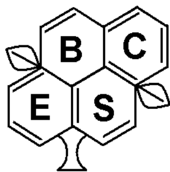

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APPLICATION OF NANOTECHNOLOGY USING WHEY PROTEIN CONCENTRATES TO IMPROVE BIOAVAILABILITY OF IRON

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ABSTRACT

Nanoparticles of whey protein concentrate-chitosan (CS-WPC) complex were prepared with the aim of developing a biocompatible carrier for the oral administration of iron as a nutraceuticals. Effects of pH, concentration of native CS-WPC and iron on the nanoparticles with sodium tripolyphosphate (TPP) prepared by ionic gelation were investigated. CS-WPC were loading with different iron concentration namely; ferrous sulphate. The surface charge of the particles was positive and negative that strongly pH dependent and showed positive charge after iron loading at low protein concentration and was negative at 8 and 12 % when the pH increased to 5.5. The association efficiency (AE) and loading efficiency (LE) of CS-WPC nanoparticles was highly sensitive to formulation pH. This adsorption can be mainly attributed to electrostatic, hydrophobic interactions and hydrogen bonding between WPC and CS. The iron release experiments showed that the nanoparticles prepared with native WPC had favorable properties to resist acid and pepsin degradation in simulated gastric conditions. When transferred to simulated intestinal conditions, the WPC shells of the nanoparticles were not degraded by pancreatin showing the same results with and without enzymes after 6 h. CS-WPC iron nanoparticles at level 0, 3, 6, 9, 12 mg/g protein showed very high bioavailability after evaluated in simulated gastric and intestinal fluids in the presence or absence of the enzymes.

Keywords: Nanoparticles; Whey proteins concentrate; Chitosan; Iron bioavailability

INTRODUCTION

Whey proteins are valuable constituent from the whey (by-product from the cheese industry) and *ß*-Lactoglobulin (*ß*-LG) is the main whey protein component. It is used widely in a variety of foods primarily for their superior gelling and emulsification properties. The physicochemical properties of the whey proteins suggest that it may be suitable for other novel food and nonfood applications. For example, whey protein gels may be used as pH-sensitive hydrogels for the controlled delivery of biologically-active substances (Gunasekaran and Ould, 2006). A hydrogel can be defined as a three-dimensional network that exhibits the ability to swell in water and retains a significant fraction of water within its structure. Whey protein concentrates (WPC) production represents the best means for the utilization of whey proteins (Morr and Foegeding, 1990). WPC are ingredients widely used in the food industry in a variety of formulated products, such as dairy, bakery, meat, beverage and infant formulas due to their excellent functional properties (Kinsella and Whitehead, 1989).

Iron is considered to be one of the essential minerals required by the human body. Although milk is a good source of minerals, its iron content is too low (0.2- 0.5 mg iron/L) to contribute significantly to daily dietary requirements (Flynn and Cashman, 1997). Fortification of milk or dairy products with iron has been considered as a potential approach to deliver this nutritionally important mineral in required quantities to the consumer. Therefore it can help in preventing iron deficiency in humans, which is a major nutritional problem worldwide (Hurrell and Cook, 1990). As a result, a number of dairy products (yogurt, cheese,) have been fortified with iron from different sources (Hekmat and McMahon, 1998; Zhang and Mahoney, 1989_a, 1989_b). However, recurrent problems are associated with iron fortification, including variable bioavailability, formation of sediments, organoleptic defects and the effect of iron on lipid oxidation. The whey protein fraction is slightly modified by iron supplementation of milk (Hekmat and McMahon 1998) but the nature of whey proteins by iron is not determined precisely modified. Some model studies on the

interaction between iron and purified *α*-La and *ß*-Lg indicated that these proteins bind iron with 6.0 and 3.5 Fe^{2+} ions, respectively (Baumy and Brule 1988). Their binding abilities decreased with lowering of pH value.

Nanoparticles are matrix systems of a dense polymeric network in which an active molecule may be dispersed throughout the matrix (Nakache, *et al.*, 2000). Since nanoparticles are submicron and sub-cellular in size, they have versatile advantages for targeted, site-specific delivery purposes as they can penetrate circulating systems and target sites (Vinagradov, *et al.*, 2002). The nanoparticles offer the feasibility to entrap drugs or bioactive compounds within but not chemically bound to them. Various biocompatible and biodegradable biopolymers have been used in the formation of nanoparticles to maximize delivery efficiency and increase the desirable benefits (Coester, *et al.*, 2000; Rhaese, *et al.*, 2003). Whey proteins may also be formed into nanoparticles and albumin nanoparticles have been extensively investigated with respect to their preparation methods and release properties (Langer *et al*., 2003; Loo *et al*., 2004). Human serum albumin (HSA) and bovine serum albumin (BSA) have been used as natural materials for delivery devices. The objectives of this research were to investigate the use of whey proteins as a natural nano-capsular vehicle to carry and improve the bioavailability of iron.

MATERIALS AND METHODS

Materials

Chitosan $(C_6\ H_{11}NO_4)_n$, molecular weight of 100,000 -300,000 D was provided by ACROS ORGANICS New Jersey; USA. Demineralised Whey Protein Concentrate was obtained from FRIESLAND Hiprotal, New York, USA. Sodium tripolyphosphate (TPP), was purchased from ACROS ORGANICS New Jersey; USA. Ferrous sulphate was obtained from SISCO Research Laboratories PVT, Ltd. Mumbai, INDIA; Pepsin 1:60000, from porcine stomach mucosa, crystallized and lyophilized and pancreatin 4X, from hog pancreas were obtained from Sigma Chemical Co.

Methods

Formation of CS-WPC nanoparticles:

WPC solutions at concentrations of 2, 4, 8 and 12 % were prepared and protein concentration measured by the absorbance at 280

nm (Nanodrop). These solutions were prepared by hydrating WPC in deionized water with agitation at room temperature for 1 h. The solution was allowed to rest for 2 h before further treatment in order to permit a good protein hydration as suggested by Beaulieu, *et al*., (2002). CS-WPC nanoparticles were prepared by the method adapted from that reported for CS nanoparticle formation by Janes *et al.,* (2001). WPC solutions at various concentrations and pH values (adjusted with 1 mg/ml HCl and NaOH) were added to CS solutions in aqueous acetic acid (0.1%) to form CS-WPC complexes with CS concentration of 2 mg/ml. Two ml of TPP solution (1 mg/ml) was added as drop wise to 5 ml of CS-WPC complexes, opalescent suspension was formed spontaneously under magnetic stirring at room temperature, and was further examined as nanoparticles. The final pH of the nanoparticle suspension was measured with a Laboratory pH meter (HANNA– instrument, 211 micro processor, USA), and the nanoparticles were characterized immediately. All experiments were performed in triplicates.

