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Nano silver based targeted drug delivery for treatment of cancer

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ABSTRACT

Based on the future drug delivery vehicle,a drug in nanoformulation based on targeted drug delivery for effective treatment of cancer is developed. The drug is a Nano formulation consisting of a Nanocarrier, Cancer specific receptor or general receptor, Sensing material to identify if the cell is normal, malignant or benign, Substance to indicate the amount of uptake of the nanomaterial by the cell for the staging of cancer, a drug wrapped in a charged molecule, stabilizing and binding agent.When an external electrical potential is applied to the solution containing the mixture of stabilizing or binding agent, drug, cancer specific receptor or general receptor, sensing material, the binding agent loses its negative charge, which causes the nanoformulation to disintegrate, releasing the drugs. The amount of drug delivered and the timing of the dose can be precisely controlled by turning the voltage on and off. The electrical signal can be remotely administered (for example, by a physician) using radio signals or other techniques that have already been developed for other biomedical devices. The formulation can carry discrete packets of drugs that can be released separately, which could be especially beneficial for chemotherapy.

Keywords: Targeted drug delivery, Nano formulation, Silver nanoparticles, Cancer.

INTRODUCTION

Nanotechnology is a most promising field for generating new applications in medicine. However, only few nanoproducts are currently in use for medical purposes. Nanotechnology has the potential to revolutionize cancer diagnosis and therapy. Advances in protein engineering and materials science have contributed to novel nanoscale targeting approaches that may bring new hope to cancer patients.Nanotechnology is the design, characterization, production and applications of structures, devices and systems by controlling shape and size at the nanometer scale.Nanometer-sized particles offer novel structural, optical and electronic properties that are not attainable with individual molecules or bulk solids. Nanoparticles are of great scientific interest as they are effectively a bridge between bulk materials and atomic or molecular structure.

Advances in nanomedicine can be made by engineering nanoparticles that are capable of targeted delivery of drugs.

Drug delivery is the method or process of administering a pharmaceutical compound to achieve a therapeutic effect in humans and animals.Drug delivery technologies modify drug release profile, absorption, distribution and elimination for the benefit of improving product efficacy and safety, as well as patient convenience and compliance.Current efforts in the area of drug delivery include the development of targeted delivery in which the drug is only active in the target area of the body (for example, in cancerous tissues) and sustained release formulations in which the drug is released over a period of time in a controlled manner from a formulation.
Several therapeutic nanocarriers have been approved for clinical use. Nanocarriers can offer many advantages over free drugs. They:

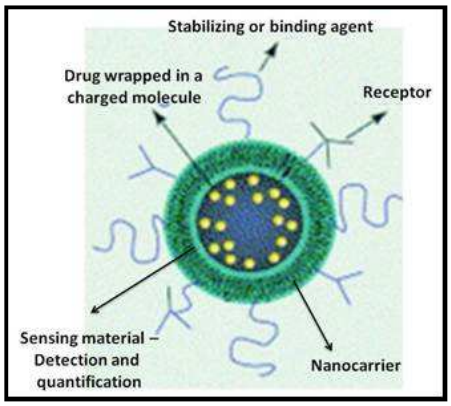
- protect the drug from premature degradation;

- prevent drugs from prematurely interacting with thebiological environment;
- enhance absorption of the drugs into a selected tissue(for example, solid tumour);
- control the pharmacokinetic and drug tissuedistribution profile;
- improve intracellular penetration.

However, to date, there are only a few clinically approved nanocarriers that incorporate molecules to selectively bind and target cancer cells. In recent years, metallic nanoparticles have drawn a lot of attention due to their unusual physical and chemical properties, which largely differ from their bulk properties. Among all metals, silver has the highest electrical and thermal conductivity.

Our present work proposes a drug in nanoformulationbased on targeted drug delivery for effective treatment of cancer.

The nanoformulation has a
a. Nanocarrier
b. Conjugation of Nano particles with PEG (Pegylation favor the pharmacokinetics of the these nano formulations prolonging the circulation periods that grant a proper time frame for the nano particles to be accumulated in the tumor micro environment although PEG is the most effective method to reduce protein adsorption invivo and to avoid the RES system clearance).
c. Cancer specific receptor or general receptor.
d. Sensing material for to identify if the cell is normal, malignant or benign.
e. Substance to indicate the amount of uptake of the nanomaterial by the cell for stagingthe cancer.
f. A drug, pemetrexed which is an FDA approved drug in the treatment of malignant Pleural Mesothelioma, a type of tumor of the lining of the lung. In combination with other drugswhose disease is unrespectable orwho are otherwise candidates for curative surgery.
g. A stabilizing and binding agent.



