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Research Article

Preparation and *In-vitro* Evaluation of Fe_3O_4 Encapsulated by Chitosan Loaded Capecitabine Nanoparticles for the Treatment of Breast Cancer

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ABSTRACT

Nanoparticulate Carriers which is biodegradable, biocompatible and bio adhesive have significant feasible applications for administration of therapeutic molecules. The present study was aimed to formulate and optimise Capecitabine loaded Chitosan-Fe_3O_4 Nanoparticles and to study the *in-vitro* evaluation by sigma dialysis method. Capecitabine loaded chitosan – Fe_3O_4 nanoparticles batches with different ratios of drug: polymer (1:1, 1:2, 1:3, 1:4, 1:5, 1:6) were prepared by ionic gelation method. Increase in polymer concentration increases the nanoparticle drug content. Entrapment efficiency was 60.12% with drug to polymer ratio F3 (1:3). *In-vitro* release was found to be 65.20% for 12 hrs. Capecitabine from chitosan-Fe_3O_4 nanoparticles SEM image reveals discrete spherical structure and particles with size range of 100-500nm. FTIR studies represent the functional groups present with no characteristics change in formulations. Samples stored at refrigerator conditions showed better stability compared with samples kept at other conditions during 8 weeks of storage.

Keywords: Chitosan, Capecitabine, Fe_3O_4 nanoparticles, Ionic gelation, biodegradable.

INTRODUCTION

Throughout the world the breast cancer is most incurable disease among the women's for about 1.7 million in the year 2012. The cancer is deadly disease status when compared to all other cancers; breast cancer is the second common among overall. Most of countries like U.S., U.K., Australia, Germany, Iceland, Switzerland, Italy, New Zealand, Ireland and some other top countries were women's commonly affected from breast cancer. Also in developing countries India and Sri Lanka women's were affected by breast cancer[1]. May be in the year 2030 the breast cancer may cross over 2 millions all over the world including the proportions of developing countries[2]. In India across the country the breast cancer incidence rates around 3 - 4 folds of variation. Mostly north-eastern part of India shows the highest rates especially in major cities like Mumbai and New Delhi[3]. According to the press in India 17% of world's population were affected by breast cancer. The main reasons for these variations are due improper education in the field of reproduction (age at first child and children numbers), lifestyle factors (consumption of alcohols, using tobacco and smoking habits) and anthropometric (adiposity). In order to create awareness to people's government had launched the national programmes such as National Cancer Control Programme (NCCP) in the year 1975. In the national programmes the disease states covered by cardiovascular disease, Cancer and stroke. The 12th five plans were launched by NPCDCS from the year 2012-2017[4]. The NPCDCS create the awareness over the highest rank in the country versus the mortality rates for breast cancer[5]. The magnetic nanoparticles which is encapsulated over by the polymers plays vital role in applications such as targeted drug delivery system, hyperthermia cancer treatment, diagnostics, tissue magnetic resonance imaging purpose and for rapid blood detoxification methods[6]. Due to the presence of magnetic field the properties are approved by their behaviour and their elevated surface area. The Fe_3O_4 (magnetite) iron oxide is core material covered by matrix or shell synthetic and natural polymer[7]. Natural polymer such as chitosan is the second most abundant among the polymers. It contains especially chitin and it deacetylation partially in the alkaline conditions. This polymer is biodegradable, non-toxic and biocompatible in nature. Chitosan contains the interesting functional groups such as amino and hydroxyl groups. It is mainly used for biomedical applications. The chitosan polymer is widely used for drug delivery system and it can be easily prepared by different methods such as spray drying, coacervation, emulsion cross linking, ionic gelation, emulsion droplet coalescence, sieving and reverse micelle preparation[8]. The present study is deals with the preparation of Fe_3O_4 encapsulated by chitosan loaded Capecitabine nanoparticles, optimization and characterization of the formulations. The Fe_3O_4 nanoparticles are prepared by chemical precipitation method. The surfactant used to stabilize the formulations and chitosan is encapsulated over the iron oxide by using cross linking agent TPP to form ionic gelation. The method of preparation is simple, non-toxic and reproducible. The iron oxide present in these formulations is nothing but super paramagnetic materials which is converged to tumour cells by applying external