Formation of CS-WPC Iron complex nanoparticles:

Ferrous sulphate solution (2%) was added to the prepared WPC solutions at various concentrations and pH values (4.5, 5.5, 6.5 and 7.5) to form whey protein iron complexes with iron concentration of (0, 3, 6, 9, 12 mg iron/g protein) Remondetto, *et al*., (2004). The WPC iron complex solutions were added to CS solutions in aqueous acetic acid (0.1%) to form CS-WPC-Iron complexes with CS concentration of 2 mg/ml (Janes, *et al*., 2001). Two ml of TPP solution (1.0 mg/ml) was added as drop wise to 5 ml of CS-WPC-Iron complexes prepared above; opalescent suspension was formed spontaneously under magnetic stirring at room temperature, and was further examined as nanoparticles. The final pH of the nanoparticle suspension was measured with a Laboratory pH meter (model pH enomenal^{TM}, pH.cond.O_2 VWR), and the nanoparticles were characterized immediately. All experiments were performed in triplicates.

Characterization of the nanoparticles:

Examination of particle size and morphology:

The freshly-prepared nanoparticles were diluted with distilled water and placed on a copper grid coated with carbon (carrier powder) and dried at room temperature. Particle size of nanoparticles and

nanoparticle morphology were examined by transmission electron microscopy, (TEM, JEOL, JEM, 1230 Japan) working at 100 KV.

Determination of surface charge:

Zeta potential of values nanoparticles were measured with Laser Zeta meter (Malvern Instruments) model "Zeta Sizer 2000" for Zeta potential measurements. Ground sample was (0.01 gram) placed in 50 ml double distilled water. The pH values were then adjusted to 4.5, 5.5, 6.5 and 7.5 respectively. The sample was shaked for 30 min, followed by recording pH and measuring zeta potential of the particles.

WPC coating properties:

To determine the association efficiency (AE) and loading efficiency (LE) of WPC on the nanoparticles, triplicate batches of nanoparticles were centrifuged at 30,000g (F21-8X50 ml, Fixed Angle Carbon Fiber Rotor for sovall, Backman, and Jouan centrifuges, SOVALL Instruments DuPont, Newton, Conn.), with temperature adjusted to 20 ºC for 30 min. The WPC content in the supernatant was determined by the absorbance at 280 nm (Nanodrop) and the pellet was vacuum dried and weighted. The AE and LE values were calculated using the following formulae as mentioned by Chen and Subirade (2005).

$$AE = \frac{\text{Total amount of Whey protein} - \text{Whey protein in the supernatant}}{\text{Total amount of Whey protein}} X\,100$$

$$LE = \frac{\text{Total amount of Whey protein} - \text{Whey protein in the supernatant}}{\text{Weight of recovered particles}} X\,100$$

Determination of iron encapsulation capacity of the CS-WPC nanoparticles

The iron encapsulated nanoparticles were prepared by incorporating ferrous sulfate into the CS-WPC complexes to a final concentration of 3, 6, 9 and 12 mg/g protein, prior to the formation of the nanoparticles. For the determination of the iron encapsulation capacity, the iron encapsulated CS-WPC nanoparticles were separated

from the aqueous suspension medium by ultracentrifugation at 30.000 g and 20ºC for 30 min. The amount of free iron in the clear supernatant was determined as mentioned by Shu and Zhu (2002) with 1, 10-Phenanthroline by measuring the absorbance at 510 nm using spectrophotometer (Nanodrop) Iron encapsulation capacity (EC) was calculated with the following equation:

$$EC = \frac{Total\ amount\ of\ Iron - Iron\ in\ the\ supernatant}{Total\ amount\ of\ Iron} X\ 100$$

In vitro release studies

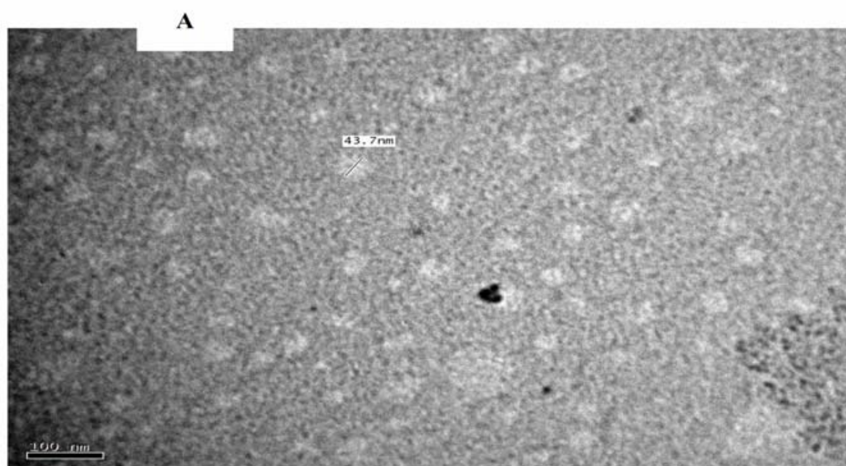
The iron encapsulated CS-WPC nanoparticles separated from 14 ml suspension were re-dispersed into test tubes with 4 ml HCl solution (pH 1.2) at 37°C under agitation for 30 min. The suspension pH was then raised to 7.5 with concentrated NaOH, and 0.2 ml phosphate buffer (0.5M, pH 7.5) was added. The mixture was adjusted to a final volume of 5 ml with distilled water. The iron release in phosphate buffer (pH 7.5) at 37 °C was carried out under agitation for 6 h. At predetermined incubation time, then samples were centrifuged (30.000 g for 30 min) and the iron released was determined by spectrophotometer (Nanodrop), as mentioned above. Following supernatant extraction, pellets were discarded (destructive sampling).

