In this paper the preparation of chitosan nanoparticles was carried out using methacrylic acid (MAA) and studied by both ultraviolet (UV)-visible transmission spectrophotometry and transmission electron microscopy (TEM). Nanoparticles with sizes as small as 17 to 25 nm were achieved. The obtained nanoparticles had a homogeneous morphology showing a quite uniform particles size distribution with a spherical shape. The solution was pH-sensitive, due to swelling and aggregation of the nanoparticles. The use of slow release fertilizer has become a new trend to save fertilizer consumption and to reduce environmental pollution. In this work, chitosan (CS) nanoparticles were obtained for the loading of NPK fertilizers. The stability of the CS-PMAA colloidal suspension was higher with the addition of nitrogen and potassium than with the addition of phosphorus, due to the higher anion charge from the calcium phosphate than the anion charges from the potassium chloride and urea. The mean diameter increase of the CS-PMAA nanoparticles in suspension with the addition of different compounds was P > K > N which indicates that the elements are being aggregated and loaded on the surface of the chitosan nanoparticles.

Key words: Nanoparticles formation, chitosan, fertilizers, nanosolution.

INTRODUCTION

The evolution of nanotechnology and the emergence of novel nanodevices and nanomaterials open up new trends in agriculture and biotechnology. Nanoparticles are molecules that are so small to fall within the nanometric range, with at least one of their dimensions being less than a few hundred nanometers. This small size rather brings about marvelous changes in their physical properties with respect to those observed in bulk materials. There are a large variety of methods that can be used in the production of nanoparticles, using techniques from both physics and chemistry. Among the first ones, arc-discharge, high-energy ball milling, laser pyrolysis and laser ablation are the most commonly used. Electrochemical and chemical vapour deposition, sonochemistry and different wet chemistry routes (for example, sol-gel, co-precipitation, inverse micelles, etc.) are also widely employed (Malendi et al., 2008).

Preparation of nanoparticles is recently under heavy
research because of its various applications, in food processing, biomedical, optical, and electronic devices (de Moura et al., 2008). The usage of natural polysaccharides in the preparation of nanoparticles has attracted attention because of their biodegradability and hydrophilic characters which are favorable characters in multiple applications. Chitosan nanoparticles emerged due to their unequalled properties (de Moura et al., 2008).

Fertilizers are chemical compounds applied to enhance plant growth (Bahera and Panda, 2009; Corradini et al., 2010). Usually, fertilizers are applied either through the soil or by foliar spreading. Artificial fertilizers are inorganic compounds devised in appropriate concentrations and combinations to supply three main nutrients: nitrogen, phosphorus and potassium (N, P and K) for multiple crops and growing conditions (Corradini et al., 2010). N (nitrogen) induces leaf growth and forms proteins and chlorophyll. P (phosphorus) enhances root, flower and fruit development. K (potassium) induces stem and root growth and synthesis of proteins (Mandal et al., 2009).

Saigusa (2000) pointed out that 40 to 70% of nitrogen, 80 to 90% of phosphorus, and 50 to 70% of potassium of the normal fertilizers are lost to the environment and not absorbed by plants, causing intrinsic economic and resource losses and dangerously increasing environmental pollution. Recently, the usage of slow release fertilizers has become a new tendency to save fertilizer consumption and to reduce environmental pollution (Wu and Liu, 2008; Corradini et al., 2010). This presents the idea of developing encapsulated fertilizers in which NPK fertilizers are loaded within nanoparticles (Teodorescu et al., 2009). Thus, the fertilizers are preserved by the nanoparticles for better survival in inoculated soils, permitting their controlled release into the soil (Saigusa, 2000). Therefore, the procedure of loading of fertilizers in polymeric nanoparticles is relatively novel, with multiple commercial applications.

Chitosan nanoparticles have been investigated as a carrier for drug delivery, meanwhile a few studies have been attempted to discover the potential of chitosan nanoparticles as controlled release for NPK fertilizers. Chitosan is a polysaccharide derived from chitin, which may be obtained from crustaceans (Boonsongrit et al., 2006). Several preferred properties have been studied, such as film-forming ability, gelatinous characteristics and bio-adhesion.

