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Research Article

Formulation and Evaluation of Chitosan Nanoparticles Coated Graphene-Gold Nanoparticles for Controlled Released of Anticancer Drug Vincristine

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Abstract: In this study chitosan nanoparticles (CSNPs) coated functionalized graphene-gold nanoparticles (F-GE-AuNPs) known as (CSNPs-F-GE/AuNPs). The prepared nanocomposites were characterized by UV-Visible spectrophotometer, XRD, FTIR, SEM, tensile strength and swelling in stimulated in different phosphate buffer medium. The model anticancer drug Vincristine (VIS) was used for controlled drug delivery purpose. From FTIR result was clearly demonstrated that the model drug VIS did not change in any molecular level at CSNPs-F-GE/AuNPs. In vitro drug release system was carried out. VIS was presented exceptional higher drug result in different pH medium. It was observed that CSNP and F-GE/AuNPs was decrease less drug release rate compared to CSNPs-F-GE/AuNPs. So that it can be clearly understood that CSNPs-F-GE/AuNPs nanocomposites have enhanced drug release activity in different pH medium.

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1. Introduction

Today cancer is a common problem for human society (Xiaomei et al., 2006). Recently most of advanced technology has been discovered for cancer diagnosis (Frangioni et al., 2008). But main problem is cure to cancer disease. The present scientific community study about cancer cell and reported hopefully result how to cure disease. Current scientific review of literature reported that nanoparticles may be cure and control to cancer cell (Rodzinski et al., 2016).

Chitosan (CS) is a natural biopolymer which is used for different purpose like biomedical, cosmetics, pharmaceutical products etc. It is very popular in pharmaceutical and medical filed for its unique properties like biodegradable, non toxic, biocompatible, antimicrobial activities etc. In present research, it is used for drug delivery applications (Rinaudo 2006; Majeti et al., 2000).

AuNPs is used as an anticancer agent. It has different type of biomedical properties such as biomedical, pharmaceutical etc. It is very well known in pharmaceutical and medical filed for its unique properties like low toxic, biocompatible, anticancer activities etc. In present research, it is used for drug delivery applications (Jain et al., 2012; Abadeer et al., 2016).

Graphene (GE) has attracted tremendous attention in recent years owing to its exceptional thermal, mechanical, biosensor and electrical properties. One of the most promising applications of this material is in polymer nanocomposites, pharmacitical field, polymer matrix composites. This polymer functionalized graphene acts as an efficient nanofiller in polymer composites to improve its drug delivery properties and a small quantity of polymer functionalized graphene improve the mechanical, electronic, optical,

thermal and magnetic properties significantly (Liu et al., 2011; Orecchioni et al., 2015).

Graphene based nanocomposites have been few studied for drug delivery. But this is first time to studies on CSNPs coated F-GE-AuNPs for controlled drug released of anticancer drug Vincristine. Generally the hydrophobicity and hydrophilicity nature of polymer is contributing a lot in controlling the drug release characteristics apart from its. However this experiment is focused on the preparation of CSNPs-F-GE/AuNPs nanocomposites for controlled drug release of anticancer drug VIS in different pH medium. This work is aimed to prepare CSNPs-F-GE/AuNPs to evaluate the feasibility of loading and release of VIS drug with applicable efficiency and to elucidate the effect of biopolymer addition.

2. Material and Method:

Graphene and chitosan were purchased from Sigma-Aldrich. HAuCl_4 was purchased from Sigma-Aldrich, USA, and other reagents like ammonium persulfate (APS), Vincristine drug, hydrochloric, sulfuric and nitric acid (Sigma Chemicals) were of analytical grade.

2.1. Synthesis of Functionalized Graphene

Functionalized graphene (F-GE) was synthesized as described in the recent literature (Li et al 2014). Typically, GE was reacted with $\text{H}_2\text{SO}_4:\text{HNO}_3$ (3:1), then tip sonicated for 30 minutes using an ultrasonic processor with amplitude at 30% and 7 s pulse to yield carboxylic acid functionalized GE (GE-COOH). The carboxylic acid group was converted to formyl chloride via reaction with thionyl chloride for 24 h at 75°C while refluxing. This resulted in functionalized GE which is referred to as GE-COCl. After the reaction was stopped, reaction mixture was cooled before centrifuging and washing to remove excess

reactants. Samples were dried overnight at 90°C and 30 in Hg (Kuila et al., 2012).

