Original Research Article Medicinal Chemistry

International Journal of Pharma and Bio Sciences ISSN 0975-6299

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

PRADIPTA DAS[1] AND UMESH KUMAR PARIDA[2*]

[1]Department of General Surgery, KIMS, KIIT University, Bhubaneswar, Odisha, India
[2]Newredmars Education Pvt. Ltd. Odisha, India*

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***

UMESH KUMAR PARIDA *

Newredmars Education Pvt. Ltd. Odisha, India

Received on: 01-07-2017
Revised and Accepted on: 21-09-2017
DOI: http://dx.doi.org/10.22376/ijpbs.2017.8.4.b307-313

INTRODUCTION

Drug delivery system (DDS) is a great challenge for pharmaceutical and medical science to treatment of cancer. In recent biostatics analysis, it was reported that second largest death caused by cancer[1-3]. 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 properties [4-5]. From different type of metallic nanoparticles, Gold have anticancer activities and suitable for drug conjugation and biosensor activities[6]. 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 nanoprticles 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 enzymes[7-13]. 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 doxorubicin[15]. 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 bioactive[16] 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 ($HAuCl_4 \cdot XH_2O$) 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 4ºC.

Synthesizing the HAL-AuNPs

AuNPs were synthesized by the reduction of $HAuCl_4 \cdot XH_2O$ 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 $HAuCl_4$) 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 parameters[17].

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 processing[19].

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).

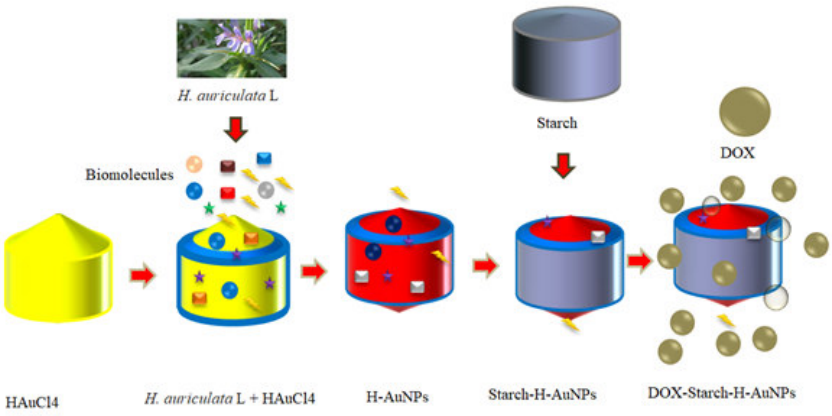


Figure 1
Schematically presented DOX-loaded starch coated H-AuNPs nanoparticles

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 particles[18].

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 $HAuCl_4$ had indeed been reduced to form of H-AuNPs by *H. auriculata* L leaf extract under reaction conditions[18].

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 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:

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

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

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.

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 $HAuCl_4$ 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 water[19]. 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^{0}C, indicating that the H-AuNPs were stable during this period.

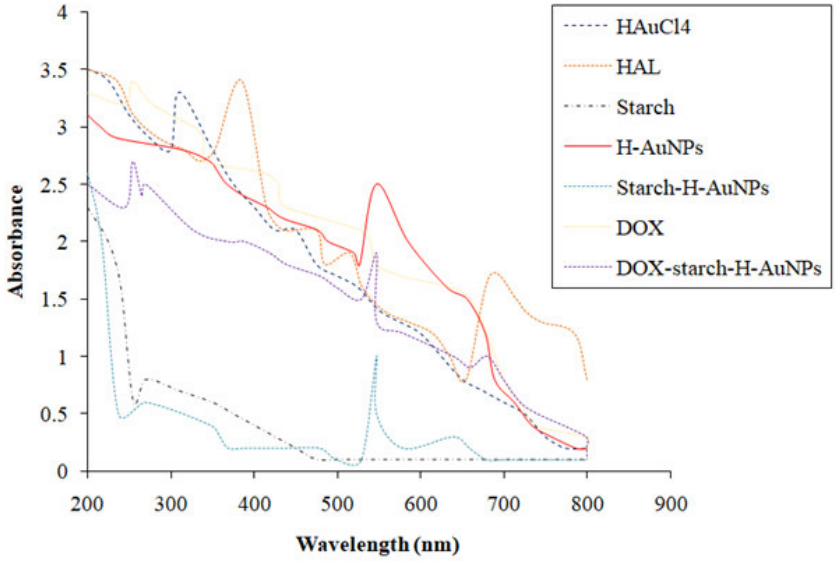


Figure 2
UV–visible spectrum of $HAuCl_4$ solution, starch, HAL, DOX, H-AuNPs, DOX-loaded starch coated H-AuNPs in water

XRD

In Figure 3, the XRD pattern of the $HAuCl_4$ 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 intensity[20]. 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.

