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Whey Processing with Nano Chitosan

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Abstract: Since maleic acid is capable of specially dissolving Chitosan, in this research the method of producing water lubricant nanoparticles in Chitosan solution, based on maleic acid has been studied. The resulting nanoparticles have a remarkable contact surface with the ordinary Chitosan form in absorbing metal ions, proteins and fat existing in Whey. After providing the 0.02, 0.03, 0.05 gr. density of Chitosan nanoparticle and ordinary Chitosan in 10cc Whey with pH 4, 5, 6 were compared in absorbing the particles and the results were observed through (UV) spectrum. Since maximum absorption was observed in pH=6 and 0.05 gr. density of Chitosan nanoparticles and ordinary Chitosan, colloidal absorbent in Whey, by atomic absorption and infrared spectrum were examined in the rest of the test. An increase in the absorption of metal ions like lead, iron and copper is among the significant points of using this kind of nonoparticles. The other items examined in this study are comparison of proteins and fats absorption after treatment of Whey in pH=6 and 0.05gr density of nano Chitosan with ordinary Chitosan in 10cc of this material.

Key words: Nanoparticles · Chitosan · Lactose · Whey

INTRODUCTION

Whey is the largest derivative of diary industries which causes environmental pollution and microscopic organisms in these substances. The rate of oxygen inside water used by B.O.D (biochemical oxygen demand) microorganisms is different in Whey (it is 30000-50000 ppm) and depending on the proportion of the milk source and lactose, it can be among largest and most important effective factor [1]. The high nutrition value of milk depends on consuming 11 gram lactose daily [1, 2]. Lactose has also significant nutrition properties that can be enumerated here; 1.Effects on nutritive materials and minerals absorption [2].

- Dietary fiber-like activities and probiotic effects [3].
- Calorific value [3].
- Glycemic index [3].
- Sweetness [2].

Many methods for isolating and purifying lactose as well as Whey products and separating particles of this valuable substance have long been established; this process includes chemical [2], physical and biological methods or integration of chemical and physical methods. Among physical methods we can mention lactose separation of Whey using heat and inverse osmosis can be mentioned. In another method that uses calcium carbonate with interaction accelerant factors, lactose is separated. It was proposed as a chemical method [4]. Nanotechnology can be defined as the production of materials, parts and useful systems with a control in nanometer longitudinal scale and utilizing properties and new phenomena resulted from such a scale. Nanotechnology make it possible to manipulate materials up to nano scale and in this area, the synthesis of nanoparticles have been on focus, especially polysaccharide physical nanoparticles due to their biological destruction properties, hydrophilic and biocompatibility.

In this research, attempt we made to not only discuss high value of Chitosan and Chitosan nanoparticles for better separation of Whey including (fat, lactose, metal particles protein and solution solid materials), but also justify the use of this material in economical plans.

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MATERIAL AND METHOD

Nano Particles Production: Chitosan is a very sensitive polymer material which is affected by heat and changes in environmental condition. So during the test, attention and accuracy in correct performance is a necessary factor for production of this material.

Chemical Materials: Potassium Persulfid ($K_2S_2O_8$), Maleic Acid ($HO_2CCH_2CHOHCO_2H$), Hydrochloric Acid (HCl) 37% which were purchased from Merck Co. Sodium Hydroxide (NaOH) and Chitosan with low molecular weight (deacitylation degree between 75-85%) prepared from shrimp shell purchased from (Sigma-Alderich Co.).

In 100ml distilled water, 0.003mol Maleic Acid was dissolved, then 0.012mol Chitosan was added and the sample was mixed by a mixer for 3 hours [5]. After that, pH was adjusted by the solution of Hydrochloric Acid and 0.1 molar Sodium Hydroxide. The sample was mixed at room temperature for 8 hours and nitrogen gas was blown onto it over the last hour. After one hour, as Nitrogen gas was blowing, 0.054gr Potassium Persulfid ($K_2S_2O_8$) was added and was Nitrogen gas was blown onto the sample in the temperature of 70 centigrade degrees for 2 hours. In the case suspended particles appeared, the resulted mixtures were cooled in ice bath and the resulting nanoparticles were being separated by the centrifuge with 16000 rpm cycle for 30 minutes. The obtained particles were dried by freeze-dryer and used for absorption tests [6].

