

SYNTHESES OF GOLD NANOPARTICLES (GNP) AND CHITOSAN-GOLD NANOPARTICLES COMPOSITE BEADS AND STUDY OF THEIR PHYSICAL PROPERTIES

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Abstract

This research work concerns with the preparation and characterization of gold nanoparticles (GNP) and chitosan-gold nanoparticles (CS-GNP) composite beads by chemical method. The gold nanoparticles (GNP) were prepared from chloroauric acid in the presence of sodium borohydride as well as sodium tetraborate used as reducing agents. The existence of prepared GNP in colloidal solutions was determined by using laser pointer by Tyndall effect and the synthesized gold nanoparticles (GNP) solutions were also characterized by UV-visible spectroscopy. On the other hand, chitosan beads were produced from different concentrations of chitosan (1 % to 5 % w/v) in acetic acid solution and sodium hydroxide solution (2 % w/v). The chitosan-gold nanoparticles composites were then prepared from gold nanoparticles and chitosan solutions (3%). The physical properties prepared chitosan beads and chitosan- gold nanoparticles composite beads were studied and also characterized by modern techniques such as FT IR, EDXRF, SEM and XRD analytical methods.

Keywords: Chitosan, gold nanoparticles, chitosan – gold nanoparticles composite beads, Tyndall effect

Introduction

Chitosan can be obtained from deacetylation of chitin. Chitin is widely available from a variety of source of shellfish wastes such as shrimps, crabs, and crawfish. It also exists naturally in a few species of fungi. Chitin is associated with proteins and, high in protein contents. Chitin is made up of a linear chain of acetyl glucosamine groups while chitosan is obtained by removing enough acetyl groups (CH₃CO-) for the molecule to be soluble in most diluted acids. This process is called deacetylation (Domard and Rinaudo, 1983). Chitosan possesses positive ionic charges, which give it the ability to chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules. Chitosan is readily soluble in dilute acetic acid

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solutions below pH 6.0. The solubility is controlled by the degree of deacetylation and it is estimated that acetylation must be at least 85 % complete in order to achieve the desired solubility (Mima *et al.*, 1983). Sodium borohydride and sodium tetraborate used as reducing agents in the present study reduced chloroauric acid solution from Au³⁺ to Au⁰. GNPs are nanospheres, nanorods, nanoshells, nanocages and surface enhance Raman scattering properties (SERS), which depend on the size, shape and physical properties (Khan, A.K *et al.*, 2014). GNPs have unique optical and physical properties. They are used as sensors in environmental science, medicine, pharmacy, engineering etc. due to their nontoxicity. GNPs display different colours such as green, orange, dark purple and ruby red etc. GNPs are currently manufactured for targeted delivery of biomolecules and drugs to selected cells (Sharma *et al.*, 2009). Gold nanoparticles are composited with chitosan to form chitosan- gold nanoparticle composite beads. Gold-nano composites are used for biomedical purposes in the areas of tissues engineering, drug delivery and cellulose therapies (Papasani *et al.*, 2012).

Materials and Methods

Materials

The chemicals used in this research work were the products from British Drug House (BDH), London, Kanto Chemical Co., Inc., Japan and chitosan (degree of deacetylation 81.13 %) was purchased from Golden Dragon Co., Ltd, FMI City, Hlaing Tharyar Township.

Methods

The appropriate reported conventional and instrumental techniques were used throughout the experimental works. Some of the instruments used in the experiments were Digital pH meter, Digital Balance, Electric Furnace (100-1100°C, Gallenkamp, West Australia), Magnetic Stirrer (Gallenkamp, England), UV spectrophotometer (UV mini 1240), XRD (Rigaku, D-max - 2200, Japan).

Preparation of Stock Solutions

All of the stocks solutions were freshly prepared before the experiments.

(i) **1 % w/v chloroauric acid solution**

A stock solution of 1 % w/v chloroauric acid was prepared by dissolving 1 g of gold (III) chloride in 100 mL of deionized water in a 250 mL reagent bottle.

(ii) **1 % w/v sodium borohydride solution**

A stock solution of 1 % w/v sodium borohydride was prepared by dissolving 1 g of sodium borohydride in 100 mL of deionized water.

(iii) **1 % w/v sodium tetraborate solution**

A stock solution of 1 % w/v sodium tetraborate was prepared by dissolving 1 g of sodium tetraborate in 100 mL of deionized water.

(iv) **1 % w/v sodium citrate solution**

A stock solution of 1 % w/v sodium citrate solution was prepared by dissolving 1g of sodium citrate in 100 mL of deionized water. This solution must be freshly prepared before the experiment.

(v) **2 % w/v sodium hydroxide solution**

A stock solution of 2 % w/v sodium hydroxide solution was prepared by dissolving 2 g of sodium hydroxide in 100 mL of deionized water.

(vi) **1 % acetic acid solution**

A stock solution of 1 % v/v acetic acid solution was prepared by dissolving 1 mL of acetic acid in 100 mL of deionized water.

Preparation of Chitosan Beads

Chitosan flakes (3 g) was weighed and placed into a 250 mL beaker. Then, 100 mL of 1 % v/v acetic acid solution was added and stirred with magnetic stirrer thoroughly until all of the solids were dissolved and allowed to stand overnight to become uniform solution. Next, 2 % w/v sodium hydroxide solution was prepared and stirred with magnetic stirrer for 30 min. When the prepared chitosan solutions were forced through syringe into the 2 % w/v sodium hydroxide solution at a constant rate of 10-12 drops per min, the chitosan beads were formed. Chitosan beads were allowed to stand for

12 h, then filtered and washed three times with distilled water. The beads were dried at room temperature for 24 h and placed in an oven at 45°C for another 24 h.

