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Investigation on the use of graphene as a unique drug delivery platform for dissimilar anticancer drugs

*Kandasamy Vinothini, Mariappan Rajan**

Biomaterials in Medicinal Chemistry Laboratory, Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai, India

Abstract: This study examines the performance of 5-flurouracil (5-FU) and cisplatin (CDDP) loaded graphene nanocarrier, which were prepared using nanoprecipitation technique to investigate the loading nature of two anti-cancer drugs. Initially, the graphite was exfoliated under ultrasonication method. The exfoliated sheets characterized and a single layer of carbon atoms like graphene sheets was confirmed. 5-FU and CDDP as an anti-cancer model drugs were entrapped on to the surface of the graphene sheets via electrostatic and π-π staking interactions. The prepared graphene nanocarrier have been thoroughly characterized by using various microscopic techniques, including Fourier transform infrared (FT-IR) spectroscopy, scanning electron microscopy (SEM), and X-ray diffraction (XRD). The encapsulation efficiency and *in-vitro* drug releasing behavior of the nanocarrier were investigated by UV-Vis spectrometry the wavelength of λ_{max} 265 nm and 267 nm. Aromatic 5-FU anti-cancer drug have higher encapsulation efficacy and sustained releasing nature. Since graphene is good platform for the entrapment of 5-FU anti-cancer drug then CDDP for chemotherapy.

Keywords:
Cisplatin; drug delivery, 5-Flurouracil, graphene, interaction

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*Correspondence to:

Mariappan Rajan, Biomaterials in Medicinal Chemistry Laboratory, Department of Natural Products Chemistry, School of Chemistry, Madurai Kamaraj University, Madurai, 625021, India; rajanm153@gmail.com

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Introduction

Cancer is currently one of the most lethal diseases and a world health concern. The most common cancer treatments are chemotherapy, radiotherapy, gene therapy and surgery[1-4]. Now chemotherapy is a major treatment of cancers and it is an essential clinical therapeutic modality for a wide range of cancers. But, herein low therapeutic efficacy, drug resistance low cellular uptake efficiency and high side effects[5-6]. The 5-Fluorouracil (5-F U) is one of the oldest but still widely used anti-neoplastic agents. It is an anti-metabolite of the pyrimidine analog type used for treating various types of solid tumors such as the cancer of stomach, intestine, colon, pancreas, ovary, breast, etc., alone or in combination. It has a plasma half-life of 10–20 min, hence high doses (400–600 mg/m^2 weekly) are required to achieve therapeutic concentration[7]. Even though, it is useful for treating various types of cancer, it has serious side effects such as bone marrow depression, gastrointestinal tract reaction, leucopenia, and thrombocytopenia[8]. Cisplatin (CDDP) is one of the most active anticancer agents, and it is used in the treatment of several cancers such as ovarian, testicular, osteosarcoma, head and neck, and small cell lung cancer. CDDP must be used in limited short-term, high-dose treatments for cancer because of its nephrotoxicity and ototoxicity. Generally, CDDP is administered through a vein (intravenously or IV) as an infusion[9].

The need of reducing the toxicity and enhancing the chemotherapy, a platform is required for delivering of cancer drug in exact places. Graphene, a two-dimensional nanomaterial reported for the first time in 2004, has been widely investigated for its novel physical properties and potential applications in various medicinal applications[10,11]. Graphite Oxide can be readily exfoliated into monolayer sheets to yield stable suspensions in water because of the hydrophilic oxygenated functional groups on its basal planes and edges[12-14]. Further, the large two-dimensional plane of Graphene sheets provides large specific surface area to carry drugs via surface adsorption, hydrogen bonding, and other types of interactions. Meanwhile, the excellent biocompatibility and nontoxicity of graphene makes it a promising material for drug carrier substances[15–17]. Nevertheless, a careful and thorough investigation of syntheses methods is required. Therefore, different methods have been explored continuously using mechanical, chemical and physical approaches for the exfoliation of graphene[18-22].