The in vitro release of iron was also evaluated in simulated USP gastric and intestinal fluids in the presence of the enzymes, using the method by Beaulieu *et al*., (2002). Iron encapsulated CS-WPC nanoparticles separated from 14 ml suspension were re-dispersed in 4 ml of 0.1N HCl in a test tube and magnetically stirred for 10 min at 37 °C. Pepsin solution, 0.05 ml (1 mg/ml, 0.1 N HCl), was added to initiate the hydrolysis. The digestion was carried out for 30 min and stopped by raising the pH to 7.5 with NaOH. A concentrated phosphate buffer (0.2 ml; 0.5M, pH 7.5) was added. The reaction was initiated by adding 0.05 ml of pancreatin enzyme (10 mg/ml) prepared in phosphate buffer (0.02M, pH 7.5). The reaction mixture was adjusted to 5 ml with distilled water, and the digestion was carried out for 6 h. The amount of iron released was expressed as a percentage of the total iron encapsulated in the nanoparticles as calculated from the EC value. The iron release experiments were repeated three times.

RESULTS AND DISCUSSION

CS-WPC nanoparticles

CS and WPC showed good miscibility in the solution and no phase separation appeared. Upon addition of TPP, the mixture of CS and WPC changed from clear solution to an opalescent solution, indicating the formation of CS-WPC particles. This result agrees with Chen and Subirade (2005). TEM photographs CS-WPC suspension [Fig 1 (a)] show that the particle size ranged between 13 and 70.6 nm, indicating that nanoparticles were formed and appeared spherical in shape with smooth surfaces. While the interior structure of CS-WPC nanoparticles (the same sample) demonstrates a circular shape consisting of a dark core and light shell [Fig 1 (b)]. Compared to samples [Fig 1 (a)], the shells of the nanoparticles were destructed to some extent in the sample treatment process, showing an irregular surface. In the preparation process, it is supposed that a CS-TPP core was initially generated as an ionic gelation involving positively charged amino groups on the CS molecular chains and negatively charged TPP (Leaver and Horne, 1993). Then WPC molecules in the bulk phase approached the CS-TPP core, readily adsorbed onto interfaces like other surface active macromolecules and a membrane of WPC was formed on the surface of the CS-TPP core through a combination of ionic, hydrophobic interactions and hydrogen bonding. Thus, the CS-WPC nanoparticles with core-shell structure were formed.



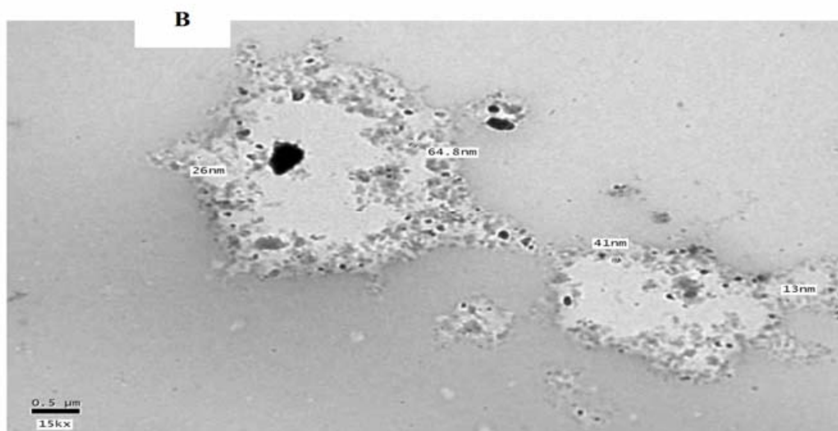


Fig (1) TEM photographs of CS-WPC nanoparticles prepared with 2% concentration of WPC at pH 5.5, A and B represents particle size ranged between 13 and 70.6 nm

Surface charge

The Zeta potential (ζ) is the electrostatic potential at the boundary dividing the compact layer and the diffuse layer. Fig. (2) shows that the effect of pH value and protein concentration on zeta potential (mV) of CS-WPC nanoparticles. The ζ-potential of CS-WPC using different concentration of protein (2, 4, 8 and 12 %) went from positive (+0.5, +16.5, +16.5 and +14.2 mV) to negative (-5.1, -1.1, -10.5 and -8.5 mV) as the pH was increased from 4.5 to 7.5. These results are inagreement with Harnsilawat *et al.,* (2006). The ζ-potential of CS-WPC changed from -9.4 to -10.5 mV and -5.1 to -8.5mV at protein concentration 8 and 12 % as the pH was increased from 6.5 to 7.5 respectively. The magnitude of the negative charge on the chitosan molecules was appreciably lower in the pH range of 4.5 – 5.5 than at higher pH values, which can be attributed to the fact that the anionic carboxylic ($-COO^-$) groups on the mannuronic and guluronic acid groups became partially protonated (-COOH) in this pH range (Draget, 2000). At relatively low pH (<6.5), chitosan was positively charged and tended to be soluble in dilute aqueous solutions, but at higher pH it tended to lose its charge and may precipitate from solution due to deprotonation of the amino groups. Delben and Stefancich, (1998) showed that chitosan can interact with proteins to form either soluble or insoluble complexes. These

interactions may be either physical (e.g. electrostatic) or chemical (e.g. Maillard) in origin. It has been shown that chitosan will form covalent complexes with lysozyme (Song, et *al*., 2002) and *β*-lactoglobulin (Hattori, et *al*., 2000) through a Maillard type reaction.