MATERIALS AND METHODS

Chemicals:
All the chemicals are procured from Sigma Aldrich, Bangalore. The main chemicals used for the synthesis of the nano formulation are Silver nitrate,Tri sodium citrate, Glucose, Polyethylene glycol (PEG) 4000s, Deionised water, Prussuian blue, Pemetrexed di sodium,sodium folate receptor, water soluble curcuminoid.

Measurement techniques:
The following measurement techniques are used to characterize the nano formulation.
1)Fourier Transform infrared (FTIR) spectroscopy: For FTIR measurements, the nanoformulation solution was centrifuged at 10,000 rpm for 30 min. The samples were dried and grinded with KBr pellets and analyzed on a JASCO FT/IR-5300 model in the diffuse reflectance mode operating at a resolution of 4 cm^{-1}.

2)Raman Spectroscopy: The Raman spectra were carried out on Jobin-Yvon U1000 Raman spectrometer with a Spectra- Physics model 165 argon-ion laser. The 90° scattering and back-scattering configurations were adopted for silver colloid system, thespectral resolution of Raman spectrometer was about 4 cm^{-1}.

3)Particle size and Zeta Potential analyzer: Zetasizer Nano ZS of Malvern is used for the studies. It can measure Maximum particle size range (diameter) of 0.3nm - 10µm, Zeta potential measurement of 3.8nm - 100µm and Molecular weight range of 342 - $2x10^7$ Da.

Zetasizer Nano series can measure three of the most important parameters for the colloid and polymer chemist:

Particle size - of particles and molecules from a maximum size range 0.3nm to 10 microns using NIBS technology and Dynamic Light Scattering.

Zeta potential - in aqueous and non-aqueous dispersions using M3-PALS technology.

Molecular weight - An absolute measurement using Static Light Scattering and the sensitivity from an avalanche-photodiode detector and fibre detection optics.

4)Scanning electron microscopy: SEM Instrument from Philips, XL30 Scanning Electron Microscope and EDAX with Magnification range X 15 - X 200,000 and Resolution of 2 nm is used for Particle / Phase analysis - Detection, analysis, morphology and size and Automatic particle counting and characterization.

5)UV Vis Spectroscopy: The instrument is from Shimadzu, double – beam spectrophotometer, UV-3600 with spectral range of 165 nm to 1000 nm with a spectral resolution of ±0.2 nm. The stray light of the instrument is 0.00005% at 340 nm.

Synthesis of Nanofomulation:
Preparation of nanosilver: Nano silver is prepared by adding 100ml of 0.01M silver nitrate solution to 100ml of 0.1M glucose solution. The mixture is continuously stirred for 5 minutes.Later 100ml of 0.005M sodium lauryl sulphate solution is added into a beaker containing above solution and kept for stirring for about 5min. After uniform solution is obtained the solution containing the mixture of trisodium citrate and sodium lauryl sulphate is added and stirred for 30 minutes. The solution turns into brown colour after 5min.

Pegylation of the nano silver: 50gms of the above prepared silver nano particle solution is transferred into a flask containing 100gm of water. 50%w/w of Poly ethylene glycol 400 is added to the above solution. UV spectra of nano silver solution is recorded to confirm the formation of nano silver with pegylation.

Addition of curcumin and Folate receptor:50% water soluble curcuminoid solution is added topegylatednano formulation. Further folate receptor is conjugated to PEG.

Pemetrexed-PEG conjugation: Pemetrexed is added to the above obtained PEG conjugate. Pemetrexed-PEG conjugate was dissolved in acetonitrile to form 1 mg/ml solution and 1ml of such solution was added drop wise to 3ml of water. The mixture is stirred for 2 hours allowing acetonitrile to evaporate. The resulting solution is filtered by centrifugal filters and then re suspended in 1ml distilled water. To physically encapsulated, 1mg of PEG was first dissolved in acetonitrile, followed by the addition of Pemetrexed pre-dissolved in 25ml of dimethyl sulfoxide.

