SEEJPH Volume XXVI, S1, 2025, ISSN: 2197-5248; Posted:05-01-2025

Developing and Testing iron oxide Encapsulated by Chitosan-Loaded the medication Capecitabine Nanomaterials for Breast carcinoma Management in Vitro

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KEYWORDS

Fe3O4 nanoparticles, chitosan, capecitabine, ionic gelation, and biodegradability.

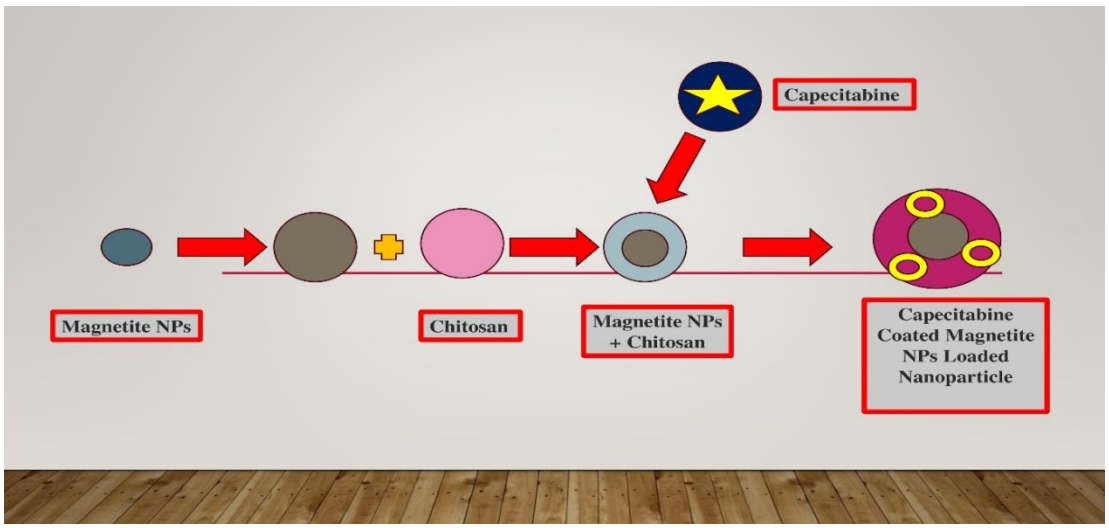
ABSTRACT:

Biodegradable, biocompatible, and bio adhesive nanoparticulate carriers offer a wide range of practical uses in the delivery of medicinal compounds. The goal of the current work was to create and optimize Chitosan-Fe3O4 nanoparticles loaded with capecitabine and investigate the in-vitro assessment using the sigma dialysis method. Ionic gelation was used to create batches of capecitabine-loaded chitosan-Fe3O4 nanoparticles with varyingratios of medication to polymer (1:1, 1:2, 1:3, 1:4, 1:5, 1:6). The drug content of nanoparticles rises with an increase in polymer concentration. When the drug to polymer ratio was F3 (1:3), the entrapment efficiency was 60.12%. The in-vitro release for 12 hours was 65.20%. Capecitabine is derived fromFe3O4-chitosan nanoparticles Particles with sizes ranging from 100 to 500 nm and a distinct spherical structure are visible in the SEM image. The functional groups found in formulas that don't alter properties are represented by FTIR investigations. During the eight weeks of storage, samples housed in a refrigerator demonstrated greater stability than samples held in other circumstances.

Introduction: In 2012, breast cancer was the most common and incurable disease among women worldwide, affecting over 1.7 million women. The disease of the breast is the second most common type of cancer overall.frequent type, making it a fatal disease. In the majority of nations, including the United States, the United Kingdom, New Zealand, the United Kingdom, Ireland, Iceland, Italy, Switzerland, Argentina, and Australia a few others, breast cancer is a prevalent disease among women. Breast cancer also impacted women in developing nations like Sri Lanka and India1. Perhaps by 2030, there will be more than 2 million cases of breast cancer worldwide, including the percentages of developing nations2. The incidence of breast cancer varies by about three to four times throughout India. The highest rates are seen in the northeastern region of India, particularly in big cities like Mumbai and New Delhi.3. The Indian news reported that 17% of people worldwide have breast cancer. The primary causes of these discrepancies are lifestyle factors (alcohol intake, tobacco use, and smoking behaviors), anthropometric factors (adiposity), and inadequate instruction on reproduction (number of children and age at first child). In the year, the government launched nationwide programs such as the National Cancer Prevention Programmes (an NCCP). to raise awareness among the populace. 1975. Cardiovascular disease, cancer, and stroke are among the disease states addressed in the national programs. NPCDCS introduced the 12th five plans between 2012 and 2017.[4]. The NPCDCS raises awareness of the nation's top ranking in relation to breast cancer death rates.[5]. Applications include tissue magnetic resonance imaging (MRI), rapid blood