In this study, a new graphene sheet was prepared by ultra-probe sonication and the abilities to binding the drug on the carrier and controlled release system was developed. The 5-FU and CDDP loaded graphene was investigated the encapsulation onto graphene *in-vitro* release behavior at different pH conditions 2.8 and 6.8 by UV–Vis spectrometry.

Materials and Methods

Materials

Graphite, ethanol, were purchased from Sigma Aldrich, Mumbai, India. 5-FU and CDDP were purchased from Alfa Aeser, Mumbai, India. All Chemicals were of analytical grade were used directly as purchased without further purification. Double distilled water was used throughout the experiments.

Synthesis of Exfoliated Graphene

0.5 g of graphite was subjected to an ultra-probe sonication treatment for 30 min (130 W) with the aid of the ultrasonication (VIBRA – Cell Ultrasonic processor), the graphite was exfoliated and thus a brown aqueous suspension of graphene nanosheets was formed[23].

Investigation of drug loaded Graphene carrier

The exploited graphene nanosheets (GNs) were used for the drug loading process. 50 mg of graphene nanosheet dispersed in 10 mL of water and 5 ml of 5-FU (1 mL/mg) were added. The mixture is stirred under magnetic stirrer with 1000 rpm for 1 h. After the reaction completion, the 5-FU loaded graphene was filter using the Whatman filter paper and dried for further characterizations. The same procedure was followed by the loading CDDP on the graphene sheet inside of 5-FU[24].

Characterization studies

FT-IR

A small quantity of Graphene, drug loaded graphene nanocarriers, was separately mixed with 100 mg KBr and compressed to form tablets. These tablets were scanned on a Fourier Transform Infrared Spectrometer (Spectrum GX-1, Perkin Elmer, and USA) in the spectral region of 4000–400 cm^{-1}.

Scanning Electron Microscopy (SEM) analysis

The morphology and surface appearance of the nano graphene sheets (before and after the drug loading) were analyzed by scanning electron microscopy (VEGA3SB, TESCAN, Czech). The instrument has the following components like electron source, condensing lens, scan coil, objective lens, detector, and monitor. One drop of the nanoparticle suspension was placed on a glass surface and dried at 40 °C. The samples are usually mounted on to the sample holder with aluminium plate. The less-conducting samples are to be gold sputtered before mounting it onto the specimen stage of the SEM. Coating was achieved at 25 mA for at least 60 s. Scanning was performed under high vacuum and ambient temperature with a beam voltage of 20–30 kV.

X-Ray diffraction spectra (XRD)

The wide-angle X-ray diffraction (XRD, Bruker AXS D8) spectra were measured on a powder diffractometer with nickel-filtered Cu $K\alpha_{\epsilon}$ X-ray beam (λ = 0.15418 nm). The scanning range was between 100 and 600 and the scanning rate was 60/min.

Drug loading efficiency

The loading content of 5-FU and CDDP was measured by UV spectrum at λ_{max} value of

226 and 265 nm. Accordingly, the drug loaded supernatants obtained were determined by UV spectrometer. The 5-FU and CDDP loading content were calculated by the following equation

$$(\%)\ EE = \frac{\text{Total amount of drug - Free amount of drug}}{\text{Weight of dry nanoparticles}} X100$$

In-vitro Drug release studies

In vitro release profiles of 5-FU and CDDP from drug-loaded nanoparticles were investigated for in the PBS solution (pH 6.8) and acidic medium (pH 2.8). The (10 g) nanoparticles in 5 mL, of release medium were put into a dialysis tube (MWCO: 12000 Da). The dialysis tube was placed in 50 mL of double distilled water at 37 °C and stirred continuously at 500 rpm. At specific time intervals, 3 mL of solution was withdrawn from the outer compartment and replaced with fresh double distilled water (3 mL). The concentration of the released 5-FU and CDDP was determined by UV spectrometer (UV 1800, SHIMADZU) at λ_{max} value 265 and 267 nm. The dialysis was performed in triplicate for each sample.

