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Zeta Potential and Turbidimetry Analyzes for the Evaluation of Chitosan/Phytic Acid Complex Formation

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Abstract

The aim of this work was to study the possible complex formation between chitosan and phytic acid. Zeta potential and turbidity measurements were used as a basis to confirm the possible complex between these two molecules. The obtained results showed that chitosan at a concentration of 0.1% (w/v) were soluble in 0.1% acetic acid solution. This concentration of the acetic acid was the lowest that allows chitosan to dissolve. A positive surface charge of chitosan was recorded in the pH interval from 1 to 7. The highest zeta potential values were obtained at pH < 5 and decreased significantly at pH 6 and 7. Regarding phytic acid, it was soluble in deionized water and acetic acid whatever the concentration of the acetic acid and in the entire pH range 2-10. Phytic acid had negative surface charge in deionized water and in 0.1% acetic acid, but was slightly positively charged in 5% acetic acid solution. The solubility of chitosan was decreased by the presence of phytic acid. The formation of chitosan/phytic acid complex as monitored by measuring the zeta potential does not allow us to conclude that the formation of this complex is possible.

Keywords: chitosan, phytic acid, zeta potential, turbidity, complex

1. Introduction

Chitosan is a linear natural polysaccharide composed of several units of D-glucosamine and N-acetyl-D-glucosamine linked by β (1-4) glycosidic bonds. It can be found in the mycelium of fungi in combination with other polysaccharides, but it is most often obtained by deacetylation of chitin in the solid state under alkaline conditions or by enzymatic hydrolysis in the presence of chitin deacetylase (Dash, Chiellini, Ottenbrite, & Chiellini, 2011). Since chitosan can be considered as a strong base because it has primary amino groups with a pKa value of 6.3, it is readily soluble in dilute acid solutions with a pH less than 6. The presence of amino groups indicates that the pH significantly changes the state of charge and the properties of chitosan. At a pH below its pKa, the chitosan is a polycation whereas at a pH ≤ 4, it is completely protonated (Jayakumar, Menon, Manzoor, Nair, & Tamura, 2010). After protonation of the amine functions, the electrostatic repulsion between the NH_3^+ groups lead to the destruction of inter-chain attractive interactions such as hydrogen bonds and hydrophobic interactions and consequently the solubility of the chitosan. On the other hand, as the amines groups become deprotonated, chitosan loses its electric charge and becomes insoluble when the pH increases above 6. Thus, the state transition soluble-insoluble chitosan occurs at its pKa value is in the vicinity of a pH between 6 and 6.5 and the value of pKa is strongly dependent on the degree of N-acetylation, the chitosan solubility depends also on the degree and the method of deacetylation used (Pillai, Paul, & Sharma, 2009). Due to its biocompatibility, chitosan is widely used for biomedical and pharmaceutical applications. Some studies have shown that the modified chitosan has an inhibitory effect on the growth of tumor cells. This property was used by conjugating chitosan with 5-fluorouracil (5-FU) to provide a macromolecular system with strong anti-tumor activity and reduced side effects. Indeed, the strong anti-tumor activity exhibited by 5-FU is accompanied by undesirable side effects. An *in vivo* study showed that chitosan-5FU has a strong chance of survival against lymphocytic leukemia in mice and it says no acute toxicity, even at high doses (Qi & Xu, 2006; Toshkova et al., 2010). It is also used in different applications such as gene and drug delivery.

Phytic acid is a ubiquitous plant component which is 1-5% by weight of most cereals, nuts, pulses, oil seeds, spores and pollen. It typically represents 60-90% of total phosphorus in the seed. It is found as a mixture of Ca/Mg/K-salts in the discrete areas of the seeds as the aleuronic layer of wheat and rice (Graf & Eaton, 1990). The appropriate chemical designation of phytic acid is myo-inositol hexaphosphoric acid. This molecule is highly charged with six phosphate groups extended from the center of the myo-inositol ring. The X-ray crystallographic analysis of the crystalline sodium phytate showed the exact structure of phytic acid as the ester of myo-inositol hexaorthophosphate with phosphates in positions C1, C3, C4, C5 and C6 in the axial position and the C2 is in the equatorial position. Phytic acid contains six functional groups as strong acids which are completely dissociated in solution (pKa 1.1-3.2), three protons as weak acids (pKa of 5.2 to 8) and three very low acidic protons (pKa 9.2 to 12). These pKa values simply that phytic acid will be highly negatively charged over a wide range of pH (Somasundar et al., 2005) and have great potential to bind positively charged species such as cations or proteins and chitosan (Konietzny & Greiner, 2003). Because of the multiplicity of reactive phosphate groups, phytic acid can complex cations (bi or trivalent) in a phosphate group itself, between phosphate groups of the same molecule or between phosphate groups of different molecules of phytic acid. So, this acid corresponds to the classic definition of a chelating compound (Cheryan & Rackis, 1980). Stability and solubility of the complexes in which phytic acid is involved depend on the cation type, the pH, the molar ratio phytate-cation and the presence of other compounds in the solution. Most phytates tend to be more soluble at low pH values. The pH value below which the solubility increases is about 5.5-6 for Ca-phytate, 7.2 to 8 for Mg-phytateand 4.3-4.5 for Zn-phytate. However, ferric phytate are in soluble at pH ranging from 1 to 3.5 at equimolar ratio Fe^{3+}-phytic acid. Their solubility increases above a pH of 4, reaching 50% at pH 10.When the molar ratio Fe^{3+}- phytic acid is increased to 3.5:1, the solubility increases to a pH < 2, with a maximum of 90% at a pH of 1.5 and alower solubility at pH values above4. The interaction of phytate with proteins depends on the pH of the medium. Phytic acid is known for its ability to form complexes with proteins in both acidic and alkaline pH. This particularity is attributable to the protein surface net electric charge.