Due to its polymeric cationic charac-teristics, chitosan may react with negatively charged molecules and polymers, showing a favorable reaction (Boonsongrit et al., 2006). The ability of controlling nanoparticle size is highly favorable for most applications in nanotechnology (de Moura et al., 2008).

Chitosan nanoparticles fabricated via different preparation protocols have been in recent years widely studied as carriers for therapeutic proteins and genes with varying degree of effectiveness and drawbacks. In general, it was demonstrated that the polyionic coacervation process for fabricating protein loaded with chitosan nanoparticles offers simple preparation conditions and a clear processing window for manipulation of physico-chemical properties of the nanoparticles (for example size and surface charge), which can be conditioned to exert control over protein encapsulation efficiency and subsequent release profile. The weakness of the chitosan nanoparticle system lies typically with difficulties in controlling initial burst effect in releasing large quantities of protein molecules (Gan and Wang, 2007).

Naturally occurring polymers, such as chitosan, have been extensively studied as carriers for therapeutic protein and gene delivery systems. β-Lactoglobulin (β-LG) is a member of the lipocalin superfamily of transporters for small hydrophobic molecules. Agudelo et al. (2013) examined the binding of milk β-Lactoglobulin with chitosan of different sizes such as chitosan 15, 100, and 200 KD in aqueous solution at pH 5 to 6, using FTIR, CD, and fluorescence spectroscopic methods.

In their study, structural analysis showed that chitosan binds β-LG via both hydrophilic and hydrophobic contacts with overall binding constants of $K_{\beta-LG-ch-15} = 4.1 \pm 0.4 \times 10^3 \text{ M}^{-1}$, $K_{\beta-LG-ch-100} = 7.2 \pm 0.6 \times 10^4 \text{ M}^{-1}$, and $K_{\beta-LG-ch-200} = 3.9 \pm 0.5 \times 10^5 \text{ M}^{-1}$ with the number of bound protein per chitosan ($n$) 0.9 for ch-15, 0.6 for ch-100, and 1.6 for ch-200. Chitosan 100 KD forms stronger complexes with β-LG than chitosans 200 and 15 KD. Polymer binding did not alter protein conformation inducing structural stabilization. Chitosan 100 is a stronger protein transporter than chitosan 15 and 200 KD (Agudelo et al., 2013).

Biodegradable chitosan of different sizes were used to encapsulate antitumor drug doxorubicin (Dox) and its N-(trifluoroacetyl) doxorubicin (FDox) analogue (Sanyakamdhorn et al., 2013). In their study, the structural analysis showed that Dox and FDox bind chitosan via both hydrophilic and hydrophobic contacts with overall binding constants of $K_{Dox-ch-15} = 8.4 \pm 0.6 \times 10^3 \text{ M}^{-1}$, $K_{Dox-ch-100} = 2.2 \pm 0.3 \times 10^4 \text{ M}^{-1}$, $K_{Dox-ch-200} = 3.7 \pm 0.5 \times 10^5 \text{ M}^{-1}$, $K_{FDox-ch-15} = 5.5 \pm 0.5 \times 10^4 \text{ M}^{-1}$, $K_{FDox-ch-100} = 6.8 \pm 0.6 \times 10^5 \text{ M}^{-1}$, and $K_{FDox-ch-200} = 2.9 \pm 0.5 \times 10^4 \text{ M}^{-1}$, with the number of drug molecules bound per chitosan ($n$) ranging from 1.2 to 0.5. The order of binding is ch-100 > 200 > 15 KD, with stronger complexes formed with Dox than FDox.

The molecular modeling showed the participation of polymer charged NH$_2$ residues with drug OH and NH$_2$ groups in the drug–polymer adducts. The presence of the hydrogen-bonding system in FDox-chitosan adducts stabilizes the drug-polymer complexation, with the free binding energy of $-3.89 \text{ kcal/mol}$ for Dox and $-3.76 \text{ kcal/mol}$ for FDox complexes. The results showed that chitosan 100 KD is a more suitable carrier for Dox and FDox delivery (Sanyakamdhorn et al., 2013).

The objectives of this study were to obtain chitosan nanoparticles by polymerizing methacrylic acid for the
loading of NPK fertilizers. The reaction and stability of chitosan nanoparticles suspension containing N, P and K were estimated by UV-visible transmission spectrophotometry and TEM.