Results and Discussion

FT-IR

Figure 1 shows the FTIR spectra of exfoliated graphene, 5-FU loaded graphene, and CDDP loaded graphene nanocarrier. For graphene spectra Figure 1(a) shows weak features, the spectra demonstrating the exfoliation of graphene with no functionality, the peak 1572 cm^{-1} corresponds to the carbon skeletal vibration of graphene sheets[25]. Figure 1(b) shows that the peak at 1275 cm^{-1} was belongs to C-F stretching band in the spectrum of 5-FU. The spectrum at 1650 cm^{-1}, 1625 cm^{-1} which peaks indicate presence of C=O stretching, 3335 cm^{-1} this peak shows that corresponds due to the N-H stretching of 5-Flurouracil[26]. Figure 1(c) this absorption is due to the N-H stretching of NH_3 group present in CDDP molecule, which was confirmed the encapsulation of CDDP on the graphene nanocarrier[27].

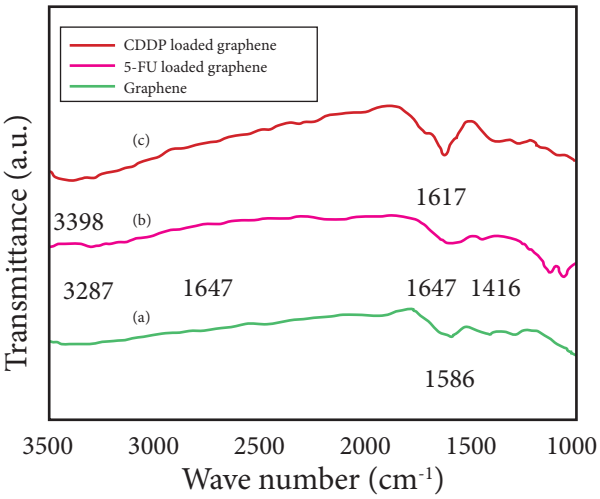


Figure 1. (a) FTIR spectra of exfoliated graphene, (b) 5-Fluorouracil (5-FU) loaded graphene, and (c) cisplatin (CDDP) loaded graphene nanocarrier

Morphological investigation

The detailed morphology of the graphite, exfoliated graphene, 5-FU and CDDP entrapped graphene was investigated by SEM analysis. Figure 2(a) and 2(b) shows that graphite and after exfoliated graphite by ultra-sonication process. The Figure 2(b) morphology is clearly indicated the exploitation of graphite and confirmation of graphene[28]. Figure 2(c) established that the CDDP entrapped graphene as white coating on the graphene structure. Figure 2(d) showed multi textured particles surfaces, which is the interaction of 5-FU and Graphene system with electrostatic or hydrophobic interaction of graphene sheets[29].

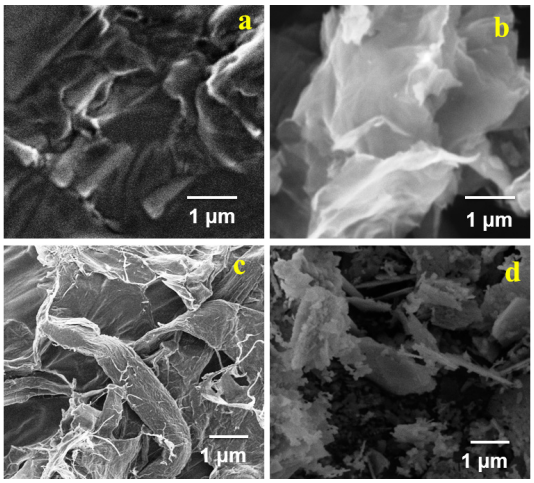


Figure 2. (a) The SEM images of the graphite, (b) graphene, (c & d) 5-FU and CDDP loaded graphene sheets

XRD analysis

Figure 3 shows the XRD patterns of the graphene nanosheets, 5-FU and CDDP loaded graphene sheets. Figure 3(a) the graphene sheets show a broad peak at 26.15, revealing that graphene was successfully synthesized[30,31]. Figure 3(b) the 5-FU loaded graphene sheets shows a sharp peaks at (15.39 θ, 16.36 θ, 24.11 θ and 30.57 θ) indicating its multi crystalline nature of 5-FU[32]. The sharp peaks appeared in Figure 3(c) be due to the platinum loaded graphene nanocarrier[33].

