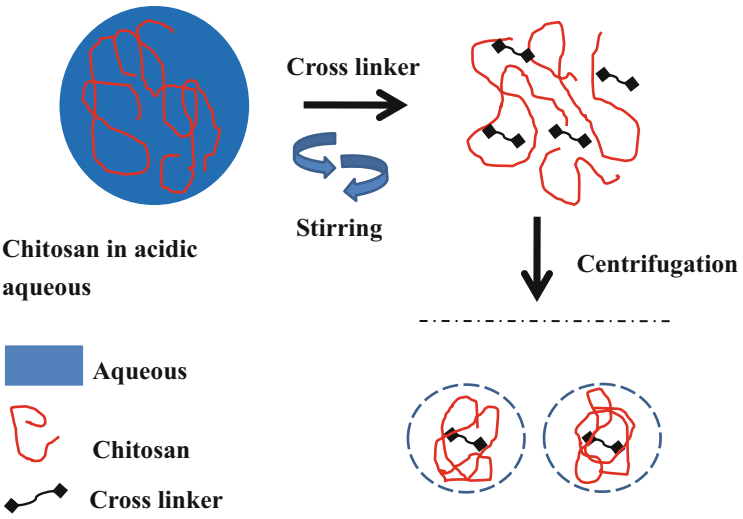

Fig. 2.5 Sieving method

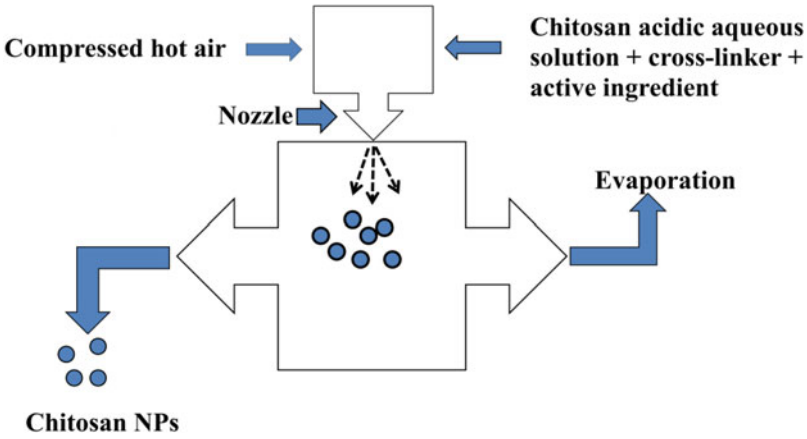

Fig. 2.6 Spray drying method

chitosan. During this process, the solvent is also evaporated and dry form of chitosan is received at the end of the reaction. Size of nozzle, velocity of compressed air, its temperature and degrees of cross-linking of chitosan are some of the vital factors which can amend the size of the chitosan NPs (He et al. 1999). This method is fast with single step, inexpensive and can be used to synthesize particles with or without cross-linking (Fig. 2.6). The encapsulation can also be performed in the same step with higher encapsulation efficiency (Table 2.1).

2.7 Factor Affecting Chitosan Nanomaterial Synthesis

As discussed in previous sections, different methods have been developed to prepare chitosan NPs. These methods have their own advantages and disadvantages based on where they are applied and what kind of physico-chemical features are required. In view of the serious issue regarding increasing toxic chemical load on ecosystem, novel strategies to develop eco-friendly, biodegradable, nontoxic nanoformulation for protection and growth of crop plants are in prime priority (Saharan et al. 2013, 2015). Therefore, more emphasis is on those methods in which stable, controllable, highly active, non-toxic, organic solvent free and easy synthesis of chitosan nanoparticles could be achieved. Amongst the various methods, ionic gelation technique has attracted considerable attention because this process is non-toxic, organic solvent free, uses minimum chemicals, easy in mass synthesis, convenient and controllable (Berger et al. 2004; Agnihotri et al. 2004). In ionic gelation, ionic interactions are ensued between the positively charged primary amino groups of chitosan and the negatively charged groups of sodium tripolyphosphate (TPP). A polyanionic sodium tripolyphosphate (TPP) is the most widely used non-toxic cross-linking agent (Shu and Zhu 2002). This physical cross-linking between chitosan

Table 2.1 Synthesis of chitosan-based nanoparticles: advantages and disadvantages (Agnihotri et al. 2004; Kashyap et al. 2015)