MATERIALS AND METHODS

Chitosan (CS) (MW 71.3 kDa, degree of deacetylation 85%) was purchased from Sigma-Aldrich (Germany). All reagents were of analytical grade. Potassium persulfate (K\textsubscript{2}S\textsubscript{2}O\textsubscript{8}) and methacrylic acid (MAA) were purchased from Sigma-Aldrich (Germany). Calcium phosphate (Ca\text{H}_{2}PO\textsubscript{4})\text{H}_{2}O, urea (CO\text{ (NH}_{2})\text{) and potassium chloride KCl} were purchased from Sigma Chemical Co. (St. Louis, USA).

Preparation of CS-PMAA nanoparticles

As recommended by de Moura et al. (2008) and Corradini et al. (2010), the CS-PMAA nanoparticles were obtained by polymerizing MAA in CS solution in a two-step process. In the first step, 0.2 g chitosan was dissolved in methacrylic acid aqueous solution (0.5%, v/v) for 12 h under magnetic stirring.

In the second step, with continued stirring, 0.2 mmol of K\textsubscript{2}S\textsubscript{2}O\textsubscript{8} was added to the solution, until the solution became clear. The polymerization was subsequently carried out at 70°C under magnetic stirring for 2 h which leads to the formation of CS-PMAA nanoparticle solution, which was thereafter cooled in an ice bath.

Description of CS-PMAA nanoparticles

The morphology and size of the CS-PMAA nanoparticles were investigated using a JEOL 1010 transmission electron microscope at 80 kV (JEOL, Japan). One drop of the nanoparticle solution was spread onto a carbon-coated copper grid and was subsequently dried at room temperature for transmission electron microscopy (TEM) analysis. The sizes of the nanoparticles were determined directly from the figure using an Image-Pro Plus 4.5 software. The value is an average size of three parallels.

The percentage transmittance of CS-PMAA nanoparticle solution was measured using UV-visible spectrophotometer in the wavelength range from 330 to 730 nm. The readings were recorded and plotted graphically.

Loading of NPK fertilizer in chitosan nanoparticles

The sources of N, P and K used were urea, calcium phosphate, and potassium chloride, respectively. These substances were used separately. The loading of NPK fertilizers in chitosan nanoparticles was obtained by dissolving suitable amounts of NPK into 100 ml of CS-nanoparticle solution under magnetic stirring for 8 h at 25°C. The following concentrations: i) 500 ppm of N; ii) 60 ppm of P and iii) 400 ppm of K were finally obtained in each solution.

The maximum P solution concentration was of 60 ppm because the solution precipitates at higher concentrations. The resulting solutions had a pH of 4.5.

Measurement of zeta potential of CS-PMAA and CS-PMAA-NPK nanoparticles

The zeta potential measurements of CS and CS-NPK nanoparticles were taken on a Zetasizer NanoZS (Malvern Instruments, Worcestershire, UK). The measurements were carried out after equi-librating the prepared samples at pH 4.5 at 25°C. All analyses were performed in triplicate.

RESULTS

Figure 1 shows a transmission electron microscopy (TEM) photo of the chitosan nanoparticles (CS-PMAA). These nanoparticles showed a rather spherical shape with a homogeneous size distribution. The mean diameter of CS nanoparticles was of approximately 20±2 nm. Figure 2 shows the UV-visible transmittance spectra of the CS-PMAA nanoparticles. The interaction between CS and PMAA may be elucidated. The CS-PMAA species presented different values at the wavelength range (from 330 nm to 580 nm) reaching a maximum value of 83.4% at 630 nm.

Careful examination of Figures 3, 4 and 5 indicates that all nanoparticles accumulated presented a homogenous morphology with regular particle size distribution and a spherical shape. TEM micrographs of nanoparticles of CS-PMAA either alone or in combination with nitrogen, potassium or phosphorus show that the mean diameter of the CS-PMAA nanoparticles in suspension was approximately 20±2 nm and increased with the addition of the different compounds. The percentage increase in the mean diameter was of 54% with the addition of phosphorus of 31% with the addition of potassium and of 19% with the addition of nitrogen. Figure 6 shows the difference in zeta potential of CS-PMAA nanosolution and the other solutions with N, P and K at pH 4.5.