The Measurement of Different Values of Whey Including Protein, Fat, Lactose and Metal Ions: Protein measurement of Whey through formal titration method: this method is known as formula method which is a very quick test for protein measurement in food like milk and Whey.

Measurement of fat content in Whey was performed by using gravimetric through the test method. This method is famous to Rose Gotlib. Lactose of Whey was measured by Lori method [4]. Measurement of ash was performed by heating the sample in a furnace with 550 °C for 30 minutes and the amount of each metal element was done by using atomic absorption.

The Determination of the Required Amount of Chitosan Nanoparticles

Spectrophotometer: Chitosan and its nanoparticles have a tendency to adhesiveness and self-assembly in pH above 6. Also, in pH lower than 4, due to the proteinization of their amino groups because of an increase in H^+ in acidic environment, the absorption process of the particles in question is ultimately blocked. Given these two facts, the range of particles absorption and the results of Whey spectrophotometer in pH 4, 5, 6 and in 0.02, 0.03, 0.05 densities were examined and measured. The results are in figures 205 and 3-5. Concerning the comparison of the absorbents, it was observed that the dispersion of numbers in Abs is between 0-0.09. Therefore, to have a better examination and more accurate conclusion, a standard sample with densities lower than Whey (0-0.09) was collected again

A Miniature Fourier Transform Spectrometer: In order to compare the amount of absorbed particles in the primary sample and the test (Whey treated with pH: 6 and 0.5 gr. Nano Chitosan) to reach a fixed weight, 10ml of each sample water was evaporated in furnace with 550 degree. The resulting ash using solid KBr, the special tablet, is produced and finally the resulting chart of each sample is evaluated.

Statistical Data Analysis: All of the tests were done in three block plans three times. Analysis and evaluation (ANOVA), using linear model (G.L.M) in SPSS18 with the probability of 5% and Danken multi-range test was done for confirming the possible difference between the means.

Atomic Absorption: After observing the ideal pH absorption and the required nano Chitosan amount for the highest amount of separation, the researchers used atomic absorption machine to measure the amount of three metals of Cu, Fe and Pb with amount of each in the primary sample and after absorption.

RESULTS AND DISCUSSION

Nanoparticles Production Examination: The results of SEM pictures related to Chitosan nanoparticles in weight proportions of 2:1, Maleic Acid/ Chitosan in pH 4, 5 and 6. In mole rate of CS/MA=2:1 are showed in (Fig. 1).

Given the fact that maleic acid is a double-agent acid, agent groups proportion is NH_2/COOH=1. As can be seen in Figure (a) the particles have smaller diameters and dimensions compared to the ones in Figure (b). This shows the positive effect of pH=4 and proportion rate of 2:1, maleic acid/ Chitosan. The Porosity rate in molecular structure of Figure (c) is also more than that of Figure (b); therefore, the desirability of PH=4 with PH=6 could be diagnosed from the results.