Methods	Advantages	Disadvantages
Emulsion cross-linking	1. Easy control of particle size	1. Need to remove oil and surfactant
	2. Good loading efficiency	2. Cross-linker can react with active ingredient
	3. Good stability of nanomaterials	3. Purification of nanoparticle is tedious process
Emulsion-droplet coalescence method	1. Higher encapsulation efficiency	1. Excess alkali induces more precipitation and leads to increased particle size
	2. No cross-linker leads to higher Zeta potential	2. Due to absence of cross-linker nanoparticles stability decreases
	3. No reactivity of cross-linker to active ingredient	
Ionic gelation method	1. Reduced the chemical side effect	1. Partial size, distribution and stability strongly affected by degree of deacetylation, MW of chitosan and molar ratio of chitosan and TPP
	2. Better control on physico-chemical feature of nanoparticles	
	3. Easy and fast	
Reverse Micellar Method	1. Stable and smaller and monodispersed nanoparticles with suitable polydispersity index	1. Cumbersome procedure
		2. Chance of side effect of reaction components (solvent, surfactant, etc.)
Seiving method	1. Very simple and rapid procedure	1. Need specialized sieve with particular size for desirable size of nanoparticles
	2. Easy mass synthesis	
Spray drying method	1. Mechanized method for mass production	1. Size of nanoparticles depends on nozzle size, flow rate and temperature of air
		2. Temperature sensitive component could not be encapsulation

and TPP evades two things – no use of chemical cross-linkers and avoids the emulsifying agents, both of which are often toxic for biological system. Due to a number of advantages, ionic gelation method is extensively researched amongst the others methods. A number of valuable informative reviews are available for specific details of various factors which affect the synthesis of chitosan-based nanoparticles (Kalotia and Bohidar 2010). Most vital thing related to chitosan NPs synthesis is the production of custom-made nanoparticles for precision applications. Hence, it is imperative to focus on the study of various factors which affect the physico-chemical characters (size, dispersity, zeta-potential, polydispersity index and solubility). Followings are some of the vital factors which affect the physico-chemical characters of chitosan-based nanomaterials.

2.7.1 Concentration of Chitosan and TPP

It is well understood that concentrations of both components affect the synthesis process and physico-chemical features of the chitosan NPs. Before getting into the details, it is important to know the different interaction systems existing in the chitosan solution. In acidic conditions, amino groups of chitosan get protonated and a strong electrostatic repulsion occurs between chitosan molecules which keeps them apart and prevents their agglomeration (Mi et al. 1999; Shu and Zhu 2002). On the other hand, an interchain hydrogen bonding (intermolecular interaction) between chitosan molecules tend to bring them together. Hence, the size of the chitosan NPs is chiefly affected by these two interactions during synthesis process (Qun and Ajun 2006). If electrostatic repulsion is more dominant, the particle size remains small, and if interchain hydrogen bond is stronger, the size of chitosan NPs becomes larger. Various research groups have concluded that certain ranges of concentrations (2.0–1.5 mg /ml and 1.0–0.5 mg /ml) of chitosan and TPP is generally able to synthesize the nanoscale particles (Calvo et al. 1997; Gan et al. 2005; Fan et al. 2012; Jaiswal et al. 2012; Saharan et al. 2015; Kashyap et al. 2015). With increase in chitosan above the critical concentration, the interchain hydrogen bonds become stronger and abundant chitosan molecules mixed up in the cross-linking making the particle size larger (Fig. 2.7). In addition to this, at higher concentrations of TPP,

