



STARCH COATED GOLD NANOPARTICLES USING *HYGROPHILA AURICULATA* L FOR CONTROLLED RELEASED OF ANTICANCER DRUG DOXORUBICIN

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ABSTRACT

In this study, we developed encapsulating doxorubicin (DOX) drug loaded starch coated *Hygrophila auriculata* L mediated gold nanoparticles (H-AuNPs) [DOX loaded starch coated-H-AuNPs]. H-AuNPs were characterized by UV-Visible spectrophotometer, XRD, TEM. The DOX-loaded starch coated-H-AuNPs was evaluated by particle size, surface charge, entrapment efficacy, and effect of pH in drug release profile. Additionally, drug entrapment efficacy (EE) was up to 43%; DOX-starch-H-AuNPs showed a pH-responsive drug release in vitro. The DOX release was nearly 79% at pH 5.4 and 68% at pH 7.4. The current work proves the potential of pharmacology that involves a fusion of advanced techniques from nanoscience to develop the biology and used in the fields of drug delivery.

KEYWORDS: *Hygrophila auriculata* L, AuNPs, starch, Doxorubicin, Drug delivery



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INTRODUCTION

Drug delivery system (DDS) is a great challenge for pharmaceutical and medical science to treatment of cancer. In recent biostatistics analysis, it was reported that second largest death caused by cancer¹⁻³. The newly emerging field of nanotechnology is gaining important role to designing anticancer drug for treatment of cancer. The new advance nanotechnology and its unique physical, chemical and biological designing have influenced to pharmaceutical and drug designing research. Nanotechnology is an interdisciplinary such as polymer science, biotechnology, molecular science etc respectively. Today in polymer science, polymeric and metallic nanoparticles have been strongly influenced to medical and pharmaceutical science due to its physical, biological and chemical properties⁴⁻⁵. From different type of metallic nanoparticles, Gold have anticancer activities and suitable for drug conjugation and biosensor activities⁶. For environment safety, the recent research has been accepted to green chemistry technology and rejected to physical and chemical synthesis process. The green synthesis of metallic nanoparticles is created revolution in polymer science as well as nanotechnology. The plant extract contains various types of bio molecules and secondary metabolism such as proteins, sugars, amino acids, enzymes and other traces of metals. These metabolites are strongly involved in the bio reduction process. The proposed reaction was Au⁺ ions reduction into metallic Au nanoparticles in the presence of metabolites and redox enzymes⁷⁻¹³. In the literature review, it has been reported that small size of AuNPs was cross-linking with Doxorubicin. Doxorubicin is an anti-cancer drug that has been shown to be more effectiveness when conjugated to hydrophilic nanoparticles that penetrate more deeply into the cell than the drug alone; nanoparticles may also enhance uptake of unbound doxorubicin¹⁵. Starch is a naturally occurring polysaccharide; it is a biocompatible and biodegradable mucoadhesive polymer that has been extensively used for a potential carrier for different therapeutic agents such as peptides, proteins, vaccines, DNA, and drugs for parenteral and nonparenteral administration. Therapeutic Agent-loaded starch were found to be more stable, permeable, and bioactive¹⁶. In this work, our idea here was to develop an innovative strategy for DOX-loaded starch coated-H-AuNPs to perform enhance the effectiveness of DOX, to overcome DOX resistance and to reduce the toxicity associated and also study it's entrap and release, in a controlled manner.

MATERIAL AND METHOD

Chemicals and Plant Material

Tetrachloro auric acid (HAuCl₄·XH₂O) was purchased from Sigma-Aldrich. Starch and Doxorubicin were purchased from Himedia and Sigma-Aldrich. All other chemicals were used as an analytical grade. Millipore Water was used in the entire experimental work. The *Hygrophila auriculata* L leaf was collected from local garden.

Preparation of *H. auriculata* L leaf Extract

The Fresh *H. auriculata* L (HAL) leaf was collected from the local garden, Mature and healthy leaf were washed and extract prepared with triple distilled water. The extract was centrifuged at 2,000 rpm for 10 minutes. The supernatant containing the extract was filtered through 0.2 µm syringe filter (EMD Millipore) and collected in separate conical flasks by standard filtration method and stored at 4°C.