2.2. Synthesis of Chitosan-Dextran sulfate (CSNPs) nanoparticles

Two percent of chitosan solution was prepared in 1% acetic acid on magnetic stirring at 1000 rpm. Similarly, 0.5 mg/mL dextran sulfate was prepared in deionized water. CSNPs nanoparticles were prepared by adding equal volume of CS solution in dextran sulfate solution on a constant stirring at 800 rpm overnight (Chaiyasan et al., 2013).

2.3. Synthesis of AuNPs

AuNPs were synthesized by green synthesis method using medicinal plant leaf extract of *Ocimum tenuiflorum* (OTE) in 100 ml of water containing 1 mM HAuCl₄ at 37 °C for 12 h. The colour change from colourless to reddish in color can be attributed to the formation of AuNPs in the reaction mixture (Philip et al., 2011).

2.4. Synthesis and Characterization of F-GE-AuNPs Nanocomposite

Initially, 100 mg F-GE was dispersed in 60 ml water by 60 min of sonication. The reaction mixture was prepared in a 250 mL round-bottom flask by dissolving 40ml of aqueous AuNPs with 60 ml of the F-GE, The mixture was stirred at 90 °C for 12 h. After 12 h, the reaction was stopped, and the resultant mixture was washed three times with water using centrifugation. The product was obtained as a pure and used for further experiments.

2.5. CSNPs coated GE-AuNPs nanoparticles

F-GE-AuNPs were mixed in CSNPs solution in 2:5 ratios and incubated on a magnetic stirrer at 800 rpm overnight. Free CSNPs particles were removed by centrifugation at 5000 rpm for 15 minutes. The CSNPs coated F-GE-AuNPs nanoparticles were collected by ultra-

centrifugation at 35000 rpm for 30 minutes at 4 °C.

2.6. Drug loading

The required amount of CSNPs coated F-GE-AuNPs sample was stirring at 200 rpm at room temperature. Then the prepared VIS drugs with different percentages like 10 wt %, 20 wt %, 30 wt %, 40 wt %, 50 wt %, 60% and 70% were placed into CSNPs coated F-GE-AuNPs solutions and constantly stirring for one hour. After preparation homogenized drug loaded nanoparticles were kept at room temperature for drying.

The VIS loaded CSNPs coated F-GE-AuNPs nanoparticles was calculated using a UV-VIS spectrophotometer. The drug loading efficiency and drug loading content of VIS was calculated

Loading efficiency

$$LE = \frac{W_a}{W_d} \times 100\%$$

Loading content

$$LC = \frac{W_a}{W_{np}} \times 100\%$$

Where LC: Loading content; LE: Loading efficiency, W_a: quantity of drug found in the drug-loaded nanoparticles, W_d: quantity of drug beginning added into the system and W_{np}: quantity of drug-loaded nanoparticles

3. Characterization

3.1. Fourier transforms infrared (FTIR spectra)

The FTIR spectra were demonstrated on a Nicolet 8700 spectrometer, in the absorbance range in between 400–4,000 cm⁻¹.

3.2. X-ray diffraction (XRD)

XRD instrument Rigaku, D/Max, 2,500 V, Cu-αα radiation: 1.54056Å° was used for experiment. The result was recorded at temperature of 30⁰C .

3.3. Scanning Electron Microscopy (SEM)

SEM of nanocomposites was demonstrated by S 4300 Hitachi, Illinois, USA. The prepared sample

was placed into a specimen holder with silver plate coated by palladium. At analysis time the accelerator potential was 10kV.