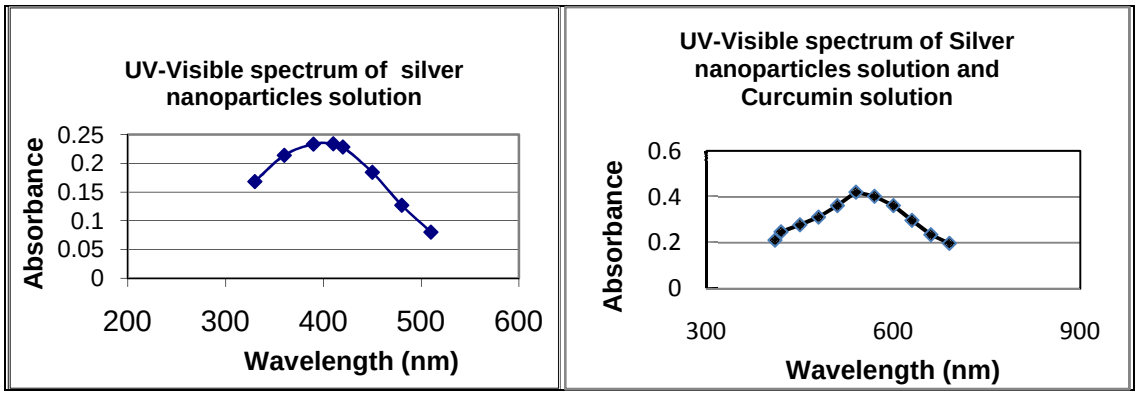
Preparation of Prussian blue: Saturated iron (III) chloride solution is prepared by dissolving 3.7g of iron chloride ($FeCl_3$) in 5 ml of distilled water and the contents are stirred to obtain a dissolved solution. Separately, a saturated solution of potassium ferrocyanide is prepared by dissolving 1.39 g of K4[Fe(CN)6] in 5ml of distilled water. Later potassium ferrocyanide solution is transferred into a beaker containing ferric chloride solution. The contents are stirred to obtain a dissolved solution.The above prepared solution is transferred into the Buchner funnel and filtered using a filter paper. The obtained Prussian blue is dried.

Final nano formulation forTargeted drug delivery:
The negatively charged Prussian blue solution and a positively charged Pemetrexed (PEGYLATED with folate receptor and curcuminoid) are mixed thoroughly for a day at room temperature for the formation of the final formulation.

RESULTS AND DISCUSSION

Results:
Synthesis of nanoformulation:
The formation of brown colored silver nano particle with a particle size around 50 nm is confirmed by the UV-Visible spectrum of the silver nanoparticle solution with a maximum absorption peak around 410 nm (shown in Fig-1) and the particle size data (as shown in Fig-5 and Fig-6).

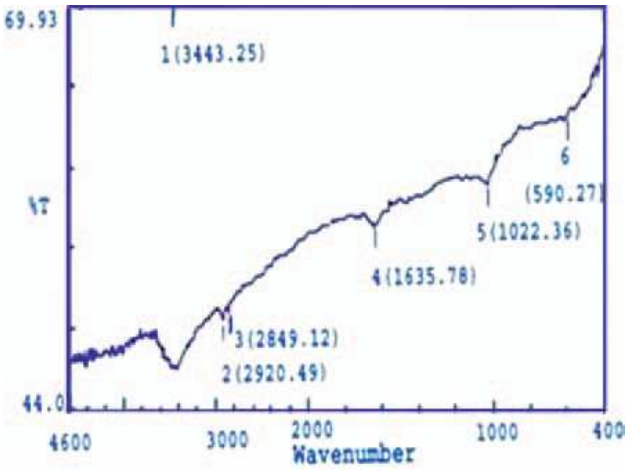
Yellowish brown colored silver nano particle solution with a peak around 540 nm (shown in Fig-2) was observed on addition of curcumin solution.