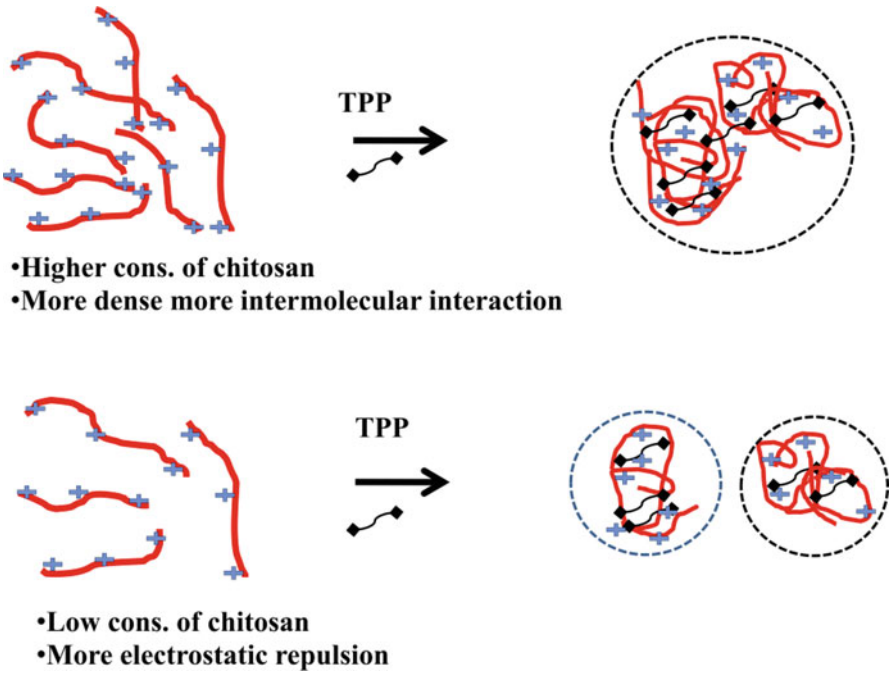

Fig. 2.7 Effect of chitosan concentration on chitosan nanoparticles

zeta-potential of the NPs decreases because TPP occupies most of the positively charged amino groups. Decrease in zeta potential also affects the stability of the NPs as electrostatic repulsion between chitosan particles reduces and particles start to aggregates and large particles appear in the reaction (Fig. 2.7). Therefore, the concentrations of chitosan and TPP play a crucial role in NPs synthesis. Further research has revealed that TPP concentration should mostly be kept equal or lower to chitosan to prevent the excess intermolecular cross-linking. All together, the stable chitosan NPs can only be synthesized at certain concentrations of chitosan and TPP (Kashyap et al. 2015).

2.7.2 Effect of the Mass Ratio of Chitosan and TPP

Effect of the mass ratio of chitosan and TPP can easily be understood by experiment done by Fan et al (2012). They mixed different volumes of TPP (0.5 ml/ml) in 10 ml of chitosan solution (0.5 ml/ml) and found that increasing TPP volume from 2.5 to 3.5 ml (mass ratio decreasing from 4.0:1 to 2.9:1) first steadily decreased the size of NPs from 172 to 133 nm and then increased to 237 nm. The zeta potential also decreased linearly (from +39 to +26 mV) due to the neutralization of protonated amino groups by TPP anions. During chitosan TPP cross-linking, researchers observed that the reaction solution showed clear appearance without visible opalescence which indicates that inadequate volume of TPP is unable to make cross-linked structure. Increasing the volume of TPP, the particle size decreased due to cross-linking in chitosan and compact structure appears and solution becomes opalescent. As TPP volume continued to increase, chitosan molecules got fully cross-linked and excess TPP got involved in intermolecular cross-linking resulting in larger particle size (Fig. 2.8). With further addition TPP in the reaction mixture, the electrostatic repulsion becomes very less due to neutralization of amino groups by TPP and larger particles start to precipitate. Overall the optimum mass ratio of chitosan and TPP has not been recommended because different research groups used different types of chitosan with respect to MW and degree of deacetylation. However, in majority of the experiments, the mass ratio of chitosan and TPP was kept from 2.5:1 to 5:1 to get chitosan NPs (Fan et al. 2012; Jaiswal et al. 2012; Saharan et al. 2015; Kashyap et al. 2015).

2.7.3 Effect of pH of the Reaction Solution

The pH of the reaction solution has very prominent role on synthesis process and quality of nanoparticles. Chitosan is insoluble at neutral and alkaline pH and easily solubilize in low concentration of acidic environment. In acetic medium, H^+ ions protonate the amino groups of chitosan, conferring a high charge density. Therefore, the surface charge density of chitosan molecules is strongly dependent