Preparation of Gold Nanoparticles Colloidal Solutions

At first, 5 mL of 1 % w/v chloroauric acid solution was diluted with 35 mL of deionized water in a 250 mL beaker. This solution was stirred for 1 min and 1 mL of 1 % w/v trisodium citrate was added into it and stirred about for 1 min. Then, 1 mL of 1 % w/v sodium borohydride solution was added into this mixture solution and stirred for 30 min. A purple colloidal solution was formed after one day later due to the formation of gold nanoparticles. The gold nanoparticles were also prepared by using 1 % w/v sodium tetraborate as the same procedure.

Characterization of the Prepared Gold Nanoparticle Solutions Confirmation for the existence of gold nanoparticles in solution by Tyndall effect

When the gold nanoparticles colloidal solutions in the bottle was pointed out with a laser pointer, the light scattered through the colloidal solution.

UV-visible spectroscopy and XRD analysis

The formation and the presence of gold nanoparticles were studied by UV-visible spectroscopy. In this experiment, the absorbance of the prepared gold colloidal solutions and chloroauric acid solutions were measured at different wavelengths ranged from 480 to 580 nm. The characteristics of gold nanoparticles were also studied by XRD analysis by using XRD diffractometer (Rigaku, D- max-2200, Japan).

Preparation of Chitosan-Gold Nanoparticles Composite Beads

When 3 g of chitosan was dissolved in 1 % v/v acetic acid solution and stirred with magnetic stirrer thoroughly until all of the solids were dissolved, and allowed to stand overnight to become uniform solution. And the prepared gold nanoparticles colloidal solution was added with sodium borohydride as reducing agent and the mixture was stirred with magnetic stirrer for 15 min to

become the homogenous chitosan gold nanoparticles solution. After that 2 % w/v sodium hydroxide was stirred with magnetic stirrer for 30 min. The prepared chitosan gold nanoparticles colloids were forced through syringe into the gently stirred sodium hydroxide solution at a constant rate of 10-12 drops per minute and the composite beads formed were allowed to stand until curing for 12 h. The beads were filtered and washed three times with distilled water followed by drying at room temperature for 24 h and placing in an oven at 45 °C for another 24 h. Similarly the chitosan gold nanoparticles composite beads were prepared by using sodium tetraborate as reducing agent according to the above procedure.

Determination of Some Physical Properties of the Prepared Chitosan Beads and Chitosan Gold Nanoparticles Composite Beads

(i) Determination of water uptake

The prepared dry beads (1 g) in 10 mL distilled water were placed in a porcelain crucible and were kept at room temperature for 9 h. After 9 h, beads were removed from water and then they were placed in a desiccator and weighed again. The weighing was repeated until a constant weight was achieved. The amount of water uptake in the chitosan bead and composite beads were then calculated.

(ii) Determination of free moisture content

Moisture content (%) was determined by the oven method at 110 ± 5 °C. An accurately weighed sample (about 1 g) was added to a pre-dried and cooled dish with a cover. The uncovered dish is placed in an electric oven, and dried at 110 ± 5 °C for 2 h. After heating the cover was placed in position and in desiccator for cooling. And weighing which was repeated until a constant weight was obtained. The moisture percent is represented by the loss in weight.

(iii) Determination of pH

The sample (about 1 g) was placed into a Pyrex 200 mL beaker and 100 mL of distilled water was added. The content of the beaker was heated at 80 °C for 10 min. The beaker and content were gently shaken and the sample

was filtered. The filtrate was cooled at room temperature and pH of the sample was determined by using a pH meter.

(iv) Determination of swelling percent

The swelling behavior of prepared beads in acidic and in phosphate buffer solutions was determined by using gravimetric method. In this method, the prepared chitosan beads (1g) was immersed in acidic solution and phosphate buffer solutions of different pH values of 1.4, 2.4, 4.4, 5.4, 6.9, 7.4, 8.4 and 9.4 for 8 h. The weight of each sample was determined after the removal of the beads from the solutions.

Characterization of Chitosan Beads and Chitosan Gold Nanoparticles Composite Beads

The prepared chitosan beads and chitosan gold nanoparticles composite beads were characterized by FT IR, EDXRF, SEM and XRD analysis.

Results and Discussion

Preparation and Characterization of Gold Nanoparticles Colloidal Solutions

(i) Tyndall Effect

When the gold nanoparticles colloidal solution in the bottle were pointed out with a laser pointer, it was observed that the light scattered. Therefore the gold nanoparticles were presented in the prepared gold colloidal solutions.

