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International Journal of Current Research
Vol. 9, Issue, 06, pp.53269-53273, June, 2017

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

ISSN: 0975-833X

RESEARCH ARTICLE

5-FLUOROURACIL ENCAPSULATED SILVER NANOPARTICLE FOR EFFECTIVE ANTICANCER DRUG DELIVERY

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ARTICLE INFO

Article History:
Received 27th March, 2017
Received in revised form
19th April, 2017
Accepted 23rd May, 2017
Published online 30th June, 2017

Key words:
Anticancer Activity,
Fourier Transform Infra-Red Spectroscopy, 5-FU, DSC,
Silver Nanoparticles,
X-Ray Diffraction,
Cytotoxicity.

ABSTRACT

In the proposed work, enhancement of anticancer potential of 5-Fluorouracil (5-FU)by encapsulated in the silver nanoparticle.5-FU shows poor solubility and low absorption rate therefore its silver nanoparticles was formed to increase solubility, absorption as well as affectivity against tumor. Various analytical methods and assay such as Particle size, Zeta potential, Atomic Force Microscopy (AFM), DSC, X-ray Diffraction (XRD) and Fourier Transform Infrared Spectroscopy (FTIR), Drug Release, *In-vitro* cytotoxicity study were employed for monitoring the formation of nanoparticles. Presence of silver nanoparticles with an average size of 28.9 nm was revealed by Atomic Force Microscopy. The crystalline nature of synthesized nanoparticles was done by X-ray diffraction. This work shows that 5-FU encapsulated silver nanoparticles shows potential activity against cancer cell.

Citation: Garg and Garg. 2017. "5-Fluorouracil encapsulated silver nanoparticle for effective anticancer drug delivery", *International Journal of Current Research*, 9, (06), 53269-53273.

INTRODUCTION

Nanotechnology can be defined as the formation, development, enhancement and exploration of nano sized materials having size range of (1-100nm). It works with the substance which have specific properties such as physical, chemical, and biological. Now a days nanotechnology works on the design and development of many novel formulations for the prevention, treatment and diagnosis of many critical diseases (El-Nour *et al*., 2010), like cancer, T.B. and cardiovascular diseases etc. Because of their specific characteristics, silver nanoparticles (AgNPs) may used as catalysts (Santos *et al*., 2012), in spectrally selective coatings for absorption of solar energy as optical sensors (Shi *et al*., 2013), in fabric tailoring as well as in electronics devices (Karni *et al*., 2012), and in various therapeutic activity of bactericidal agents (Prabhu *et al*., 2012).

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AgNPs have a important role in prohibiting microbial (bacterial) growth in solid and liquid culture media because of its high reactivity (Singh *et al*., 2014). AgNPs prepared by green synthesis phenomena is effective in biological environmental systems (Ravindren *et al*., 2013). 5-Fluorouracil (5-FU)is widely used in the treatment of cancer and effective against solid tumors, mainly has a better therapeutic effect on gastrointestinal cancer (Yin *et al*., 2013). However, 5-Fu has heavy toxic side effects, little affinity to tumor cells, a short plasma half-life, so it was administered by intravenous infusion (Manar *et al*., 2012; Hala *et al*., 2014). The drug was enfolded or adsorbed on the surface of carriers, nanoparticles has advances in changing the distribution of drugs in the body and the release rate, increasing bioavailability and the permeability of the membrane (Wang *et al*., 2009). Recently, in order to reduce 5-Fu associated side effects and improve its therapeutic index by use of incorporation of 5-Fu into particulate carriers (Zhang *et al*., 2006; Li *et al*., 2008). We select cationic natural polymer chitosan and anionic sodium alginate as the carriers, prepared 5-Fu nanoparticles use the theory of polyelectrolyte complexation (You *et al*., 2006).

MATERIALS AND METHODS

Material

5-Fluorouracil obtained as a generous gift sample from Neon Pharmaceuticals Ltd., Mumbai, India. $AgNO_3$, acetone, isopropyl alcoholand other chemicals was purchased from Chemical drug house, New Delhi, India and other chemical used analytical reagent grade.