Synthesizing the HAL-AuNPs

AuNPs were synthesized by the reduction of HAuCl₄·XH₂O by *H. auriculata* L. Leaf extracts. 50 ml of triple distilled water was heated to 60°C in a boiling flask, followed by the addition of 500 µL of *H. auriculata* L. Leaf extract with constant stirring for 10 minutes. Then, 500 µL (0.1 M HAuCl₄) was added dropwise to the mixture. The colour of the reaction mixture changed to purple pink, indicating the synthesis of H-AuNPs. The reaction was stopped by immediate cooling on ice. H-AuNPs with different sizes were obtained by changing the reaction parameters¹⁷.

Starch-coated H-AuNPs

The Starch of 1gm is made 50ml of distilled water at 200rpm stirring for 10 minute. To the above solution was added H-AuNPs in 1:3 ratio and continuing constant stirring without heat supplied for 2 hours. Then prepared starch coated H-AuNPs were ready for further processing¹⁹.

DOX loading in Starch coated H-AuNPs

DOX-loaded starch coated H-AuNPs nanoparticles (1 mg/mL) were mixed and incubated on a magnetic stirrer at 800 rpm overnight. Free starch particles were removed by centrifugation at 4000 rpm for 10 minutes. The DOX-loaded starch coated H-AuNPs nanoparticles were collected by ultra-centrifugation at 30000 rpm for 15 minutes at 4 °C. Based on the above preparation of H-loaded starch coated H-AuNPs, we have drawn a scheme of the mechanism of action for starch coated H-AuNPs for controlled drug released (Figure 1).

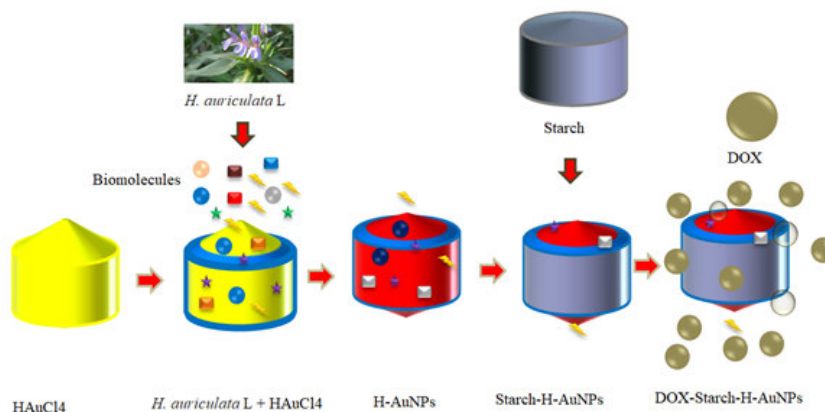


Figure 1
Schematically presented DOX-loaded starch coated H-AuNPs nanoparticles

Characterization

UV-visible spectral analysis

The UV-visible spectrum from 200 to 900 nm of *H. auriculata* L, starch, H-AuNPs, starch coated H-AuNPs and DOX-loaded starch coated H-AuNPs with different pH medium were recorded in an EPOCH™ Multi-Volume Spectrophotometer System (Biotek Instruments, Inc., Mumbai, India). H-AuNPs displayed maximum absorbance at 547 nm with predomination of smaller size particles¹⁸.

X-ray diffraction analysis (XRD)

The lyophilized powders of the H-AuNPs were used for XRD study (powder X-ray diffractometer, Bruker, USA). The diffracted intensities were reported from 0 to 80° at 2θ angles. The diffraction pattern corresponds to starch, H-AuNPs, starch coated H-AuNPs, and DOX-loaded CS coated H-AuNPs. The received results illustrate that HAuCl₄ had indeed been reduced to form of H-AuNPs by *H. auriculata* L leaf extract under reaction conditions¹⁸.

Transmission electron microscopy (TEM)

Morphological characterization of H-AuNPs was carried out by TEM (JEOL-JEM 2100, 1.4 Angstrom Unit, Tokyo, Japan). The H-AuNP and DOX-loaded starch coated H-AuNPs was made by air-drying drops of diluted H-AuNP solutions on carbon films

Then, percentage of DOX content in starch coated H-AuNPs

$$\text{DOX drug loading} = \frac{L}{L_0} \times 100$$

DOX release from starch coated H-AuNPs-DOX

To analysis, DOX from starch coated H-AuNPs, 50 mg of each formulation was incubated in 5 ml of 1x PBS at pH 7 at room temperature. At different time intervals, 5 ml of the supernatant was withdrawn and followed with fresh buffer. Additionally, measurement of effect pH on DOX release from starch coated H-AuNPs, two different buffer solutions: 1X PBS pH 7.4 (physiological pH) and pH 5.4 (pH of tumor tissue) were used to study the effect of pH on drug release. The absorbance of each solution was calibrated by using a UV-Spectrophotometer at a wavelength of 254 nm. All experiments were presented in triplicate.

supported by copper grids. TEM images were visualized at 120 kV under the microscope.

