GREEN SYNTHESIS OF GOLD NANOPARTICLES AND CHITOSAN-GOLD NANOPARTICLES COMPOSITE BEADS AND THEIR APPLICATION

Ma Ohn Kyi[1], KhinThan Yee[2], Ma Hla Ngwe[3]

Abstract

This research work concerns with the green synthesis and characterization of gold nanoparticles (GNP) and chitosan-gold nanoparticles (CS-GNP) composite beads. The gold nanoparticles (GNP) were prepared from chloroauric acid in the presence of the aqueous extract from fresh papaya leaf as well as dry papaya leaf used as reducing agents, respectively. The existence of prepared GNP in colloidal solutions was determined by using laser pointer, noted as GNP (G) and GNP (D), Tyndall effect and the synthesized gold nanoparticles (GNP) solutions were also characterized by UV-visible spectroscopy, TEM, SEM, EDXRF and XRD analyses. On the other hand, chitosan beads were produced from different concentrations of chitosan (1 % to 5 % w/v) in 1 % v/v acetic acid solution and in 2 % w/v sodium hydroxide solution. The chitosan-gold nanoparticles composites were then prepared from gold nanoparticles and 3 % w/v chitosan solutions. These composites solutions were forced through by syringe into 2 % w/v sodium hydroxide to form beads, were designed as CS-GNP (G) and CS-GNP (D).The physical properties of prepared chitosan beads and chitosan-gold nanoparticles composite beads were studied, such as water content, moisture content, pH and swelling percent in different pH of buffer solutions at different contact times, and these samples were also characterized by using FT IR, EDXRF, SEM and XRD analyses. Then these prepared composite beads were applied in drug delivery system. Aspirin (ASA) was used as a model drug. The absorption of aspirin by the composite beads was determined by using UV-visible spectrophotometer. The physical properties of drug loaded chitosan-gold nanoparticles composite beads such as ASA-CS-GNP (G) and ASA-CS-GNP (D) were studied and these drug loaded samples have been characterized by FT IR, EDXRF, SEM and XRD analyses. Then drug release behaviors of such drug loaded composite beads were investigated in simulated gastrointestinal fluid pH of buffer solutions and acute toxicity of the prepared chitosan-gold nanoparticles colloidal solutions was examined.

Keywords: Chitosan, gold nanoparticles, chitosan-gold nanoparticles composite beads,Tyndall effect, drug release behaviors, acute toxicity

Introduction

Chitosan can be obtained from deacetylation of chitin which is widely available from shrimps, crabs, and crawfish. It also exists naturally in a few species of fungi and is associated with proteins (Domard and Rinaudo, 1983). Chitosan, positive ionic charges chemically bind with negatively charged fats, lipids, cholesterol, metal ions, proteins, and macromolecules. Chitosan is readily soluble in dilute acetic acid solution below pH 6.0. The solubility is controlled by the degree of deacetylation (Mima *et al.*, 1983). Aqueous extract fresh and dry *papaya* leaves were used as reducing agents in the present study and reduced chloroauric acid solution from Au^{3+} to Au^{0} (Sumit *et al*., 2012). GNPs are nanospheres, nanorods, nanoshells, nanocages and Surface Enhance Raman Scattering properties (SERS), which depend on the size, shape and physical properties (Kirubha and Alagumuthu, 2014). GNPs have unique optical and physical properties and used as sensors in environmental science, medicine, pharmacy and

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engineering due to their non-toxicity (Solomon, S. 2007). GNPs display different colours such as green, orange, dark purple and ruby red etc and 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 solution followed by the composite beads which are used for biomedical purposes in the areas of tissues engineering, drug delivery and cellulose therapies (Papasani *et al.,* 2012). In this research, acute toxic effect of green synthesized chitosan-gold nanoparticles composites was studied on albino mice, and they were applied in drug delivery system.

Materials and Methods

Materials

The chemicals used in this research work were the products from British Drug House (BDH), London and Kanto Chemical Co., Inc., Japan. Fresh *papay*a leaf was collected from University of Medicine Campus, Taunggyi, Myanmar and chitosan (degree of deacetylation 81.13 %) was purchased from Golden Dragon Co., Ltd, FMI City, HlaingTharYar Township, Yangon, Myanmar.

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 (100-1100ºC, Gallenkamp, West Australia), Magnetic Stirrer (Gallenkamp, England), UV spectrophotometer (UV mini 1240), XRD (Rigaku, D-max -2200, Japan).

Preparation of Fresh and Dry *Papaya* Leaves Extracts

The collected fresh *papaya* leaves were washed thoroughly three times with the distilled water and once in the deionized water. These leaves were dried for 5 days at room temperature to obtain dry leaves. The fresh and dry *papaya* leaves were used to make the respective aqueous extract which was prepared by taking 10 g of finely leaves or leaf powder in a 500 mL Erlenmeyer flask with 100 mL deionized water followed by boiling the mixture for 5 min, separately. Then the extracts were filtered through Whatman filter paper No.1 and kept in the refrigerator at 4ºC for further experiments (Farooquimaqdoom *et al.,* 2013).

Green Synthesis of Gold Nanoparticles Using 1 % w/v Chloroauric Acid with Aqueous Extracts

5 mL, 10 mL and 15 mL of each of the aqueous extract from the fresh leaves and dry leaves were taken into 20 mL test tubes separately. 1 mL of 1% w/v chloroauric acid solution added drop-wise into each of extract with the ratios of 5:1, 10:1 and 15:1, respectively. Within 10 min they changed colour from light reddish brown to deep purple and kept at room temperature for 24 h.