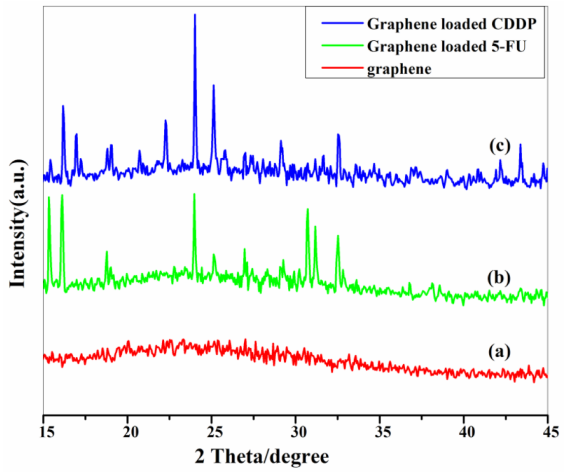


Figure 3. (a) The XRD patterns of the graphene, (b & c) 5-FU and CDDP loaded graphene sheets

Encapsulation Efficiency

Encapsulation efficiency is an important parameter to determine the encapsulating behavior 5-FU and CDDP entrapped graphene sheets. In this regard, we carried out the encapsulation studies by using the UV-Spectrometry studies by the λ_{max} value of 265 and 267 nm using UV-Vis Spectrum. The absorption intensity of 5-FU and CDDP (λ_{max} ~ 265, 267 nm) decreases with time due to the encapsulation on 5-FU and CDDP on Graphene nanocarrier. The maximum drug entrapped efficiency was observed on 5-FU & Graphene system compared then CDDP & graphene. The encapsulation efficiency of 5-FU and CDDP on graphene was 99% and 96.08% obtained at 3 h and 5 h respectively. Based on the results 5-FU entrapped graphene nanocarrier shows an awesome performance toward encapsulation enhanced than CDDP entrapped graphene nanocarrier. Because, the hydrophobic, π-π stacking interaction of graphene sheets and 5-FU drug.

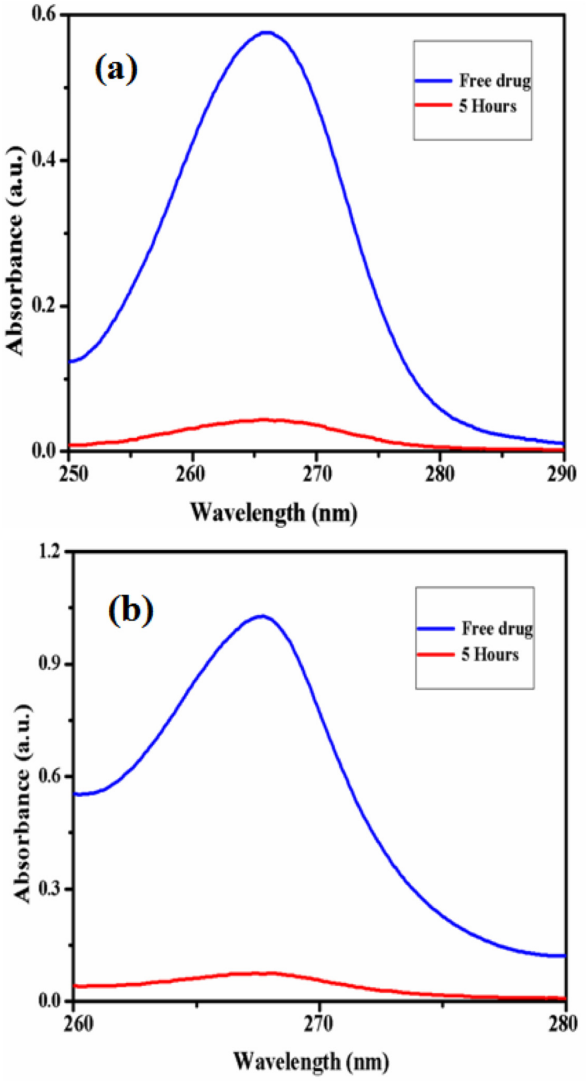
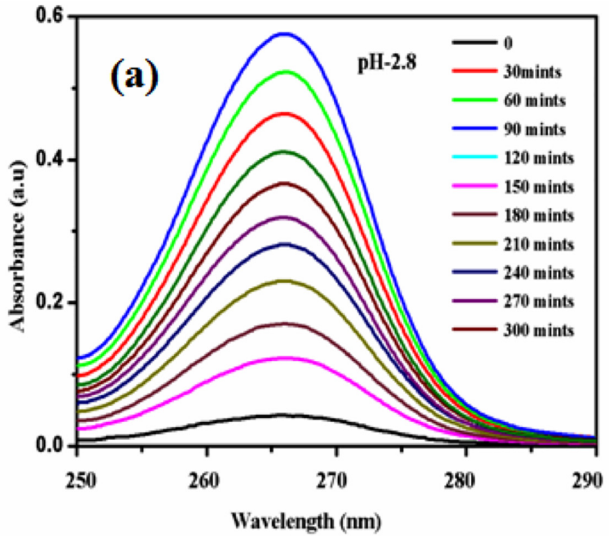