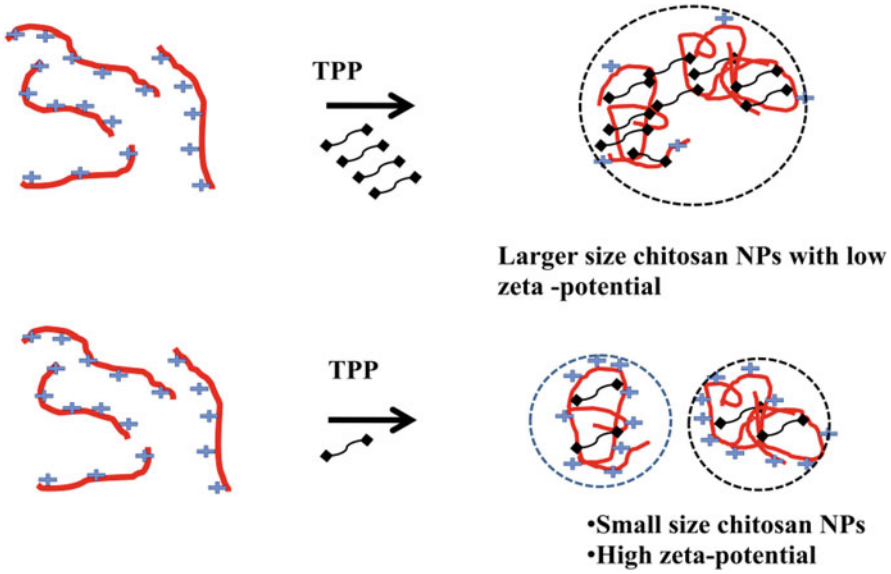

Fig. 2.8 Effect of TPP concentration on chitosan NPs

on pH of the solution. Hence the change in pH of the solution will have strong impact on cross-linking process during reaction. Gan et al. (2005) have explained the effect of pH on the size and zeta-potential of chitosan nanoparticles. It was observed that particle size and zeta-potential are very sensitive to the changing pH of the aqueous solution. During the cross-linking reaction of chitosan and TPP (mass ratio 4:1), with a gradual increase in pH above 5.5, a sharp increase in particles size and precipitation was noticed. It is suggested that with gradual increase in pH from 5.5, the degree of protonation decreases due to increase in OH^- ions which leads to decreased electrostatic repulsion between the particles thereby increasing the probability of particle aggregation. When pH is steadily decreased below 5.0, it is not easier to achieve proper cross-linking by TPP, and as a result there is absence of compact chitosan nanostructures in the highly repulsive conditions in chitosan solution. Variation of pH also influences the zeta-potential of nanoparticles. It steadily lowers down as pH of the reaction mixture is increased. This is due to the neutralization of amino groups of chitosan. When pH of the reaction mixture gets down from 5.0, a sharp increase in zeta-potential is noticed due to the protonation of chitosan (Figs. 2.9, and 2.10). It is clearly evident that a pH around 4.5–5.5 is ideal to get lower average size of particles with net positive zeta-potential (Fan et al. 2012).