Among the most important properties of phytic acid, one can distinguish its anticancer activity. This action has been demonstrated both in vivo and in vitro studies (Verghese, Rao, Chawan, Walker, & Shackelford, 2006). It has been found that this acid has an action against a broad range of cancers such as cancer of the colon, pancreas, liver, and skin, prostate in a variety of animal models. A study showed that oral administration of phytic acid (1-2 g/100 mL) in water significantly reduces aberrant crypt foci induced by azoxymethane in Fisher 344 male rats(Verghese et al., 2006). An increase in LDH release, fragmentation of DNA associated with histones and budding of the cell membrane of carcinoma cells and human colon Caco-2 treated with phytic acid (IP6) were also observed. This indicates that phytic acid can exert anticancer activity through induction of apoptosis. The anticancer activity of phytic acid is based on the hypothesis that exogenous administered IP6 can be internalized and dephosphorylated IP1-5, IP3 and IP4 that can affect cell cycle regulation, growth and cell differentiation malignant when they are involved in signal transduction pathways (Somasundar et al., 2005). Another potential mechanism for the anticancer activity of phytic acid may be via its antioxidant properties. This function of IP6 occurs by chelation of Fe^{3+} and suppression of the formation of hydroxyl radicals. This activity seems to be closely related to its unique structure that resides in the phosphate groups in positions 1, 2, 3 (axial-equatorial-axial) used especially in interaction with iron to inhibit its ability to catalyze the formation of hydroxyl radicals (Konietzny & Greiner, 2003). The anticancer action of IP6 can still be linked to its ability to bind minerals. IP6 by binding with Zn^{2+}, can affect the activity of thymidine kinase, an enzyme essential for DNA synthesis (Somasundar et al., 2005).

Considering the aforementioned information, one can hypothesizes that a complex of phytic acid with cationic polysaccharide chitosan could be a good candidate as a natural drug for cancer treatment. Thus, the study of the possible formation of such complex under different conditions seems to be a key for the application of phytic acid/chitosan complex in the cancer treatment.

2. Materials and Methods

2.1 Chemicals

Certified low and high molecular weight chitosan with average molecular weight of 150 and 600 kDa, respectively, were purchased from Fluka Bio Chemica (Steinheim, Switzerland). The chitosan samples were used without additional purification. Phytic acid of 98% purity in a form of a dodecasodium salt was purchased from Sigma Chemical Co (Sigma-Aldrich, St-Louis, MO, USA). Glacial acetic acid was purchased from the Sigma-Aldrich Co (MO, USA). Hydrochloric acid and sodium hydroxide were purchased from Merck & Co. Inc. (Darmstadt, Germany). HPLC-grade water was used to prepare and dilute all stock solutions.

2.2 Solution pH Adjustment

The pH of the analyzed solutions was measured with a pH meter (SP20 SympHony, VWR) and adjusted to the desired values with either 0.1 N HCl or NaOH solutions. Syringes (2.5 mL) were used for the injection of the chitosan D-glucosamine and oligomers solutions in the Zetasizer 2000.

2.3 Zeta Potential Measurements

The zeta potential measurements of the chitosan, phytic acid and the mixture of the both components were carried out using a Zetasizer 2000 system (Malvern Instruments Ltd., Worcestershire, UK) equipped with a Photon Correlation Spectroscopy (PCS) system. The voltage applied to the driving electrodes of the capillary electrophoresis cell was 150 V. The calibration of the Zetasizer 2000 was made using a standard (DTS5050, Malvern instruments) with standard zeta-potential of -50 ± 5 mV at 25 °C. To ensure the stability of the measurements, before each use, the Zetasizer 2000 cell was rinsed with HPLC-grade water. All experiments were conducted at a stable measurement temperature which was maintained at 25 °C.

2.4 Turbidity Measurements

The possible complex formation between chitosan and phytic acid under different pH and component ratios conditions was determined by measuring the solution turbidity with a Hachturbidimeter (model 2100AN, Hach Company, Colorado, USA) as adapted from (Quoc et al., 2006).

2.5 Experimental Protocol

Stock solutions of each chitosan type (high and low MW-chitosan) and phytic acid (sodium phytate) were prepared by dissolving 5 g of each component in HPLC-grade water for phytic acid and 1% acetic acid to prepare chitosan stock solutions. A total of 100 mL of each solution was prepared. Work solutions were prepared by adjusting the pH of 10 mL of the stock solution by adding few drops of 0.1 N HCl or NaOH. The solution was well homogenized and equilibrated for 30 min before any measurements. This time was considered sufficient to allow all the susceptible groups to react to be completely functionalized. After that, the targeted solution of chitosan, phytic acid and a mixture of the both molecules were injected into the measuring cell of the Zetasizer. This procedure was also used to measure the solutions turbidity. Each analysis was realized in a triplicate and mean values were used for data analysis and comparisons.