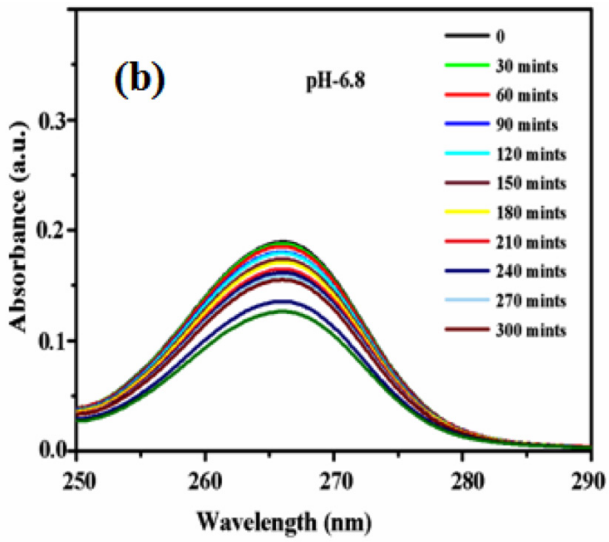
Figure 4. (a) Encapsulation efficiency of 5-FU and (b) CDDP entrapped graphene

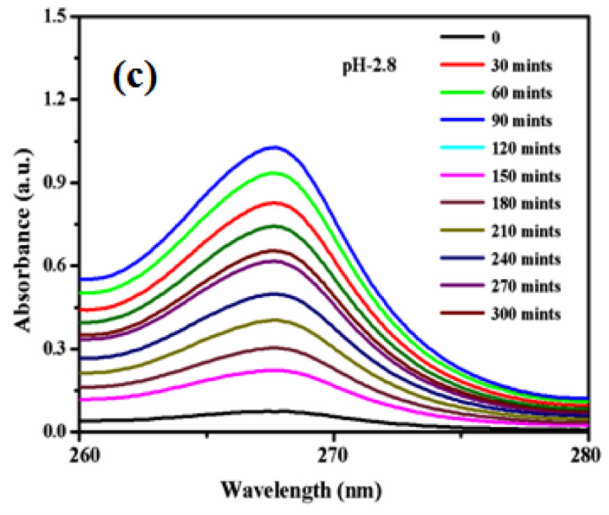
***In-vitro* drug release studies**

Following the synthesis, drug loading, surface conjugation, and characterization of nanoparticles, drug release studies are performed to study the rate at which the loaded drug is released into the different physiological environment. Drug release studies are performed at biologically relevant pH 2.8 and 6.8. In-vitro 5-FU, CDDP drug release profile from the graphene carriers was assessed using the dialysis technique at pH 2.8 (acidic pH) and pH 6.8 (basic pH) at 37 °C. In the 5-FU & Graphene system higher 95% of drug released at pH 2.8, when compared to basic medium 84% at pH 6.8. But in the CDDP & Graphene system having lower releasing mechanism was perceived, it is due to the weak interaction of CDDP and graphene. The synthesized carrier preparation was based on pH dependent manner and the basic medium

having controlled releasing properties. Hence the carrier with 5-FU can move through blood stream and body fluids without any loss of drug concentration. From the above information, we strongly suggest the 5-FU and graphene system very useful for cancer environment[34, 35].