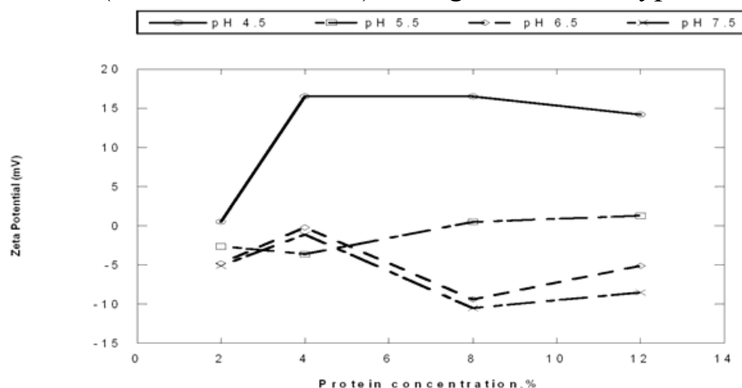
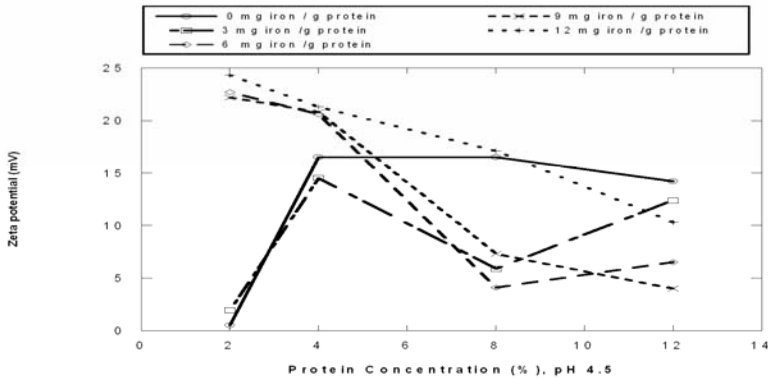
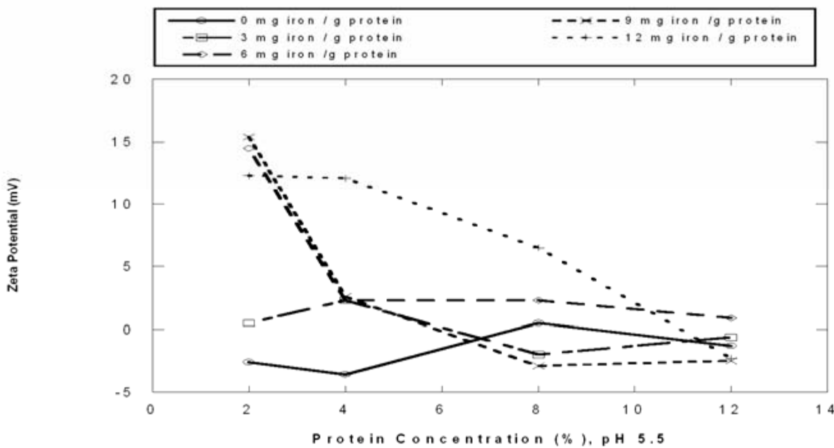


Fig. (2) Effect of protein concentration and pH value on Zeta potential (ζ, mV) of CS-WPC nanoparticles.

The data illustrated in Fig. (3) (A, B, C, D) show the effect of pH and protein concentration on Zeta potential (mV) of CS-WPC iron complex. It was reported that one of the major factors influencing the electrostatic interaction of charged biopolymers in aqueous solutions is the pH, since this affects both the sign (−/+) and magnitude of the charge of them (Guzey and McClements 2006). The effect of pH on the interaction of WPC with chitosan indicated that at pH 4.5 the charge was positive and when loading the iron at different concentrations (3, 6, 9 and 12 mg/g protein) also the positive charge was occurred. The charge increased from +1.9 to +24.3 when the concentration of iron was increased from 3 to 12 respectively at 2% WPC concentration and pH 4.5. The Zeta potential increased when the WPC increased to be 4 and 12% (+14.5, +12.4 in order) but decreased when the concentration was 2 and 8% (+1.9, +5.9 in order). At pH 5.5 the negative charge appears in 8% protein concentration and 9 mg iron /g protein to be -2.9. It also appeared in 12% protein concentration with 9 and 12 mg iron /g protein to be -2.5 and -2.3 respectively. The negative charge increased to -9.12 and -13.5 at pH 6.5 and protein concentration of 8 and 12% in order with 3 mg iron/g protein. When the pH increased to be 7.5 zeta potential was negative at 3 mg iron /g protein and 2% protein concentration but when the concentration of

iron was increased the corresponding positive charge obtained was +10.7, + 0.8 and +20.1 mv. When the protein concentration increased from 4 to 8 and 12% at the same pH value (7.5) the negative charge appeared and increased from -11.1 to -11.6 and then decreased to -5.8 at 12 % protein concentration. Zeta potential reach to minimum negative charge (-0.4) at pH 6.5, 3 mg iron / g protein and 2 % protein concentration but maximum negative charge (-13.5) was recorded at pH 6.5, 3 mg iron / g protein and 12 % protein concentration. The maximum positive charge (+24.3) was observed at pH 4.5, 2% protein concentration and 12 mg iron / g protein but the minimum positive charge (+0.9) was at pH 5.5 ,7.5, 12% and 4% protein concentration or 6 and 12 mg iron / g protein respectively.





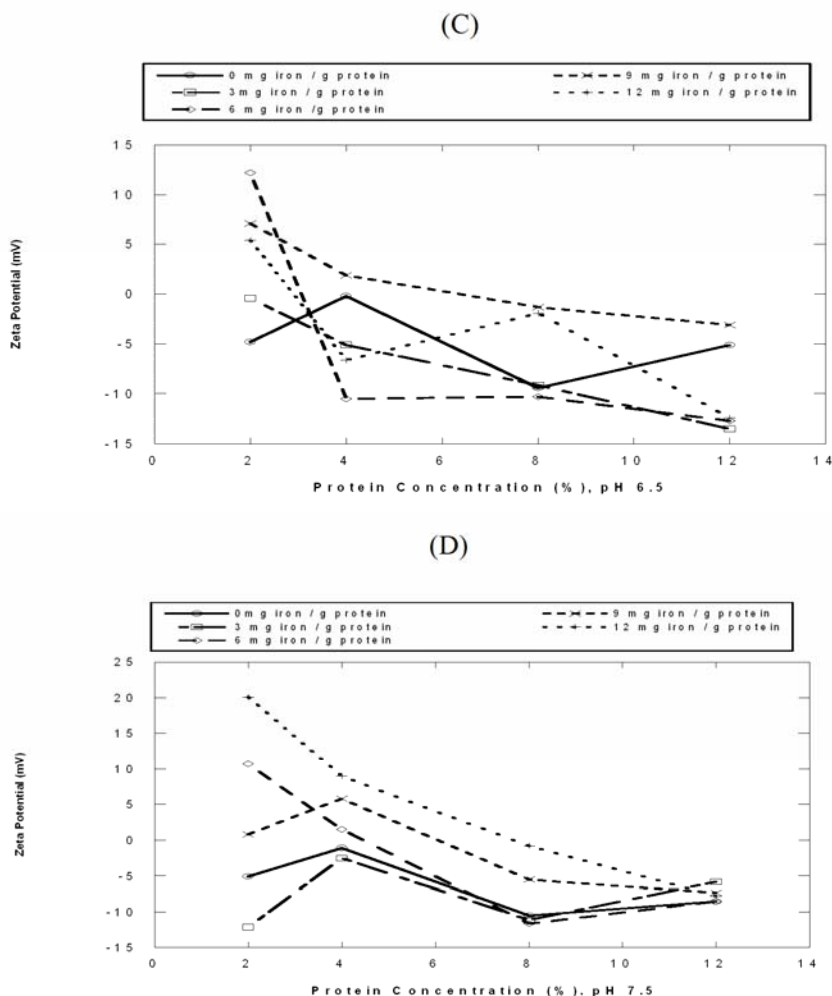


Fig. (3) Effect of protein concentration and pH value on Zeta potential (ζ, mV) of CS-WPC complex in iron nanoparticles form.