Particle size analysis & zeta-potential measurements

Dynamic light scattering (DLS) was used to estimate the hydrodynamic diameter, and laser Doppler anemometry was used to determine zeta-potential (mV). The DLS and laser Doppler anemometry analyses were shown using a Zetasizer Nano ZS (Malvern Instruments, Worcestershire, UK). To define the particle size and zeta-potential, a dilute suspension of H-AuNPs (100 μl diluted to 1 ml Milli-Q water) was sonicated in an ice bath for the 30s and subjected to particle size and zeta potential determination.

Entrapment Efficiency and Drug Content

During the preparation of DOX encapsulated in starch coated H-AuNPs, the amount of DOX encapsulated into the nanoparticles was calculated by EE formula. A calibration curve was developed using the known standard concentrations of DOX dissolved in DI water. The spectrophotometric quantification was achieved by taking the absorbance at 254 nm. The EE was calculated using the following formula:

STATISTICAL ANALYSIS

Data were expressed as mean ± SD. Statistical analysis was done by one-way ANOVA where appropriate with Graph Pad Prism 5.0 (Graph Pad Software Inc., San Diego, CA, USA).

RESULTS AND DISCUSSION

UV-Vis Spectroscopic

Figure 2 shows the UV-visible absorption spectrum of the HAuCl₄ solution, starch, *H. auriculata* L leaf

extract (HAL), DOX, H-AuNPs, starch coated H-AuNPs, DOX-loaded starch coated H-AuNPs in water. The UV-visible spectrophotometer analysis of the product obtained displayed peaks and bands for SPR absorption. It was observed that the optimum reaction mixture SPR absorbance peak at 547 nm which is also clearly observed in starch coated H-

AuNPs in water¹⁹. The stability of the H-AuNPs and starch coated H-AuNPs -DOX was determined by measuring the absorption spectrum at 24-h intervals for 90 days. No significant changes in absorbance were determined during storage time at 37°C, indicating that the H-AuNPs were stable during this period.

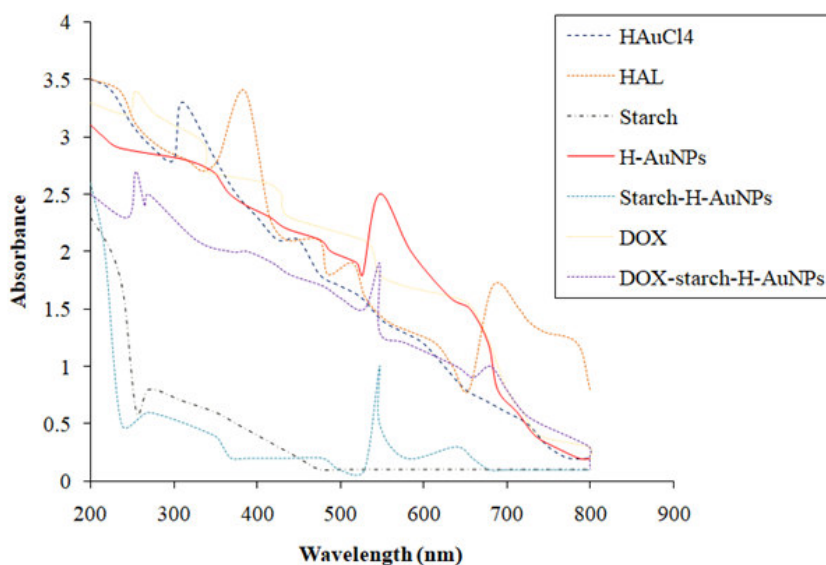


Figure 2
UV-visible spectrum of HAuCl₄ solution, starch, HAL, DOX, H-AuNPs, DOX-loaded starch coated H-AuNPs in water

XRD

In Figure 3, the XRD pattern of the HAuCl₄ solution, starch, *H. auriculata* L leaf extract (HAL), DOX, H-AuNPs, starch coated H-AuNPs, DOX-loaded starch coated H-AuNPs are explained. The diffraction peaks for starch coated H-AuNPs was found at 19, 45 and 57°, which is different from starch, HAL and H-AuNPs with higher in intensity²⁰. The result was

manipulated to study the crystal structure of H-AuNPs, and starch coated H-AuNPs. The specific X-Ray diffraction pattern of the H-AuNPs obtained from green rout was obtained by Bragg reflections corresponding to the (111), (200), (220), (311) and (222) sets of lattice planes are observed that may be indexed by the fcc structure of H-AuNPs.