2.6 Statistical Analyses

The used full factorial experimental design was entirely randomized. Statistical analysis of the obtained data was performed with SAS software (V8.0, SAS Institute Inc., Cary, NC) at a 95% confidence level. The ANOVA procedure was used to analyze the variance and to determine the significance of the effect of each independent variable.

3. Results and Discussion

3.1 Effect of on Zeta Potential

Statistical analysis of the obtained data showed a high significance of the pH on the zeta potential of chitosan (low and high MW) and phytic acid ($p < 0.001$). As expected, whatever the molecular weight (low or high), chitosan was insoluble in deionized water at a concentration of 0.1% (w/v) even at pH 5 or below, which is less than its pKa (≈ 6.2). However, it was easily soluble in acid 5% (v/v) acetic acid at the same concentration. Regarding phytic acid, it was readily soluble in deionized water at a concentration of 0.1% whatever the solution pH. Thus, zeta potential of the LMW and HMW chitosan was measured as a 0.1% (w/v) solution in a 5% (v/v) acetic acid and the zeta potential of the phytic acid was measured in water and acetic acid.

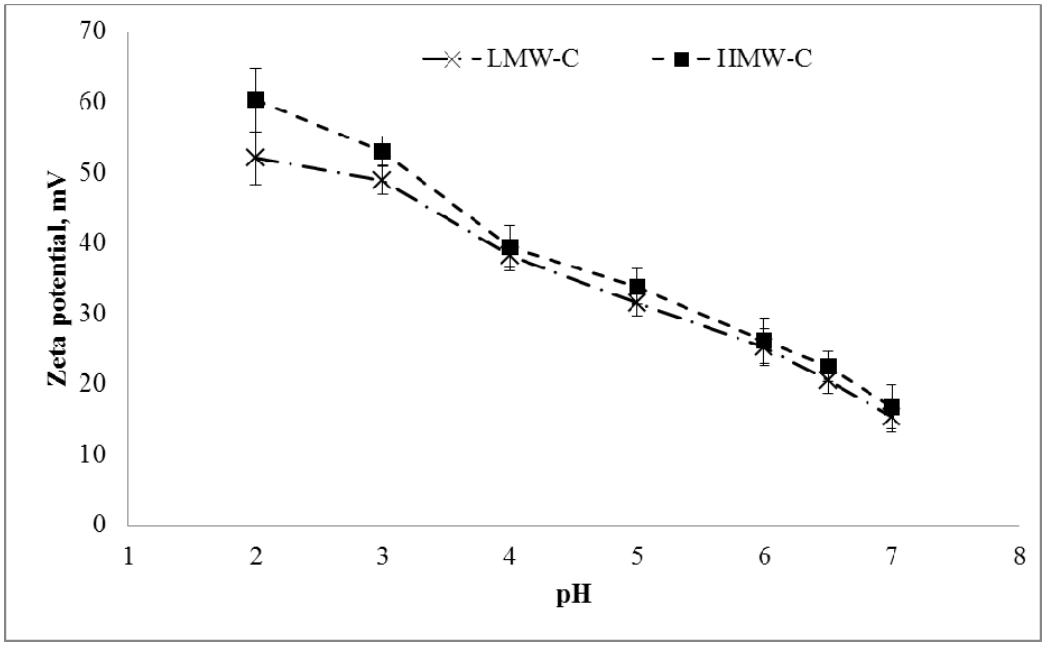

Figure 1. Zeta potential of low molecular weight (LMW) and high molecular weight (HMW) chitosan as a function of pH at chitosan 0.1% (w/v) concentration in 5% acetic acid solution

The effect of pH on the zeta potential of chitosan is shown in (Figure 1) and the statistical analysis of the obtained results did not show any significant difference ($p > 0.05$) of the molecular weight on the values of the zeta potential at each given pH value. At the same time, the effect of pH on chitosan zeta potential was highly significant ($p<0.001$). The highest values were obtained at pH 2 with an average value of 56.20 ± 5.80 mV. By increasing the pH, the zeta potential of chitosan deceased following a quasi-linear manner to reach the lowest value at pH 7 with an average zeta potential of 16.10 ± 1.15 mV. It is important to mention that at pH >5, the positive surface charge of the chitosan it not enough to give strong electrostatic interactions because the zeta potential at pH 5 and 6 is about 32.75 ± 1.62 and 21.55 ± 1.34 mV, respectively. The positive charge of chitosan is obtained after its solubilization in acetic acid by protonation of the amino groups in the form of NH_3^+. The mechanism of this protonation was described by (Rinaudo, Pavlov, & Desbrières, 1999). Being a weak acid, acetic acid is dissociated in an aqueous medium in the following manner: $CH_3COOH + H_2O \rightarrow H_3O^+ + CH_3COO^-$. In acidic medium, chitosan (Chit-NH_2) which is a weak base reacts with protons H_30^+ issued from the dissociation of acetic acid to give the protonated form of chitosan (Chit-NH_3^+) according to the following equilibrium reaction: Chit-$NH_2 + H_3O^+ \rightarrow NH_3^+ +$ Chit-H_2O. The decrease in zeta potential of chitosan as the pH was increased can be explained by the charge neutralization of chitosan due to the addition of NaOH which is a source of strong OH hydroxyl groups that can interact with proton the H^+ of the amine group of chitosan. Thus, the amine groups become deprotonated and chitosan loses its charge to become insoluble from pH 6.5. This insolubility is manifested by the change in the appearance of the chitosan solution that becomes turbid. These results are consistent with the literature where it has been mentioned that at a pH above its pKa (6-6.5), chitosan becomes deprotonated and thus loses its solubility (Konietzny & Greiner, 2003).