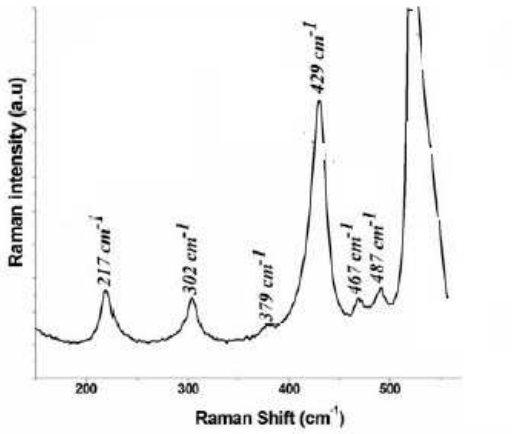
(Fig-1) UV Visible spectrum of Silver nanoparticles solution (Fig-2) UV-Vis spectrum of silver nanoparticles solution & Curcumin Solution

Fourier Transform Infrared and Raman spectrum:

The FT-IR spectrum (Fig-3) shows major peaks at 3443.25, 2920.49, 2849.12, 1635.78, 1022.36 and 590.27cm^{-1}. The major peak shows the formation of colloidal silver and minor peaks suggest the presence of organic moieties with carbonyl functional groups (curcuminoids and presence of PEG). The Raman Spectrum (Fig-4) further confirms the FT-IR results.



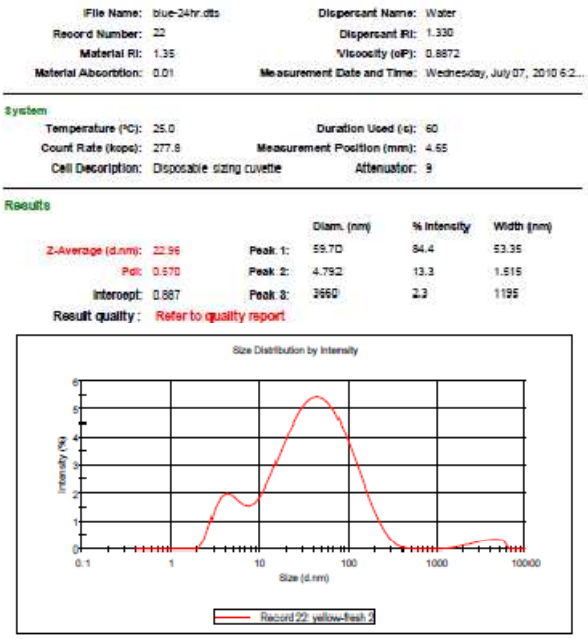
(Fig-3) FTIR spectrum of Colloidal silver and other organic moieties



(Fig-4) Raman spectrum

Particle size:

The particle size of the colloidal silver obtained is around50nm (Fig-5)whereasthe final formulation with curcumin, PEG and other additives had a particle size around 230nm (Fig-6). This confirms the formation of desired nano formulation.

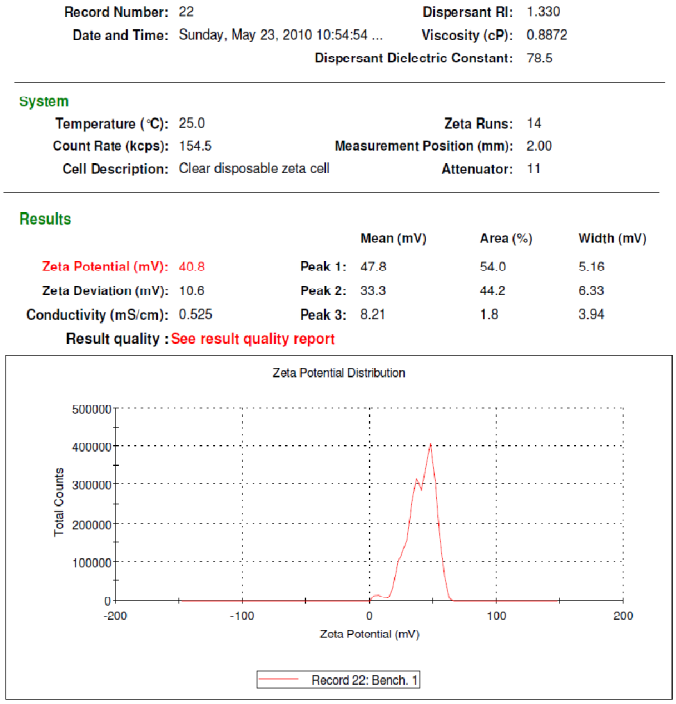


(Fig-5) Histogram of Colloidal Silver

(Fig-6) Histogram of Final nano formulation

Zeta potential:

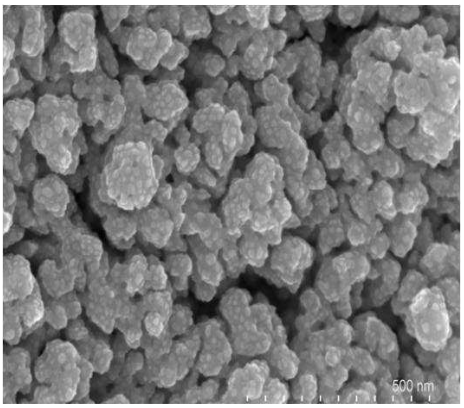
The Zeta Potential of the product before adding Prussian Blue shows a positive charge around 40 mV (Fig-7) suggesting that the nano formulation is positively charged, hence facilitating addition of negatively charge Prussian blue.



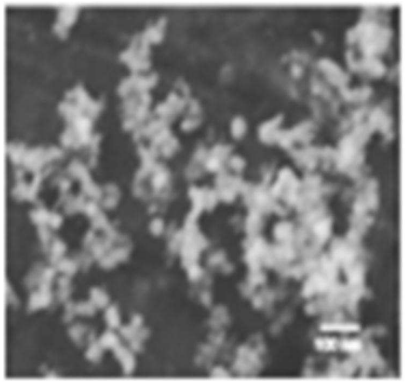
(Fig-7)

Scanning Electron Microscopy:

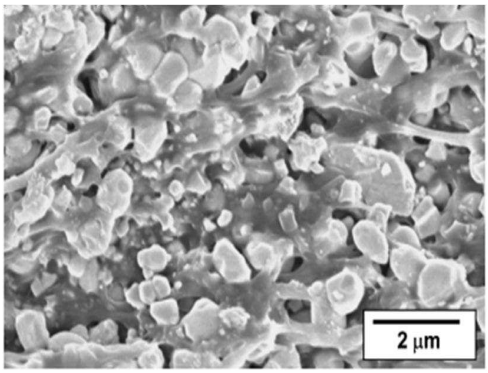
The formation of the nano particles and the particle size is further confirmed by Scanning Electron Microscopy. The Fig (8) recorded in 500 nm resolution shows the formation of silver nanoparticles with a particle size of 50 nm. The Fig (9) shows the formation of Pegylatednano silver particles with the drug in the particle size range of 100 nm. The final formulation (Fig-10) before addition of Prussian blue shows nano particle size around 200 nm recorded in the 2 micron resolution range. The illuminated particles suggest the formation of a charged particle.



(Fig-8)



(Fig-9)



(Fig-10)

DISCUSSION

In recent years, metallic nanoparticles have drawn a lot of attention due to their unusual physical and chemical properties, which largely differ from their bulk properties. Among all metals, silver has the highest electrical and thermal conductivity.

Silver nanoparticles as an arch product from the field of nanotechnology, has gained interest because of distinctive properties, such as good conductivity, chemical stability, catalytic, antibacterial activity, antifungal, anti-viral, anti-inflammatory.

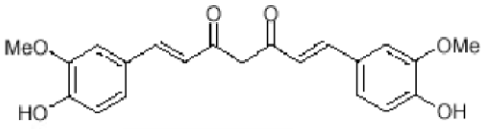
Nano silver[1] acts as a carrier for specifically targeting the cancerous cells. Usually the pores in the cell wall of normal cell are less than 10 nm and the pores in the cell wall of cancerous cells are10 to 100 nm. The nano carrier prepared by us has a particle size of around 50 nm and has an enhanced permeability to enter into cancerous cells and does not touch the normal cell.

Cancer specific receptor used by us was folate receptor which specifically targets the cancerous cells since folate receptors are over expressive only in the epithelial cancerous cells. The Folate receptor has two glycosyl phosphatidylinositol – anchored isomers and folate receptor expression is amplified in epithelial cancers, where asfolate receptor expression is found in myeloid leukemia and activated marcophages associated with chronic inflammatory diseases.Conjugates of folic acid and anti folate receptor antibodies are taken by cancer cells via receptor mediated endocytosis, thus providing a mechanism for targeted delivery to folate receptor cells. Substantial targeting efficacy is found *in vitro* studies. Therefore, the folate receptor[2] tags only to the cancerous cells.