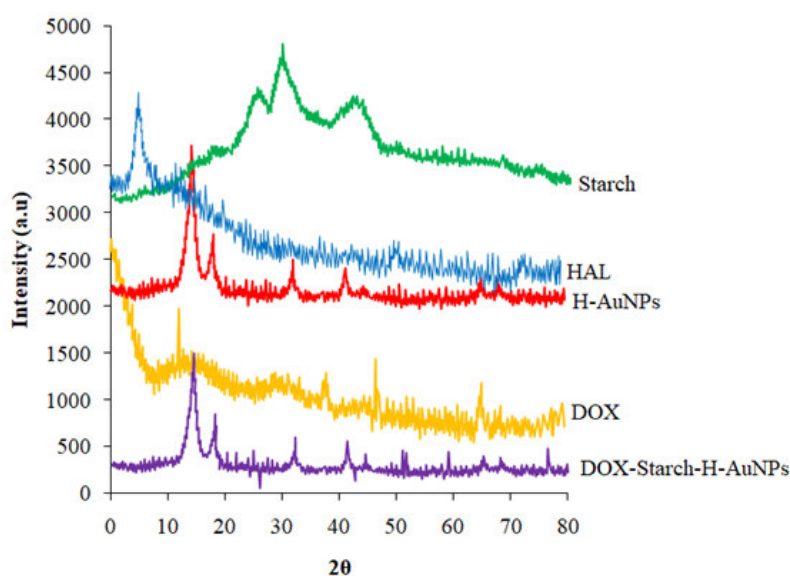


Figure 3
X-ray diffraction analysis of HAuCl₄ solution, starch, *H. auriculata* L leaf extract (HAL), DOX, H-AuNPs, starch coated H-AuNPs, and DOX-loaded starch coated H-AuNPs

DLS

TEM was presented on the H-AuNPs, to visualize the morphology of nanoparticles. The images were shown that particles are spherical and polydispersed with a size ranging from 35 to 50 nm in (figure 4). Next, DLS analysis exhibited that the formulated H-AuNPs and DOX-loaded starch coated H-AuNPs had an average diameter of 15.7 ± 1.4 nm and 27.4 ± 1.4 nm in (table-1).

Zeta-potential

The data in Table.1 shows the zeta-potential to be positive, with a value of 2.151 ± 2.1 Mv of H-AuNPs and 2.361 ± 4.3 mV of starch coated H-AuNPs. The neutral starch effectively coats the positively charged surface of the H-AuNPs, but the particles retain their positively charged characteristic. This data proves that the DOX-loaded starch coated H-AuNPs are homogenously coated.

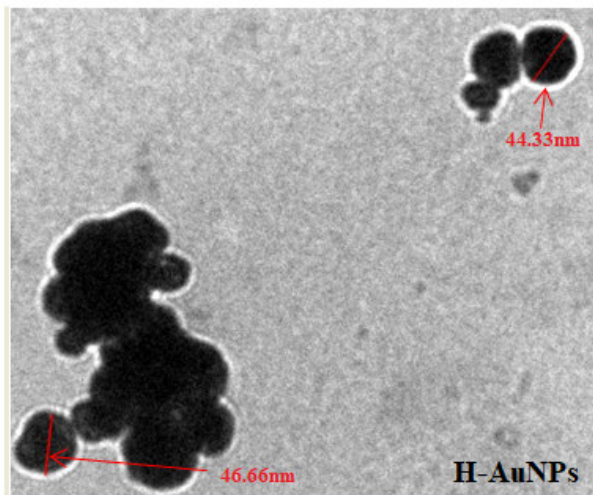


Figure 4
TEM of H-AuNPs

Table 1
Zeta-potential and DLS of starch coated H-AuNPs

Sample	Zeta potential	DLS
H-AuNPs	2.151 ± 2.1	15.7 ± 1.4 nm
Starch-H-AuNPs	2.361 ± 4.3	27.4 ± 1.4 nm

Effect of pH on Drug release

The pH dependent in vitro drug release from DOX-loaded starch coated H-AuNPs was carried out in different buffered solution with pH 7.4 (physiological pH) and 5.4 (similar to tumor tissue pH) at 37°C. Figure 5 shows initial sustained release of DOX from starch coated H-AuNPs. Figure 5 shows highly

different DOX release profile from starch coated H-AuNPs in same pH condition about 68% for pH 7.4 and 79% for pH 5.4. It also seen both release profiles exhibited rapid burst effect during the first 7 to 8 h. This result was suggested that pH independent nature of the Sample