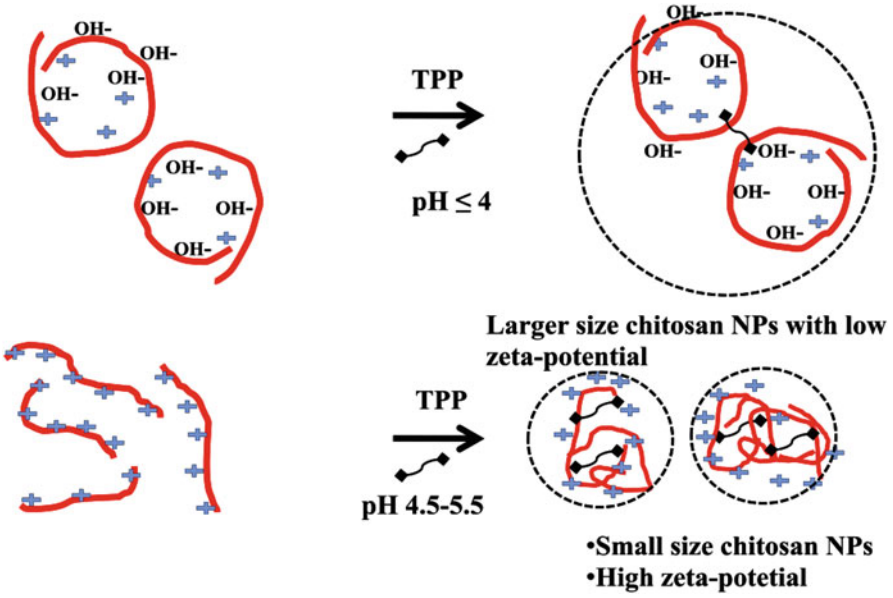

Fig. 2.9 Effect of pH on chitosan NPs

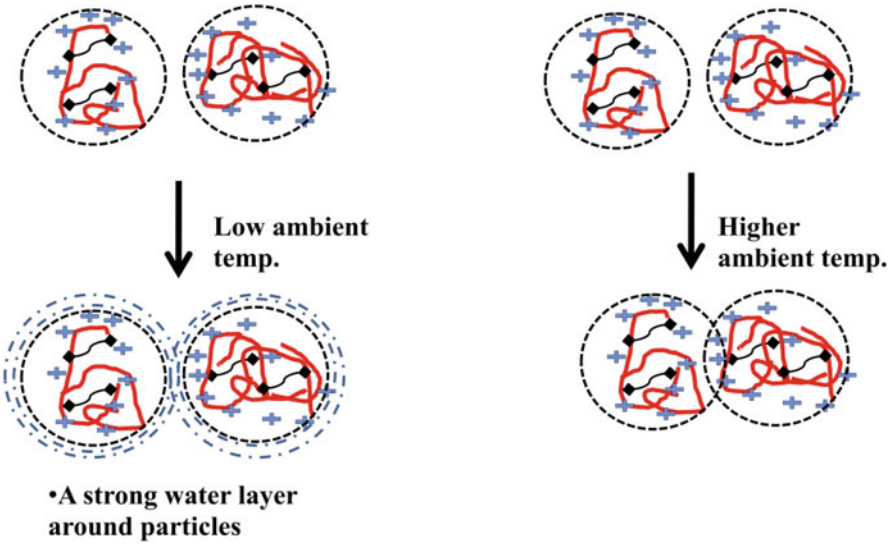

Fig. 2.10 Effect of temperature on NPs synthesis

2.7.4 Effect of the Concentration of Acetic Acid

Acetic acid is primarily used to dissolve chitosan in aqueous. Fan et al. (2012) did experiment to investigate the effect of different concentrations of acetic acid (0, 0.1, 0.2, 0.5 and 0.8 mg/ml) to dissolve chitosan (0.50 mg/ml in aqueous) for formation of chitosan NPs. At lower acetic acid concentration (0.1 mg/ml), a two centric (bimodal) particle distribution around 164 nm and 850 nm size was observed. This indicates varying interaction of TPP and chitosan due to insufficient cross-linking. Further, increasing concentration to 0.2 mg/ml exhibited monodispersed nanoparticle in reaction solution. As we discussed that protonation of chitosan is imperative for cross-linking with TPP to form nanoparticles, thus acetic acid increases the H^+ ions concentration that makes the solution conductive (high protonation) and TPP cross-linked to chitosan to form nanoparticles. In case of lower concentration of acetic acid, lesser number of H^+ ions are available for protonation of amino groups, thus an insufficient number of interaction sites are available for TPP. Albeit at lower concentration of acid, the cross-linking is not uniform to obtain monodispersed nanoparticles. Increasing the acetic acid concentration from 0.5 mg/ml to 0.8 mg/ml increases the amount of smaller particles but also increases the number of larger particles which results in a decrease in the monodispersity of the particles. To prepare chitosan nanoparticles at higher acetic acid concentration, NaOH is added to reaction mixture to bring pH to 4.7–4.8. At this conductive pH, nanoparticles could be synthesized in unimodal fashion at higher acetic acid concentration. However, higher concentration of acetic acid indirectly causes an increase in counter ions (CH_3COO-), which shields the cross-linking points to be accessed by TPP. Thus the intramolecular electrostatic repulsive forces decreases, which make the molecules contracted (Cho et al. 2006; Tsaih and Chen 1997). Contracted conformations of chitosans are not in proximity to accumulate more nanoparticles as compared to their extended conformation. Thus a greater number of smaller nanoparticles are formed. On the other hand, shielding effect of counter ions will lower down the electrostatic repulsion between particles and reduce the thickness of surface hydration layer on the particles. As a result, a number of particles start to aggregate resulting in a wider particle size distribution. Small size monodispersed chitosan nanoparticle synthesis can't be possible at too higher and too lower concentration of acetic acid. Optimum concentration of acetic acid needs to be standardized.

2.7.5 Effect of Temperature of Chitosan Solution

Temperature of the cross-linking reaction also has role in amending the monodispersity and size of particles. During the synthesis process, flexibility, rigidness and hydrogen-bonded hydration layer in chitosan molecules are directly influenced by temperature (Chen and Tsaih 1998; Colic et al. 1998). Narrow unimodal small-sized

chitosan nanoparticles could be obtained at low ambient temperature (0–4°C). It is noticed that as the temperature of chitosan solution decreases, there is an increase in specific volume of chitosan molecule and a decrease in chitosan chain flexibility. The hydrogen bond between the polar groups of chitosan and water molecules increase speedily which build a hydration shield around the particles which prevent the particle aggregation. Under low ambient temperature, the rigidness of the chitosan is high which is favorable for the stabilization of the nanoparticle structures (Fig. 2.10).