3.4. Cyclic Voltammetry (CV)

Cyclic Voltammetry (CV) is a type of potentiodynamic electrochemical measurement, recording a relationship of current vs. voltage when the potential at the working electrode is ramped linearly versus time (at a certain scan rate) to a set potential and reversed back to the original potential at the same sweep rate. CV curves can be also used to compute the specific capacitance of the active material using the relationship shown below. The area under the current–potential curve can be estimated by integration, and then the area is divided by the sweep rate (v , V s⁻¹), the mass of active material of the electrode (m , g), and the potential window ((V_a-V_b) , V), giving a specific capacitance (C) in F g⁻¹

3.5. Tensile strength

The tensile properties of the sample were incorporated on a universal testing machine (CMT4104, Shenzhen SANS Test Machine Co. Ltd, CIPET, and Odisha) with a tensile rate of 5 mm/min.

3.6. Swelling studies

This study was performed in both 7.4 and 3.4 pH in phosphate buffer (PBS) at 37°C on copolymer pellets (100 mg, 2.55 mm thickness and 13 mm diameter). The sample was 25mm diameter; 50 mm height which was immersed 20ml phosphate buffer. The weights of the swelled sample at predetermined time intervals were calculated after wiping the mesh containing the swelled polymer with a tissue paper. Then a graph was drawn between the degree of swelling ($= (W_t-W_0)/W_0$) and time, where W_t and W_0 are the weights of the sample after and before swelling, respectively.

Table-1 Sample preparation of CSNPs coated F-GE-AuNPs nanoparticles composite with different % of drug loading (n)

Sample	% of Drug	Loading Content of VIS (%)	Loading Efficiency (%)
CSNPs -F-GE-AuNPs-1	10	6.12± 0.19	61.2± 0.56
CSNPs -F-GE-AuNPs-2	20	17.39± 0.41	86.95± 0.76
CSNPs -F-GE-AuNPs-3	30	27.82± 0.31	92.73± 1.39
CSNPs -F-GE-AuNPs-4	40	35.27± 0.86	88.17± 0.62
CSNPs -F-GE-AuNPs-5	50	45.21± 1.43	90.42± 1.74
CSNPs -F-GE-AuNPs-6	60	56.31± 0.74	93.85± 2.43
CSNPs -F-GE-AuNPs-7	70	66.49± 0.32	94.98± 1.63
CSNPs	70	53.11± 0.12	91.2± 0.36
F-GE-AuNPs	70	55.10± 0.11	89.2± 0.46

3.7. In vitro drug dissolution study

In vitro drug dissolution study was made in 7.4 and 3.4 at room temperature with 500 RPM. The amount of model drug released was estimated by UV spectrophotometer in different time interval. The absorbance of pure VIS drug was calculated to be 295 nm.

3.8. Statistical analysis

The experimental data are measured by one-way analysis of variance (ANOVA). All data analysis was executed using the SPSS Statistics 17.0. All experimental data were introduced as a mean value with its standard deviation (mean $\pm$ SD). *P*-values less than 0.05 were recommended to be statistically significant.

4. Result

4.1. UV-Vis Spectroscopic

Fig.1 explained the UV-visible absorption spectrum of the CS, AuNPs, OTE, F-GE, F-GE/AuNPs, CSNPs, and CSNPs-F-GE/AuNPs. The UV-visible scanning analysis of the product obtained displayed peaks and bands for SPR absorption. It was observed that the optimum reaction mixture for the best yield of the AuNPs was 1:6 ratios as at this point there was highest band intensity, and peaks in SPR absorbance peak at 557 nm which is also clearly observed in CSNPs coated F-GE/AuNPs. The symmetric peak symbolizes that the solution does not contain any aggregated particles. The stability of the CSNPs-F-GE/AuNPs was determined by measuring the absorption spectrum at 24-h intervals for 90 days. No significant changes in absorbance were identified during storage time at 37°C, indicating that the CSNPs-F-GE/AuNPs were stable during this period. F-GE and CSNPs solutions did not show any peak at 557 nm (Philip et al.,2011).