Synthesis of silver nanoparticle

A 0.017gm of silver nitrate was accurately weighed and dissolved in 100 ml of double distilled water and stored in amber coloured bottle until further use**.** A 100 ml of 1mM silver nitrate (AgNO3) was heated to boiling. 30 mg of 5-FU was dissolved in mixture of acetone and isopropyl alcohol (9:1). To this appropriate solution of silver nitrate, 10 ml of 5-FU solution was poured for reduction of silver ions (Ag+), then kept at room temperature for 1 hour, the change in colour of solution was examined timely. The color change from yellow to yellowish brown indicated the formation of silver nanoparticle. It observed that the aqueous silver ions could be reduced by the 5-FU solution to generate very stable silver nanoparticles named 5Fu-AgNPs. The prepared silver nanoparticle was lyophilized for further use.

CHARACTERIZATION PARAMETERS

Fourier Transform Infra-Red Spectroscopy (FTIR analysis)

FTIR Spectra of 5Fu-AgNPswas determined with a FT-IR spectrophotometer (Cary- 630 FTIR, Agilent Technologies).

Particle Size and Zeta potential Determination

The particle size and zeta potential of silver nanoparticle of 5Fu-AgNPswas determined using particle size analysis instrument (Malvern Instrument, United Kingdom).

Morphology

Atomic Force Microscopic analysis

The structure and surface characteristics of 5Fu-AgNPswas determined by AFM (AIST-NT Smart SPM 1000, CA). AFM of the nanoparticles was accomplished at glass substrate in AC mode.

X-Ray Differaction (XRD) analysis

Crystalline nature of silver nanoparticle of 5Fu-AgNPswas confirmed by X-ray diffractometer (Bruker, Munich, Germany). The scanning rate was 2θ/min over a 2θ range of 0–40º and with an interval of 0.02º.

Differential Scanning Colorimetric (DSC) analysis

DSC study of silver nanoparticle of 5Fu-AgNPswas performed by using DSC instrument (Mettler Toledo DSC 822e). 5Fu-AgNPswere weighted into aluminum pan and confined with a pin-holed lid, then thermo grams was observed under nitrogenenous atmospheric condition from ambient to 350ºC at a heating rate of 700ºC in 7 min.

Hemolytic toxicity

Whole human blood was collected and stored in HiAnticlot blood collection vials as described in our previous paper(Bhadra *et al*., 2005).The human blood was centrifuged then red blood cells were separated and suspended in normal saline solution (10% hematocrit). One ml of the red blood cell suspension was individual incubated with 5 mL of distilled water (taken as 100% hemolytic standard) and 5 mL of normal saline (taken as blank for spectrophotometric evaluation). Further, 1 mL of adequately diluted 5Fu-AgNPswas added to 5.0 mL of normal saline and interacted with RBC suspension. The suspension was centrifuged for 10 min at 2000 rpm and the absorbance of supernatants was measured at 540 nm, which was used to evaluate the percentage hemolysis using distilled water as 100% hemolytic standard.

Drug Release Study

Ten milligrams of 5Fu-AgNPswas dissolved/dispersed in 5 mL of PBS (pH 7.4) and then placed into a dialysis tube (MWCO 2000 Da). The dialysis tube was placed into 100 mL of aqueous recipient medium of PBS (pH 7.4). The medium was placed in the stirring condition at 100 rpm at 37 ± 2°C, then the sample are released through the dialysis tube continuously and then the sample were withdrawn at specific time interval and analyzed on HPLC and the same volume of fresh PBS (pH 7.4) was added to maintain the sink condition. Then the samples were examined by using HPLC.

In-vitro cytotoxicity assay

Cellular cytotoxicity was assessed by tetrazolium dye-based MTT assay following a previously reported procedure (Garg *et al*. 2014, Kolhe *et al*. 2003). SKOV-3, MDA-MB-435 and A-549 cells were maintained in RPMI-1640 medium supplemented with 10% heat inactivated fetal bovine serum and antibiotics at 37°C in a humidified incubator containing 5% CO2. The cells were treated with 5Fu-AgNPsin various concentrations (10, 20, 40, and 80 lg/mL) for 24 h. The amount of formulation needed to prepare molar equivalents of 5-FU was calculated, based on the drug content in the formulation. Control was taken without any drug treatment. Subsequently, MTT was added and plates were then incubated for another 3h, the medium was pipetted off and DMSO was added. The absorbance of individual wells was noted at 570nm via an ELISA plate reader at 25°C. Average values from triplicate were subtracted from average value of control and the survival fraction of cells was calculated by the formula.