Characterization of the Synthesized Gold Nanoparticles Solutions

Confirmation for the existence of gold nanoparticles in solution by Tyndall effect

When the synthesized gold nanoparticles solutions (5 mL, 10 mL and 15 mL) in the test tubes were pointed out with a laser pointer, the light scattered through the solutions.

UV-visible spectroscopy, EDXRF, XRD, SEM and XRD analyses

The formation and the presence of gold nanoparticles were studied by using UV-visible spectroscopy. In this experiment, the absorbance of the synthesized gold nanoaparticles solutions (5 mL, 10 mL and 15 mL) as well as the chloroauric acid solution was measured at different wavelengths ranged from 480 to 580 nm. The gold element present in such samples was determined by EDXRF analysis and the crystallite sizes of the gold nanoparticles were investigated by XRD analysis using XRD diffractometer (Rigaku, D- max-2200, Japan).The morphology and structure of synthesized gold nanoparticles were examined by TEM (JEOL JEM, 3010) and SEM (JSM 5610, JEOL Ltd Japan).

Preparation of Chitosan Beads, Chitosan-Gold Nanoparticles Composite Beads and Determination of their Physical Properties

Chitosan flakes (1g, 2 g, 3 g, 4 g and 5 g) were placed into each of 250 mL beakers. 100 mL of 1 % v/v acetic acid solution was added into each beaker and stirred with a magnetic stirrer thoroughly until the solids were dissolved and allowed to stand overnight to become uniform solution. Then 2 % w/v sodium hydroxide solution was prepared and stirred witha magnetic stirrer for 30 min. When the prepared chitosan solutions was forced through by a syringe into the 2 % (w/v)sodium hydroxide solution separately at a constant rate of 10-12 drops per min, irregular and regular shape of chitosan beads were formed. However the regular shapes of chitosan beads were selected and allowed to stand for 12 h followed by filteration and washing three times with distilled water. The beads were dried at room temperature for 24 h and placed in an oven at 45ºC for another 24 h. Similarly chitosan-gold nanoparticles composite beads were prepared as the same procedure by using 3 g of chitosan and the synthesized gold nanoparticles colloidal solutions. These prepared chitosan beads and chitosan-gold nanoparticles composite beads were subjected to study their physical properties such as water uptake, moisture content, pH and the degree of swelling in different pH (1.4, 5.4, 6.8 and 7.4) of different buffer solutions on contact time 8 h.

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 analyses.

Determination of Acute Toxicity of Chitosan–Gold Nanoparticles Colloidal Solutions

Acute toxicity of different doses of chitosan-gold nanoparticles colloidal samples was evaluated by the method of OECD Guidelines for the Testing of Chemicals 423. According to the test description, 15 total number of adult female albino mice, weighing 25-30 g were selected and divided into five groups. Each group contained three animals. They were fasted for 18 h before giving the chitosan-gold nanoparticles colloidal samples. Four groups were orally administrated with 5000 mg/kg body weight dose and 2000 mg/kg body weight dose of each sample, respectively. One group performed as a control group and they were treated with clean water and normal laboratory animal food of Laboratory Animal Services Division, Department of Medical Research. All groups of mice were kept in the five mouse cages in the separated room at the room temperature of 26 ± 1º C. After administration of chitosan-gold nanoparticles colloidal samples on each group of animals, the animals were observed first 6 h continuously for mortality and behavior changes. Then the animals were checked each 24 h for fourteen days. The mortality during this period was noted (Nil or percent death).

Extraction Preparation of Aspirin solution

The aspirin crystals were extracted from aspirin tablets. Three tablets of aspirin (80 mg) were placed into 50 mL beaker and 10 mL of ethanol was added and stirred followed by filtered into a porcelain crucible and heated in an oven at 45± 5º C for 1 h. After heating the aspirin were obtained and was covered and cooled in desiccators for 30 min. When the obtained aspirin crystals crystal (0.003 g) was dissolved in 100 mL ethanol, 30 mg/L (30 ppm) of aspirin solution was obtained.

Determination of Absorbance of the Prepared Aspirin Solution by Using UV-Visible Spectrophotometer and Construction of Calibration Curve

The absorbance of prepared aspirin solution was investigated by UV visible spectroscopy. From the results it was found that the maximum absorbance was at the wavelength 276 nm which was agreed with the literature. Then a series of different concentrations of aspirin solutions (30, 15, 7.5, 3.7, 1.85, 0.925 and 0.463 mg/L) in ethanol were prepared. The absorbance of these solutions was measured by UV-visible spectrophotometer followed by plotting the calibration curve.

Preparation of Aspirin Loaded Chitosan-Gold Nanoparticles Composite Beads and Determination of their Physical Properties

Each of the wet chitosan-gold nanoparticles composite beads (about 1 g) was separately immersed in 100 mL of 30 mg/L prepared aspirin solution for 24 h, and filtered and dried at room temperature for 36 h. The amount of drug loaded on composite beads was determined by using UV-visible spectrophotometer. Some physical properties (free moisture content, pH and swelling percent) of the prepared drug loaded chitosan-gold nanoparticles composite beads were also determined.

Characterization of the Aspirin Loaded Chitosan - Gold Nanoparticles Composite Beads

The drug loaded chitosan-gold nanoparticles composite beads were characterized by EDXRF, XRD, SEM, EDXRF and FT IR analyses.