Zeta potential of the phytic acid as a function of pH is shown in (Figure 2) and the obtained data indicates the high significance of the effect of the pH on the surface charge of this molecule. Analysis of the phytic acid zeta potential curve indicated 3 distinctive zones: the most important one is in the pH range 4-10 in which the phytic acid is characterized by a strong negative surface charge which varies from -29.40 ± 1.18 and -62.25 ± 3.48 mV at pH 4 and 10, respectively. At pH 2 and 3, the net surface charge of the phytic acid molecule is very close to zero and a slight positive charge was observed at pH 1 with an average value of 19.69 ± 2.16 mV. This molecule acquires the negative surface charge due to its solubilization which is followed by a loss of Na^+ ions from the phosphate groups. These ions react in the presence of water molecule by the following equation: $Na_2^+ \; 2 \; H_2O \rightarrow 2NaOH + H_2$. The formation of NaOH after this reaction explains the initial pH of the basic solution of phytic acid, which was around 10.5. The change in the zeta potential is explained by the fact that during the acidification of the phytic acid solution by adding hydrochloric acid, the H^+ protons of the acid compete with the Na^+ ions to

interact with the water molecules to form the hydronium ion H_3O^+. Thus, Na^+ ions are associated again to the phytic acid molecule which is negatively charged, a fact which causes the decrease in the negative surface charge of this molecule.

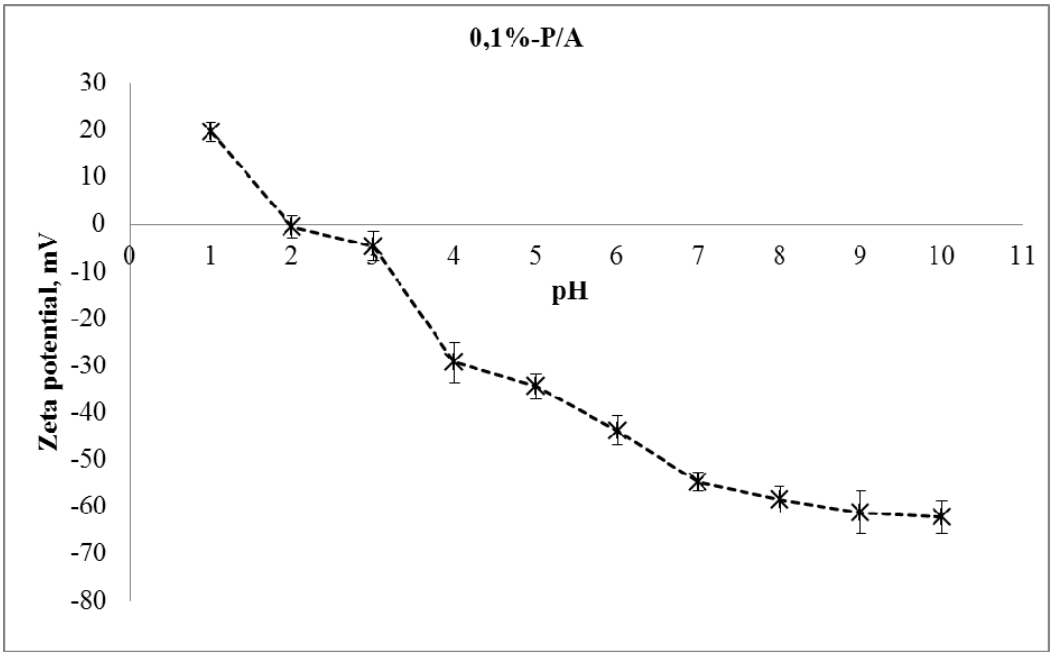

Figure 2. Zeta potential of phytic acid as a function of pH at 0.1% (w/v) concentration in water

3.2 Chitosan Phytic Acid Mixture

After determination of the zeta potential of chitosan and phytic acid, the formation of chitosan/phytic acid complex was considered as being possible since the chitosan is positively charged and that phytic acid has a negative surface charge. We chose to study the possibility of forming the complex with low molecular weight chitosan, because it is easily absorbed in the human intestine more than the high molecular weight one. The results of the variation of the zeta potential of chitosan/ phytic acid mixtures at different ratios are shown in Figure 3. Data show that the surface charge of the particles suspended in the 5% acetic acid solution is positive over the entire range of pH 2 to 7 and for all of the selected concentrations. This charge decreases by increasing the solution pH. The results also showed that the surface charge of these particles has not been affected by the variation of the concentration of phytic acid. In addition, the zeta potential of these solutions along the entire pH range of 2-7 is quite similar to that of the 0.1% solution of chitosan in acetic acid. This may lead one to hypothesize that the chitosan/phytic acid complex is not formed because it was expected that the zeta potential of the complex potential is lower than chitosan alone at each pH value.