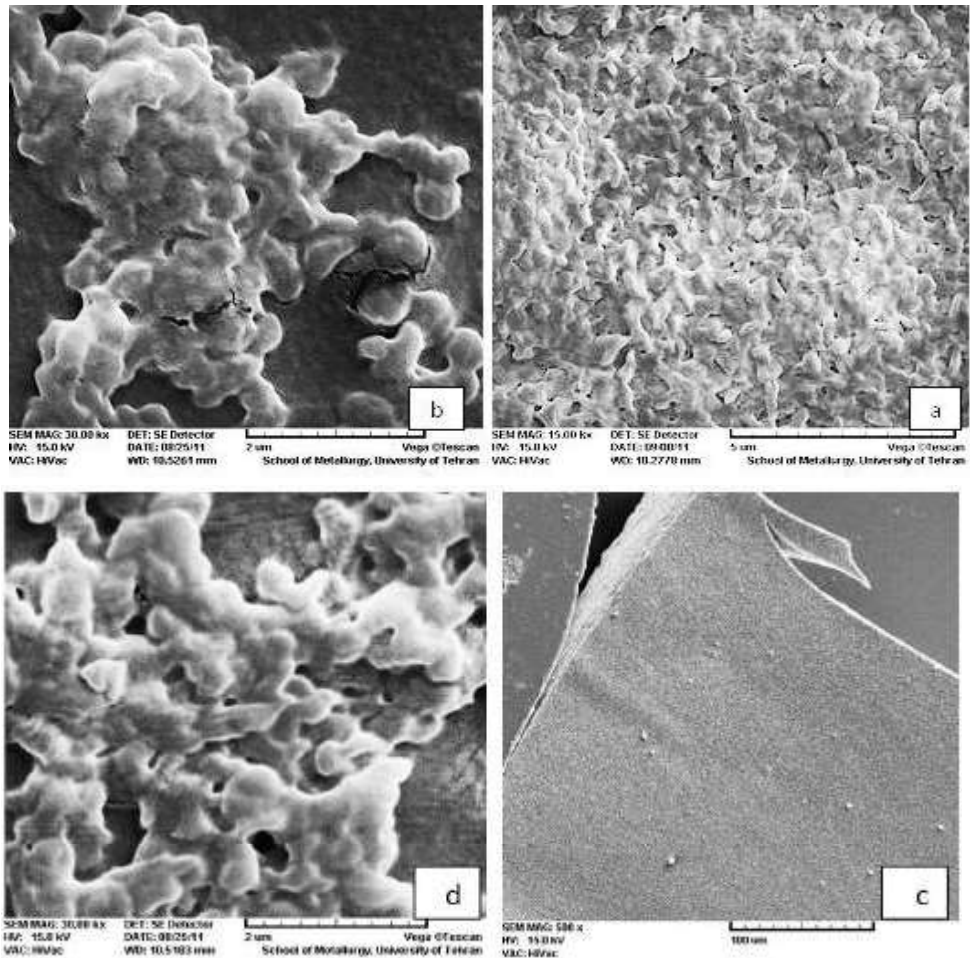

Fig. 1: Compare of different Nano Chitosan. a) Nano particle in pH=4 b) Nano particle in pH=6 c) Nano particle in pH=4 d) Nano particle in pH=5

The production of Chitosan particles out of Chitosan with high molecular weight was done. It was observed that distribution of nano Chitosan particles size with gr. rate CS/MA=2:1 in pH: 3, 4, 5, 6 are various and different from each other.

In pH=4, the distribution of particles is narrower than in pH=3. This shows that in the said pH, the particles are less assembled and the majority of the particles are formed separately. In pH=5, the particles are completely separated and are larger than the two previous forms. It is also the case in acidic pH of the amino Chitosan group and protonated carboxyl acid maleic group, in which case the size of nanoparticle is affected. Therefore, as pH increases from 3 to 6, the mass of the nanoparticles too increases. There might be two reasons for that.

First, with an increase in pH, the ionization degree and load density of maleic acid molecules also increase; as a result, inner-molecules repulse electrostatic forces of amleic acid increase hence it causes distention in nanoparticles and an increase in their size (Fig. 1) shows this fact. Second, with an increase in pH, the solubility of Chitosan is decreased, causing an increase in the adhesion and aggregation of nanoparticles. So, with regards to the studies carried out in the past, in this study with only two pH, 4 and 6 are taken into account for producing Chitosan nanoparticles from Chitosan with low molecular weight. The results are as follows:

The Result of Spectrometry: In order to select the standard sample, first the Whey concentrations from 0.1 up to 0.9 were prepared; Fig. 2 (a) was observed, while the comparison of the resultant absorptions was taken into account. The range of the observed figures is between 0-0.9 covering the rate of absorption of nanoparticles in Whey.