Application of the Aspirin Loaded Chitosan-Gold Nanoparticles Composite Beads in Drug Delivery System

Determination of aspirin release from aspirin loaded chitosan-gold nanoparticles composite beads using buffer solutions at different pH

0.1 g of drug loaded chitosan-gold nanoparticles composite beads were immersed in 100 mL buffer solutions at different pH (1.4, 5.4, 6.8 and 7.4) and was shaken with an orbital shaker at 75 rpm for 1 h. Then 5 mL of the above solution was withdrawn and the absorbance was measured by using a UV- visible spectrophotometer. This procedure was carried out in simulated gastric fluids at pH 1.4 for 2 h followed by carrying out in simulated intestinal fluids at pH 6.8 for 10 h. The total residence time in buffer solution was 12 h. The concentration of aspirin released was determined spectrophotometrically at 276 nm and the amount of aspirin released from such composite beads.

Results and Discussion

Green Synthesis of Gold Nanoparticles and Characterization

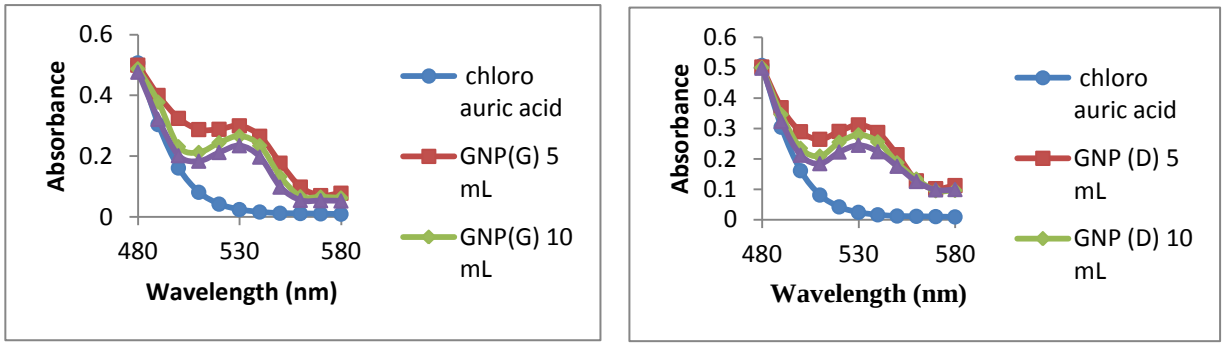
The gold nanoparticles solutions were synthesized from 1 % w/v chloroauricacid with 5 mL, 10 mL and 15 mL aqueous extracts of fresh *papaya* leaves as well as dry leaves, respectively. When chloroauric acid was added drop-wise into the leaves extracts used as reducing agents, the reduction of gold ions into gold particles in solution would happen and followed by colour change to deep purple colour in aqueous solution due to the surface plasmon resonance phenomenon within 10 min as the following equation (Nagajyothi *et al*., 2012).

$HAuCl_4$+ Plant extract $\longrightarrow$ Au^0 (Green synthesis)

The existence of gold nanoparticles (GNPs) in solution was investigated by Tyndall effect and was characterized by UV- visible spectroscopy, EDXRF, XRD, SEM and TEM analyses.

According to the Tyndall effect, it was observed that the light scattered by particles in a solution indicating the presence of gold nanoparticles in solution.

By UV-visible spectroscopy, the resulting GNP colloidal solutions and chloroauric acid were examined by UV- visible spectroscopy, are shown in Figure 1. From figures, it was observed that the maximum wavelength (λ_{max}) of each sample was existed at 530 nm whereas the wavelength range of the absorbance for chloroauric acid was from 480 to 580 nm. In this process, 5 mL of aqueous extract *papaya* leaves showed the λ_{max}value at 530 nm in 10 min of reaction at room temperature, which was superior other aqueous extracts (10 mL and 15 mL) under the same reaction conditions. It might be due to the rapid reduction and formation of gold nanoparticles between chloroauric acid and 5 mL of *papaya* aqueous extract while that of 10 mL as well as 15 mL aqueous extract were significantly slow and after 50 min the purple colour has appeared. The results obtained were interesting in the context of time taken for the synthesis of gold nanoparticles (Kavitha and Harini, 2013). So GNP solutions containing 5 mL of aqueous extract of fresh *papaya* or dry *papaya* leaves with chloroauric acid were chosen to use in this research.



(a) (b)

Figure 1 UV- visible absorption spectra of 1 % w/v chloroauric acid solution and synthesized gold nanoparticles solutions using 5mL, 10 mL and 15 mL aqueous extracts of (a) fresh *papaya* leaves (b) dry *papaya* leaves

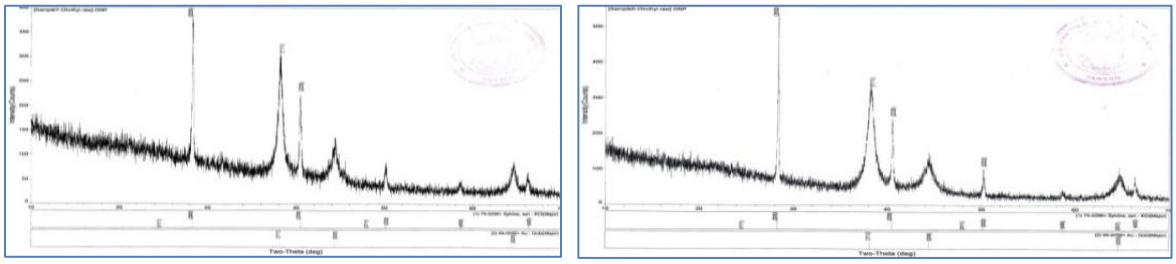
According to the EDXRF analysis, the presence of gold in such samples was determined by EDXRF analysis and the results are shown in Table 1. From this table, it was noted that the relative abundance of gold atom in GNP (G) was 51.5 % and 42.9 % in GNP (D).