RESULT AND DISCUSSION

FTIR analysis of 5Fu-AgNPs

FTIR analysis was done to measure the characterization and conjugation of potent biomolecules of 5-FU with silver nanoparticles. According to the FTIR spectra of 5Fu-AgNPs(Figure 1) different peaks was obtained which indicates different functional groups and intermolecular bonding of 5Fu-AgNPs. The spectra are obtained at 1178, 1247, 1343, 1409, 1555, 1705, 2689 and 2910cm-1 indicates the presence of C-N stretching of aromatic amine group and C-N stretching ester and ethers in the residual solution and NO3- group, aromatic C-H bending,, C=C bonding, C=O stretch and O-H carboxylic acid, C-H alkene stretch respectively.

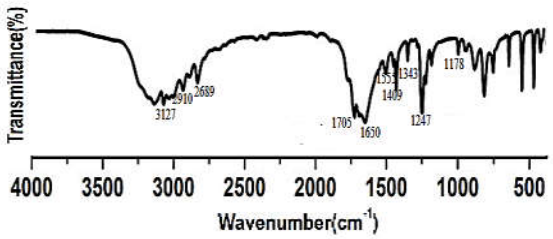

Figure 1. FTIR of 5Fu-AgNPs

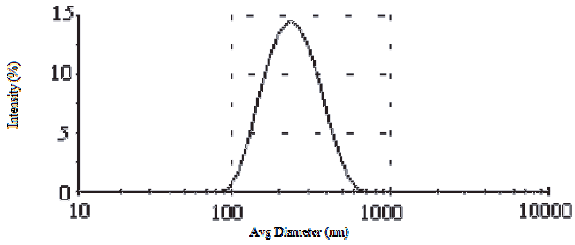

Figure 2. Particle size study of 5Fu-AgNPs

The variety of functional groups indicated in FTIR spectra are mainly obtained from heterocyclic substances that are generally water soluble compounds presents in the formulation. So it can be understood that various water soluble heterocyclic compounds like alkaloids and flavonoids works as the capping ligands for the formation of 5Fu-AgNPs (Geetha *et al*., 2014).

Particle Size and Zeta potential Determination

The average particle size of 5Fu-AgNPs were found to be 28.98 (Figure 2) and distribution of particles size of 5Fu-AgNPs were found to be 71.3% of 20.31nm, 17.5% of 16.67nm and 11.2% of 14.5nm and zeta potential of 5Fu-AgNPs was found to be -10.5 mV (Figure 3).

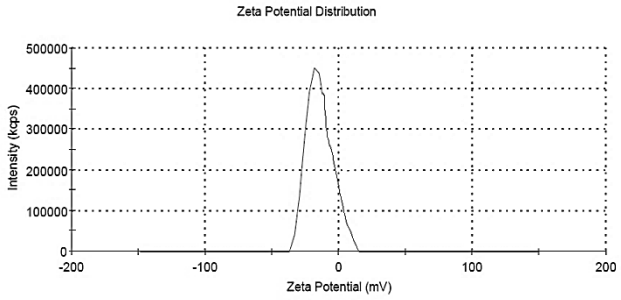

Figure 3. Zeta potential study of 5Fu-AgNPs

Morphology

Atomic Force Microscopic analysis

The structure and surface characteristics of5Fu-AgNPs was characterized by atomic force microscope and image was obtained. The 5Fu-AgNPs was visualized in spherical shaped and in nanometric size range (23nm) as estimated by AFM image (Figure. 4).

XRD analysis of 5Fu-AgNPs

XRD is a very potent technique that is normally used to analyze the characteristics and details of structure nanoparticles.