4.2. XRD

Fig. 2 shows the XRD pattern of the CS, F-GE, CSNPs, AuNPs, F-GE/AuNPs, CSNPs- F-GE/AuNPs. The XRD pattern of CSNPs coated F-GE/AuNPs composite revealed all the peaks pertaining to AuNPs and F-GE and matched well. Presence of all the peaks of AuNPs and F-GE in the composite structure confirmed the success of the prepared of the CSNPs- F-GE/AuNPs composite. The diffraction peaks at 22.3° corresponds to (011) planes of CS, the peak at 27.3° can be ascribed to the periodicity perpendicular to the CSNPs nanoparticles (Wu et al 2015). The diffraction peak at 17.5° can be related to f-GE in the composite structure with a d-spacing of 0.31 nm. Additionally the diffraction peak at 6.2°, 15.4°, can be related to AuNPs in the composite structure. The diffraction peaks at 7.1°, 13.4°, 22.6°, and 33.6° and 49.8° correspond to (111), (200), (220), (311) and (222) planes of CSNPs-F-GE/AuNPs, respectively(Philip et al.,2011).

4.3. FTIR

Fig.3 showed FTIR of pure CS, a broad -OH stretch absorption band between 3500 and 3100 cm^{-1} and the aliphatic C-H stretch between 2990 and 2850 cm^{-1} . The peak at 1384 cm^{-1} represented the -C-O stretch of primary alcoholic group (-CH₂-OH) .

The FTIR of prepared CSNPs, a broad -OH stretch absorption band between 3500 and 3100 cm^{-1} and the aliphatic C-group shifted to 2783 cm^{-1} . The peak was changed at 1312 cm^{-1} represented the -C-O stretch of primary alcoholic group (-CH₂-OH).

The FTIR of AuNPs, a strong -OH stretch absorption band between 3500 and 3100 cm^{-1} and the C-H absorption band at 2923 cm^{-1} . The peak at 1484 cm^{-1} represented the -C-O primary alcoholic

group ($-\text{CH}_2\text{-OH}$) (Philip et al., 2011). The peak at 1254 cm^{-1} represented the $-\text{C-O}$ strong.

In F-GE was shows dominant peaks at 3447 cm^{-1} , 2894 cm^{-1} , 1692 cm^{-1} , 1385 cm^{-1} , 1110 cm^{-1} , 891 cm^{-1} , 615 cm^{-1} .

The FTIR of F-GE/AuNPs, the broad peak at 3449 cm^{-1} (interlayers and interlayer H-bonding upon stretching). The peak at 2932 cm^{-1} was lower frequency value which was indicates an electrostatic interaction between AuNPs and F-GE . The FTIR of CSNPs coated F-GE/AuNPs showed both patterns of F-GE/AuNPs and CSNPs. The expected peaks of CSNPs-F-GE/AuNPs backbone was at 3517 cm^{-1} and 1482 cm^{-1} ($-\text{OH}$ stretching and $-\text{C-O}$ stretching), respectively. Further, the absorption peak vibration of F-GE at 2946 cm^{-1} .

4.4. SEM

Fig. 4 shows SEM images of CSNPs-F-GE/AuNPs composite .The SEM images of both the CSNPs and F-GE/AuNPs revealed rough like structure. At same time CSNPs-F-GE/AuNPs was changed structure promoting for F-GE and AuNPs. The presence of AuNPs was dispersed throughout the F-GE matrix suggesting the successful incorporation of f-GE and AuNPs into CSNPs composite.

4.5. Tensile properties

In fig.5 identified tensile strength CSNPs and CSNPs-F-GE/AuNPs composites films. The result of CSNPs-F-GE was indicated progress in mechanical properties with the increase in f-GE concentration up to 3% ($35.12 \pm 54\text{ MPa}$). Similarly CSNPs-F-GE/AuNPs (2:3:4) was increased tensile strength up to $35.78 \pm 62\text{ MPa}$ which was highest mechanical strength comparing with other compositions.