DISCUSSION

During the preparation of chitosan nanoparticles, it was observed that the chitosan solution in methacrylic acid (MAA) changed from a clear to a semiturbid suspension. This conversion is an evidence of the formation of chitosan nanoparticles with MAA. According to the mechanism proposed by de Vasconcelos et al. (2006), the formation of nanoparticles occurs via inter and intra-molecular linkages between PMAA carboxyl groups and amino groups of chitosan during the process of polymerization of MAA.

CS molecules in solution are in cationic electrolytic form, which tends to make the formation of specific structures via electrostatic reactions with MAA easy and leads to the makeup of CS-PMAA nanoparticles through polymerizing MAA in the presence of CS (de Moura et al., 2008; Zhang et al., 2010). This is in agreement with results for similar systems using acrylic acid reported in the literature (de Vasconcelos et al., 2006). It is known that the interplay of other reactions may also contribute to the stability of systems formed by CS and PMAA, such as hydrophobic association of methyl groups proceeding (Chen et al., 2007; de Moura et al., 2008).

CS-PMAA nanoparticles were formed by the reaction
between positively charged CS and negatively charged PMAA (de Moura et al., 2008). These results are very interesting, since they do not only show that CS-PMAA nanoparticles are pH sensitive, but that by controlling the pH, their surface may be charged either positively or negatively, which is of key value to know the solution stability state as well as for their applications (Wu et al., 2006; de Moura et al., 2008).
Figure 3. TEM micrograph obtained for chitosan nanoparticles loaded with potassium (K).

Figure 4. TEM micrograph obtained for chitosan nanoparticles loaded with nitrogen (N).
Figure 5. TEM micrograph obtained for chitosan nanoparticles loaded with phosphorus (P).

Figure 6. Zeta potential of CS-PMAA and CS-PMAA-NPK nanosolutions.
The values of percentage increase in nanoparticle diameter (listed above in the results section) are related to the size of the species separated in the colloidal suspension, and species separated from the urea have smaller value than the accumulated species from the KCl and calcium phosphate. These results are not in accordance with what had been reported in Corradini et al. (2010) study with regard to potassium and nitrogen.

The stability of a colloidal dispersion is removed as a result of the electrical double layer on the surface of the colloidal particles. When an electrolyte is added to a colloidal dispersion, it suppresses the diffused double layer and reduces the zeta potential. This reduces the electrostatic repulsion between the particles and precipitates the colloid. The colloid is so sensitive to ions of opposite sign. A positively charged colloidal dispersion is precipitated by negative ions, these ions are incorporated into the fixed portion of the double layer, reducing the total charge of the particle and the reverse to this is true. The ion with the same charge of the colloidal particle has little effect on precipitation.

The highest Zeta potential value was recorded for CS-PMAA- K solution, that of 85.4 mV which indicates the higher stability of the solution in comparison with that of N (45.3 mV) and that of P (33.6 mV). The zeta potential of the K-nanosolution was higher than that of the chitosan nanosolution (81.3 mV) which proves the high stability of the resulting nanosolution of K. It was found that for the colloidal dispersions of CS-PMAA with phosphate, the addition of 60 ppm of phosphate makes the stability of the colloidal system under the conditions studied reaches a maximum. The dispersions of CS-PMAA combined with 500 ppm of N were higher in stability compared with that of phosphorus. Corradini et al. (2010) showed that above 500 ppm of N a reduction of positive charges is occurring in the colloidal dispersion of CS-PMAA, which is due to the presence of negative groups from the urea molecules. For dispersion with potassium, the stability of solution is confirmed with the addition of 400 ppm. This shows that the presence of CI ions (from KCl) did not affect the stability of colloidal dispersion with the addition of up to 400 ppm (Wu et al., 2006; de Vasconcelos et al., 2006; de Moura et al., 2008; Corradini et al., 2010).

Further studies are needed to understand the mechanism and to optimize the loading of the N and P elements into the CS-PMAA nanoparticles to reach rather stable solutions and to study the effects of nanofertilizers and their applications on plant growth.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES