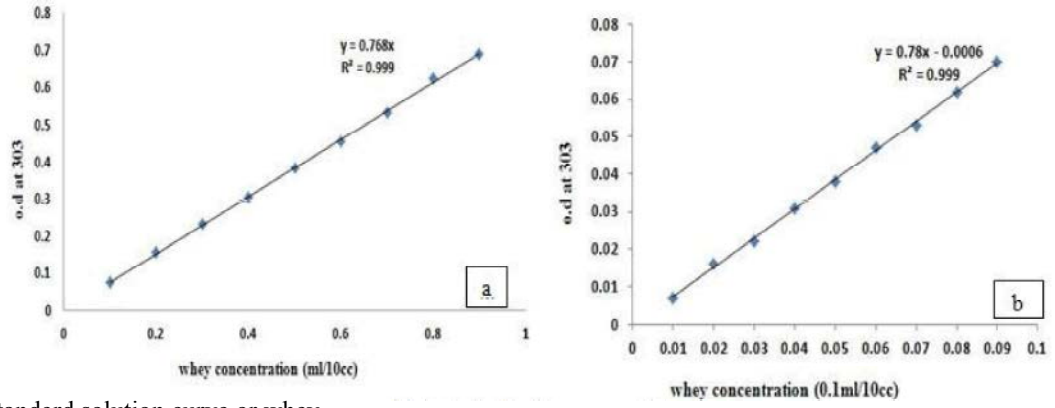

Fig. 2: Standerd solution curve or whey

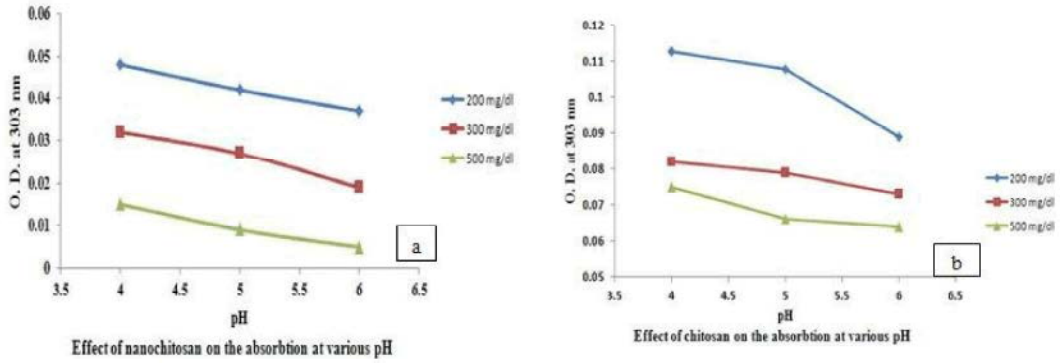

Fig. 3: Effect of nano paricle and Chitosan in absorption a) nano Chitosan b) Chitosan

So, for the sake of better investigation and more accurate conclusion, a standard sample was collected again from pure Whey, with the concentrations of 0-0/0.09. In (Fig. 2) two matters are illustrated (b). Chitosan and its nanoparticles tend to assemble and adhere in pH more than 6 in the pH lower than 4, result of this treatment will be due to decrees in absorbent. So in this study to determine effective absorbent doze, which determines the capacity of the absorbent, certain tests were carried out by chitisan nanoparticles for examining the effect of the absorbent amount on the absorption process of protein ions and suspending solid particles in Whey. In order the range of absorbent amount was determined in various amounts including 0.02, 0.03, 0.5 gr. on 10 ml. of Whey in pH 4, 5, 6, by using spectrophotometer (Fig. 3).

Figure 3 shows the absorption of the particles in question, the range of particles absorption and the results of Whey spectrometry. The effect of pH 4, 5, 6 and the viscosities of 0.02, 0.03 and 0.05 on particles absorption of Whey.