Table 1 Relative Abundance of Gold in Prepared GNP (G) and GNP (D)

Sample	Relative abundance of gold (%)
GNP (G)	51.5
GNP (D)	42.9

* GNP (G) = Gold nanoparticles using chloroauric acid with aqueous extracts fresh *papaya* leaf
* GNP (D) = Gold nanoparticles using chloroauric acid with aqueous extracts dry *papaya* leaf

The synthesized gold nanoparticles were also examined by XRD analyses are shown in Figure 2 and Table 2. From these, it was found that the gold nanoparticles were formed andpossessed a face centered cubic (fcc) structure at 2θ value which can be indexed to the (111) orientation. Then the sizes of gold nanoparticles were calculated by using Scherer's equation (Haiss *et al*., 2007). It was observed that the size of GNP (G) was 10.75 nm and that of GNP (D) was 9.65 nm.



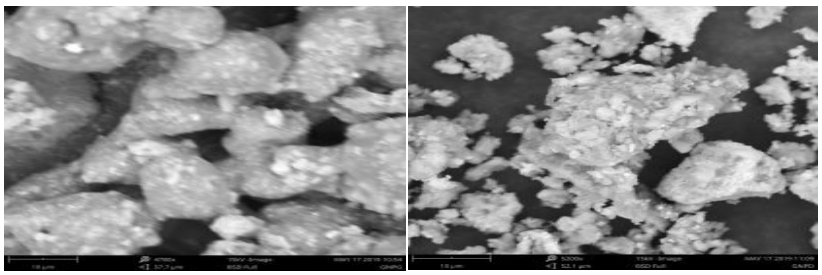
GNP (G) GNP (D)

Figure 2 XRD diffractograms of synthesized gold nanoparticles by green synthesis

Table 2 Crystallite Sizes of the Synthesized Gold-Nanoparticles Using 1 % w/v Chloroauric Acid and Aqueous Extracts of Fresh and Dry *Papaya* Leaves

Sample	2θ (deg)	d (Å)	h k l	FWHM (deg)	Size (nm)	Structure
GNP (G)	38.239	2.3517	(1 1 1)	0.542	10.75	Cubic(a=b=c)
GNP (D)	38.260	2.3505	(1 1 1)	0.606	9.65	Cubic(a=b=c)

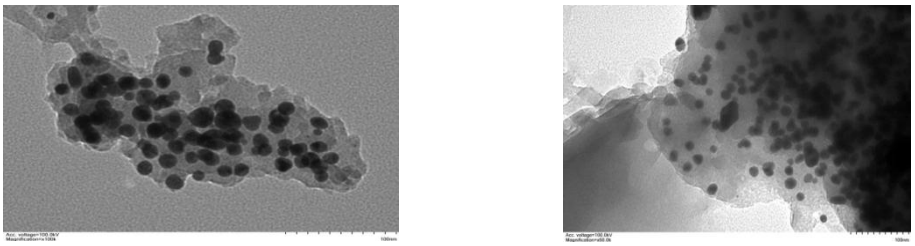
The SEM images are shown in Figures 3 (a) and (b) for the synthesized gold nanoparticles. From images, some porous or cavities and small cracks were found on the surfaces of gold nanoparticles.



GNP (G) GNP (D)

Figure 3 SEM images of the synthesized gold nanoparticles GNP (G) and GNP (D)

The TEM images shown in Figures 4 (a) and (b) are for the synthesized gold nanoparticles. From the results, general observations indicated that the size of GNP (G) was bigger than that of GNP (D). The particles size of GNP (G) was within 100 nm while that of GNP (D) was within 50 nm.



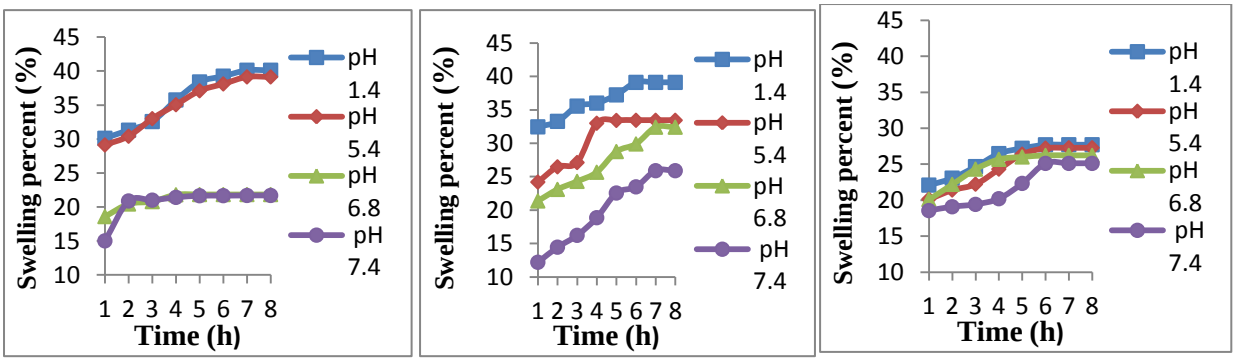
GNP (G) GNP (D)

Figure 4 TEM images of the synthesized gold nanoparticles GNP (G) and GNP (D)

Chitosan Beads and Chitosan-Gold Nanoparticles Composite beads

According to the preparation of chitosan beads, 1 % and 2 % chitosan solutions have nearly liquid so they formed irregular shape of beads with more porous structures in sodium hydroxide solution. On the other hands, a high concentration of chitosan (>3 % w/v) was not feasible because they were too viscous to extrude through the syringe. Thus 3 % w/v chitosan solution was chosen to prepare beads in this study. Similarly, chitosan-gold nanoparticles (CS-GNP) composite beads were prepared when the prepared chitosan-gold nanoparticles colloidal solutions were forced through by using a syringe into gently stirred 2 % w/v sodium hydroxide solution.