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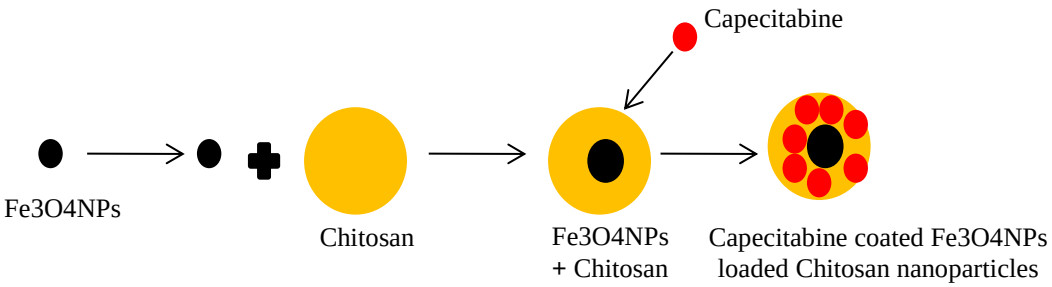

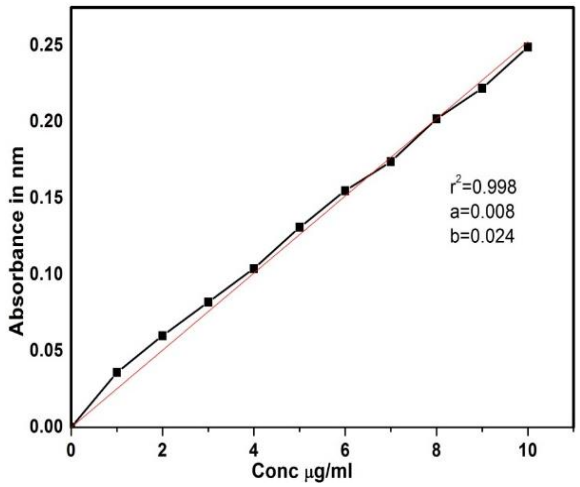

Figure 1: showed the linearity plot for the pure drug capecitabine with regression co-efficient values.

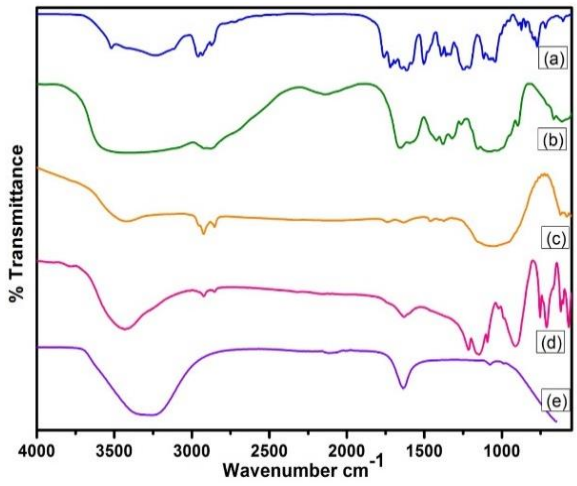

Figure 2: showed the FTIR spectrum of (a) pure drug Capecitabine, (b) Chitosan, (c) Fe3O4 nanoparticles, (d) sodium tripolyphosphate (TPP) and (e) Formulation F3.

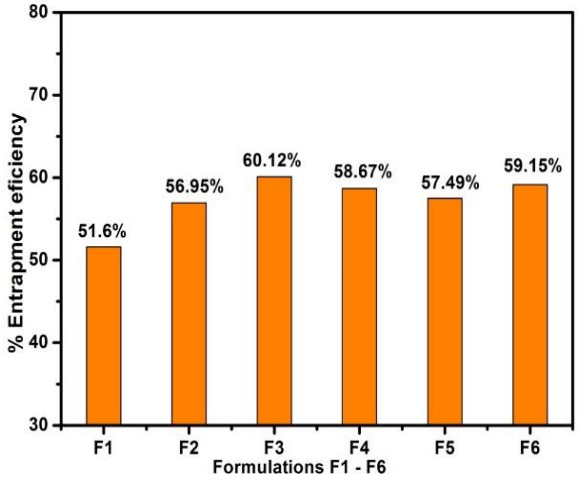

Figure 3: indicate the % Entrapment Efficiency of all formulations F1-F6.