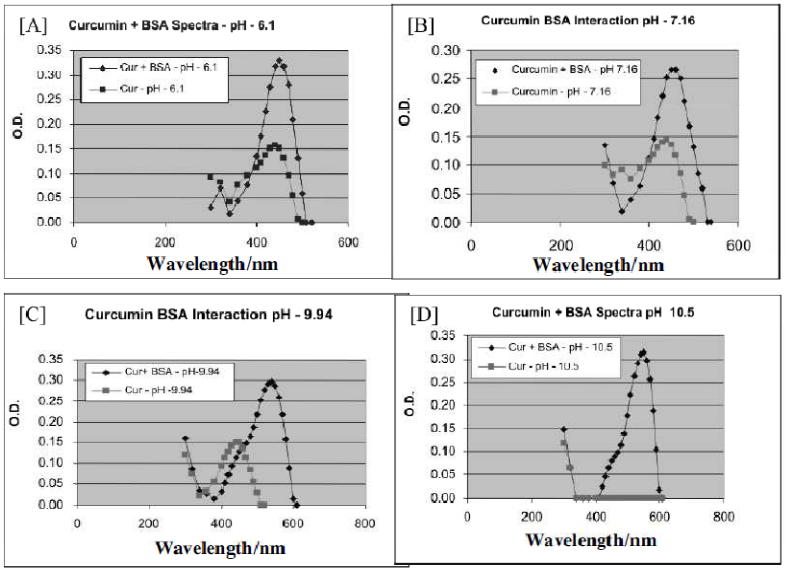
[1]FDA Approves First Drug for Rare Type of CancerFDA news release, P04-14, February 5, 2004

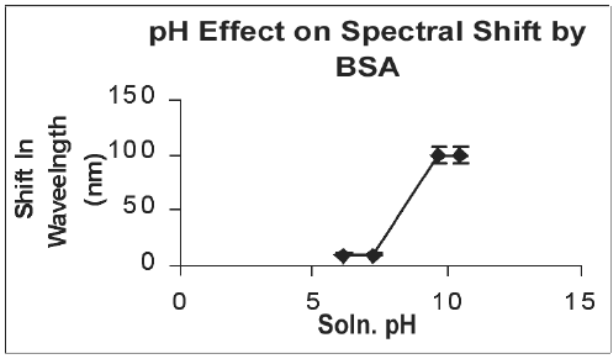
[2]Utilizing the folate receptor for active targeting of cancer nanotherapeutics, Nano Reviews 2012, **3**: 18496, *Grant L. Zwicke, G. Ali Mansoori, Constance J. Jeffery*

Once the nano particle tags to the cancer cell, curcumin in the solution changes its color based on the pH of the solution, which is confirmed by our *in vitro* studies.



Curcumin





Curcumin also acts as an antioxidant agent, thus clearing any oxygen free radicals which enhances the cancerous conditions inside the cell. The antioxidant property is further proven by the oxidation potential curcumin.Curcumin also has anti-inflammatory properties. Because free-radical-mediated peroxidation of membrane lipids and oxidative damage of DNAand proteins are believed to be associated with a variety of chronic pathological complications such as cancer, curcumin is thought to play a vital role against these pathological conditions.

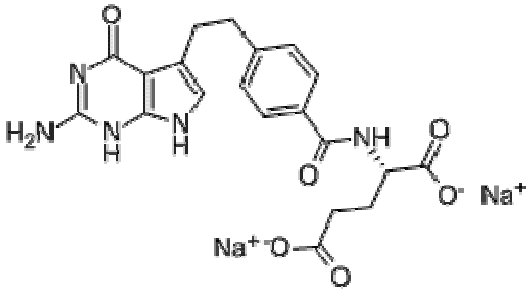
Curcumin has proved its credentials as a wonderful chemo preventive agent against a variety of cancers. Curcumin, being a powerful antioxidant, quenches free radicals, thereby decreasing the cellular damage. It also helps in reducing blood lipid levels, particularly cholesterol. Curcumin, with its potent anti-inflammatory activity, reduces the inflammation, promotes fibrinolysis, and inhibits the leukotriene formation, which are important steps in the prevention of atherosclerosis.