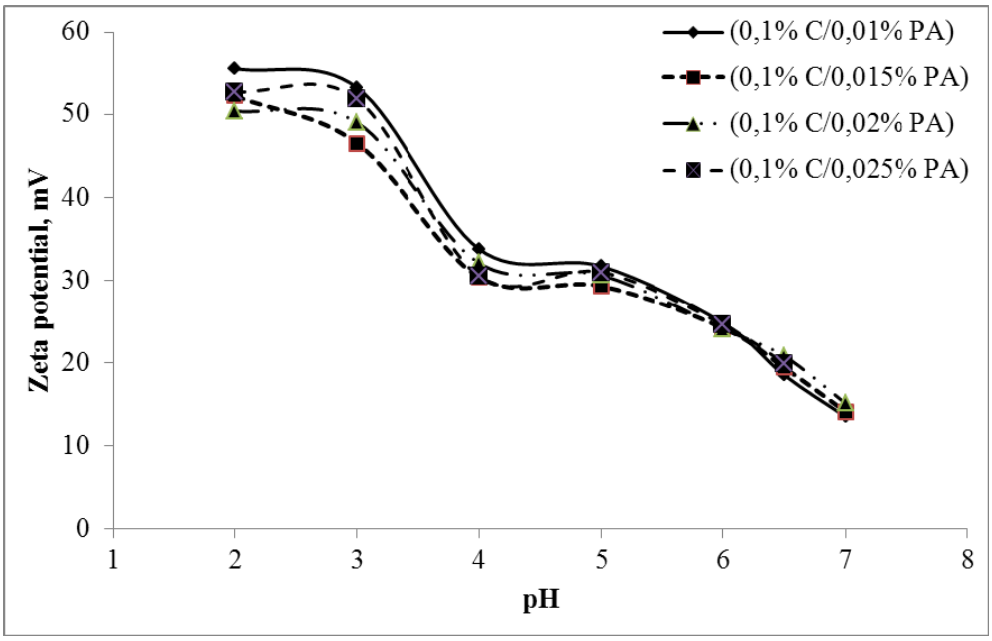

Figure 3. Zeta potential of the mixture chitosan (C) with phytic acid (PA) as a function of pH at chitosan concentration of 0.1% (w/v) and 0.01, 0.015, 0.02 and 0.025% (w/v) phytic acid

Chitosan and phytic acid solutions were prepared alone and were used as a second method of contacting between chitosan and phytic acid by mixing equal volumes of each solution and the pH was adjusted to the desired value. The results of the zeta potential measurements are shown in Figure 4. From this figure, it was found that there is no significant difference between the zeta potential of chitosan dissolved in acetic acid and that of the particles obtained by mixing chitosan with phytic acid in the pH range from 2 to 7. These results are similar to those obtained by the first mixing method which consisted of a dry mix of the two components followed by dissolving the mixture in acetic acid. This leads us to make the same assumption indicating that the chitosan/phytic acid is probably not formed. To confirm or refute this hypothesis, zeta potential measurements of phytic acid dissolved in 5% acetic acid at 0.03% over the pH range from 2 to 7 were carried out. The results showed that phytic acid was unchanged at pH 2-5 and had a very weak positive surface at pH 5-7 whereas it was negatively charged when it was dissolved in deionized water at the same concentration. This charge reversal of phytic acid can be explained by the fact that in the presence of acetic acid, the majority of water molecules have been converted into hydronium ion, which reduces the probability of Na^+ ions of the phosphate groups in the phytic acid molecule to be dissociated to be able to NaOH. This is confirmed by the initial pH of the acid solution of phytic acid dissolved in 5% acetic acid at a rate of 0.03% which was 2.55. These results may confirm the hypothesis of absence of a complex formation since it was found that in 5% acetic acid, chitosan and phytic acid have positive surface charges, a condition which is not favorable tom form complex.