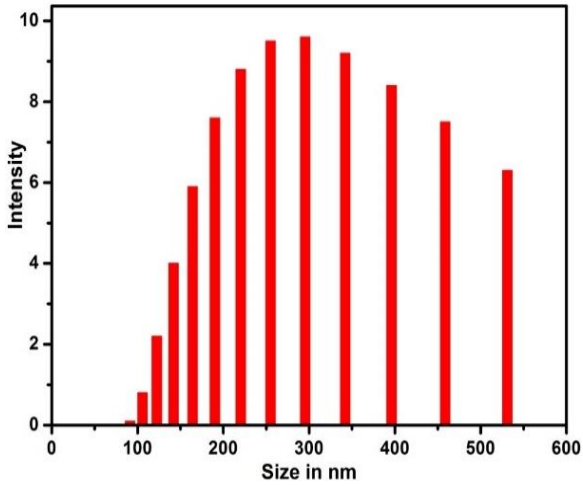

Figure 4: showed the particle size distribution of the formulations F3.

magnetic field. After the applied magnetic field the iron oxides (Fe_3O_4 Nanoparticles) get heated to 37ºC - 40ºC and the tumour cells get destroyed. The diagrammatic representation of Fe_3O_4 encapsulated by chitosan loaded Capecitabine nanoparticles are as follows,

MATERIALS AND METHODS

Capecitabine was purchased from Burgeon Pharmaceutical Company-Chennai as a gift sample. Chitosan and TPP were purchased from the sigma Aldrich-Bangalore. Ferrous chloride, Ferric Chloride and Ammonium hydroxide (25%) are purchased from SD fine chemicals - Mumbai. Other chemicals used for these studies were of analytical grades.

Preparation of Standard curve calibration curve

10mg of the pure drug capecitabine were weighed accurately and dissolved in10ml standard flask of phosphate buffer 6.8 solutions. Then 1ml of solution is withdrawn and makes up to 10ml with buffer solutions. Further, the serial dilutions were made as 1, 2, 3, 4, 5, 6, 7, 8, 9 and10µg/ml in the ratios and make up with 10ml buffered solutions. Finally, the samples were subjected into the UV spectrophotometric analysis at 240nm. According to the Beer's Lambert laws the plot was made.

Table 1: shows the optimization of the formulations at variable drug polymer ratios.

Formulation code	Amount of drug (mg)	Amount of Chitosan (mg)	Amount of TPP (%)	Amount of Fe_3O_4 NPs (mg)	Drug: polymer ratios
F1	10	10	0.25	5	1:1
F2	10	20	0.25	5	1:2
F3	10	30	0.25	5	1:3
F4	10	40	0.25	5	1:4
F5	10	50	0.25	5	1:5
F6	10	60	0.25	5	1:6

Preparation of Fe_3O_4 Nanoparticles

The most familiar method of preparation Fe_3O_4 nanoparticles by using 6.1 g of $FeCl_3.6H_2O$ and 4.2 g of $FeSO_4.7H_2O$ were dissolved in 100 ml deionized water. Further, it was placed in a three-necked flask subsequently added 25% ammonium hydroxide solution (10ml) with continuous stirring at 50 - 55^{o}C under a N_2 atmosphere condition. Adjust the pH with ammonium hydroxide to 11.0 –12.0. Then the temperature was increased to 65^{o}C and the process was then mixed for about 2 hours maintaining at same temperature. Then, adjust pH to 6-7 by adding dilute HCl solution and further the temperature was gradually raised to 80^{o}C with constant stirring for 1 hour. Finally, the pH slowly reduced to 3.4–4.0[9]. Moreover, the black precipitate were washed with D.I. water for several times, filtered, air dried and collected the nanoparticles. The Fe_3O_4 nanoparticles were yielded and stored for further preparation of formulations.