2.7.6 Effect of the Stirring on Cross-Linking

Stirring during the ionic interactions between amino group and TPP is recommended to evade particle aggregation and to achieve narrow size distribution. It is manifested that stirring of cross-linking reaction endows equal chance to functional group of chitosan and TPP to link in symmetry. Even though stirring speed comprehensively narrows down the size distribution, increasing the speed from 200 to 600 rpm significantly lessen the size of nanoparticles. This happens because stirring at particular rate does not allow the excess chitosan and TPP to participate in cross-linking and also prevent intermolecular cross-linking. In spite of this, excess stirring with higher RPM may wipe out repulsive forces between the particles and induces bigger size particles synthesis. Albeit, stirring at higher RPM may disturb the symmetrical interactions of chitosan and TPP.

2.8 Characterization Techniques

After the synthesis of nanoparticles, it is most important to measure various key characters like mean size, distribution, charge density (zeta-potential), polydispersity index (PDI), chemical bonding, shape and topography of the particles. Elemental analysis is another key technique to find out the elements involved in NPs makeup. Chitosan nanomaterials have been used for the encapsulation of various other materials which could be characterized by atomic absorbance spectrophotometer and other *in vitro* release kinetics. The state-of-the-art techniques can be used to infer physico-chemical characterization of chitosan nanoparticles.

2.8.1 Particle Size, Zeta-Potential and Polydispersity Index

Dynamic light scattering (DLS) is a technique used to measure the size of particles in suspension. Nanoscale-sized particles are governed by electromagnetic forces and gravitational forces are insignificant. The nanoparticles in suspension follow the

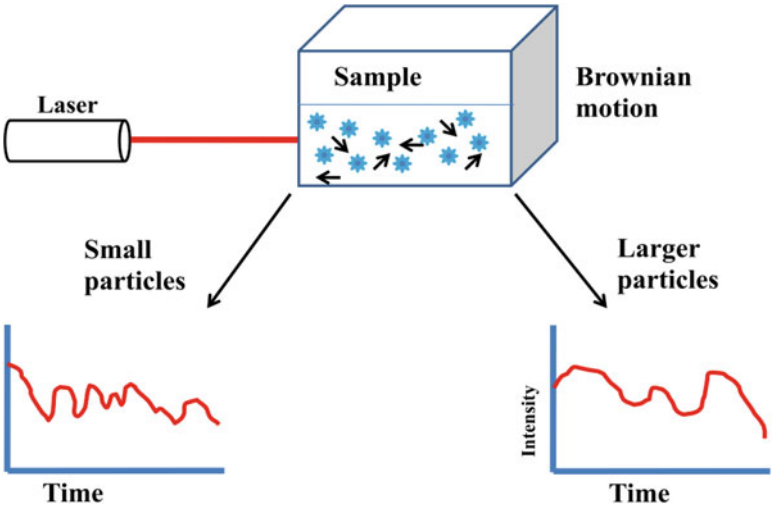

Fig. 2.11 Brownian motion, particle size and intensity fluctuations

Brownian motion caused by collision of solvent molecules. The technique of DLS measures the speed of particles undergoing Brownian motion which is influenced by particle size, viscosity and temperature. The smaller particles show rapid Brownian motion as compared to larger particles. The velocity of Brownian motion is defined by the translational diffusion coefficient (D) which can be converted into particle size using the Stokes-Einstein equation (where $d_H =$ hydrodynamic diameter, $k =$ Boltzmann's constant, $T =$ absolute temperature, $\eta =$ viscosity and $D =$ diffusion coefficient)

$$d_H = \frac{kT}{3\pi\eta D}$$

DLS estimates the time-dependent fluctuations in the scattering intensity to determine the translation diffusion coefficient (D) and subsequent hydrodynamic size. The rate of intensity fluctuation is dependent upon the size of the particles (Fig. 2.11). The mean size measured by the DLS is the mean hydrodynamic diameter. Hydrodynamic diameter is diameter of particle along with the layer of water molecules or ions or media components on the surface (Fig. 2.12). Chitosan nanoparticles having amino groups provide a net positive charge. The cationic nature of chitosan nanoparticles has more probability to interact with media components particularly to anionic substances. These interactions affect its diffusion in the medium and subsequently module hydrodynamic diameter. PDI gives a value which indicates the size distribution of particles. Its value range from 0 to 1, value 1 or above signify highly polydispersed particles and less than 1 indicates monodispersed particles. Chitosan nanoparticles display variability in