Association efficiency (AE) and loading efficiency (LE).

The association efficiency (AE) obtained as a function of pH for CS-WPC nanoparticled prepared with different concentrations of WPC is displayed in Figure (4). The AE obviously increased with increasing pH value until reach to the maximum at pH 6.5 and then decreased at pH 7.5. AE values for native CS-WPC increased steadily until pH value of 6.5. When AE value increased sharply to the

maximum of 68.83, 62.13, 46.02 and 47.02 with protein concentration of 2, 4, 8 and 12 % respectively. The changes of the AE value as a function of pH exhibit three association dominations corresponding to three kinds of interactions between WPC and CS. As a cationic polyelectrolyte, CS (90.1%) was positively charged due to protonation of amino groups on the molecular chain at pH below 9.0. While the net charge on a protein is dependant on pH and interaction of a protein with CS-TPP core surface will therefore vary with pH, which leads to different AE values. Chen & Subirade (2005) explained these changes in the AE when they used *ß*-lg as an amphoelectrolyte with pI of 5.3. When pH value was lower than 4.3, both CS and *ß*-lg were positively charged, strong repulsion prevents association of *ß*-lg on CS-TPP core. When pH value increased in the range of 4.3 to 5.9, where the pI of *ß*-lg exists, intraionic attractions between COO^- and NH_3^+ resulted in seldom residual ionic groups on *ß*-lg. In this pH range, hydrophobic interactions and hydrogen bondings between *ß*-lg and CS are supposed to dominate to explain the steadily increase of the AE value. In fact, it is revealed that the hydrophobic interactions are the most important aspect of protein adsorption onto the nanoparticle surfaces. Further increase of the pH value above 5.9, CS is positively charged, while *ß*-lg becomes negatively charged, the driving force for *ß*-lg association thus, changed from hydrophobic interactions gradually to electrostatic attractions.

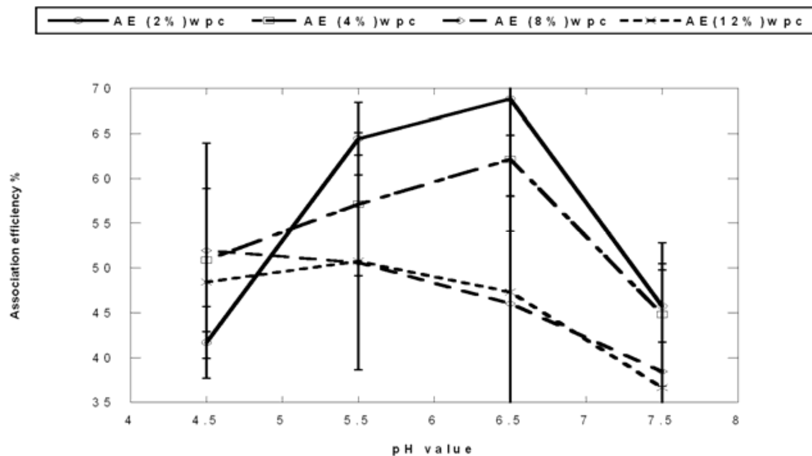


Fig. (4): The association efficiency (AE) obtained as a function of pH for CS-WPC nanoparticles prepared with different concentrations of WPC.

The LE values which measure the amount of WPC on unit weight of nanoparticles were also strongly pH dependent and showed similar changing tendency of AE values as function of pH, as demonstrated in Fig. (5). However, the maximum LE values were recorded at pH 6.5 and with further increase of the pH, LE values decreased. A reasonable explanation for this decrease could be a conformation change of the absorbed WPC. As globular protein, near pI, native WPC apparently adsorbs onto hydrophobic surface in an end on orientation, but with increasing pH value, the surface charge of the WPC increases accordingly, and the high charge density opposite that of the positively charged CS surface leads to switch of the orientation of the WPC on the CS-TPP core surface to side-on in order to maximize electrostatic interactions, which is then followed by decrease of the LE value. The LE value was enhanced dramatically by increasing the concentration of WPC from 2 to 12 %, and then reached to the maximum at concentration 8% but at 12% the LE decreased and reached the minimum value at this concentration. Regardless of the WPC concentration, optimal LE value was achieved at pH 6.5. However, at low pH values (pH 4.5), the adsorption equilibrium reached at concentration of 8 % , while low WPC was coated on CS-TPP core above WPC concentration of 8 % due to strong electric repulsion between native WPC and CS molecular chains, where both of them are strongly positively charged.

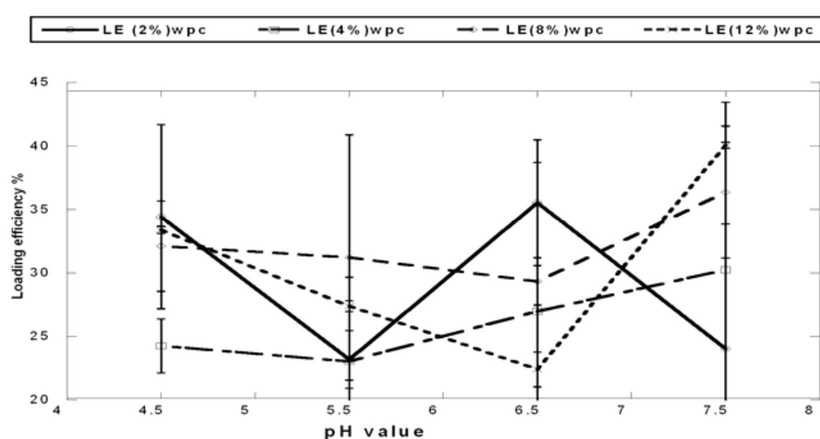


Fig. (5): The loading efficiency (LE) obtained as a function of pH for CS-WPC nanoparticles prepared with different concentrations of WPC.