Preparation of Fe_3O_4 Encapsulated by Chitosan Loaded Capecitabine Nanoparticles

Chitosan Nanoparticles were prepared according to the Ionic gelation method. The chitosan was the polymer contains positively charged amino groups and TPP in the form sodium TPP negatively charged molecules combine together to produce gelation. TPP is nontoxic, multivalent and able to form gelate through ionic interaction between opposite charged particle or molecules. Capecitabine loaded chitosan – Fe_3O_4 nanoparticles batches with different ratios of drug: polymer (1:1, 1:2, 1:3, 1:4, 1:5, 1:6) were prepared by these above methods. Depending upon the pH of the formulation the charge density of both chitosan and TPP can be controlled[10]. 0.1% of Glacial acetic acid was used to dissolve the weighed chitosan and stirred. Separately dissolve TPP in D.I. Water. Moreover, Tween 20 was also dissolved in D.I. water followed by adding to the top of solutions. Further, the Fe_3O_4 nanoparticles (5mg/5ml) were slowly added simultaneously to the prepared formulations. The solutions (TPP & Tween20) were then added drop wise simultaneously under constant magnetic stirring to the top of solutions. The process of stirring is continued for about 3-5 hours. Meanwhile the Capecitabine is dispersed in phosphate buffer (pH-6.8) and added drop wise to the chitosan nanoparticles.

Fourier Transforms Infrared Spectroscopy (FTIR)

The spectrum obtained from the FTIR analysis of Capecitabine, Chitosan, sodium tripolyphosphate and Fe_3O_4 Encapsulated by Chitosan Loaded Capecitabine Nanoparticles formulations were recorded to verify the drug - polymer interaction and also to verify the physio-chemical properties of Chitosan, Capecitabine (pure drug) and sodium tripolyphosphate (TPP) were triturated with 1:3 ratios of KBr. Every sample was subjected to compress the pellet for IR analysis[11,12]. The spectrum of these samples were analyzed on a Perkin Elmer FT-IR Spectrometer USA, with region around 450 to 4000 $cm^{-1}$and were interpreted[13].

Determination of Entrapment Efficiency

The prepared formulations were taken and centrifuged for about 30 minutes at 12000rpm. The sample was separated from supernatant solution as it has unentrapped drug. Then the remaining entrapped solution was re-dispersed into buffer solution and centrifuged, repeat the procedure 2-3 times and further analyzed by using UV spectrophotometrically at 240nm. The Capecitabine concentration is analyzed and calculated[14]. The Entrapment efficiency (EE %) was calculated from the following equations:

$$\% \text{ Entrapment Efficiency} = \frac{\text{Total amount of drug} - \text{Entrapped drug X 100}}{\text{Total amount of drug}}$$

Determination of Particle Size

In order to obtain the particle size distribution of the formulation, Malvern analyzer was used. The drop of the sample was taken and diluted to 1ml with D.I water and placed in the sample cuvette. The prepared formulation F_3 was taken for particle size analysis and found the average particle size of the formulation is around 308nm.

Determination of Surface Morphology

The particle size of the formulation of was Fe_3O_4 Encapsulated by Chitosan Loaded Capecitabine Nanoparticles viewed and photographed using Scanning Electron Microscopy (SEM) (QUANTA 3D FE- SEM). The prepared Nanoparticles are dropped on a double sided carbon tape. The solution evaporated slowly at room temperature. The image was captured on SEM mode at desired magnification. The SEM image shows the particles of Capecitabine loaded chitosan - Fe_3O_4 nanoparticles is spherical in shape and size approximately around 100nm to 500nm.