Data Analysis for Obtaining the Concentration and Optimized pH for Performing Subsequent Tests: According to the (Table 1), the variance analysis with probability of %95 was observed and investigated. There is a significant difference between the concentrations and pH. Performing the comparison test between the means in Table 2, the researchers observed that pH=6 and the concentration 0.05 gr. of nano Chitosan in 10cc Whey has the best absorption. In order to perform the other tests, the same pH and concentration were used.

Table 1: variance analysis of the effect of changing concentrations and pH on absorption caused by Nano Chitosan and Chitosan

Change Reference	Free Rate	Total of Squares	The Average of Squares	F-Value	Probability
Nano Chitosan	2	0.002	0.001	24.334	0.001
Chitosan	2	0.002	0.001	13.634	0.006
Failure	6	0.000	0.000		
Total	8	0.002			

Table 2: The effect of Nano Chitosan, Chitosan viscosities and pH on particles absorption in 10ml Whey

concentration (ncs.cs) gr. in 10 ml Whey	pH	Absorption of Nano Chitosan (Abs)	Absorption by Chitosan
0.02	4	0.045[c]	0.113[c]
0.02	5	0.042[b]	0.108[b]
0.02	6	0.037[a]	0.089[a]
0.03	4	0.032[c]	0.082[c]
0.03	5	0.027[b]	0.079[b]
0.03	6	0.019[a]	0.073[a]
0.05	4	0.015[c]	0.075[c]
0.05	5	0.009[b]	0.066[b]
0.05	6	0.005[a]	0.064[a]

The means having similar letters in a column have no significant difference based on Danken test with 95% probability.

Table 3: The rate of absorption and cooper concentration in Whey and Whey treated with nanoparticles

No.	Test Material	Concentration (ppb)	Absorption
1	Distilled Water	0	2.21
2	Standard 1	0	2.286
3	Standard 2	0	2.829
4	Standard 3	0	3.257
5	Whey 1	0.1413	2.241
6	Treated Whey	0.0657	2.207

Table 4: The rate of absorption and Iron concentration in Whey and Whey treated with nanoparticles

No.	Test Material	concentration (ppb)	Absorption
1	Distilled Water	0	0.003
2	Standard 1	0	0.005
3	Standard 2	0	0.006
4	Standard 3	0	0.006
5	Whey 1	181.7247	0.113
6	Treated Whey	19.0716	0.009

Table 5: The rate of absorption and density of lead in Whey 1 and Whey treated with nanoparticles

No.	Test Material	Density (ppb)	Absorption
1	Distilled water	0	0
2	Standard 1	0	0
3	Standard 2	0	0.047
4	Standard 3	0	0.001
5	Whey 1	101/968	0.011
6	Treated Whey	0	0

Table 6: Measuring metal ions in pure Whey and Whey treated with Nano-Chitosan

Different values of metal ions	Pure Whey	Whey treated in Nano-Chitosan (ppb)
Pb	101/968	0
Fe	181/7247	19.0716
Cu	0/1413	0.0657

Atomic Absorption: In studies carried out by Mukhopadhyay *et al.* (2006) for isolating metal ions, fat and protein by using Chitosan gel with deacitylation more than 90% and high molecular weight, the rate of metal ions Fe, Cu and Pb respectively reached to 0.001, 0.0019, 0.0004 gr. in 100 ml. Finally, the total of metal ions reached to 0.1 gr. in 100 ml [17], but when nanoparticles were used with low molecular weight and lower deacitylation (0.75-0.85) through the research, its rate reached the lowest amount. The results of each metal can be seen in Fig. 3, Fig. 4 and

The Study of Metal Ions Absorption: Metal ions absorption occurs by one or a combination of ion exchange mechanisms, chelating and electrostatic absorption [7]. Many studies have shown that the most important parameter for heavy metal absorption by absorbent is the pH primary solution that has a high impact on metal-absorbing spots on the absorbents surface and chemical structure of metal in water [8]. In this study, the effect of primary pH solution, within the range of 4-6, on the ratio of metal ion absorption of Pb, Cu and Fe was considered. In pH less than 3, Chitosan nanoparticles are solved and in pH more than 7, metal ion precipitation occurs as metal hydroxide. Metal ion absorption is highly depends on protonation and non-protonation of amino groups and carboxylates exists in Chitosan nanoparticles [9].

