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In-vitro drug release study: preparation and evaluation of chitosan anchored nanoparticles

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ABSTRACT

Cancer has become a solemn threat to the life of human beings universally. Various strategies are available to steadfastness cancer; however they are not so effective owed to their serious side effects, noxious effect to healthy cells and non- specificity to cancer cells targeting. To tenacity above facts we try to deed inherent characters of cancer cells. HA was used as a targeting agent for drug delivery to breast cancer cells. In this work nanoparticles were equipped using chitosan and sodium tripolyphosphate encapsulating methotrexate. Methotrexate (Mtx) a folic acid antagonist that inhibits dihydrofolatereductase (DHFR) and blocks conversion of dihydrofolic acid (DHFA) to tetrahydrofolic acid (THFA) of the cell cycle. Chitosan anchored nanoparticles were prepared by ionotropic gelation method by means of sodium tripolyphosphate and evalauted for i*n-vitro* drug release study with dialysis membrane. Result depicts that drug releases from chitosan nanoparticles in sustained manner over a prolonged episode of time from the NPs as the medium acidity enhanced at the target site, not in plasma. In conclusion, chitosan anchored nanoparticles of MTX could be well thought-out as probable candidate for drug delivery in the treatment of breast cancer.

Keywords: Breast cancer, Methotrexate, Chitosan, TPP and Nanoparticles.

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INTRODUCTION

Cancer is merely the gain of oncogenes and the loss of tumour suppressor genes. Genetic abnormalities found in cancer classically affect two general classes of genes. Cancer-promoting oncogenes are naturally activated in cancer cells, giving those cells new properties, such as hyperactive growth and division, protection against programmed cell death, loss of respect for normal tissue boundaries, and the capability to become established in varied tissue environments. One in eight women in the United States will be diagnosed with breast cancer in her lifetime.[1] Breast cancer is the most frequently diagnosed cancer in women. It is the second leading cause of cancer death among women. Each year it is estimated that over 252,710 women in our country will be diagnosed with breast cancer and more than 40,500 will die. Although breast cancer in men is rare, an estimated 2,470 men will be diagnosed with breast cancer and approximately 460 will die each year. On average, every 2 minutes a woman is diagnosed with breast cancer and 1 woman will die of breast cancer every 13 minutes. In current years, perhaps coinciding with the failure in prescriptive hormone replacement therapy after menopause, we have seen a gradual reduction in female breast cancer incidence rates among women aged 50 and older.[2]

Delivery of drugs through nanoparticulate grounded drug carriers has reached an attractive courtesy, mainly in the treatment of different types of malignancies. These sub-micron sized particles have a potential to target the tumour sites passively, through enhanced permeation and retention effect. Nanoparticles are multipurpose nanosized structures which have found many claims in medical practice. Functionality of nanoparticles can be derived from their inherent properties as a result of their sizes. For example, nanoparticles of within a specific size range can easily evade the reticulo endothelial system (RES) and increase the biological half life of their drug payload. Nanoparticles also offers advantage of targeted or site specific drug delivery to of chemotherapeutics and other drugs to affected cells over extended period of time thereby reducing toxic side effects. Advances in the technology assists drug to be delivered passively to the target site, preserving their efficacy and creating a host of prospects for cancer chemotherapy [3-10]

Polymeric NPs are considered as optimal carriers for drug delivery due to their appropriate characteristics. One of the most polymers used in the field of nano-drug delivery systems is Chitosan.

Many applications and attentions to this polymer in drug delivery field is because of its properties such as

formulations and techniques of the production conformed by various types of drugs (e.g. hydrophilic or hydrophobic molecules or macromolecules), biodegradability and biocompatibility, FDA approval in drug delivery systems for medical applications, protection of drug from degradation, possibility of sustained release, facility in modifying surface properties, resulting in better interaction with biological materials and facility in targeting NPs to specific points [11]. Therefore, chitosan NPs have potential to improve drug delivery systems and overcome many problems related to the treatment of breast tumors.

MTX, a folic acid antagonist, generally used as clinical chemotherapy agent and highly efficacious antineoplastic drug [12-13]. MTX inhibits Dihydrofolate Reductase (DHFR) and interfere with tumor cell DNA, RNA and consequently protein synthesis, leads to inhibition of the proliferation of tumor cells [14]. However, using methotrexate has been restricted because of limitations such as low half-life in plasma, low penetration into the brain and dose-dependent systemic side effects. To overcome this problem, MTX can be loaded in various NPs were prepared by various authors. [15-18]

MATERIALS AND METHODS

Materials:

Methotrexate was received as gift sample from Khandelwal Laboratories Pvt. Ltd. Pune, India. Chitosan was obtained from Central Institute of Fisheries, Cochin; TPP was purchased from Advance scientific Labs. Bhopal. All the chemicals and solvents were analytical grade.