In-Vitro Release Studies

The release studies of prepared formulations were carried out by using Dialysis bag membrane method. Donor compartment contains the Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles and phosphate buffer in the receptor compartment. The experiment as done at room temperature withdrawing 2/3ml of sample for every 1hr for a period of 12hrs and is replaced with refresh medium after every withdrawal. The sample was analysed spectrophotometrically at 240nm the cumulative % release

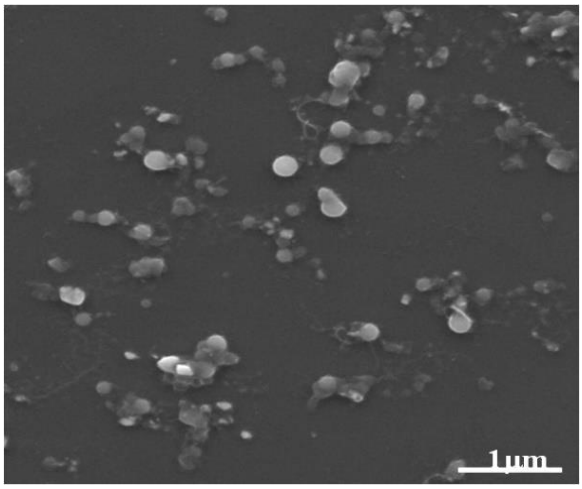

Figure 5: SEM images shows the particles obtained were round, spherical and discrete in shape.

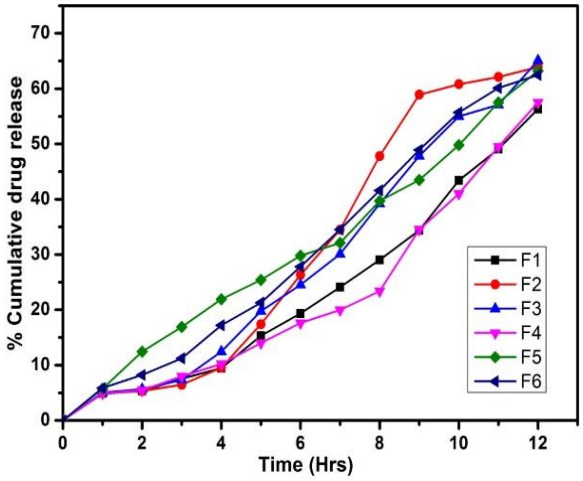

Figure 6: represent the *in-vitro* drug release for all formulations F1-F6.

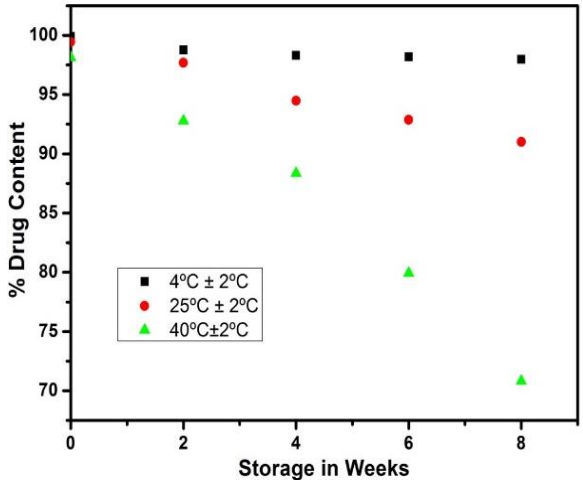

Figure 7: Shows the storage of formulation F3 at different temperatures.

was calculated for the prepared formulations F1-F6 as shown in the figure 6.

Stability Studies

The drug content was studied to ensure the stability of Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles[15]. The best formulation F3 was stored at (4ºC), (25ºC) and at 40ºC for the time of 8 weeks. It remained stable throughout the precise extent of time.