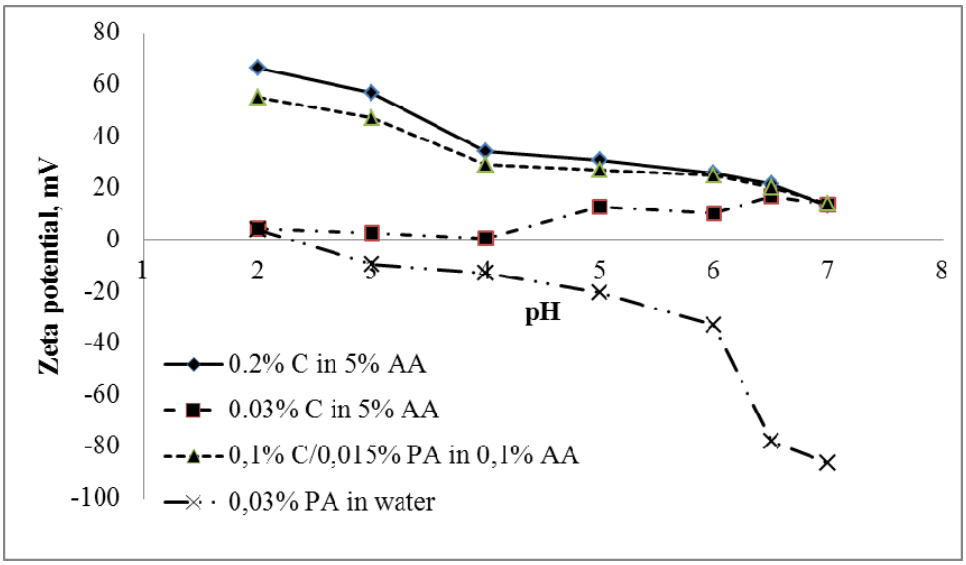

Figure 4. Zeta potential of chitosan (C) alone in acetic acid (AA), chitosan (C) with phytic acid (PA) in 5% acetic acid (AA), phytic acid (PA) alone in 5% acetic acid (AA) and phytic acid alone (PA) in water

3.3 Turbidity Measurement

The first method used for the implementation of the contact between the low molecular weight chitosan and phytic acid consisted of mixing the two powders together in a beaker and then 5% acetic acid solution was added. Several solutions were thus prepared by maintaining the concentration of the chitosan at 0.1% (w/v) and varying the concentration of the phytic acidin the mixture in the range of 0.01-0.1% (w/v).The obtained turbidity measurements of these solutions were performed and the results are shown in (Figures 5, 6). It was found that when the concentration of phytic acid in the mixture decreases, the solutions become increasingly clear. This is confirmed by the instrumental measurements of turbidity as shown in Figure 5. The turbidity of the solutions was decreased from 287 NTU for phytic acid concentration of 0.1% down to 5.12 NTU for phytic acid concentration of 0.01%. The turbidity of these solutions was due to the insolubility of chitosan in the presence of high concentrations of phytic acid. Since phytic acid dissolves faster than chitosan (a few seconds for phytic acid compared to an hour or more for chitosan) and high levels of phytic acid, it was possible that a steric hindrance occurred between the insolubilized chitosan molecules and phytic acid. This phenomenon makes hydronium ion H_3O^+, resulting from the dissociation of acetic acid, inaccessible to the amine groups of chitosan, a fact that prevented the solubility of chitosan. Therefore, this phenomenon will be reduced at low levels of phytic acid in the mixture, a fact which may explain the good solubility of chitosan at phytic acid concentrations which gave solution turbidity of 30 NTU and less.

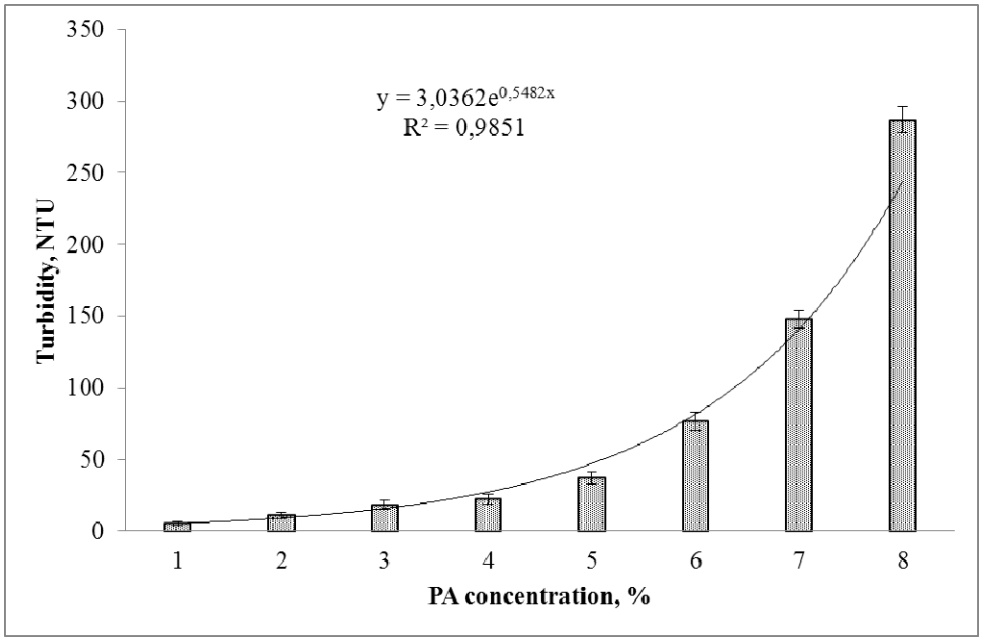

Figure 5.Variation of the 0.1% (w/v) chitosan solution turbidity solubilized in 5% acetic acid as a function of the added phytic acid (PA) concentration