(ii) UV-visible Spectroscopy

Gold nanoparticles were prepared as colloidal solutions from chloroauric acid reduced by using 1 % sodium tetraborate as well as by 1 % sodium borohydride reducing agents. The existence of gold nanoparticles in the solution was confirmed by UV- visible spectroscopy (Table 1). The plots of absorbance versus wavelength for chloroauric acid and gold nanoparticles colloidal solutions prepared by using sodium borohydrate as well as by sodium tetraborate reducing agents are described in Figure 1. According to these results, the wavelength of maximum absorption was found at λ_{\max}

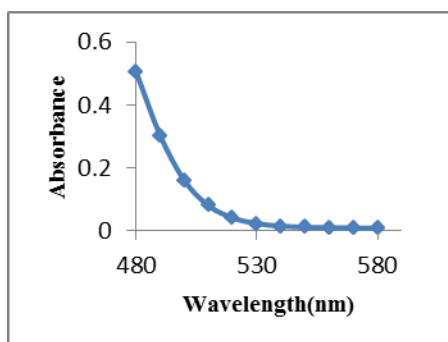
530 nm for both gold nanoparticles colloidal solutions, however, no increase occurred in the absorbance of chloroauric acid in the wavelength ranged from 480 to 580 nm. Therefore, it can be inferred that gold nanoparticles were formed in the solutions because of the λ_{max} occurred at 530 nm. (Khan, A.K *et al.*, 2014).

Table 1: Absorbances of 1 % Chloroauric Acid and the Prepared Gold Nanoparticles Colloidal Solutions by Using 1 % NaBH₄ and 1 % w/v Na₂B₄O₇ as Reducing Agents

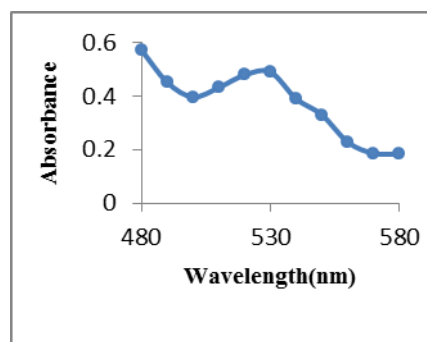
Wavelength (nm)	Absorbance		
	(1 % w/v HAuCl ₄)	GNP (SBH)	GNP (STB)
480	0.507	0.573	0.544
490	0.304	0.452	0.452
500	0.161	0.397	0.415
510	0.081	0.432	0.456
520	0.042	0.479	0.518
530	0.024	0.490	0.533
540	0.016	0.390	0.441
550	0.012	0.300	0.309
560	0.011	0.228	0.221
570	0.010	0.186	0.152
580	0.009	0.184	0.150

GNP (SBH) = Gold nanoparticles colloidal solution prepared by using 1 % NaBH₄

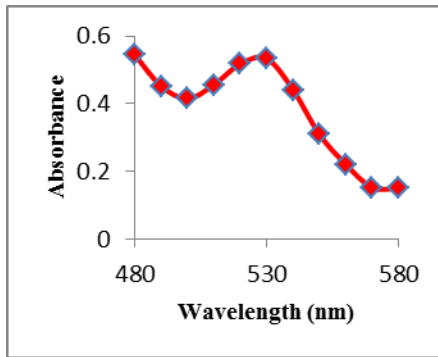
GNP (STB) = Gold nanoparticles colloidal solution prepared by using 1 % w/v Na₂B₄O₇



(a)



(b)



(c)

Figure 1: (a) Plot of absorbance Vs wavelength for 1 % w/v HAuCl_4
 (b) Plot of absorbance Vs wavelength for GNP (SBH)
 (c) Plot of absorbance Vs wavelength for GNP (STB)

(iii) XRD analysis

The XRD diffractograms of gold nanoparticles prepared by using sodium borohydride as reducing agent is illustrated in Figure 2. The XRD diffractogram exhibited the broad maximum extending 2θ values from 37.789° to 64.194° , indicating the presence of gold nanoparticles which was found to be cubic and the average size was 32.81 nm (Table 2).

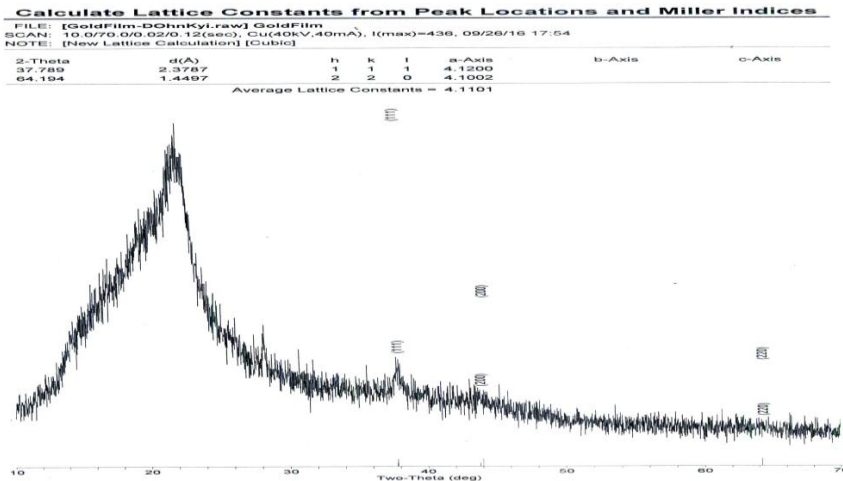


Figure 2 : XRD diffractogram of gold nanoparticles prepared by using 1 % NaBH_4

Table 2: Crystallite Size of Gold Nanoparticles

2θ ($^{\circ}$)	$d(A^{\circ})$	h	k	l	FWHM	Sizes (nm)
37.789	2.3787	1	1	1	0.220	26.88
64.194	1.4497	2	2	0	0.171	38.74

Average crystallite size = 32.81 nm

Chitosan Beads and Chitosan Gold Nanoparticles Composite Beads

To prepare the chitosan beads, chitosan flake was firstly dissolved in 1 % acetic acid to give 3 % chitosan solution. This solution was added dropwise into 2 % sodium hydroxide solution to produce chitosan beads. The resultant chitosan beads are illustrated in Figures 3(a), (b) and (c). In the case of chitosan gold nanoparticles composite beads, the composite beads were prepared by adding the mixture of chitosan (3 %) and colloidal gold nanoparticles solution into 2 % sodium hydroxide solution. The observed composite beads are shown in Figures 4 and 5.