Encapsulation capacity (EC)

The encapsulation capacity (EC) of CS-WPC iron complex nanoparticles prepared with different concentration of WPC and iron was determined and displayed in Table (1). No significant differences in encapsulation efficiency approximately for all sample studied at different protein and iron concentrations at different pH value. Sugiarto *et al*., (2009) demonstrated that WPC had sites to bind iron (8 sites) and compact structure of whey proteins (globular proteins) may be reasoned in their strong ability to bind added iron.

The encapsulation efficiencies ranged between 99.92 and 99.99 in all samples. It was reported that the solubility of iron was considerably greater in the presence of whey protein isolate (WPI) than in the absence of protein but the solubility decreased gradually with increasing levels of ferrous sulfate in the mixture (Sugiarto *et al*., 2009). The interactions of iron with whey protein molecules in whey protein did not cause significant precipitation of iron-WPI complexes. Some variations in the solubility of WPI in the iron-WPI mixtures, the solubility varied between 80% and 90% with concentrations of added iron. Sugiarto *et al*., (2009) found that whey protein molecules can bind added iron but the extent of binding was much lower than for sodium caseinate.

α-Lactalbumin (α-La) and *ß*-Lactoglobulin (*ß*-Lg), the two major proteins that constitute WPI, have been shown to bind metal ions. α-La can bind up to 6.0 ferrous ions, whereas *ß*-Lg is > 3.5 ferrous ions (at pH 6.6, ionic strength <0.01 M) (Jackson and Lee, 1992). Sugiarto *et al*., (2009) reported that WPI showed maximum binding of approximately 6 mol iron/mol protein (18 mg iron/g protein). Acidification of iron-WPI mixtures caused a change in the amount of iron bound to WPI approximately 8 mg iron/g protein was bound at pH 7.0. This amount decreased markedly to approximately 1 mg iron/g protein as the pH decreased markedly to 5.5 and was only approximately 1-2 mg iron/g protein at pH≤ 5.5. It was found that a decrease in pH from 7.0 to 3.0 caused only a slight change in the solubility (80-90% soluble) of the whey proteins and the iron in iron-WPI mixtures. The acidification caused also a marked decrease in the ability of whey protein to bind iron as the pH lowered to 3.0. Changes in pH generally affected the complex formation between metal ions and proteins as hydrogen ions complete with the metal ions for

binding to protein. At low pH, the reactive side chains of amino groups tend to become protonated, which decreases their affinity for cations, thus reducing their complexation with the protein. The protonation of ionic amino acids at low pH reduced the ability of whey proteins to bind iron. In addition, the change in the pH of the system can lead to reversible conformational changes in the proteins, thereby altering their metal-binding capacity. The conformational changes in the whey proteins at low pH might also alter their ability to bind iron.

Table (1): The encapsulation capacity (EC) obtained as a function of pH for CS-WPC-Iron nanoparticles prepared with different concentrations of WPC and Iron.

Iron Con. (mg/g protein)	Protein Con. (%)			
	2			
	pH value			
	4.5	5.5	6.5	7.5
3	99.9212	99.99192	99.9899	99.9899
6	99.91971	99.9601	99.99243	99.99293
9	99.98047	99.99495	99.99394	99.99495
12	99.98643	99.99433	99.99773	99.99823
	4			
3	99.96363	99.98082	99.99259	99.98249
6	99.98608	99.99141	99.99553	99.99511
9	99.99764	99.98849	99.99743	99.99777
12	99.96844	99.99596	99.99717	99.99768
	8			
3	99.98737	99.98222	99.98687	99.98923
6	99.99495	99.99596	99.99646	99.99495
9	99.99697	99.99697	99.99731	99.99641
12	99.9976	99.99663	99.99621	99.99646
	12			
3	99.98182	99.99293	99.98586	99.98653
6	99.99293	99.99394	99.98687	99.99343
9	99.87767	99.90741	99.97711	99.98721
12	99.7458	99.84277	99.9468	99.99343

Iron release

In vitro release properties of iron into simulated gastric-intestinal tract were evaluated for CS-WPC nanoparticles formed with a CS of 2 mg/ml for WPC Fig. (6) Displays the release profiles of iron in the

absence of digestive enzymes. In the simulated gastric pH (1.2) less than 1% of the iron was released from nanonative after 6 h incubation at 37°C. The iron was released after 6 h from nanonative very slowed down may be need more time than 6 h to released more from CS-WPC.

In order to investigate the release profiles of the CS-WPC nanoparticles in the presence of digestive enzymes, iron release in the simulated gastro-intestinal fluid in the presence of pepsin, or in the presence of both pepsin and pancreatin were studied and demonstrated in Fig. (6). The solid lines present iron release in the simulated gastric fluid (pH 1.2) with pepsin for 6 h and then transferred into the intestinal buffer (pH 7.5, without pancreatin) for 6 h. The dash lines present iron release in the simulated gastric fluid (pH 1.2) with pepsin for 6 h and then transferred into the intestinal buffer (pH 7.5 with pancreatin) for another 6 h. Almost the same release profiles of iron from all kinds of nanoparticles in the simulated gastric fluid in the presence of the pepsin were observed compared to those in the absence of pepsin. The results obtained with and without enzyme didn't observe any differ and after 6 h no release rate from iron to less than 1% for all studied nanoparticles samples.

It is interesting to notice that iron was very weakly released from native CS-WPC nanoparticles at different concentration of protein suggesting a coating of protein with firmer structure on the surface of the CS-TPP core. As demonstrated in Fig. (6) no differences in release profiles were observed for N-native, indicating that the WPC shell could resist degradation of pepsin in the gastric tract. Pepsin is known to preferentially attack peptide bonds involving hydrophobic aromatic amino acids. In its native structure, WPC is known to be resistant to pepsin since its hydrophobic amino acids are located in the internal corn of its calyx-like structure (Morr and Ha 1993). However, the nature of the whey proteins modified by iron is not determined precisely. From above results, it seems that nanonative has potential to be applied as oral administration carriers for nutraceuticals due to desirable properties to resist both acid and pepsin degradation in the gastric tract (Chen and Subirade 2005).

It can be concluded that nanotechnology improved the bioavailability of iron and open the door to new functionalities and applications for nanoparticle delivery systems.