According to the determination of physical properties of such samples, water uptake percent and moisture content percent of chitosan beads were 58.82 % and 20.27 % while CS-GNP (G) composite beads showed 42.18 % of water uptake and 15.02 %of moisture and CS-GNP (D) composite beads showed 38.20 % water uptake and 12.12 % of moisture content. As a result, the water uptake percent and moisture content percent of gold nanoparticles composite beads were found to be lower than chitosan beads. From the determination of pH, all prepared beads were neutral. The swelling behaviours of all prepared beads were investigated at different pH 1.4, 5.4, 6.8 and 7.4 for 8h and are shown in Figure 5. From these investigations, it was found that the swelling percent of chitosan beads (CS) was 40.12 % in pH 1.4 whereas that of CS-GNP (G) was 39.11 %and that of CS-GNP (D) was 27.71 %. However, the swelling percent of prepared chitosan beads and chitosan-gold nanoparticles composite beads decreased significantly in increasing pH. Therefore the prepared beads had higher swelling property in acidic solution than in alkaline condition due to the protonation of amino group of chitosan in acidic solution.



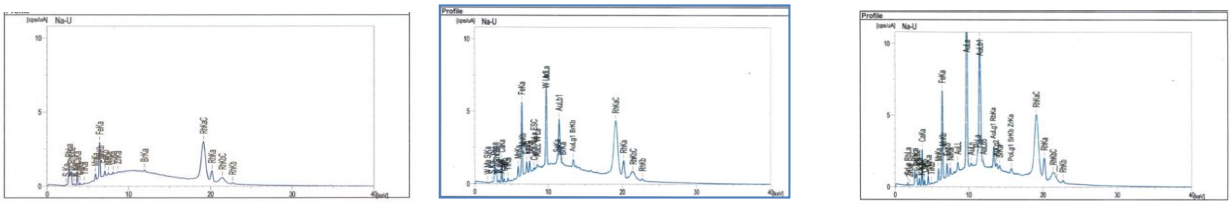
(a) (b) (c)

Figure 5 Swelling percent Vs contact time of (a) CS (b) CS-GNP (G) (c) CS-GNP (D)

Characterization of Chitosan Beads and Chitosan-Gold Nanoparticles Composite Beads

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

The EDXRF spectra of prepared samples are shown in Figure 6. From figures, it was noted that the relative abundance of gold percent in chitosan-gold nanoparticles composite beads was 0.102% in CS-GNP (G) and 0.144% in CS-GNP (D), however there is no gold element present in chitosan beads.



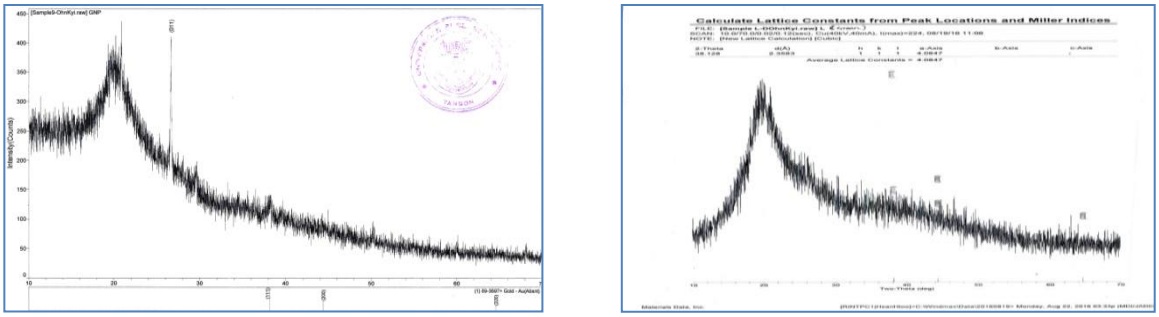
CS CS-GNP (G) CS-GNP (D)

Figure 6 EDXRF spectra of chitosan and chitosan-gold nanoparticles composite beads

Table 3 Relative Abundance of Elements Present in CS-GNP (G) and CS-GNP (D)

No	Elements	CS-GNP (G) (%)	CS-GNP (D) (%)
1	Ca	0.173	0.154
2	Au	0.102	0.144
3	Fe	0.055	0.056
4	Mn	0.014	0.015
5	K	0.013	0.019
6	Ti	0.006	0.007
7	Ag	0.004	-
8	Se	0.002	0.001
9	Br	0.002	0.001
10	Ni	0.002	0.004
11	Zr	0.001	-
12	Cu	-	0.001
13	Si	-	0.473
14	COH	99.627	99.158

The X-ray diffractogramsof prepared chitosan-gold nanoparticles composite beads are shown in Figure 7 and Table 4. The crystallite size of such chitosan-gold nanoparticles composite beads were 12.25nmfor CS-GNP (G) and 16.82 nm for CS-GNP (D), indicated that the attractive forces were formed between chitosan and gold nanoparticles.