As the pH of solution increases, amino groups in the compound of Chitosan nanoparticles become protonated in different degrees; consequently, accessible spots for chelating metal ions are reduced, leading to electrostatic removal of metal cations [10]. In low pH, because of the competition among H^+ ions and metal cation, H^+ ion dominates on absorption places and the accessibility of cations over these spots is limited, leading to a reduction of absorption percentage [10]. In fact, the effect of pH on absorption efficiency related to ligand protonation and metal ion in solution. Theoretically, metal ions in solution exist in various types that depend on the solution's pH. In low pH amounts, the majority of metal ion varieties are cations, while metal hydroxide varieties have high amounts of pH. In addition, because in low pH amin groups are protonated, thus the capability for making protonated ligand links with metal cations is less than the capability for making non-protonated molecule links with metal hydroxide in high pH amounts. On the other hand, in low pH, protons with metal cations compete with amino groups for interaction, which leads to the formation of RNH^{3+} and positive load on the absorbent surface [11].

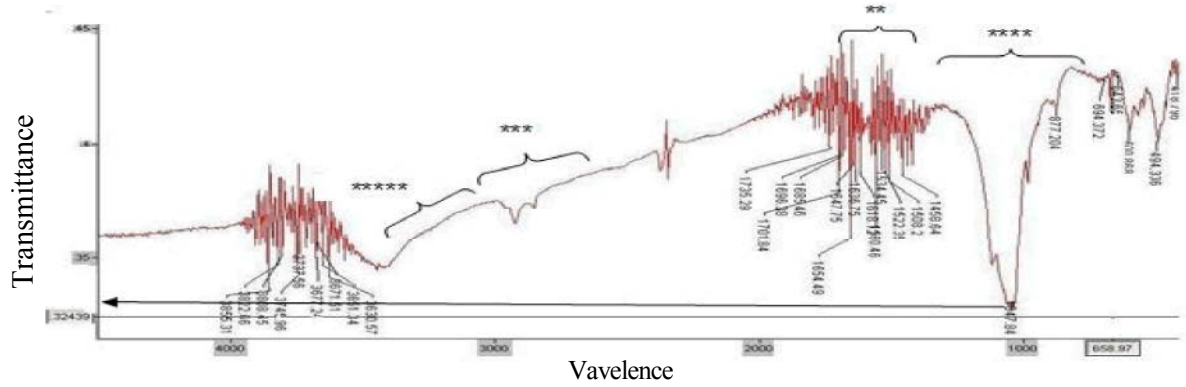

Fig. 4: Infrared spectroscopy of Whey in natural pH

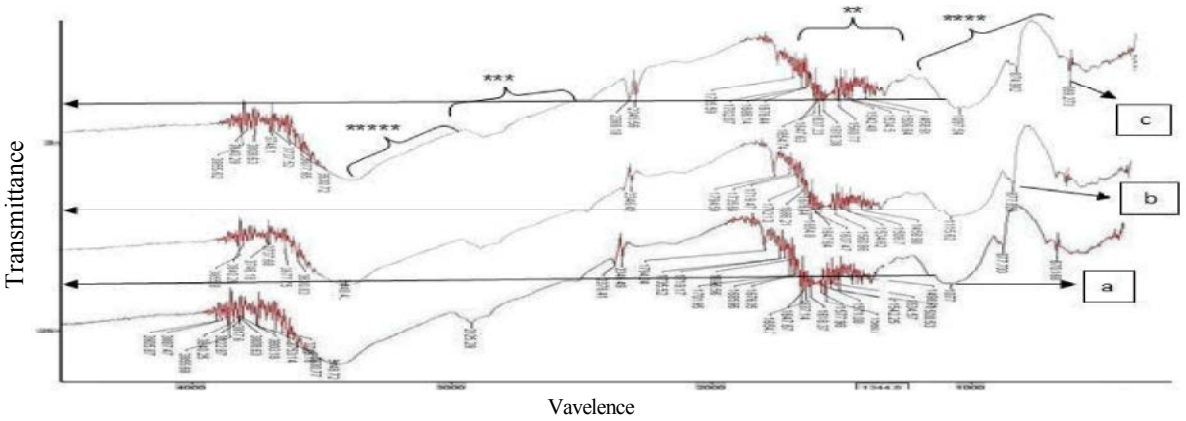

Fig. 5: Infrared spectroscopy of Nano particles after absorption in different pH a) pH=4 b) pH=5 c) pH=6

Table 7: In the following table, the amount of each parameter is after filtration

Type of used Chitosan for absorption in Whey	Protein (in100ml Whey)	Fat (in100ml Whey)	Ash (in100ml Whey)	Lactose (in100ml Whey)
pure Whey	0.26gr	0.1gr	0.56r	4.4r
Chitosan particles	0.18gr	0.08gr	0.28gr	4.2gr
Nano Chitosan particles	0.08gr		0.04	0.096gr
			Fe^{*}=19.071	
			Cu^{*}=0.0657	
			Pb^{*}=0	4.2gr
			Ppb*	