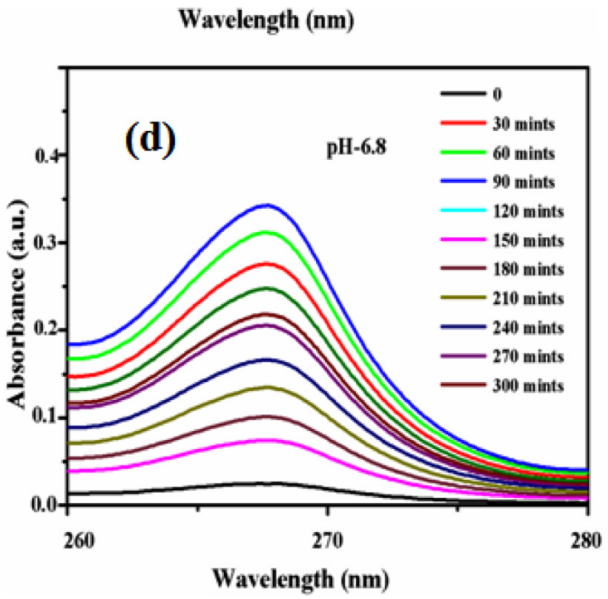


Figure 5. (a & b) *In-vitro* drug release profile of 5-FU and (c & d) CDDP loaded graphene

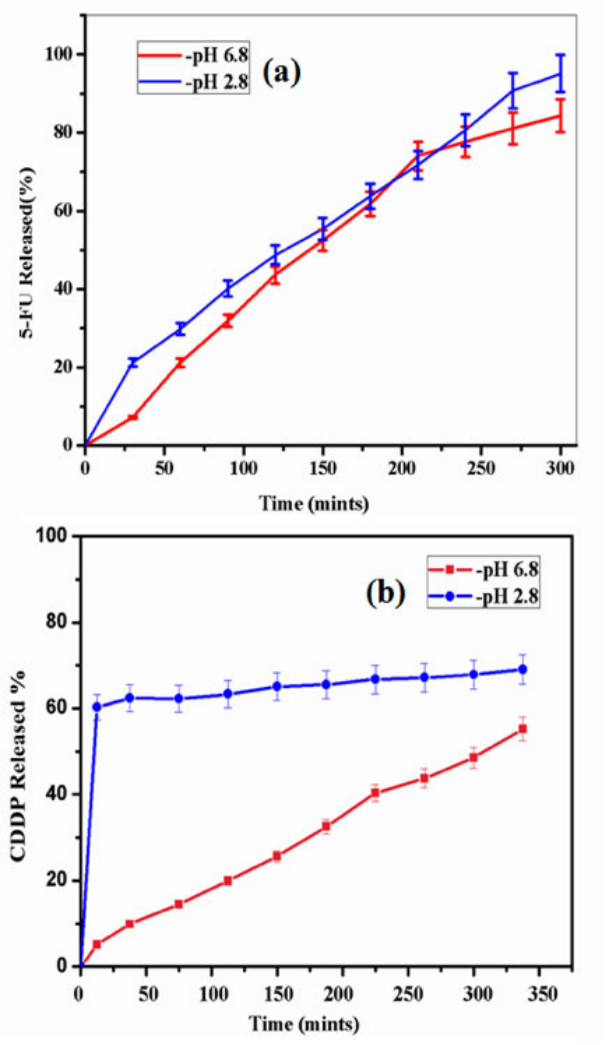


Figure 6. (a) The release profile of 5-FU, (b) CDDP from graphene sheets in buffer solutions with pH 2.8 and pH 6.8

Conclusion

We have successfully entrapped 5-FU and CDDP on the graphene sheets. The interaction of 5-FU and CDDP on the graphene carrier was investigated, the interaction is expected electrostatic and π-π stacking on the surface of graphene sheets and the drugs. With the observation of physicochemical characterization, entrapment and releasing nature, the 5-FU having higher interaction compared to cisplatin. Then the releasing nature of drug form the carrier is very compatible for cancer treatment. Since graphene is good platform for the entrapment of 5-FU anti-cancer drug then CDDP for chemotherapy.