detoxification, cancer treatment for hyperthermia, tailored drug delivery systems, and diagnostics. techniques depend heavily on the magnetic nanoparticles that are encased by polymers.6. Because of the magnetic field, the qualities are authorized by their higher surface area and behavior. Magnetite iron oxide, or Fe3O4, is the core material that is encased in a matrix or shell of natural and synthetic polymers7. The second most prevalent type of polymer is a natural one, such chitosan. In particular, it includes chitin, which partly deacetylates in alkaline environments. This polymer is naturally non-toxic, biodegradable, and biocompatible. Interesting functional groups including hydroxyl and amino groups may be found in chitosan. Applications in biomedicine are its primary usage. The chitosan polymer is frequently utilized in drug delivery systems and is readily synthesized using a variety of techniques, including ionic gelation, emulsion droplet coalescence, coacervation, spray drying, and emulsion cross linking. Reverse micelle preparation and sieving8. The creation contains Fe3O4 surrounded by Capecitabine nanoparticles filled with chitosan, as well as formulation optimization and characterization, are the topics of the current work. The process of chemical precipitation is used to create the Fe3O4 nanoparticles. By employing theagent that cross-links Chitosan and any surfactant that stabilizes the mixtures are enclosed over the iron oxide to produce ionic gelation using TPP. The preparation process is straightforward, non-toxic, and repeatable. These formulations contain iron oxide, which is nothing more than super paramagnetic substance that converges on tumour cells when external magnetic field. Following the application of a magnetic field, the tumor cells are eliminated when the iron oxides (Fe3O4 nanoparticles) are heated to 37 to 40 degrees Celsius. The following is a diagrammatic illustration of Fe3O4 enclosed in Capecitabine nanoparticles loaded with chitosan:

Materials & Methods: Capecitabine it was bought as a gift sample from Bionic Enterprises in Lucknow. In Prayagraj, Uttar Pradesh, India, the Geetraj Corporation sold the chitosan and TPP. We buy ammonium hydroxide (25%) and ferrous and ferric chlorides from Bionic Enterprises in Lucknow. Analytical-grade substances were also employed in these investigations. The standard curve calibration curve was prepared by precisely weighing 10 mg of the pure medication capecitabine and dissolving it in a 10-mlThe phosphate buffer 6.8 percent solution measuring flask. After that, 1 ml of the solution is removed, and buffer solutions are added to form 10 ml. Furthermore, 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 µg/ml were the serial dilutions created in the ratios and use 10 millilitres of buffered solutions to make it up. At 240 nm, the samples were lastly put through a UV spectrophotometric examination. The narrative was created based on Beer's Lambert rules.