But in higher pH, the existing ligands in the absorbents like COO^{-}, increase the density of negative load on ligands surface, as a result of which, electrostatic attraction of positively loaded metal ions increases on ligand surface and the percentage of absorption will be increased. As pH increases due to compression of OH-ions, the precipitation of metal ions appear as hydroxide and reduces absorption percentage [12]. In (Table 6), the absorption percentage of amounts resulted from metals is summarized.

Infrared Spectroscopy: Fig. 4 and Fig. 5 show results from infrared spectroscopy and two diagrams of treated and pure Whey. Considering the diagram, we can contend that nano Chitosan particles can absorb metal, fat and protein particles.

Absorption and Tension Examination of Protein Groups: As stated above, the spectrum is related to the existence of protein groups and single-capacity and double-capacity amide proteins with wavelength

(1650-1450) CM^{-1}. Thus, in (Figure 4), the wavelength in question, the existence of this dispatcher in nanoparticles and also extending the range of 1650-1450 related to proteins tension in Chitosan nanoparticles (Figure 5, part **) is confirmed. Therefore, based on (Figure 5), the existence of proteins in nanoparticles in all pH is proven, but if we observe the rate of absorption on vertical axis, the greatest amount of absorption is in density 0.05 gr. Nano Chitosan in 10cc. Whey with pH=6. This can be a proof for examining optimum pH and gives an appropriate density.

Absorption and Tension Examination of Fats and Fat Acids: The spectrum is related to the existence of fat acid groups (-C-H) and fats with wavelength 2800-3000 CM^{-1}. As can be seen in (Figure 4), in examining wavelength (***) the existence of this dispatcher confirms nanoparticles and extending the range of 2800-3000 related to the tension of fat acids in Chitosan nanoparticles (Figure 5) part ***). Thus, according to (Figure 5), the existence of fats in nanoparticles in all pH is proven, but if we observe the rate of absorption on vertical axis, the greatest amount of absorption is in pH=6. Thus, it gives us another proof for choosing the most optimum pH as well as absorption of particles in Nano Chitosan network.