RESULTS AND DISCUSSIONS

The Fe_3O_4 nanoparticles were also successfully prepared by chemical precipitation method. The formulations Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles were effectively prepared. The linearity plot of the drug capecitabine was plotted against concentration (µg/ml) versus absorbance (nm) as shown in the figure 1. The curve obeys Beer's Lambert laws. As per FTIR spectrum analysis Figure 2 (a) showed the characteristics peak of pure drug capecitabine at the wave number of 3528 cm^{-1} shows O-H stretching where the free hydroxyl group is present, the characteristic peak at 3248 cm^{-1} indicates the N-H stretching vibrations, at 2968cm^{-1} shows the C-H stretching, at wave number 2855 cm^{-1}shows the presence of aldehyde group (C-H=O), at 1770 and 1629 cm^{-1} indicates the presence of C=O carbonyl group of stretching vibrations, at 1507cm^{-1} shows the N=O bending vibrations and further at 1247cm^{-1}shows the C-N bending vibrations. From the Figure 2(b) the characteristics peaks of chitosan at wave number 3441 cm^{-1} for O-H stretching, 2899 cm-1 for C-H Stretching, 1629 cm^{-1} for C-N stretching, 1215 cm^{-1} for O-H bending and 905 $cm^{-1}$for C=O bending vibrations. Figure 2(c) the Fe_3O_4 nanoparticles shows the Characteristic peak at 3417cm^{-1} indicates the N-H stretching vibrations, at 2851cm^{-1} the presence of aldehyde group (C-H=O) with stretching vibrations, at 1757cm^{-1} shows the presence of C=O carbonyl group with stretching vibrations, at 1453cm^{-1} indicates the (N=O) nitro groups with bending vibrations and at 1042cm^{-1} shows the presence of C-N group with bending vibrations. The FTIR spectrum of sodium tripolyphosphate represents in the figure 2(d) at wave number 1074 cm^{-1} for PO_4 group ions. Figure 2(e) the F3 Formulation shows the characteristics peak at wave number 3254 cm^{-1} indicates the OH stretching vibrations, 2115cm^{-1} C=C shows alkenes/ alkynes stretching vibrations, 1636cm^{-1}C-N stretching vibrations and 1076cm^{-1} C=O carbonyl group with stretching vibrations. Overall, the FTIR spectrum of the capecitabine, Fe_3O_4 nanoparticles and F3 formulation

represents no interaction between the molecules. The functional groups present in the drug and polymer also exists in the formulation but without any interactions between them. The Entrapment Efficiency of the prepared formulations were analyzed as shown in the figure 3 and were found to be F1-51.6%, F2-56.95%, F3-60.12%, F4-58.67%, F5-57.49% and F6-59.15% respectively. The particle size distribution of the prepared formulation F3 shows the particle size around 80-530nm as shown in the figure 4. Further, the average particle size of the F3 formulations was found to be 296nm. Scanning Electron Microscope (SEM) images figure 5 confirmed the nanosized of the formulation F3. Capecitabine from chitosan- Fe_3O_4 nanoparticles SEM image reveals discrete spherical structure and particles with size range of 100-500nm. The *in-vitro* drug release studies of different formulations of Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles were performed and the cumulative percentage drug release was observed as shown in the figure 6. The formulations 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 with different ratios showed different drug release of 56.35%, 63.90%, 65.20%, 57.20%, 63.20% and 62.50% respectively and reported for about 12 hours as represents figure 6. An initial rapid release suggests that some drug was localized on the shell of the Nanoparticles. Formulation F3 (1:3) showed better controlled release as compared to other formulations and confirms the best. Stability studies for the formulation F3 represent in the figure 7. In refrigeration condition at 4ºC±2ºC there was no remarkable change in the drug content compared to room temperature condition 25ºC±2ºC and higher temperature 40ºC±2ºC. The formulations kept at higher temperature 40ºC±2ºC gets degraded gradually compared to room and refrigerator temperature conditions. On the whole, the formulation F3 was most stable in refrigeration condition at 4ºC±2ºC.

CONCLUSION

Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles was effectively prepared by using ionic gelation method. Different ratios of drug: polymer was used to develop various formulations of Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles and were evaluated for drug entrapment efficiency of which formulation F3 ratio shows higher drug entrapment efficiency. *In-vitro* release profile shows that Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles was capable of releasing the drug in a sustained manner with 65.20% for 12 hrs. SEM image reveals that the nanoparticles have a discrete spherical and round in shape. From the present research it would be concluded that Fe_3O_4 encapsulated by chitosan loaded capecitabine nanoparticles are successful carriers for the design of targeted drug delivery for the treatment of breast cancer. In future studies, the cell cytotoxicity, *in-vitro* cell lines and *in-vivo* studies were planned.

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