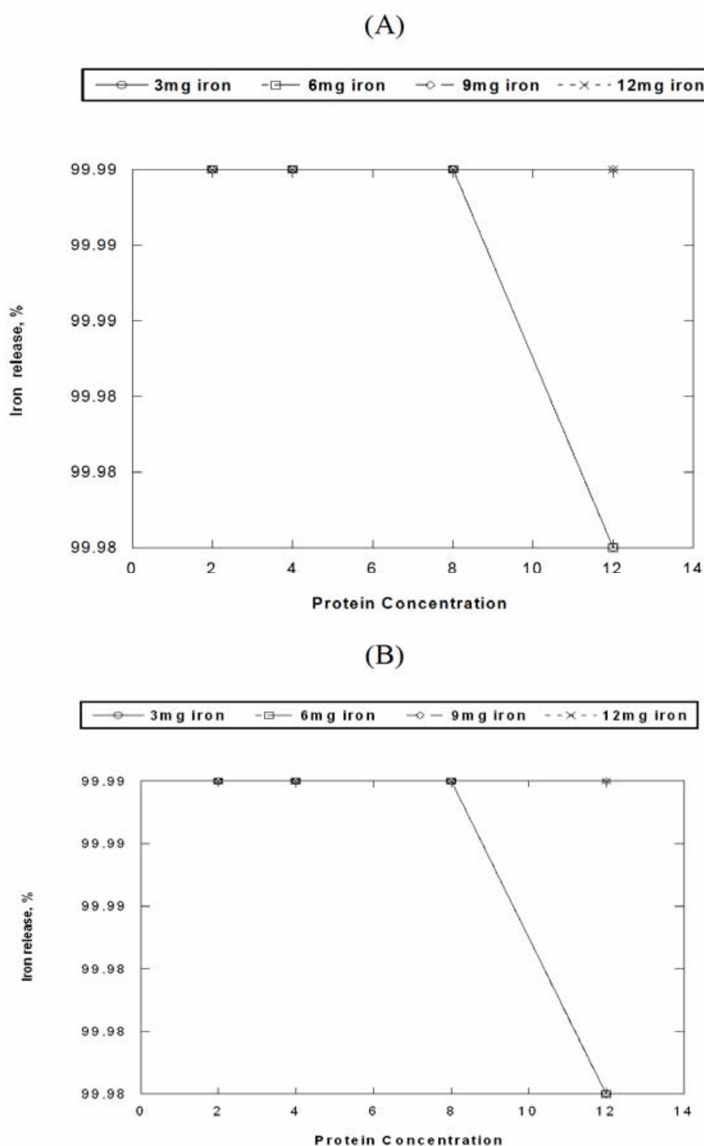


Fig. (6) Release profiles of iron from CS-WPC nanoparticles containing iron in simulated gastric fluids after agitation for 6 h (with (A) or without (B) enzyme) at pH 7.5 and 37°C.

REFERENCES

Baumy, J.J. and Brule, G. (1988) 'Binding of bivalent cations to α-lactalbumin and *β*-lactoglobulin: Effect of pH and ionic strength' Lait, 68: 33–48.

Beaulieu L.; Savoie, L.; Paquin, P. and Subirade, M. (2002). Elaboration and characterization of whey beads by an emulsification/cold gelation process: application for the protection of retinol. Biomacromolecules, 3:239–48.

Chen, L. and Subirade, M. (2005). Chitosan/β-lactoglobulin core-shell nanoparticles as nutraceutical carriers. Biomaterials; 26: 6041 – 6053.

Coester, C.; Kreuter, J.; Briesen, H. V. and Langer, K. (2000). Preparation of avidin-labelled gelatin nanoparticles as carriers for biotinylated peptide nucleic acid (PNA). International Journal of Pharmaceutics, 196:147–149.

Delben, F. and Stefancich, S. (1998). Interaction of food polysaccharides with ovalbumin. Food Hydrocolloids, 12: 291–299.

Draget, K. I. (2000). *Alginates.* In G.O. Phillips, & P.A. Williams (Eds.), Handbook of hydrocolloids (pp. 379-395). Boca Raton, FL: CRC Press, 379-395.

Flynn, A., and Cashman, K. (1997). Nutritional aspects of minerals in bovine and human milks. In P. F. Fox (Ed.) (2nd ed. Advanced dairy Chemistry (Vol. 3,pp. 257–302). New York: Chapman and Hall.

Gunasekaran, S.; Xiao, L., and Ould Eleya, M. M. (2006). Whey protein concentrates hydrogels as bioactive carriers. Journal of Applied Polymer Science, 99: 2470–2476.

Guzey, D. and McClements, D. J. (2006). Characterization of β-lactoglobulin–chitosan interactions in aqueous solutions: A calorimetry, light scattering, electrophoretic mobility and solubility study. Food Hydrocolloids; 20: 124-131.

Harnsilawat, T.; Pongsawatmanit, R. and McClements, D.J. (2006). Characterization of *β*-lactoglobulin-sodium alginate interactions in

aqueous solutions: A Calorimetry, light scattering, electrophoretic mobility and solubility study. Food Hydrocolloids; 20: 577-585.

Hattori, M.; Numamoto, K.;. Kobayashi, K. and. Takahashi, K. (2000). Functional changes in beta-lactoglobulin by conjugation with cationic saccharides. Journal of Agricultural and Food Chemistry; 48:2050–2056.

Hekmat, S. and McMahon, D. J. (1998). Distribution of iron between caseins and whey proteins in acidified milk. Lebensmittel-Wissenschaft and Technologie, 31: 632–638.

Hurrell, R. F., and Cook, J. D. (1990). Strategies for iron fortification of foods. Trends in Food Science and Technology, 9: 56–61.

Jackson, L. S. and Lee, K. (1992). The effect of dairy products on iron availability. Critical Reviews in Food Science and Nutrition, 31, 259-270.

Janes K.A; Fresneau M.P; Marazuela A; Fabra A. and Alonso M.J. (2001). Chitosan nanoparticles as delivery systems for doxorubicin. Journal of Control Release, 73:255–267.

Kinsella, E. and Whitehead, D. M. (1989). Proteins in whey: chemical, physical and functional properties. Advances in Food and Nutrition Research, 33: 343–438.

Langer, K.; Balthasar, S.; Vogel, V.; Dinauer, N.; Briesen, H. V. and Schubert, D. (2003). Optimization of the preparation process for human serum albumin (HSA) nanoparticles. International Journal of Pharmaceutics, 257, 169–180.