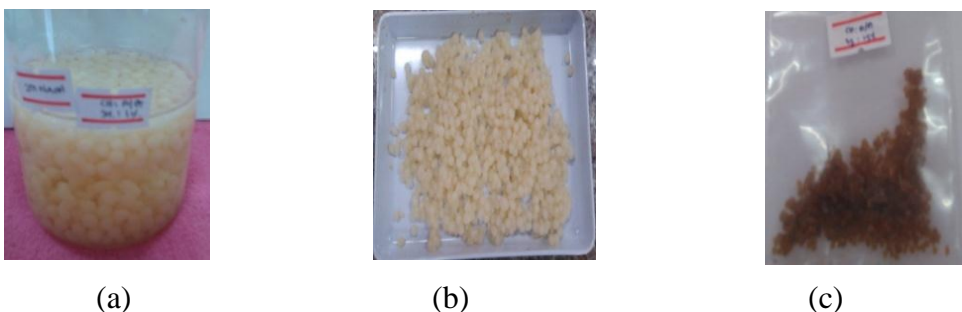


Figure 3: Prepared chitosan beads (CS) (a) in 2 % NaOH solution (b) wet chitosan beads (c) dry chitosan beads

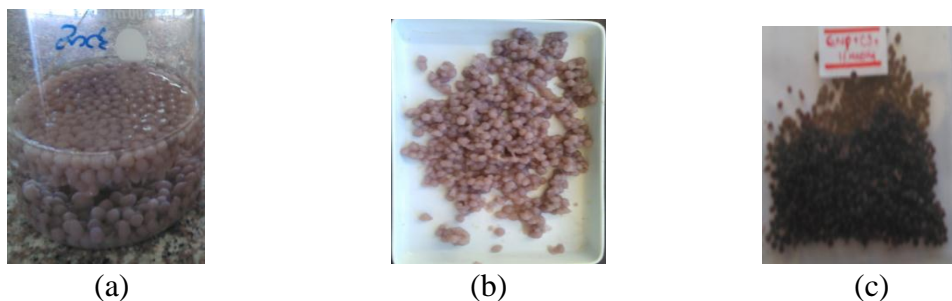


Figure 4:Chitosan gold nanoparticles prepared by using 1 % sodium borohydride (a) in 2 % NaOH solution (b) wet beads (c) dry beads

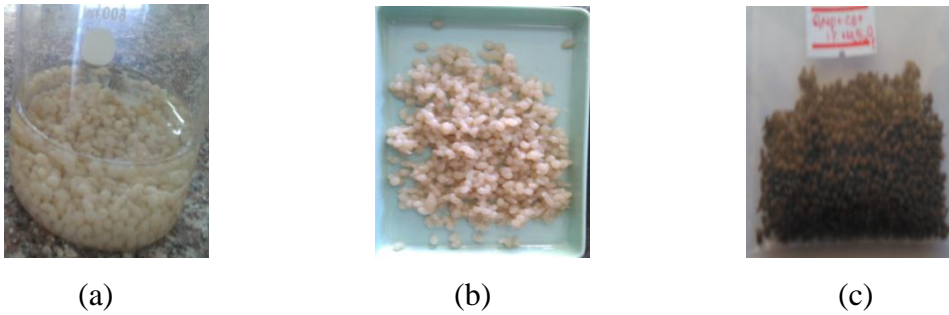


Figure 5: Chitosan gold nanoparticles prepared using sodium tetraborate (a) in 2 % NaOH solution (b) wet beads (c) dry beads

Physical Properties of Chitosan and Chitosan Gold Nanoparticles (CS-GNP) Composite Beads

The physical properties of all of the prepared beads were determined such as water uptake %, moisture content % and pH. The results are shown in Table 3.

Table 3: Physical Properties of Chitosan Beads and Chitosan Gold Nanoparticles Composite Beads

Sample	Water Uptake (%)	Moisture Content (%)	pH
CS	58.82	20.27	6.84
CS-GNP (SBH)	40.21	13.32	6.81
CS-GNP (STB)	39.84	13.21	6.83

CS = chitosan beads

CS-GNP (SBH) = chitosan gold nanoparticles prepared by using 1 % w/v sodium borohydride

CS-GNP (STB) = chitosan gold nanoparticles prepared by using 1 % w/v sodium tetraborate

From Table 2, it was found that the water uptake percent and moisture percent of prepared chitosan gold nanoparticles composite beads by using 1 % w/v sodium borohydride and 1 % w/v sodium tetraborate were decreased compared with prepared chitosan beads due to the reduction of gold ions by reducing agents. Moreover all prepared beads have neutral from the determination of pH.

Determinations of swelling percent of all prepared dry beads were also carried out at different contact times and at different pH of phosphate buffer solutions. The observed data are shown in Tables 4, 5 and 6 and Figure 6.