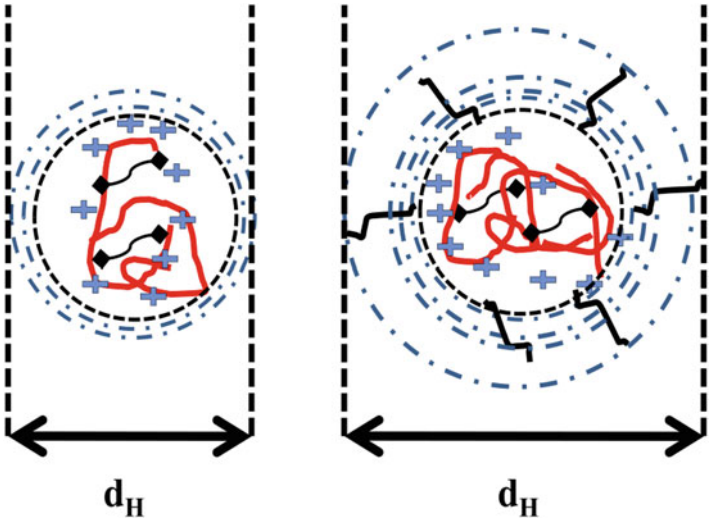

Fig. 2.12 Hydrodynamic diameter

PDI values in different experiments due to different mass ratio and concentrations of chitosan and TPP during particles preparation. Chitosan-based nanoparticles have been synthesized by various groups using various methods. The mean size of the chitosan nanoparticles ranges from 50 to 750 nm depending on various factors and different encapsulation ingredients. Chitosan-based nanomaterials have been used to encapsulate various drugs, micronutrients, biological molecules and metals. The information regarding the use of chitosan nanomaterials in plant is limited. Most of the available information deal with pure chitosan nanoparticles or encapsulation of metals salts and/or other micro/macro nutrients. Therefore the size and particle distributions are diverging. It is manifested that being a polymer, the size and shape of the chitosan nanoparticles are variable and not confined. The other pivotal character is the surface charge also known as zeta-potential. Its value for chitosan nanoparticles always remain positive due to amino groups. It is very crucial to have higher value of zeta-potential for many things. First is the stability of nanoparticles, higher the positive charge on the particles, higher is the repulsion between particles. Hence the particles remain apart and no agglomeration occur. Second is the biological activity of the particles, higher the zeta-potential, higher is the biological activity. Being positively charged, chitosan have higher affinity towards biological membranes and other anions. The values of zeta-potential in chitosan nanoparticles vary from +20 to +90 mV, and it is evident that higher zeta-potential is more valuable in biological system. Other decisive factor is PDI which represents the homogeneity of the particle distribution. A low PDI value indicates the monodispersed nature of chitosan nanoparticles. The chitosan nanoparticles are usually synthesized by cross-linking with TPP. The further functionalization

of particles is done by other components. The interactions of various components within the particles are found out by Fourier transformation infrared spectroscopy (FTIR). In FTIR, IR rays are passed through the particle sample where some of the infrared radiations are absorbed by the bond(s) existing in the sample and some of it are passed out (transmitted). The resulting spectrum represents the molecular absorption and transmission and creates a molecular fingerprint of the particles. A specific absorption at particular vibration indicates a specific bond energy. A plot of % transmittance (E) versus vibration frequency in wavenumbers (cm^{-1}) can easily map the interactions in chitosan nanoparticles. In chitosan nanoparticles, FTIR clearly showed specific peaks to denote amide -$CONH_2$, anhydrous glucosidic and primary amine (-NH_2) groups. Further addition of functional component to particles can easily be found out by redistribution of vibration frequency (Fig. 2.13).