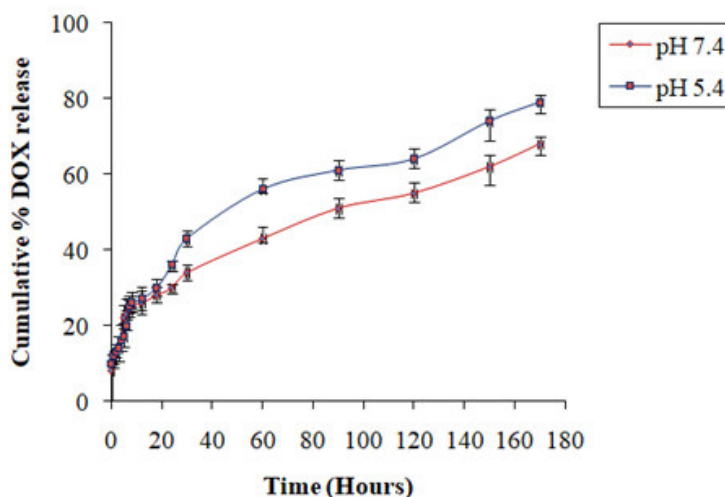


Figure 5
Effect of different pH (5.4 and 7.4) on starch coated H-AuNPs nanocarrier for controlled drug release of DOX at 37°C (means; n=3)

Kinetic analysis of in vitro release data

The data from the in vitro release studies was subjected to kinetic analysis, that is, zero-order and first-order. To determine the mechanism that best represented the release of the drug from the

$$M_t / M_\infty = k t^n$$

Where M_t / M_∞ is the fraction of drug released (using values of M / M_∞) at time t , and k is a constant incorporating the structural and geometric properties of the release device. When $n = 0.5$, Case I or Fickian diffusion is indicated, $0.5 < n < 1$ for anomalous (not-Fickian) diffusion, $n = 1$ for Case II transport (Zero order release), and $n > 1$ indicates (Costa and Sousa Lobo 2001). The experimental research data of k , n , and R^2 (coefficient of determination) have been obtained using the PCP Disso V 2.08 software as presented in Table 2. The values of n obtained by the linear regression of log

formulations, the data was also fitted to the Higuchi matrix model and Korsmeyer–Peppas equation. The release exponent (n) describing the mechanism of drug release from the matrices was calculated by regression analysis, using the Peppas equation²¹.

(M_t / M_∞) versus $\log t$, were between 0.5 to 1 for all formulations, indicating non-Fickian diffusion as the release mechanism, and close to 0.5 in the case of DOX- starch coated H-AuNPs, to follow first order kinetics. Peppas model was the best, and highest R^2 for DOX- starch coated H-AuNPs, the results of R^2 for the Higuchi matrix model and First order model for DOX- starch coated H-AuNPs was greater than Zero order model, indicating matrix-diffusion controlled release from the hydrophilic polymer matrices by first order kinetics.

Table 2
Kinetic study of in vitro drug release data from starch coated DOX loaded H-AuNPs

Release model		DOX-Starch-H-AuNPs
Zero Order	R^2	0.8916
	k	0.2642
First Order	R^2	0.9723
	k	0.0811
Higuchi Matrix	R^2	0.9737
	k	0.0942
Peppas	R^2	0.9914
	k	1.1112
	n	0.7364

CONCLUSION

The study involved the green method for the production of H-AuNPs and is the reduction of the carboxylic acid groups. The work also describes the synthesis and surface functionalization of H-AuNPs, starch coated H-AuNPs, and DOX- starch coated H-AuNPs. The results of the H-AuNPs synthesis and their functionalization were checked by using XRD, TEM, DLS, and UV/Vis spectroscopy techniques. The resulting H-AuNPs are hydrophilic and show little aggregation, even when conjugated to somewhat

hydrophobic molecules such as doxorubicin. This is significant because the hydrophobicity of many anti-cancer drugs is a barrier to their effective use. As a result, many encapsulated and conjugated nanoparticle preparations have been prepared. A new strategy of the DOX- starch coated H-AuNPs was successfully synthesized by green synthesis method.

CONFLICT OF INTEREST

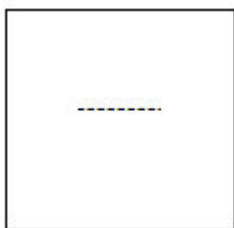
Conflict of interest declared none.

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