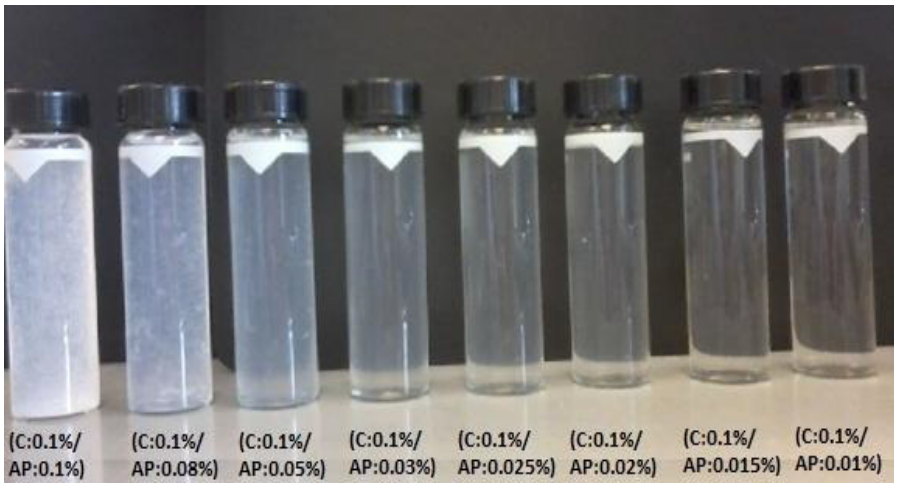

Figure 6.Visual observation of the chitosan (C) phytic acid (PA) mixture solution prepared in a 5% acetic acid

3.4 Turbidity/Zeta potential Combination

From the measurements of the turbidity of chitosan and phytic acid mixtures in different concentrations of the latter, solutions with turbidities below 30 NTU to monitor the zeta potential as a function of pH. In these solutions, chitosan concentration was fixed at 0.1% (w/v) while the concentration of phytic acid was varied in the following interval: 0.1%, 0.025%, 0.02%, 0.015% and 0.01%. Before measuring the variation of the zeta potential of these solutions as a function of pH, turbidity measurements were performed to ensure that the chitosan in the presence of phytic acid remains soluble in the same pH range in which chitosan solution alone was soluble (Figure 7).

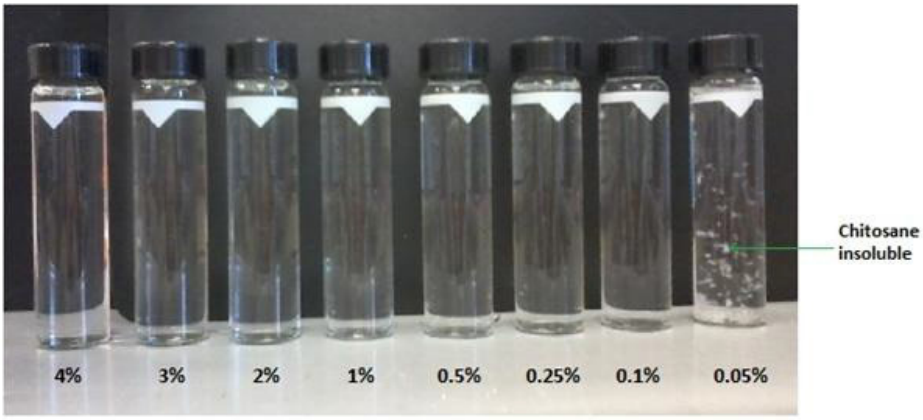

Figure 7. Visual validation of the solubility of chitosan (C) mixed with phytic acid (PA) in a 5% acetic acid

At the same time, Figure 8 shows that the phytic acid solution at a concentration of 0.1% in deionized water has a very low turbidity compared to that of chitosan and mixtures of chitosan/phytic acid over the entire pH range. The maximum turbidity reached by this solution was 0.32 NTU at pH 2, while the minimum turbidity was 0.089 NTU at pH 7. For the 0.1% chitosan solution in 5% acetic acid, the turbidity was almost constant at pH ranging from 2 to 6. This turbidity reached a minimum value of 0.748 NTU at pH 5 and a maximum value of 0.874 NTU at pH 6. At pH 7, the turbidity increased sharply to 22.6 NTU. This increase is an indication of insolubility of chitosan as the pH of the medium exceeded the pKa of chitosan. In the case when mixtures of chitosan and phytic acid were used, it has been observed that for all concentrations, turbidities were far superior to those of the chitosan solution for the entire range of pH from 2 to 7 but remained below 30 NTU. It was also noticed that at each pH in the range from 2 to 6, the higher the concentration of phytic acid in the mixture was the higher the turbidity of the solution was observed. However, the turbidity was minimal for all concentrations studied at pH 5. At pH 7, the turbidity exceeds 30 NTU for all concentrations and reached 60 NTU at a ratio Chitosan: 0.1%/Phytic acid 0.02%. Thus, the turbidity of the mixture of chitosan/phytic acid was significantly affected by the pH of the medium when the latter exceeds the pKa of chitosan.