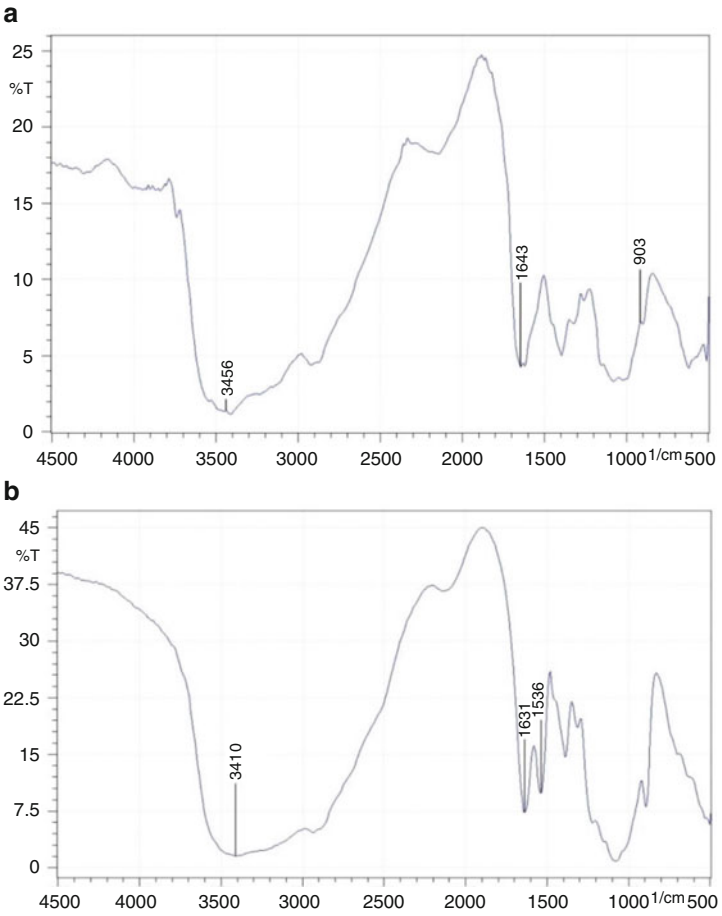

Fig. 2.13 FTIR spectra. (**a**) Bulk chitosan, and (**b**) Nanochitosan

The scanning electron microscope (SEM) uses a focused beam of high energy electrons to generate an array of signals at the surface of particles. The signals derived from sample-electron interactions provide information about the external morphology (texture), chemistry and crystal structures. A two-dimensional image is generated that provides size, shape, surface morphology and elemental analysis data. Chitosan is not conductive in nature and to make it conductive a layer of metal is applied by sputter coating on it. To collect the backscatter electrons (BSC), a separate EDS (Energy dispersive X-ray spectroscopy) analyser is used. EDS analysis provides the quantitative data of elements inside the samples. Size, shape and topography also play decisive role in the application of chitosan nanoparticles. The analysis of size shape and topography is done by transmission and scanning electron microscopy. The TEM study reveals the internal structure of the particles whereas SEM gives surface topography. TEM and SEM are also used to verify the size of the nanomaterials. Various groups have illustrated different shapes and topography of the chitosan nanoparticles varying from ovule shape to highly porous (Fig. 2.14).

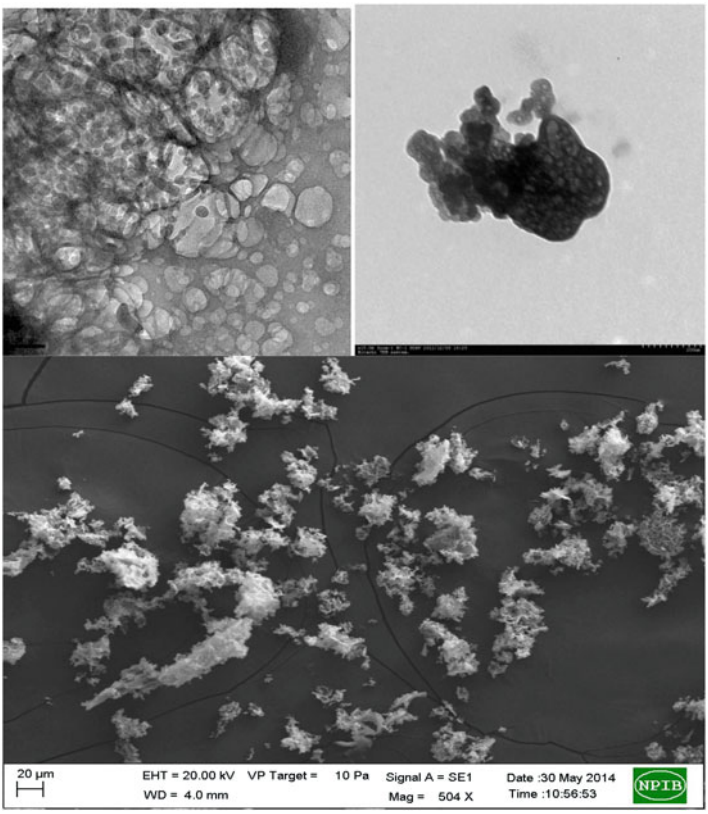

Fig. 2.14 Chitosan NPs TEM and SEM Micrograph