Upon the addition of Prussian blue to Pemetrexed, the pigment called Prussian blue sand witches the drug molecule and holds them in place (Part of the reason the researcher chose to work with Prussian Blue is that the FDA has already found it safe for use in humans.).

Anti cancer drug, Pemetrexed is used to effectively kill the tumor. Pemetrexed is chemically similar to folic acid and is in the class of chemotherapy drugs called folate antimetabolites. It works by inhibiting three enzymes used in purine and pyrimidine synthesis—thymidylate synthase (TS), dihydrofolate reductase (DHFR), and glycinamide ribonucleotide formyltransferase (GARFT). By inhibiting the formation of precursor purine and pyrimidine

nucleotides, pemetrexed prevents the formation of DNA and RNA, which are required for the growth and survival of both normal cells and cancer cells.

Similar to the natural folates, polyglutamated forms of pemetrexed are better retained in the cell and have higher affinity than parent compound for certain folate-dependent enzymes. Inhibition of several of these enzymes by pemetrexed and its polyglutamates causes depletion of nucleotide pools that is associated with growth inhibition and possibly cell death.



When an external electrical potential is applied to the solution(mixture of Prussian blue, Pemetrexed, PEG, folate receptor and curcuminoid), the Prussian Blue loses its negative charge, which causes the nanoformulation to disintegrate, releasing the drugs.The amount of drug delivered and the timing of the dose can be precisely controlled by turning the voltage on and off. The electrical signal can be remotely administered (for example, by a physician) using radio signals or other techniques that have already been developed for other biomedical devices.

The formulation can carry discrete packets of drugs that can be released separately, which could be especially beneficial for chemotherapy.

CONCLUSION

In summary,the nano carrier prepared, has a particle size of around 50 nm (usually the pores in the cell wall of cancerous cells are between 10 to 100nm, and the pores in the cell wall of normal cell are less than 10nm) and has an enhanced permeability to enter into cancerous cells and does not touch the normal cell. The cancer specific receptor, which is the folate receptor upon addition,targets specifically the cancerous cells since folate receptors are over expressive only in the epithelial cancerous cells. Curcumin acts as a pH sensing material and is usually an antioxidant and has anti-inflammatory properties.

Once the silver nanoparticle tags to the cancer cell, curcumin changes its color based on the pH of the solution.

After the external electrical potential is applied to the solution containing the mixture of Prussian blue, Pemetrexed, PEG, folate receptor and curcuminoid, the Prussian Blue loses its negative charge, which causes the nanoformulation to disintegrate, releasing the drugs. The amount of drug delivered and the timing of the dose can be precisely controlled by turning the voltage on and off.

The advantages of the developed method by us to deliver the drug to the targeted cancerous site are presented below.

Reduced turmoil to the patient: A distinct advantage of this approach is that the results are displayed in real time. The physician and patient do not have to wait for pathology results, which on average take approximately two months from first exam to final diagnosis. Consequently, the intense anxiety that many patients experience while waiting for a final determination can be eliminated.

Early treatment – increased survival chances - If the measurements indicate readings that are indicative of cancer, the physician will have a significant option to start treatment immediately.

No specific prior knowledge required fordevelopment of the system: Our detection system that is based on selective interactions of the cancerous cell with nanoparticle based sensor elements that does not require any previous knowledge of intracellular or extracellular biomarkers.

Real time observation: The developed technique tracks the progress of various therapies by providing the physician with information regarding the status of tumor recession.

Can be extended to staging of cancer using a suitable marker.

No toxicity to the systematic route: As nanocarrier specially targets the cancerous cell, the normal cells are not affected. This results in100% dosage reaching the cancerous cells which is not possible with non nano drug delivery, Effective therapy, Less number of sittings to the patient, Reduced cost of treatment, reduced turmoil to the patient, Earlier Phase II failed drugs, which are extremely toxic to the normal cell and highly potent on the cancerous cells than the present drugs in usage can be used for the treatment. More effective treatment and increased survival chances.

As the nanoformulation is basic, anytype of receptor, drug can be attached, instead of silver, metal like Iron or gold can be used which enhance the image in MRI and CT (& US) respectively, slight modification in the formulation and enhanced imaging is possible.

Acknowledgements
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