Figure.1 UV-visible spectrophotometer of OTE, F-GE, CSNPs, AuNPs, CS, CSNPs-F-GE-AuNPs, F-GE-AuNPs

Figure.2 XRD of F-GE, CSNPs, AuNPs, CS, CSNPs-F-GE-AuNPs, F-GE-AuNPs

Figure.3 FTIR of OTE, F-GE, CSNPs, AuNPs, CS, CSNPs-F-GE-AuNPs, F-GE-AuNPs

Figure.4. SEM of F-GE, F-GE-AuNPs, CSNPs-F-GE-AuNPs

Figure.5. (a) Tensile strength of CSNPs with different percentages of F-GE (b) F-GE-CSNPs with different percentages of AuNPs

4.6. Electrochemical studies

Fig. 6 shows the cyclic voltammeter (CV) measurements of CSNPs, CSNPs-F-GE, CSNPs-F-GE/AuNPs, composite in the voltage range from -1 to +1 V, at a scan rate of 10mVs^{-1} . For CSNPs, CSNPs-F-GE/AuNPs composite, a prominent anodic and cathodic peak was revealed at -0.58 and +0.55 V, respectively. CSNPs-F-GE/AuNPs composite showed greater electrochemical activity than CSNPs coated F-GE. The enhanced capacitive performance of the CSNPs-F-GE/AuNPs composite can be described to homogeneous mixing of AuNPs in the composite system, improving the charge storage ability.

4.7. Swelling Index

In Fig. 7 shows the swelling behaviour of CS, CSNPs, F-GE/AuNPs and CSNPs-F-GE/AuNPs with different percentages of the drug. The swelling response of the nanocomposites influences its bioadhesive character. The rate of adhesion increases with the degree of hydration until a point where over hydration influences to a direct drop in adhesive strength due to disentanglement at the nanocomposites interface. The rate and the extent of nanocomposites hydration and swelling also affect matrix adhesion and consequently the drug release from the nanocomposites. The formulations CSNPs-F-GE/AuNPs have reported a slower rate of swelling. The presence of CSNPs, a hydrophilic

polymer, increases the extent of swelling; therefore, maximum swelling among the nanocomposites is obtained for the formulation CSNPs-F-GE/AuNPs.

4.8. In vitro drug released

To study the outcome of pH on the swelling of composite like CSNPs-F-GE/AuNPs, the % cumulative release in both pH 7.4 and 3.4 media was measured. The percentages of cumulative release result were introduced at pH from 7.4 to 3.4 in fig.8. A significant increase in percentages of cumulative release was recognized for all formulated drug loaded nanocomposites. From fig.8a and b, it can be seen that the 70% drug loaded polymer matrix have determined longer drug release rates than the other polymer matrix. Thus, drug release depends upon the nature of the polymer matrix as well as pH medium. It has been reported that pH 7.4 = 69% and pH 3.4= 73% Cf.

4.9. Release Kinetics Parameters

From the result, the kinetics of drug release was evaluated by plotting percentages of Cumulative frequency (c.f) data verses time at selected to an exponential equation-1. The results have evaluated the value of k for all formulations which are presented in table 2. The value of n and k were shown dependence on the % drug loading into the CSNPs-F-GE/AuNPs. Values of n was calculated by varying the quantity of drug containing 10, 20, 30,40,50,60 and 70 wt% and keeping constant CSNPs-F-GE/AuNPs, ranged from 0.37-1.72 proposing shifting of drug molecules transport from non-fickian to anomalous. The value of n was introduced in between 0.36 and 1.77 which was indicated swelling and diffusion controlled release by anomalous transport. The value 1 was indicated case-II transport due to nanocomposites relation during the swelling of the nanocomposites. The value near about 0.33 was indicated that the drug release from nanocomposites due to fickian

diffusion in table 2. However, the value of n more than 1 may be in the regions of low micro viscosity inside of the nanocomposites of micro cavities during the swollen state of the CSNPs-F-GE/AuNPs. Further similar the result has also been introduced wherein the effect of different CSNPs-F-GE/AuNPs ratios and other factors on dissolution kinetics were summarized.

5. Discussion

Nanotechnology is one approach to overcome challenges of conventional drug delivery systems based on the development and fabrication of nanostructures (Liu et al., 2014). Some challenges associated with the technology as it relates to drug effectiveness, toxicity, stability, pharmacokinetics and drug regulatory control are discussed in this review. Clearly, nanotechnology is a welcome development that is set to transform drug delivery and drug supply chain management, if optimally developed (Ghosh et al., 2008; Potts et al., 2011; Singh et al.,2013; Liu et al., 2014; Sayyar et al., 2015).