CS-GNP (G) CS-GNP (D)

Figure 7 XRD diffractograms of chitosan-gold nanoparticles composite beads

Table 4 Crystallite Sizes of Chitosan-Gold Nanoparticles Composite Beads

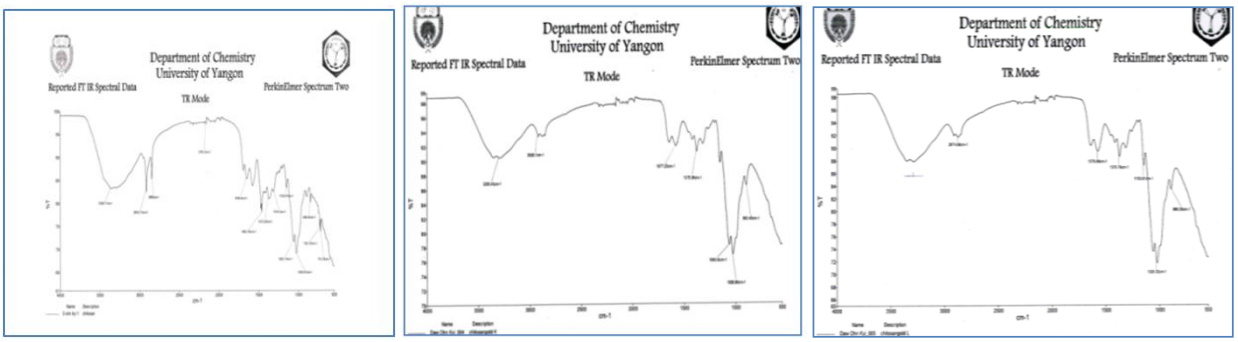
Sample	2θ (deg)	d (Å)	h k l	FWHM(deg)	Size (nm)
CS-GNP(G)	38.262	2.3504	(1 1 1)	0.455	12.25
CS-GNP (D)	38.128	2.3583	(1 1 1)	0.141	16.82

The SEM images of prepared beads are shown in Figure 8. From the SEM images, it was obvious that the surface of prepared samples has small pores with cracking so the surface morphology of all prepared beads was porous nature and had rough surface.

CS CS-GNP (G) CS-GNP (D)

Figure 8 SEM imagesof chitosan beads and chitosan-gold nanoparticles composite beads

The FT IR spectra of prepared chitosan beads and chitosan-gold nanoparticles composite beads are presented in Figure 9. From Figures, the -NH and -OH stretching vibrations were observed at 3358 cm^{-1}in CS, 3293 cm^{-1}in CS-GNP (D) and 3280 cm^{-1} in CS-GNP (G).The C-H symmetric and antisymmetric stretching of CH, CH_2 and CH_3 groups were appeared between 2852 cm^{-1} to 2920 cm^{-1}. Both the C=O stretching in amide bonding and -CH antisymmetric deformation were respectively observed at 1648 cm^{-1} and 1462 cm^{-1} in the spectrum of chitosan beads. However there is no peak formation in composite beads at this wave number. It can be found that the band assigned around 1576 to 1577 cm^{-1} showed new peak was appeared. The bands appeared below 700 cm^{-1} was due to metal-nitrogen stretching or metal-oxygen stretching to produce metal chelates compound (Barbara and Stuart, 2006). It showed that chitosan can bind to gold nanoparticles through free amine group in composite beads.



CS CS-GNP (G) CS-GNP (D)

Figure 9 FT IR spectra of chitosan and chitosan-gold nanoparticles composite beads

Acute Toxicity of Chitosan-Gold Nanoparticles Colloidal Solution

The prepared chitosan-gold nanoparticles composite samples were subjected to examine the toxicity on albino mice model in Department of Medical Research (Ygn) for 14 days. After 14 days, all albino mice were observed still alive and did not show any visible symptom of toxicity like restlessness, respiratory disorders, convulsion, aggressive activities, coma and death up to 5000 mg/ kg body weight dose. For such prepared composite beads were applied in drug delivery system.

Application of Chitosan-Gold Nanoparticles Composite in Drug Delivery System

Aspirin was used as a model drug. Firstly 0.003 g of aspirin was dissolved in 100 mL ethanol; 30 ppm of aspirin solution was obtained and determined the maximum absorptionby using UV- visible spectrophotometer (Shimazu UV-1240).The maximum absorption of aspirin solution was observed at 276 nm is agreed with the literature. The series of different concentrations of aspirin solutions (30, 15, 7.5, 3.75, 1.88, 0.94, 0.46, 0.23 and 0.115mg/L) were prepared and measured the respective absorbance by using UV-visible spectrophotometer followed by constructing the absorbance versus concentrations of aspirin calibration curve. It was used to determine the aspirin contents adsorbed and released by chitosan-gold nanoparticles composite beads.

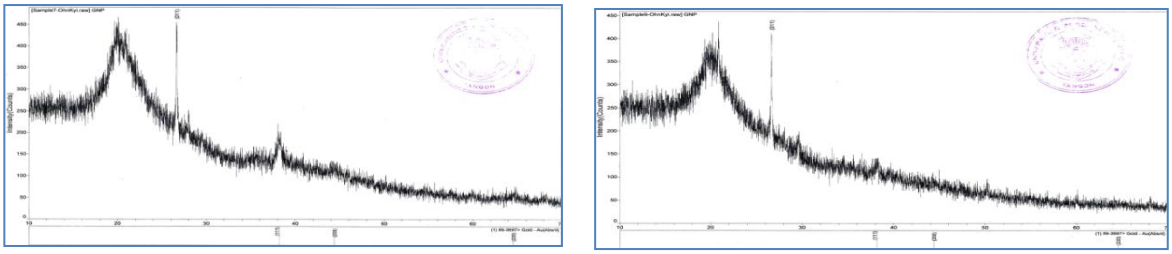
Absorption of aspirin by chitosan-gold nanoparticles composite beads

The composite beads (CS-GNP (G) and CS-GNP (D) 1 g of each were immersed in 30 mg/L aspirin solution for 12 h to become saturated aspirin loaded composite beads and filtered followed by drying at room temperature. The obtained aspirin loaded composite beads [ASA-CS-GNP (G) and ASA-CS-GNP (D)] were found to be rigid and deep reddish brown colour due to the absorption of aspirin into composite beads matrices.