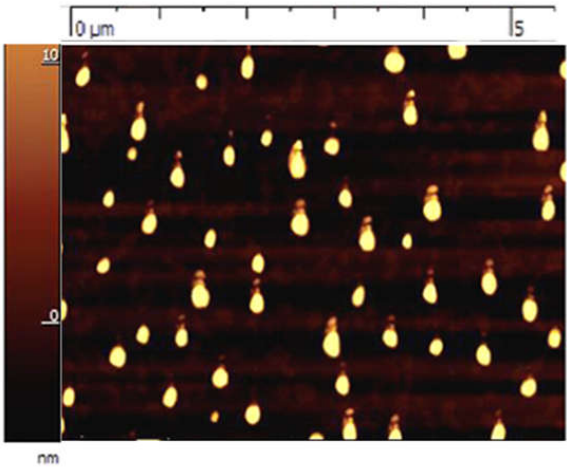

Figure 4. AFM image of 5Fu-AgNPs

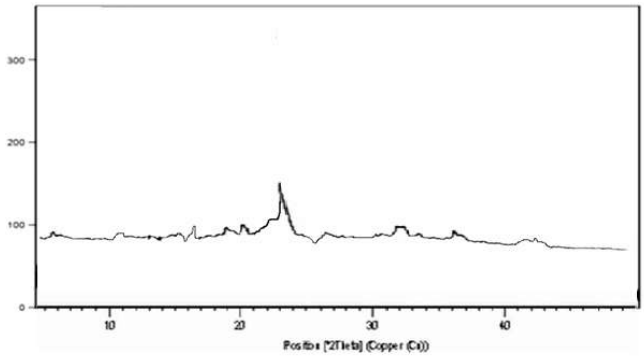

Figure 5. XRD image of 5Fu-AgNPs

The XRD patterns are observed by measuring the angles at which an X-ray beam is diffracted by the crystalline phases in the object. The XRD pattern of synthesized 5Fu-AgNPs is shown in figure 5, the following prominent peaks at 5°, 11°, 17°, 19°, 21°, 23°, 32°, 37° and 42° (2θ) indicated the crystalline property of silver nanoparticles, from the XRD pattern it is known that 5Fu-AgNPs formed and crystalline in nature.

DSC analysis

DSC thermogram of 5-FU and 5Fu-AgNPs shown in Figure 6, in the case of 5-FU, a sharp exothermic peak was observed 284ºC. In case of 5Fu-AgNPs, an exothermic peak was obtained at 47 ºC, and endothermic peak was obtained at 183, 240 and a sharp peak was visualized at 284ºC.However, the endotherm of 5-FU was detected at 284ºC in the DSC thermogram of drug loaded NPs (5Fu-AgNPs), indicating that the drug exists in crystalline form inside the nanoparticles.

Hemolytic toxicity

Hemolytic toxicity study was executed to evaluate the hemotoxic effect of the5Fu-AgNPs. The plain 5-FU and5Fu-AgNPs have exhibited hemolytic toxicity upto14.10±1.5% and 2.98±0.8% respectively. The 5-FU concentration for silver nano particulate formulations was determined on the basis of the drug content. Feasibly, the encapsulation of drug molecules in the AgNPs and consequential delayed release results in a significant reduction in the hemolytic toxicity.

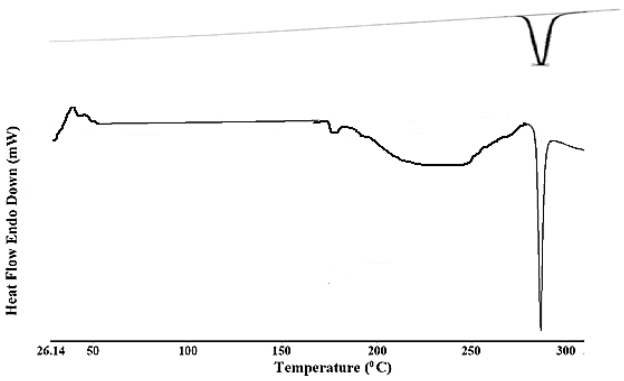

Figure 6. DSC image of5-FU and5Fu-AgNPs

Moreover, 5Fu-AgNPs demonstrated lesser hemotoxicity in comparison with plain 5-FU.