Table 4: Swelling Percents of the Prepared Chitosan Beads at Different Contact Times and at Different pH Values

pH	Swelling Percent (%)							
	1h	2 h	3 h	4 h	5 h	6 h	7 h	8 h
1.4	30.11	31.30	32.57	35.75	38.40	39.25	40.12	40.12
2.4	29.13	30.41	33.00	35.05	37.10	38.11	39.14	39.14
6.9	13.52	15.41	15.55	16.01	16.22	16.42	16.42	16.42
7.4	13.44	14.21	14.50	14.52	14.57	14.57	14.59	14.59

Table 5: Swelling Percents of the Prepared CS -GNP (SBH) Composite Beads at Different Contact Times and at Different pH Value

pH	Swelling Percent (%)							
	1	2 h	3 h	4 h	5 h	6 h	7 h	8 h
1.4	30.	38.52	46.3	57.1	57.4	58.53	58.7	58.71
2.4	29.	37.11	45.2	50.1	51.6	52.00	52.20	52.20
6.9	13.	15.23	16.1	16.4	16.8	17.00	17.21	17.21
7.4	9.7	10.31	11.1	11.9	12.2	13.00	13.72	13.72

Table 6: Swelling Percents of the Prepared CS-GNP (STB) Composite Beads at Different Contact Times and at Different pH Value

pH	Swelling Percent (%)							
	1	2 h	3 h	4 h	5 h	6 h	7 h	8 h
1.4	30.01	35.41	42.1	48.4	48.96	50.1	52.24	52.24
2.4	28.71	32.4	37.5	42.3	48.44	50.0	51.48	51.48
6.9	13.40	16.1	16.4	16.9	17.00	17.1	17.18	17.18
7.4	9.52	10.1	10.7	11.9	12.07	12.9	13.21	13.21