Characterization of aspirin loaded chitosan-gold nanoparticles composite beads

The aspirin loaded chitosan-gold nanoparticles composite beads [ASA-CS-GNP (G) and ASA-CS-GNP (D)]were characterized by XRD, SEM and FT IR analyses.

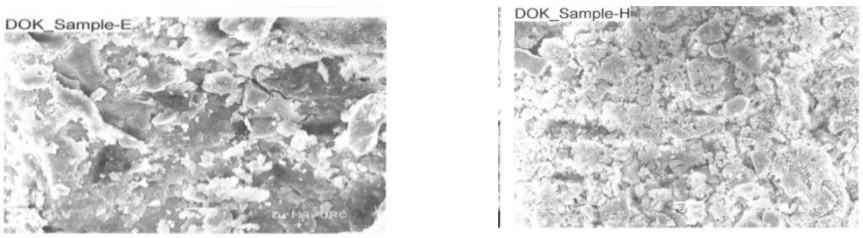
XRD diffractograms of aspirin loaded composite beads are shown in Figure 10. The average crystallite sizes of aspirin loaded chitosan gold nanoparticles composite beads were observed to be 29.07 nm for ASA-CS-GNP (G) and 21.12 nm for ASA-CS-GNP (D). It confirmed that the aspirin was totally covered on the surface of composite beads due to increase in size and applied in drug delivery system.



(a) (b)

Figure 10 XRD diffractogramsof (a) ASA-CS-GNP (G) (b) ASA-CS-GNP (D)

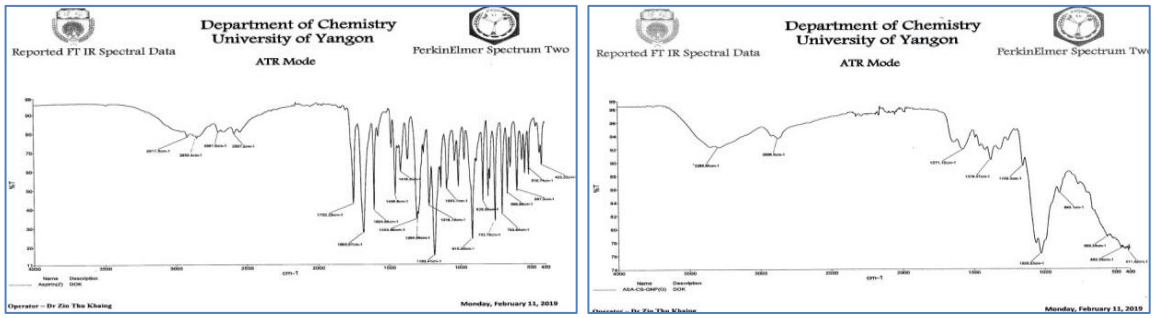
The SEM images of aspirin loaded composite beads are shown in Figure 11.The aspirin loaded composite beads were approximately spherical in shape and had a rough surface. The surface was highly wrinkled and many cavities were in the internal zones. As a result, the aspirin was found to penetrate easily into the beads and the surfaces of composite beads were almost covered with aspirin.



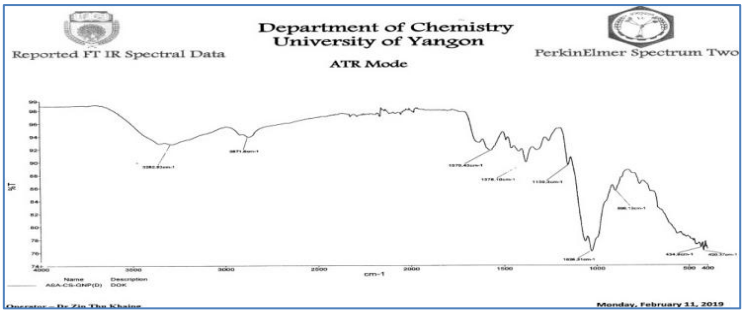
(a) (b)

Figure 11 SEM images of (a) ASA-CS-GNP (G) and (b) ASA-CS-GNP (D)

FT IR spectra of aspirin and aspirin loaded composite beads are shown in Figure 12. From figures, it was observed that the broad band appeared at 3288-3358 cm^{-1}in aspirin loaded composite beads due to -NH and -OH stretching vibration although there is no peak around this band in aspirin due to the absence of these functional groups. Then some of bands at 2918 cm^{-1}, 2849 cm^{-1}, 1681 cm^{-1} and 1604 cm^{-1}in aspirin were observed to be shifted in aspirin loaded composite beads. These stretching shifted were due to cover the most surface area with drug and also occur the intermolecular forces between aspirin and composite beads. It was found that the peak below 700 $cm^{-1}$was observed in metal oxygen or nitrogen stretching (Barbara and Stuart, 2006).