Absorption and Tension Examination of Amino Groups: The place of tension vibrations of NH^{3+} is in 3000-3130 cm^{-1} (these values are for solid state and wide spectrum) and the intensity of absorption strips in this place is more medium and the vibration is tensional. (Figure 5) shows the tension vibration of amino groups in Chitosan nanoparticles in comparison with pure Whey (****). Absorption strips are usually in 2000-2500 (but not always). Bending vibrations in-NH^{3+} group are absorption strips having the wavelength of 1500-1600 with the absorption strip intensity of *S* (strong).

Absorption Examination in Proteins, Fat and Ash in Whey by Nanoparticles: Amino acids in acidic environment and high density of hydrogen ion are dominant cations and in alkaline environment are dominate in an anion state. In a special range of pH, amino acids exist as a zwitterions and called isoelectric points. Isoelectric point of most proteins is 5/5. Protein absorption through electro static relations in pH=6 and protein electronegative in pH helps in absorption and coagulation process.

In pH higher than 7, nanoparticles begin to assimilate and connect with each other and the points with connectivity decrease. In pHs lower than 6, the connected groups among nanoparticles amines and tripoly phosphoric groups in proteins lead to deproteinization, so the absorption of the particles decreases in this situation [13].

Milk fat globule membrane fragments (MFGM) exits because of the negative charge in phosphate groups and in phospholipids membrane of Whey fat cells. Given the fact that the positively charged amine groups are in Chitosan nanoparticles structures, in acidic pH absorbing nano Chitosan amine groups by phospholipids groups of fatty and protonated cells lead to floccules and isolation of fat from the liquid phase [14]. The amount the ash of Chitosan and nano particle samples is decreased compared to that of pure Whey because of the absorption salts and minerals by the nanoparticles.

Lactose Isolation: After the examination of absorption rate and the effect of Chitosan and its nanoparticles on different parameters of concentration and pH, the amounts of protein, metal ions, fat and ash are measured again for each of them by certain methods and finally the percentage of existing lactose is calculated with a high purity. The molecules of lactose are non-ionic surfactants, so it is not affected by pH. Thus, charged particles of Chitosan and nanoparticles do not have any effect on it.

The decreasing amount of lactose in comparison to the primary state is because of fermentation during the reactions after the examination of the effect of Chitosan and Chitosan nanoparticles on Whey while there is no connection with nanoparticles. So, high purity of lactose is because of Chitosan and Chitosan nanoparticles' effect on the absorption of other materials forming Whey including protein, fats, metal ions and charged particles suspended in Whey. The average of data resulting from particles absorption before the treatment of samples and after Whey was treated with normal Chitosan and its nanoparticles (Table 7).

CONCLUSION

In this research, the effect of parameters such as pH and gram ratio of Chitosan and maleic acid on the size and morphology of nanoparticles was examined. The results show that synthesis of nanoparticles with the mole ratio of 2:1 of maleic acid to Chitosan in pH=4 yielded a more appropriate condition compared to other particles. Also, the activity of parameters such as solution pH, the

amount of absorbent on metal ions absorption, proteins and impurities caused by Chitosan and its particles were examined and the results show that with a pH increase from 4 to 6, the absorption capacity too increases. Also, as the absorbent amount increases from 0.02 to 0.05 gr on 10 cc of the sample, the capacity of absorption increases. The results of spectrophotometer show that if pH=6 and the amount 0.05gr of Chitosan nanoparticles is used for absorption, in comparison with the other ratios, it can be more effective. Also, with same amount under same conditions with normal Chitosan, the capacity of absorption is about two times greater. Any decrease in ash is due to a decrease in metal ions and proteins caused by nanoparticles. Carboxylic nanoparticles make significant connection for amino groups with proteins, fat and metal ions, thus they have special capability against Chitosan. A decrease in the amount of fat is caused by flocculation and perception which are resulted from binding nanoparticle with the membrane of fat globule. Considering the findings of the research, the Chitosan nanoparticles are suitable absorbents for removing impurities, heavy metals, fats and existing protein in Whey. They can turn into lactose with high purity after these particles are isolated. Finally, they can be used in food and drug consumptions.

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