Acknowledgment

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Conflict of interest

The authors declared that there is no conflict of interest.

References

1. Zheng M, Zhao P, Luo Z, *et al*. Theranostic nanoparticles for folate targeted cancer imaging and highly effective photo thermal therapy. *ACS Appl Mater Interfaces* 2014; 6(9); 6709−6716.

2. Misra R, Acharya S, Sahoo SK. Cancer nanotechnology application of nanotechnology in cancer therapy. Drug Discov Today 2010; 15(19−20): 842−850.

3. Alexis F, Rhee JW, Richie JP. New frontiers in nanotechnology for cancer treatment. Urol Oncol Semin Orig Investig 2008; 26(1): 74−85.

4. Shapira A, Livney YD, Broxterman HJ. Nanomedicine for targeted cancer therapy: Towards the overcoming of drug resistance. Drug Resist Updat 2011; 14(3): 150−163.

5. Jing YK, Wang L, Xia LJ, *et al*. Combined effect of all-trans retinoic acid and arsenic trioxide in acute promyelocytic leukemia cells *in vitro* and *in vivo*. Blood 2001; 97(1): 264–269.

6. Chen GQ, Shi XG, Tang W, *et al*. Uses of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia (APL): As_2O_3 exerts dose-dependent dual effects on APL cells. Blood 1997; 89(9): 3345–3353.

7. Kamm YJL, Peters GJ, Hull WE, *et al*. Correlation between 5-fluorouracil metabolism and treatment response in two variants of C26 murine colon carcinoma. Br J Cancer 2003; 89: 754–762.

8. Francini G, Petrioli R, Aquino A, *et al*. Advanced breast cancer treatment with folinic acid, 5-fluorouracil, and mitomycin C. Cancer Chemother Pharmacolo 1993; 32(5): 359–364.

9. O'Dwyer PJ, Stevenson JP, Johnson SW. Clinical status of cisplatin, carboplatin, and other platinum-based antitumor drugs. In: Lippert B (editor). Cisplatin, chemistry and biochemistry of a leading anticancer drug. Zürich: Verlag Helvetica Chimica Acta; 1999. p. 31–72.

10. Chung C, Kim YK, Shin D, *et al*. Biomedical applications of graphene and graphene oxide. Acc Chem Res 2013; 46(10): 2211–2224.

11. Fan H, Wang L, Zhao K, *et al*. Fabrication, mechanical properties, and biocompatibility of graphene-reinforced chitosan composites. Biomacromolecules 2010; 11(9): 2345–2351.

12. Liu L, Li C, Bao C, *et al*. Preparation and

characterization of chitosan/graphene oxide composites for the adsorption of Au (III) and Pd(II). Talanta 2012; 93: 350–357.

13. Tang LC, Wan YJ, Yan D, *et al*. The effect of graphene dispersion on the mechanical properties of graphene/epoxy composites. Carbon 2013; 60: 16–27.

14. Zhu Y, Murali S, Cai W, *et al*. Graphene and graphene oxide: Synthesis, properties, and applications. Adv Mater 2010; 22(46): 3906–3924.

15. Yang H, David B, Tao L, *et al*. Carboxymethyl chitosan-mediated synthesis of hyaluronic acid-targeted graphene oxide for cancer drug delivery. Carbohydr Polym 2015; 135: 72–78.

16. Zhang L, Xia J, Zhao Q, *et al*. Functional graphene oxide as a nanocarrier for controlled loading and targeted delivery of mixed anticancer drugs. Small 2010; 6(4): 537–544.