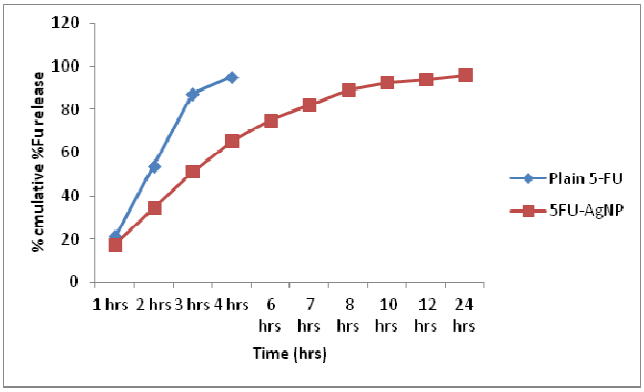

Figure 7. Drug release pattern of plain 5-FUand 5Fu-AgNPs

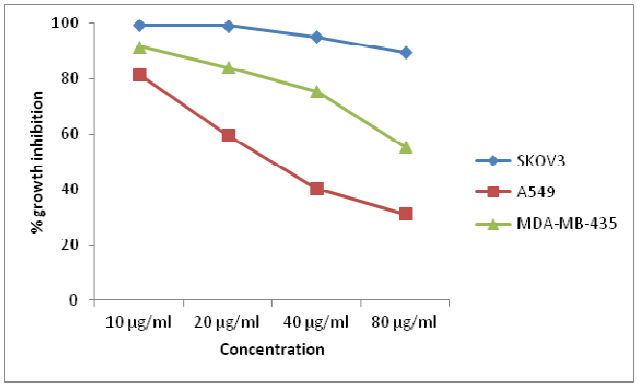

Figure 8. *In-vitro* percentage control growth of 5FU-AgNPs in SKOV 3, A549 and MDA-MB-435 cancer celllines

In-vitro drug release

The release of 5-FU from the 5Fu-AgNPs demonstrated that the AgNPs have sustained release property (Figure 7). The conclusions established the fact that there was a prominent time prolongation of 5-FU release from 5Fu-AgNPs system. 5Fu-AgNPs were able to release 96.12% 5Fu-AgNPs in 24hrs whereas the plain 5-FU was released 92.31% in 4 hrs.

In-vitro cytotoxicity assay

SKOV-3, MDA-MB-435 and A-549 cells was investigated by MTT assay. The results clearly suggested a dose-dependent cytotoxicity, i.e. reduced cellular viability upon increasing the concentration of 5-FU.

The survival fraction of cells upon incubation of plain 5-FU and 5Fu-AgNPs formulation in varying concentration is shown in Figure 8. After incubation, 5Fu-AgNPs formulation shows inhibitory effect on cell growth, the % control growth was reduced with the increasing the concentration of 5-FU. Further, the cell viability decreased when the concentration of5-FU either in free form or inside the 5Fu-AgNPs was increased. In the concentration range of 10–80 lg/mL,5Fu-AgNPs were cytotoxic to a greater degree in comparison to plain 5-FU. This is concordant with carbohydrate receptor-binding capacity exhibited by AgNPs. These results demonstrated that 5Fu-AgNPs cytotoxicity induced by 5-FU to the tumor cells were dose dependent and had agreater cytotoxic effect on the tumor cells than did plain 5-FU.

Conclusion

In conclusion, in this study, 5-FU can be widely and effectively used in the formulation of silver nanoparticles and drug delivery. The shape and size of nanoparticles can be very easily controlled with the use of plants. In the present study we observed that This approach for synthesis of AgNPs of 5FUhas many advantages including the ease with which the process can be scaled up and its economic viability. The use of such eco-friendly nanoparticles as a anticancer agent in medical applications, renders this method potentially exciting for a large-scale synthesis of other inorganic materials (nanomaterial). The effect of anticancer activity of 5-FUcan be enhanced via formation of silver nanoparticle by nanotechnology process. Results revealed that 5Fu-AgNPsshowed effective anticancer activity towards cancer cell lines. Hence it can be concluded that nanosize particle of 5-FU was more effective in cancer inhibitory better than the larger size particles.

Acknowledgement

We thank SAIL, RGPV for providing us particle size, Zeta potrential and DSC the research facility and STIC cochine, for providing us XRD study. We also thank to TMH Mumbai, for providing in vitro cell line assay.

Declaration of interest

The authors report no conflicts of interest.

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