Leaver, J. and Horne, D.S. (1993) In: Dickinson E, Walstra P, editors. Food colloids and polymers: stability and mechanical properties. Cambridge, UK: Royal Society of Chemistry;.pp. 332-340.

Loo, C.; Lin, A.; Hirsch, L.; Lee, M. H.; Barton, J. and Halas, N. (2004). Nanoshell-enabled photonics-based imaging and therapy of cancer. Technology in Cancer Research & Treatment, 3: 33–40.

Morr, C. V. and Foegeding, E. A. (1990). Composition and functionality of commercial whey and milk protein concentrates and isolates: A status report. Food Technology, 44: 100–112.

Morr, C.V. and Ha, E.Y.W. (1993). Whey protein concentrates and isolates: processing and functional properties. Food Science and Nutrition. 33: 431-76.

Nakache, E.; Poulain, N.; Candau, F.; Orecchioni, A. M., and Irache, J.M. (2000). Biopolymer and polymer nanoparticles and their biomedical applications. In H. S. Nalwa (Ed.), Handbook of nanostructured materials and nanotechnology: organics, polymers ,and biological materials (pp. 577–635). San Diego: Academic Press

Remondetto, G. E.; Beyssac, E. and Subirade, M. (2004). Iron availability from whey protein hydrogels: an in vitro study. Journal Agriculture of Food Chemistry; 52: 8137 – 8143

Rhaese, S.; Briesen, H. V.; Rubsamen,W. H.; Kreuter, J. and Langer, K. (2003). Human serum albumin-polyethylenimine nanoparticles for gene delivery. Journal of Controlled Release, 92:199–208.

Shu, X.Z and Zhu, K.J. (2002). The influence of multivalent phosphate structure on the properties of ionically cross-linked chitosan films for controlled drug release. European Journal of Pharmaceutics and Biopharmaceutics ; 54: 235-43.

Song, Y.; Babiker, E.E.; Usui, M.; Saito, A. and Kato, A. (2002). Emulsifying properties and bactericidal action of chitosan–lysozyme conjugates. Food Research International 35:459–466.

Sugiarto, M.; Ye, A. and Singh, H. (2009). Characterisation of binding of iron to sodium caseinate and whey protein isolate. Food Chemistry, 114:1007-1013.

Vinagradov, S. V.; Bronich, T. K., and Kabanov, A. V. (2002). Nanosized cationic hydrogels for drug delivery: preparation, properties and interactions with cells. Advanced Drug Delivery Reviews, 54: 223–233.

Zhang, D. J. and Mahoney, A. W. (1989a). Bioavailability of iron–milk–protein complexes and fortified Cheddar cheese. Journal of Dairy Science, 72: 2845–2855.

Zhang, D. J., and Mahoney, A. W. (1989b). Effect of iron fortification on quality of Cheddar cheese. Journal of Dairy Science, 72: 322–332.

تطبيق النانوتكنولوجى باستخدام مركز بروتينات الشرش لتحسين الإتاحة الحيوية للحديد

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تم تحضير معقد من الشيتوزان ومركز بروتينات الشرش فى صورة جزيئات متناهية فى الصغر (نانو) بهدف تحسين النقل الحيوى(الإتاحة) للمغذيات التى يتم تناولها عن طريق الفم مثل الحديد. لذلك تم تحضير جزيئات النانو بطريقة ionic gelation مع ثلاثى صوديوم عديد الفوسفات ، ثم دراسة تأثير الرقم الهيدروجينى و تركيز جزيئات النانو لمعقد الشيتوزان ومركز بروتينات الشرش وتم تقدير كل من كفاءة التحميل(LE) وكفاءة التجميع (AE) ، كما تم تحميل الحديد فى صورة كبريتات حديدوز على مركز بروتينات الشرش و الشيتوزان بتركيزات مختلفة مع دراسة تأثير الرقم الهيدروجينى و تركيز جزيئات النانو لمعقد الشيتوزان ومركز بروتينات الشرش و الحديد، كما تم دراسة تجربة معملية (In Vitro) فى وجود أو عدم وجود إنزيم الببسين والبنكرياتين على إنفراد الحديد.

و أظهرت النتائج أن الشحنة السطحية للجزيئات تعتمد على الرقم الهيدروجينى. حيث أظهرت التركيزات المختلفة لمركز بروتينات الشرش مع الشيتوزان وجود شحنات موجبة كانت عند الرقم الهيدروجينى 4.5 فى حين أظهرت وجود شحنات سالبة عند الرقم الهيدروجينى من5.5 - 7.5 . وعندما تم تحميل الحديد على مركز بروتينات الشرش و الشيتوزان أظهر زيادة الشحنات الموجبة بزيادة تركيز الحديد وذلك عند الرقم الهيدروجينى 4.5. فى حين زادت الشحنات السالبة على تركيز من 8 و 12 % لمركز بروتينات الشرش عند الرقم الهيدروجينى من5.5 - 7.5.

أظهرت نتائج التحميل أن كفاءة التحميل وكفاءة التجميع لمعقد مركز بروتينات الشرش مع الشيتوزان تعتمد على الرقم الهيدروجينى. حيث ترتفع كفاءة التجميع وتنخفض كفاءة التحميل بزيادة الرقم الهيدروجينى حتى 6.5 ثم يحدث إنخفاض تدريجى حتى رقم هيدروجينى7.5.

أظهرت النتائج لتجربة In Vitro أن معقد جزيئات النانو المحضرمن مركز بروتينات الشرش والحديد و الشيتوزان كانت لها خواص بقاء(ثبات) جيدة حيث لم يتم إنفراد الحديد بعد 6 ساعات مع التقليب المستمر على رقم هيدروجينى 7.5 وحرارة 37° م فى ظروف الهضم الحامضية و الإنزيمية . كما أظهرت النتائج عدم حدوث إنفراد للحديد فى وجود أو عدم وجود إنزيمات الببسين والبنكرياتين بعد 6 ساعات من معاملة جزيئات النانو من مركز بروتينات الشرش والحديد و الشيتوزان.

يتضح من ذلك ان معقد جزيئات النانو للحديد و الشيتوزان ومركز بروتينات الشرش على مستويات صفر ، 3 ، 6 ، 9 و 12 ملجم حديد/جم بروتين أظهرت إتاحة حيوية مرتفعة جدا بعد تقييمها فى السائل المعدى و المعوى فى وجود أو عدم وجود الأنزيمات.