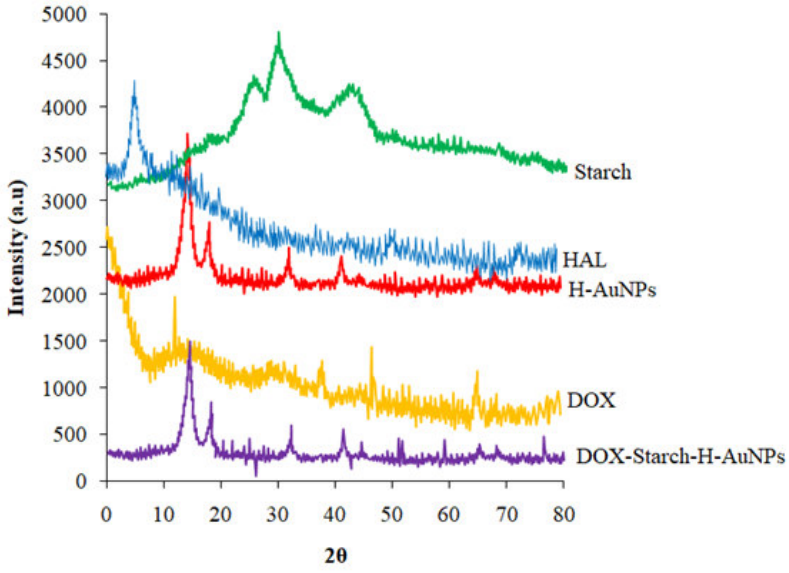


Figure 3
X-ray diffraction analysis of $HAuCl_4$ solution, starch, H. auriculata L leaf extract (HAL), DOX, H-AuNPs, starch coated H-AuNPs, and DOX-loaded starch coated H-AuNPs

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 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 ± 4.3mV 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.

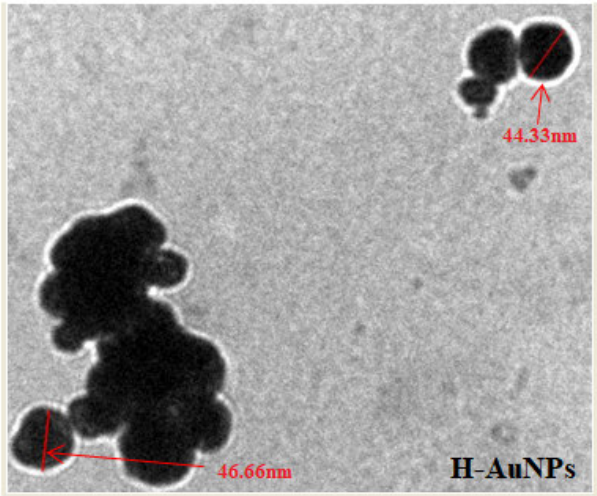


Figure 4
TEM of H-AuNPs

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 37^0C. 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

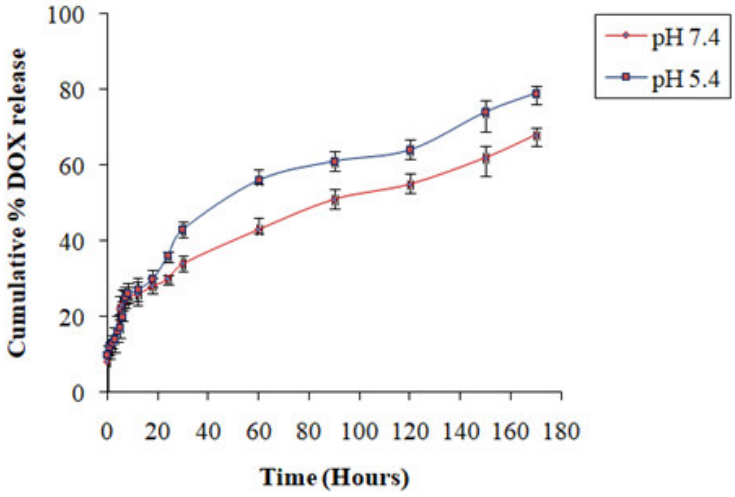


Figure 5
Effect of different pH (5.4 and 7.4) on starch coated H-AuNPs nanocarrier for controlled drug release of DOX at 37^0C (means; n=3)

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 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 equation[21].

$$Mt / M\infty = ktn$$

Where Mt / 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 experimtal 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 (Mt / M∞) versus log t, were between 0.5 to 1 for all formulations, indicating no-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

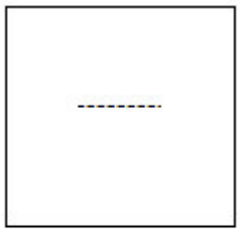
Conflict of interest declared none.