17. Liao KH, Lin YS, Macosko CW, *et al*. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. ACS Appl Mater Interfaces 2011; 3(7): 2607–2615.

18. Park S, Ruoff RS. Chemical methods for the production of graphenes. Nat Nanotechnol 2009; 4: 217–224.

19. Dreyer DR, Park S, Bielawski CW, *et al*. The chemistry of graphene oxide. Chem Soc Rev 2010; 39(1): 228–240.

20. Loh P, Bao Q, Ang PK, *et al*. The chemistry of graphene. J Mater Chem 2010; 20(12): 2277–2289.

21. Joseph D, Seo S, Williams D, *et al*. Double-stranded DNA-graphene hybrid: Preparation and anti-proliferative activity. ACS Appl Mater Interfaces 2014; 6(5): 3347–3356.

22. Marcano DC, Kosynkin DV, Berlin JM, *et al.* Improved synthesis of graphene oxide. ACS Nano 2010; 4(8): 4806–4814.

23. Stankovich S, Dikin DA, Dommett GHB, *et al*. SnO_2/graphene composite with high lithium storage capability for lithium rechargeable batteries. Nature 2006; 442: 282–286.

24. Fessi H, Puisieux F, Devissaguet JP, *et al*. Nanocapsule formation by interfacial polymer deposition following solvent displacement. Int J Pharm 1989; 55: R1–R4.

25. Murugan AV, Muraliganth T, Manthiram A. Rapid, facile microwave-solvothermal synthesis of graphene nanosheets and their polyaniline nanocomposites for energy strorage. Chem Mater 2009; 21(21): 5004–5006.

26. Olukman M, Şanlı O, Solak E. Release of anticancer drug 5-Fluorouracil from different ionically crosslinked alginate beads. J Biomater Nanobiotechnol 2012; 3(4): 469–479.

27. Lei D, Liu J, Ye F, *et al*. Synthesis, characterization and aqueous self-assembly of octenylsuccinic corn dextrin ester with high molecular weight. Food Hydrocolloids. 2014; 41: 250–256.

28. Roger E, Kalscheuer S, Kirtane A, *et al*. Folic Acid Functionalized Nanoparticles for Enhanced Oral Drug Delivery Pharmaceutics 2012; 9(7): 2103−2110.

29. Rajan M, Krishnan P, Jeyanthinath M, *et al*. Magneto-Chemotherapy for cervival cancer treatment with camptothecin loaded Fe3O4 functionalized β-Cyclodextrin nanovechicle. RSC Advance 2017; 6: 006615. In Press

30. Zhang YH, Zhang N, Tang ZR, *et al*. Graphene transforms wide band gap ZnS to a visible light photocatalyst. The new role of graphene as a macromolecular photosensitizer. ACS Nano 2012; 6(11): 9777−9789.

31. Dong PY, Wang YH, Guo LN, *et al*. Adherence to healthy lifestyle and cardiovascular ciseases in the Chinese population. Nanoscale 2012: 4(15): 4641−4649.

32. Rajan M, Raj V, Al-Arfaj AA, *et al.* Hyaluronidase enzyme core-5-fluorouracil-loaded chitosan-PEG-gelatin polymer nanocomposites as targeted and controlled drug delivery vehicles. Int J Pharm 2013; 453(2): 514–522.

33. Rajan M, Murugan M, Ponnamma D, *et al*. Poly-carboxylic acids functionalized chitosan nanocarriers for controlled and targeted anti-cancer drug delivery. Biomed Pharmacother 2016; 83: 201–211.

34. Fan XJ, Jiao GZ, Gao L, *et al*. The preparation and drug delivery of a graphene–carbon nanotube–Fe_3O_4 nanoparticle hybrid. J Mater Chem B 2013; 1(20): 2658–2664.

35. Balakrishanan MH, Rajan M. Size-controlled synthesis of biodegradable nanocarriers for targeted and controlled cancer drug delivery using salting out cation. Bull Mater Sci 2016; 39(1): 69–77.