3.5 Optimizing the Acetic Acid Concentration to Form the Complex

As noted from the previous experiences, phytic acid had a positive surface charge in 5% acetic acid. Thus, the behavior of phytic acid in 5% acetic acid is not favorable for the complex formation chitosan/phytic acid as the two molecules have positive surface charges a fact that will favorable for electrostatic repulsions rather than attraction. To overcome this problem, an objective was defined to find the optimal concentration of acetic acid in which chitosan is soluble and phytic acid will have negative surface charge. To do this, different solutions of acetic acid concentrations of less than 5% were prepared and the lowest concentration in which chitosan was soluble has been selected to be used for the complex preparation. Chitosan was solubilized in the following acetic acid solutions: 4%, 3%, 2%, 1%, 0.5%, 0.25%, 0.1% and 0.05%. Chitosan was soluble until a concentration of 0.1% acetic acid (Figure 8). The obtained data showed that phytic acid has a negative surface charge when it was dissolved in 0.1% acetic acid solution at a concentration of 0.015% in the pH range from 3 to 10. It had a slight positive charge of 6.2 mV at pH 2. Acetic acid dissociates in water to form H_3O^+ as explained above. But since its concentration is low (0.1%), enough H_2O molecules will be available to react with the sodium ion Na^+ of the phytate molecule the following reaction: $2Na^+ + 2H_2O \rightarrow 2NaOH + H_2$. This allows phytic acid to acquire a negative surface charge. At the same time chitosan will still maintain a positive surface charge over the entire pH range from 2 to 7. From these results, it appears that the formation of chitosan/ phytic acid complex is possible and more precisely at pH 5 and 6. At pH 5, the zeta potential of the chitosan was + 51.6 mV and that of phytic acid was -11.6 mV whereas at pH 6 the zeta potential of the chitosan was + 31.6 mV and that of the phytic acid was -26.1 mV.

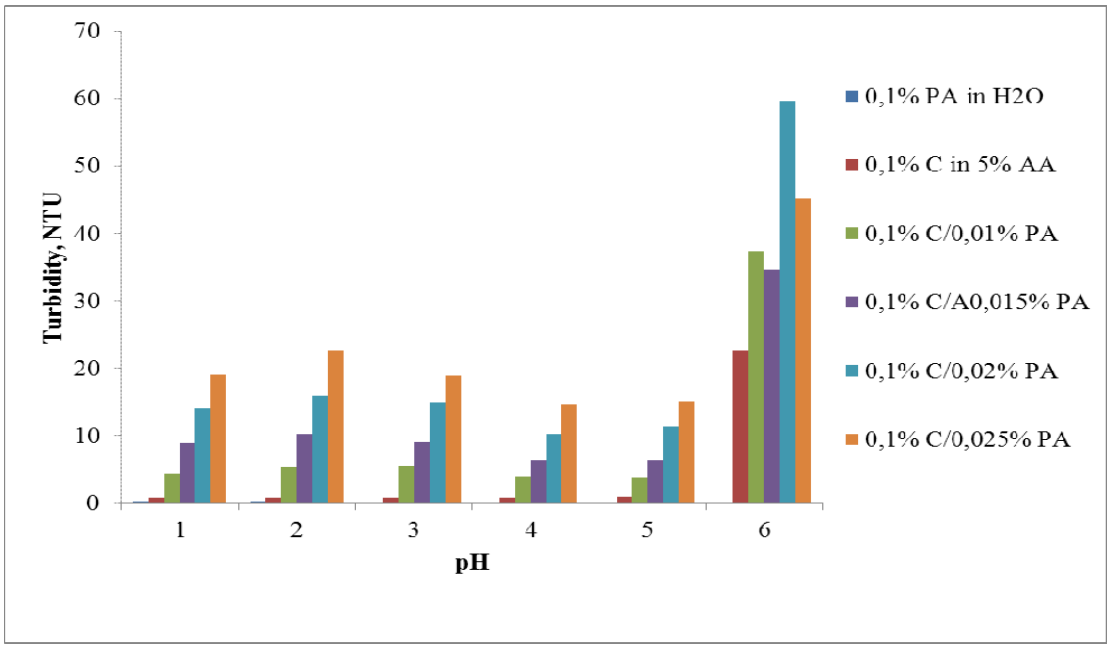

Figure 8. Turbidity of a phytic acid solution at a concentration 0.1% in deionized water, chitosan at a concentration of 0.1% in 5% acetic acid and chitosan solutions mixed with phytic acid at various concentrations as a function of pH

4. Conclusion

The obtained results in this study showed that chitosan at a concentration of 0.1% (w/v) was insoluble in deionized water what even the pH but was soluble in 0.1% acetic acid solution. This concentration of the acetic acid was the lowest that allows chitosan to dissolve. A positive surface charge of chitosan was recorded in the pH interval from 1 to 7. The highest zeta potential values were obtained at pH < 5 and decreased significantly at pH 6 and 7. Regarding phytic acid, it was soluble in deionized water and acetic acid whatever the concentration of the acetic acid and in the entire pH range 2-10. Phytic acid was characterized by a negative surface charge in deionized water and in 0.1% acetic acid. It was slightly positively charged when it was dissolved in 5% acetic acid solution. In addition, the solubility of chitosan was affected by the presence of phytic acid. The formation of chitosan/phytic acid complex as monitored by measuring the zeta potential does not allow us to draw a real conclusion about the possible formation of this complex. Further analyzes are required to confirm the assumption the chitosan can bond to phytic acid.

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