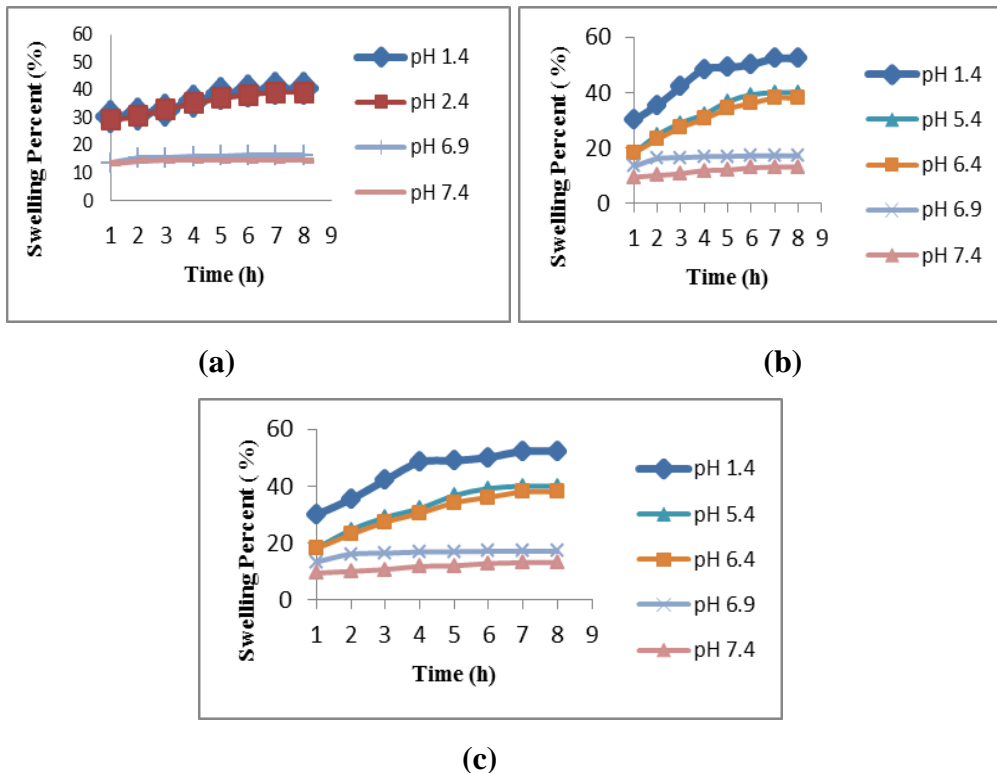


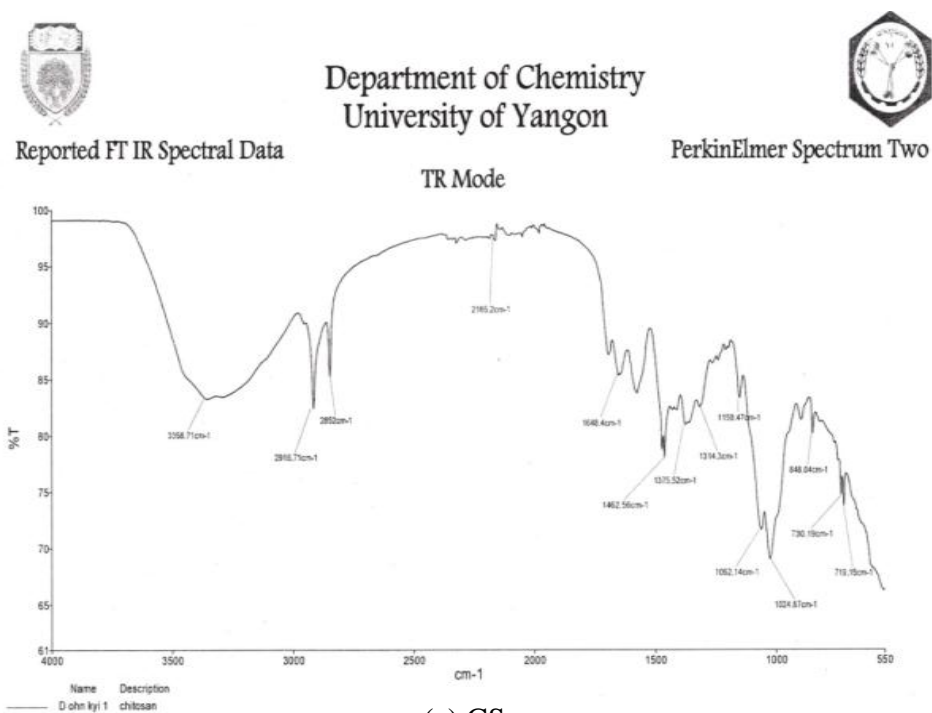
Figure 6: Swelling percent Vs contact time of chitosan beads and chitosan gold nanoparticles composite beads at different pH

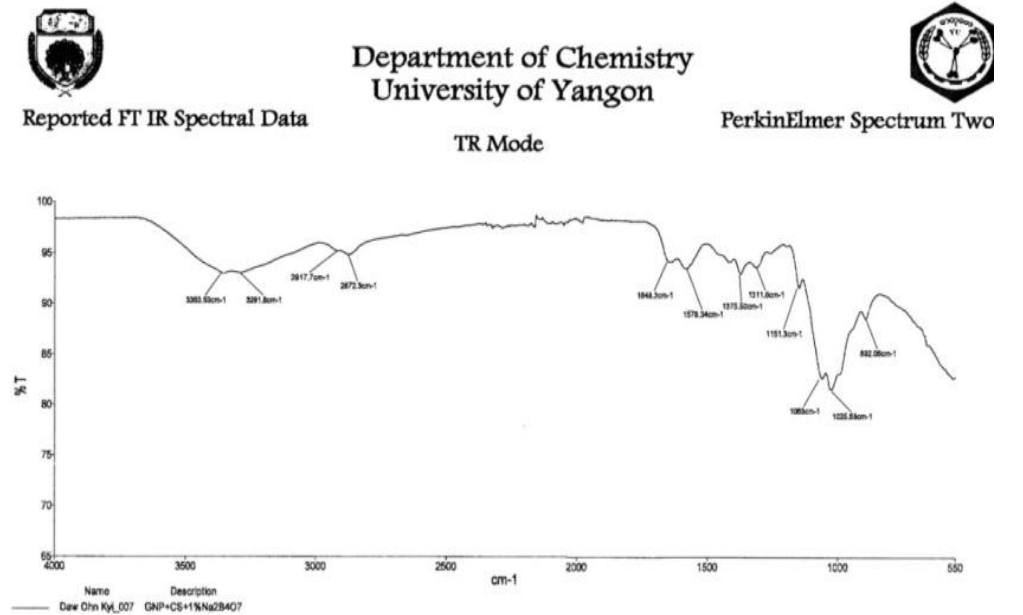
From figure 6, the prepared chitosan beads was gradually increased in swelling percent with increasing the contact times at pH 1.4 due to the protonation of amino group in chitosan. And the prepared chitosan gold nanoparticles composite beads were also increasing swelling percent with increasing the contact times in acidic condition due to the protonation of NH_2 group and reduction of gold ion by reducing agents.

Characterization of the Prepared Chitosan Beads and Chitosan Gold Nanoparticles Composite Beads

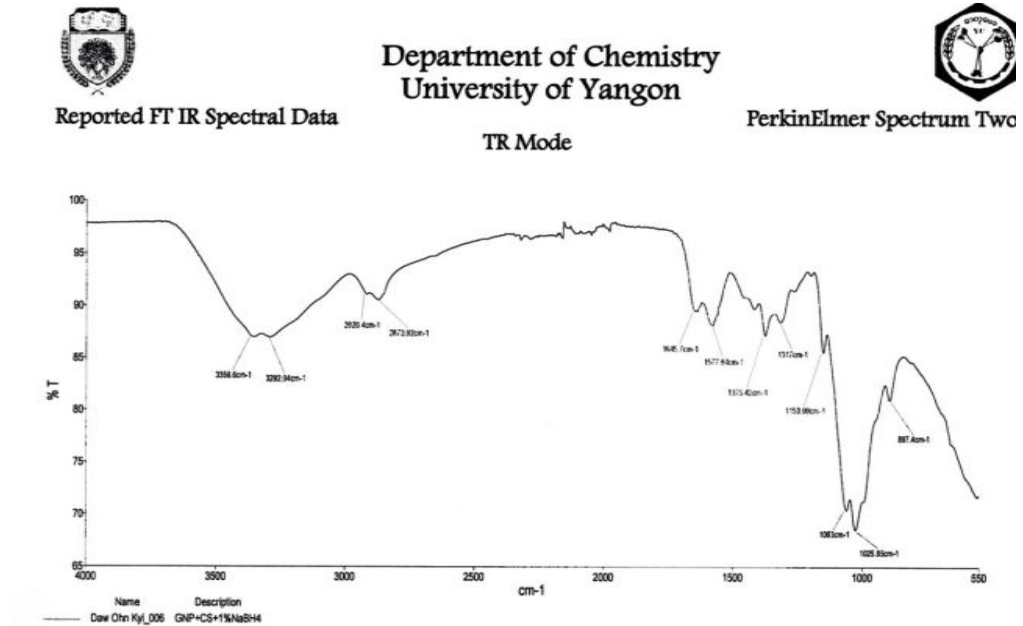
FT IR spectra of the prepared chitosan beads (CS) and chitosan gold nanoparticles composite beads such as CS-GNP (SBH) and CS-GNP (STB) are presented in Figure 7, and the $-\text{NH}_2$ and $-\text{OH}$ stretching vibrations were respectively observed at 3358 cm^{-1} , 3293 cm^{-1} and 3280 cm^{-1} . The CH_2