REFERENCE

1. Bazak R, Houri M, El Achy S, Kamel S, Refaat T., Cancer active targeting by nanoparticles: a comprehensive review of literature. J Cancer Res Clin Oncol. 2015 May 1; 141(5):769-84.
2. Tiwari M., Nano cancer therapy strategies. J Cancer Res Ther. 2012 Jan 1;8(1):19.
3. Tang M, Lei L, Guo S, Huang W. Recent progress in nanotechnology for cancer therapy. Chin J Cancer. 2010 Sep 5;29(9):775-80.
4. Chowdhury SM, Lee T, Willmann JK. Ultrasound-guided drug delivery in cancer. Ultrasonography. 2017 Jul;36(3):171.
5. Ramos AP, Cruz MA, Tovani CB, Ciancaglini P. Biomedical applications of nanotechnology. Biophysical reviews. 2017 Apr:1-1.
6. Kumar A, Huo S, Zhang X, Liu J, Tan A, Li S, Jin S, Xue X, Zhao Y, Ji T, Han L. Neuropilin-1-targeted gold nanoparticles enhance therapeutic efficacy of platinum (IV) drug for prostate cancer treatment. ACS nano. 2014 Apr 18;8(5):4205-20.
7. Khan ZU, Khan A, Chen YM, Shah NS, Muhammad N, Khan AU, Tahir K, Khan FU, Murtaza B, Hassan SU, Qaisrani SA. Biomedical applications of green synthesized Nobel metal nanoparticles. Journal of Photochemistry and Photobiology B: Biology. 2017 May 27.

8. Zheng Y, Lai L, Liu W, Jiang H, Wang X. Recent advances in biomedical applications of fluorescent gold nanoclusters. Advances in Colloid and Interface Science. 2017 Feb 16.
9. Shi W, Sahoo Y, Swihart MT., Gold nanoparticles surface-terminated with bifunctional ligands. Colloids Surf A Physicochem Eng Asp. 2004 Oct 1;246(1):109-13.
10. Kumar KM, Mandal BK, Sinha M, Krishnakumar V., Terminalia chebula mediated green and rapid synthesis of gold nanoparticles. Spectrochim Acta A Mol Biomol Spectrosc. 2012 Feb 29;86:490-4.
11. Haley B, Frenkel E. Nanoparticles for drug delivery in cancer treatment. Urol Oncol. 2008 Feb 29 (Vol. 26, No. 1, pp. 57-64). Elsevier.
12. Dreaden EC, Austin LA, Mackey MA, El-Sayed MA. Size matters: gold nanoparticles in targeted cancer drug delivery. 2012; 3(4): 457–478.
13. Ghosh P, Han G, De M, Kim C.K., Gold nanoparticles in delivery applications. Advanced Drug Delivery Reviews. 2008 Aug 17; 60(11):1307-15.
14. Tomuleasa C, Soritau O, Orza A, Dudea M, Petrushev B, Mosteanu O, Susman S, Florea A, Pall E, Aldea M, Kacso G, Cristea V, Berindan-Neagoe I, Irimie A., Gold nanoparticles conjugated with cisplatin/doxorubicin/capecitabine lower the chemoresistance of hepatocellular carcinoma-derived cancer cells. J Gastrointestin Liver Dis. 2012 Jun 1; 21(2).
15. Amreddy N, Muralidharan R, Babu A, Mehta M, Johnson E.V, Zhao Y.D, Munshi A, Ramesh R., Tumor-targeted and pH-controlled delivery of doxorubicin using gold nanorods for lung cancer therapy. Int J Nanomedicine. 2015; 10: 6773.
16. Ceballos H, Kawuki RS, Gracen VE, Yencho GC, Hershey CH., Conventional breeding, marker-assisted selection, genomic selection and inbreeding in clonally propagated crops: a case study for cassava. Theor Appl Genet. 2015 Sep 1; 128(9):1647-67.
17. Wang C, Mathiyalagan R, Kim YJ, Castro-Aceituno V, Singh P, Ahn S, Wang D, Yang DC., Rapid green synthesis of silver and gold nanoparticles using Dendropanax morbifera leaf extract and their anticancer activities. Int J Nanomedicine., 2016; 11:3691.
18. Rout A, Jena P. K., Sahoo D, Parida U. K. and Bindhani B. K., Green Synthesis and Characterization of Silver Nanoparticles for Antimicrobial Activity Against Burn Wounds Contaminating Bacteria. International Journal of Nanoscience. 2014 Apr: 13(02):1450010 .
19. Arunachalam KD, Annamalai SK, Hari S., One-step green synthesis and characterization of leaf extract-mediated biocompatible silver and gold nanoparticles from Memecylon umbellatum. Int J Nanomedicine. 2013; 8:1307.
20. Elavazhagan T, Arunachalam KD., Memecylon edule leaf extract mediated green synthesis of silver and gold nanoparticles. Int J Nanomedicine.2011; 6:1265.
21. Peppas NA. Analysis of Fickian and non-Fickian drug release from polymers.1985;60:110-1.

Reviewers of this article



Rtd. Prof. Dr. G. S Roy, Ph.D

Department of Material Science, CET,
Bhubaneswar, Odisha, India

Prof.Dr.Prapurna Chandra Rao

Assistant Proffessor, KLE University,
Belgaum, Karnataka, India

Prof.Dr.K.Suriaprabha

Asst. Editor , International Journal of Pharma and Bio sciences.

Prof.P.Muthuprasanna

Managing Editor , International Journal of Pharma and Bio sciences.

We sincerely thank the above reviewers for peer reviewing the manuscript