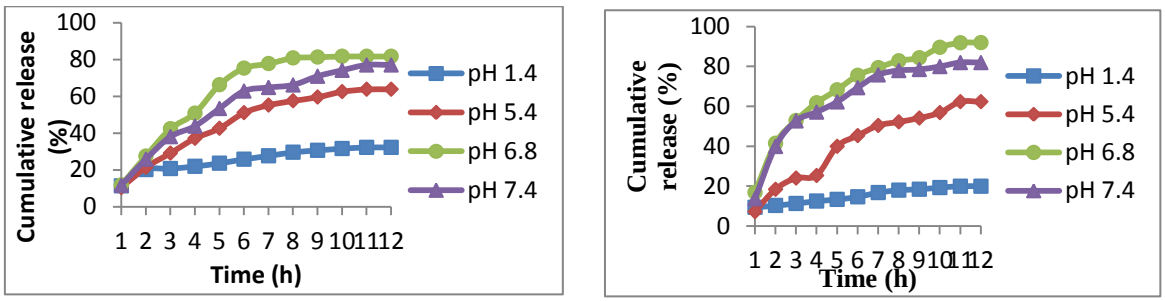
(a) (b)



(c)

Figure 12 FT IR spectra of (a) ASA (b) ASA-CS-GNP (G) (c) ASA-CS-GNP (D)

According to the investigation of aspirin loaded composite beads in drug delivery system within 12 h, *in vitro* drug release properties of chitosan-gold nanoparticles composite beads is shown in Figure 13. It was investigated the percentage of the aspirin released from the composite beads at pH 1.4 were32.2 % in CS-GNP (G) as well as 19.9 % in CS-GNP (D) while the aspirin released at pH 6.8 were found to be increased significantly as 81.7 % in CS-GNP (G) and 92 % in CS-GNP (D). From these results, it was found that aspirin released from CS-GNP (D) composite beads was minimal at pH 1.4 while the aspirin released at pH 6.8 increased significantly. In addition, the results clearly suggested that CS-GNP (D) composite beads could hold the drug better at low pH (1.4) and released more drugs at pH 6.8 than the CS-GNP (G) composite beads so CS-GNP (D) showed excellent pH sensitivity.



(a) (b)

Figure 13 Cumulative release curves of aspirin from (a) CS-GNP (G) composite beads and (b) CS-GNP (D) composite beads at various pH

In addition to investigate the sequential drug release circumstances, chitosan-gold nanoparticle composite beads were immersed in simulated gastric fluid for initial 2 h, and then moved to simulated intestinal fluid. Figure14 indicates that the drug release properties of chitosan-gold nanoparticles composite beads are pH dependent. The amount of aspirin released from CS-GNP (G) composite beads at pH 1.4 was very low (20 %) within 2 h while 15 % aspirin released from the CS-GNP (D) composite beads. However at pH 6.8, the amounts of aspirin release not only increased significantly to 81 % from CS-GNP (G) and 91 % from CS-GNP (D) after 10 h but also increased in release rate in the intestinal tract. This shows that it confirms the sustained drug release which is necessary to increase the drug bioavailability and prolonging therapeutic effect (Bharathi *et al.*, 2011). So the release rate of drugs from composite beads is affected by changing in pH. The increase in release rates could be due to an associated increase in the fluid filled cavities created by dissolution and diffusion of the drug particles near the surface, which in turn results in increase in the permeability of the drug (Bharathi *et al.*, 2011). In

this study, the effect of drug release was dependent upon the factors such as swelling and drug solubility that are determiner of the drug release.

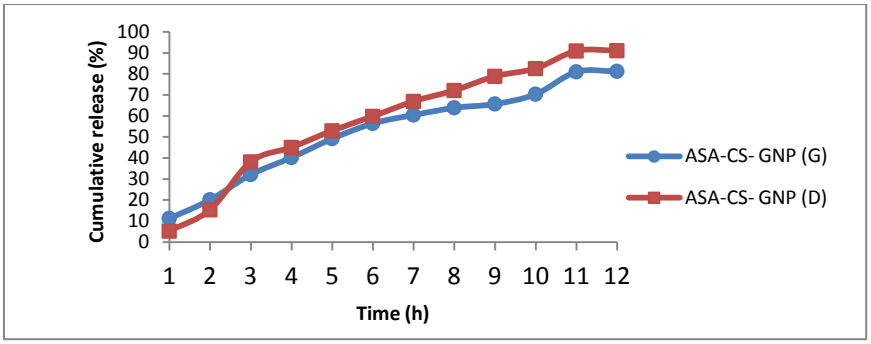


Figure 14 Sequential aspirin release from the aspirin loaded chitosan-gold nanoparticle composite beads in the simulated gastric fluid (pH 1.4) and in the intestinal fluid (pH 6.8) for 12 h

Conclusion

Present investigation deals with the synthesis of gold nanoparticles (GNP) using chloroauric acid as a metal precursor with papaya leaf extract as a reducing agent. Rapid reduction of gold ions was observed in the formation of gold nanoparticles in solution appearing the colour of deep purple under normal conditions for a long time. The dispersion of light through the solution by Tyndall effect confirmed to the existence of colloidal GNP in solution and characterized by UV, TEM, SEM and XRD analyses. From these result, it was observed that the maximum UV – visible absorption peak of colloidal GNP was appeared at 530 nm and the crystallite sizes calculated from XRD diffatograms by the Scherrer's equation were found to be 10.75 nm and 9.65 nm. Chitosan beads and chitosan-gold nanoparticles composite beads were prepared by ionotropic gelation method and studied their physical properties such as water uptake, moisture content, pH and the degree of swelling followed by characterized them by SEM, FT IR, EDXRF and XRD analyses. Aspirin was used as a model drug. The prepared composite beads were coated with aspirin and studied their characterization as well as application into drug delivery system using the different buffer solutions on contact time 12 h. From this result, it was found that aspirin loaded chitosan-gold nanoparticles composite beads using dry *papaya* leaf extract was more release drug (91 %) in alkaline solution (pH 6.8) than that of aspirin loaded chitosan-gold nanoparticles composite beads using fresh *papaya* leaf extract.

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