In this study suggest that CSNPs-F-GE/AuNPs have drug delivery properties for controlled drug released of anticancer drug. Recently, researcher reported that F-GE is able of reducing tumor-cell growth in vivo and in vitro (Battogtokh et al.,2017;Boyles et al.,2015). In the present report, we have exposed for the first time that CSNPs coated F-GE/AuNPs exhibit drug delivery applications and determined the mechanism of action in different approaches (Osváth et al., 2015;Sayed et al., 2015;Yang et al., 2014;Li et al., 2014;Hashemi et al.,2017;Muddineti et al.,2015;Duan et al.,2014;Amanulla et al.,2017;Bao et al.,2015).

This paper we prepared CSNPs coated F-GE/AuNPs, which showed remarkable stability, uniform, excellent surface charge, excellent size

and good physicochemical properties distribution throughout the experiment analysis period in fig. 2,3,4,5.

CV is known to be a powerful tool in order to determine the redox behaviour and electron transfer kinetics of any system in fig.6.CV plays an important role in characterizing the performance of various electrical energy storage devices, such as batteries, fuel cells and electrochemical capacitors.

In swelling study, the rate and the extent of polymer matrix hydration and swelling also affect matrix adhesion and consequently the drug release from the polymer matrix. The formulations CSNPs-F-GE/AuNPs have reported a slower rate of swelling.

Additionally the outcome of pH on the swelling of composite like CSNPs-F-GE/AuNPs, the % cumulative release in both pH 3.4 and 7.4 media was measured. The percentages of cumulative release result were introduced at pH from 3.4 to 7.4 in fig.8. A significant increase in percentages of cumulative release was recognized for all formulated drug loaded CSNPs-F-GE/AuNPs.

However the kinetics of drug release was evaluated by plotting percentages of c.f data verses time. The results have evaluated the value of k for all formulations which are presented.

It was clear that above research data analysis, we have drawn a scheme of the mechanism of action and signalling for CSNPs-F-GE/AuNPs in fig.9.

Figure.6 CV of CSNPs, F-GE-CSNPs, CSNPs-F-GE-AuNPs

Figure.7 Swelling study of CS, CSNPs, CSNPs-F-GE, CSNPs-F-GE-AuNPs, F-GE-AuNPs +40% VIS, F-GE-AuNPs +50% VIS, F-GE-AuNPs +60% VIS, F-GE-AuNPs +70% VIS.

Figure.8 Cumulative frequency of F-GE-AuNPs with different percentages of drug loading at pH 7.4 (a) and F-GE-AuNPs with different percentages of drug loading at pH 3.4

Figure.9. Schamatic analysis of preparation and characterization of CSNPs-F-GE-AuNPs for controlled drug released of Vincristine

Table. 2. Release kinetics Parameters of different Formulations at pH 7.4 and pH 3.4

Sample Code	K		n		Co-ordination-coefficient , R ²	
	pH 7.4	pH 3.4	pH 7.4	pH 3.4	pH 7.4	pH 3.4
10 wt%	0.11	0.14	1.22	0.26	0.9525	0.9552
20 wt%	0.13	0.16	1.25	1.29	0.9654	0.9536
30 wt%	0.15	0.17	1.28	1.37	0.9652	0.9547
40 wt%	0.18	0.23	1.33	1.39	0.9652	0.9514
50 wt%	0.21	0.26	1.36	1.42	0.9751	0.9566
60 wt%	0.31	0.33	1.32	1.35	0.9127	0.9432
70 wt%	0.22	0.28	1.33	1.43	0.9343	0.9515

6. Conclusion

CSNPs-F-GE/AuNPs has been synthesized and characterized for controlled released of VIS for oral drug delivery. The drug release rate was greater in 3.4 than in 7.4 due to enhanced CSNPs-F-GE/AuNPs swelling in acid medium and lower with CSNPs-F-GE/AuNPs carrier compared to CSNPs and F-GE/AuNPs. The observed values of power law exponents indicated a mixed pure diffusion and non-Fickian mechanisms for drug release in simulated biological fluids. This data was suggested here that CSNPs-F-GE/AuNPs may be suitable for used as a carrier for anticancer drugs.

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Conflict Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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