symmetric and asymmetric stretching vibrations of prepared beads appeared at 2916 cm^{-1} , 2920 cm^{-1} and 2874 cm^{-1} . The C = O stretching in amide bonding of chitosan beads were observed at 1648 cm^{-1} and the CH_2OH antisymmetric deformation in chitosan gold nanoparticles were not observed. The band observed at 848 cm^{-1} and 893 cm^{-1} are the absorption of $\beta - \text{D}$ glucose unit. In figure 7 (b) and (c), it can be found that the band assigned around 700 cm^{-1} showed metal – nitrogen stretching. (Figure 7(b) and (c) Barbara, H and Stuart, B .2006)





(b) CS-GNP (STB)



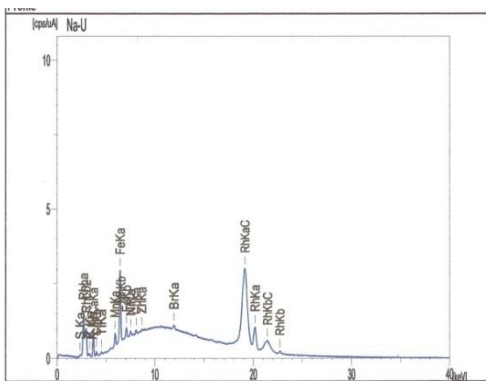
(c) CS-GNP (SBH)

Figure 7: FT IR spectra of (a) CS beads (b) CS-GNP (STB) (c) CS- GNP (SBH)

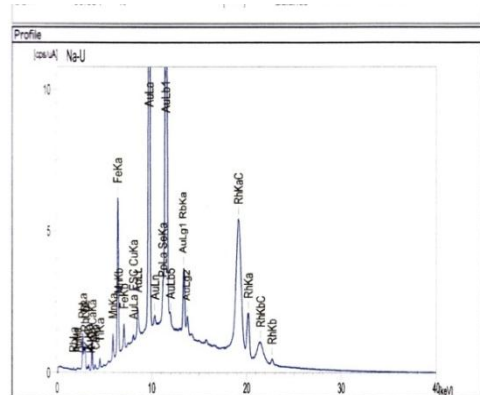
Table 7: Relative Abundance of Gold in the Prepared Samples by EDXRF

Element	Relative abundance (%)	
	CS-GNP (SBH)	CS-GNP (STB)
Ca	0.100	0.194
Au	0.227	0.260
Fe	0.051	0.072
Mn	0.011	0.016
K	0.033	0.038
Ti	0.014	0.009
Se	0.001	-
Br	0.002	0.003
Ni	0.002	-
Ba	-	0.030
COH	99.554	98.392

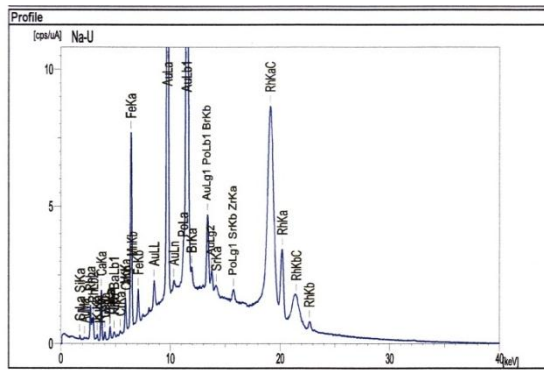
Figure 8 shows the EDXRF spectra of prepared chitosan beads and chitosan- gold nanoparticles composite beads. The relative abundance of gold in prepared CS-GNP composite beads described in Table 7. From the results, it was obvious that the relative abundance of gold percent in composite beads such as CS-GNP (SBH) and CS-GNP (STB) was 0.227% and 0.260%, respectively. However gold was not present in CS beads.