Method

Preparation of chitosan nanoparticles

Chitosan Nanoparticles were prepared by using ionotropic gelation technique. A requisite quantity of chitosan was dissolved in 0.1 % glacial acetic acid and water in the ratio of 9:1 under magnetic stirring at 3000 rpm for 4 hrs at room temperature for complete dissolution. Chitosan (Cs)solution was adjusted to pH 6.2 was performed by addition of appropriate volumes of 0.1 N NaOH, such pH value maintains chitosan in its soluble form at all examined concentration (Jain *et al.* 2008). Then solution of TPP was prepared at different concentrations in 0.1 N sodium hydroxide and this solution was added drop by drop in the above prepared chitosan solution with continuous stirring for 30 hr. Then required quantity of methotrexate (Mtx) was dissolved in 0.1 N sodium hydroxide to make 1% solution and gradually added in above chitosan solution drop wise. The mixture was stirred at room temperature for 30 min to obtain Nps. Also, spontaneously formed NPs were separated by centrifugation at 10000 rpm for 10 min and supernatant discarded and sediment was poured in dialysis bag to dialyzed three times with phosphate buffer to remove free drug from the formulation which was then determined spectrophotometrically at 307 nm by UV spectrophotometer Shimadzu 1800 to determine directly the amount of drug loaded in the system. The dialyzed formulation was lyophilized for further characterization.

Preparation of hyaluronic acid (HA) anchored chitosan nanoparticles

Finely requisite quantity of HA (10 mg) was dissolved in 10 ml PBS (pH 6.0) with continuous stirring and the reaction was performed at room temperature using EDC (1- ethyl-3-(3-dimethylanminopropyl) carbodiimide hydrochloride for 1.5hrs under magnetic stirring at 3000 rpm. This solution was added drop wise in above solution with continuous stirring.

Nanoparticles were formed on solvent interface. Then solvent was evaporated at room temperature. The resulting suspension of nanoparticles was filtered through 0.45 µm membrane filter (Millipore) and centrifuged for 45 min at 10,000 rpm (Remi, Mumbai, India). The supernatant was discarded and pellet containing hyaluronic acid (HA) anchored chitosan nanoparticles were lyophilized and kept preserved. The amount of HA on the surface of nanoparticles was assessed by change in zeta potential. The two process variables (incubation time and total nanoparticulate formulation to HA weight ratio) were optimized by measuring change in zeta potential (the surface charge density)

Characterization

***In-vitro* drug release study**

In-vitro drug release profile was performed by using dialysis bag method with the help of dialysis membrane (Himedia, Mumbai India) molecular weight cut off 12000-14000 Da. The release rate of drug from formulation depends on various factors like polymer ratio, polymer degradation or erosion, solubility. In the present study nanoparticles dispersion was filled in the dialysis tube and immersed in Phosphate saline buffer (pH 7.4) under continuous magnetic stirring and the temperature should be maintained at 37±1ºC throughout the procedure. At specific time intervals, the samples were taken and diluted to determine the concentration UV spectrophotometer (Shimadzu 1800) at 307 nm and shown in the table 1 and figure 2.

Table 1: *In-vitro* cumulative percent drug release from formulations in phosphate buffer (pH 7.4)

Time (in hrs)	Cumulative % Drug release from hyaluronic acid anchored chitosan
0.25	0.89 ± 0.32
0.50	1.88 ± 0.42
1	6.14 ± 0.38
2	18.24 ± 0.28
4	30.85 ± 0.42
8	43.42 ± 3.25
12	50.42 ± 2.14
24	54.13 ± 3.42
48	59.42 ± 2.17
72	63.85 ± 1.62
84	68.74 ± 1.90
96	72.87 ± 2.54

(n=3) Mean ± S.D.

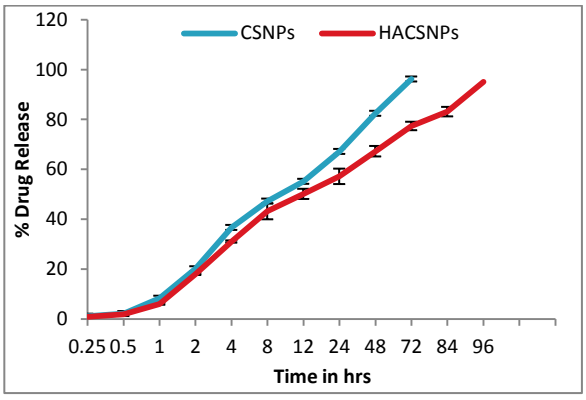

Figure 2: *In-vitro* Cumulative % Drug release from hyaluronic acid anchored chitosan nanoparticles and blank chitosan nanoparticles

RESULTS

In-vitro drug release study was performed using dialysis bag method with the help of dialysis membrane. The release studies were carried out by plotting the graph. *In-vitro* drug release studies Drug release pattern of blank chitosan nanoparticles and hyaluronic acid anchored chitosan nanoparticles was studied at pH 7.4, and it is confirmed from Fig. 4.2 that the release of drug from polymeric nanoparticles was sustained as compared to blank chitosan nanoparticles. Interestingly, more drug release was observed at cancer cell pH of 5.6 and least at plasma pH of 7.4 from the hyaluronic acid anchored chitosan nanoparticles. However, there was no such distinction of release pattern in blank formulation was obtained. This finding is unique and of significance, that the drug will be released at the target site, not in plasma.

CONCLUSION

This study reports the preparation of hyaluronic acid anchored chitosan nanoparticles via ionotropic gelation method for the delivery of the anticancer drug to breast cancer cells. The prepared nanoparticles were in nm range and *In-vitro* release profile show sustained behavior due to slow diffusion of drug from polymeric matrix follow non-fickian diffusion mechanism. The results indicated that hyaluronic acid anchored chitosan nanoparticles could be effective in controlled release of drug for prolonged period of time. Therefore, it is concluded that it could be considered hopeful carrier for targeted drug delivery to breast cancer cells.

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