(a) CS



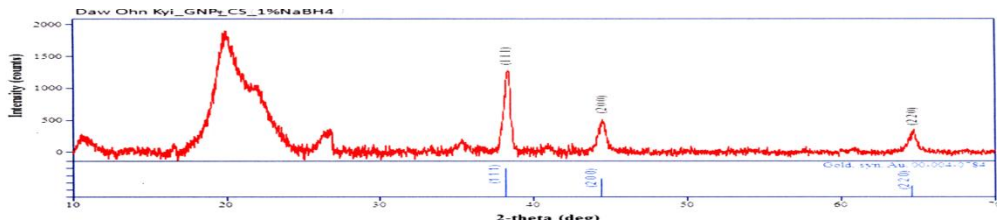
(b) CS-GNP (SBH)



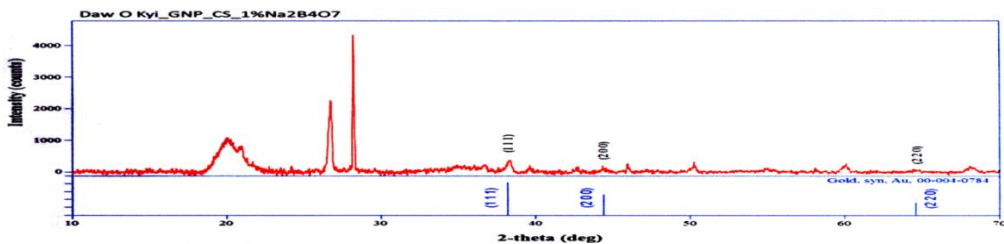
(c) CS-GNP (STB)

Figure 8: EDXRF spectra of (a) CS beads (b) CS-GNP (SBH) (c) CS-GNP (STB)

The X-ray diffraction patterns of the prepared chitosan –gold nanoparticles composite beads by using sodium borohydride and sodium tetra borate are shown in Figure 9 and Tables 8 and 9. The XRD patterns exhibit sharp and 2θ values are 38.2874° , 44.5027° and 64.7585° for CS- GNP (SBH) whereas 38.927° , 44.392° and 64.5859° for CS-GNP (STB). They are an indication of cubic and 17.33 nm (CS-GNP (SBH) and 28.16 nm (CS-GNP (STB) in size.



(a) CS-GNP (SBH)



(b) CS-GNP (STB)

Figure 9: XRD spectra of (a) CS-GNP (SBH) (b) CS-GNP (STB)

Table 8: Crystallite Size of Chitosan Gold Nanoparticle Composite Beads Prepared by Using NaBH₄

2θ (°)	d(Å)	h k l	FWHM	Size (nm)
38.2874	2.3489	1 1 1	0.4676	18.78
44.5027	2.0342	2 0 0	0.5076	17.66
64.7585	1.4384	2 2 0	0.6319	15.54

Average crystallite size = 17.33 nm

Table 9: Crystallite Size of Chitosan-Gold Nanoparticle Composite Beads Using Na₂B₄O₇

2θ (°)	d(Å)	h k l	FWHM	Size (nm)
38.927	2.3545	1 1 1	0.3263	26.91
44.392	2.0391	2 0 0	0.3263	27.47
64.5859	1.4418	2 2 0	0.3263	30.09

Average crystallite size = 28.16 nm

Scanning electron micrographs showing the surface morphology of the prepared chitosan beads and chitosan gold nanoparticles composite beads are given in Figure 10. It is obvious that there are rough surface with small pores was observed on the surface of prepared beads.

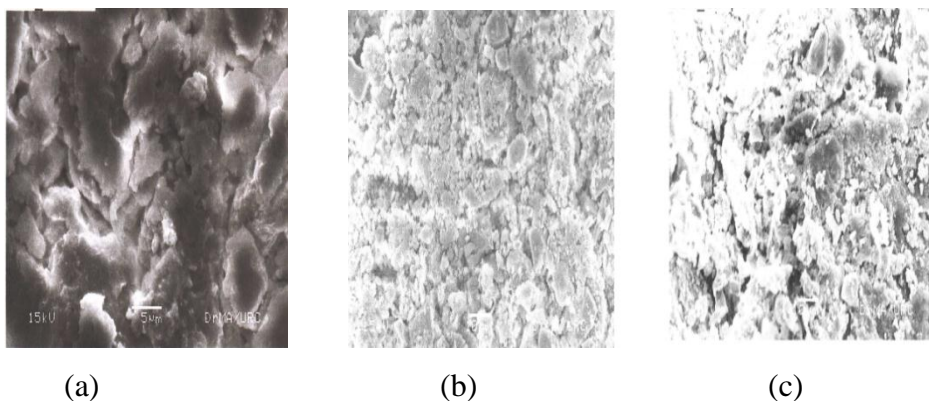


Figure 10: SEM microphotographs of (a) CS (b) CS-GNP (SBH) (c) CS-GNP (STB)

Conclusion

Chitosan with degree of deacetylation 81.13 % was purchased from Golden Dragon Co. Ltd., FMI city, Hlaing Thar Yar Township, Yangon Region. Chitosan gold nanoparticles composite beads were prepared from 3 % w/v chitosan solution and 1 % w/v chloroauric acid solution by using reducing agents 1 % w/v sodium borohydride and 1 % w/v sodium tetra borate solution. The prepared chitosan beads and chitosan gold nanoparticles composite beads were studied their physical properties such as water uptake (%), moisture content (%), swelling behavior and pH and also characterized by SEM, FT IR, EDXRF and XRD analytical methods.

From SEM analysis, the prepared samples have small racks and pores. The porous natures were found in the SEM microphotographs of CS-GNP composite beads. According to the XRD diffractogram, prepared samples exhibited three broad maximum peaks having 2θ values, 38° , 44° and 64° respectively. This was an indication of cubic. From EDXRF analysis, the relative abundance of gold percents in composite beads was found to be 0.227 % CS-GNP (SBH) and 0.260 % CS-GNP (STB).

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