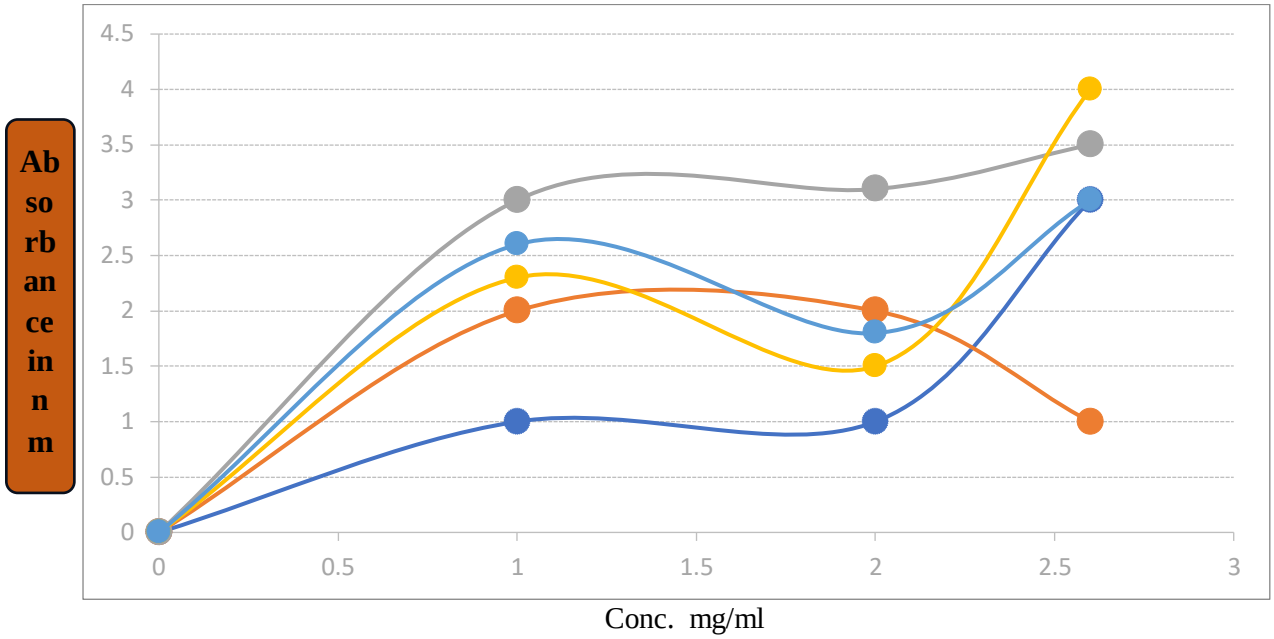


Figure 1: shown the regression coefficient values and the linearity plot for the pure medication capecitabine.

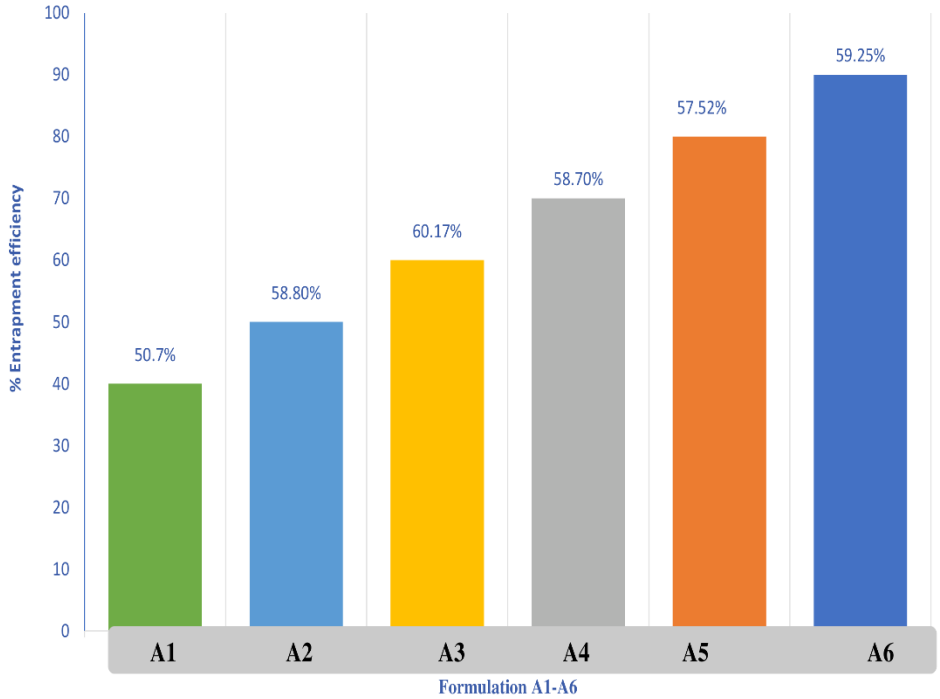


Figure 2: Show the percentage of entrapment efficiency for each of the A1–A6 formulations.

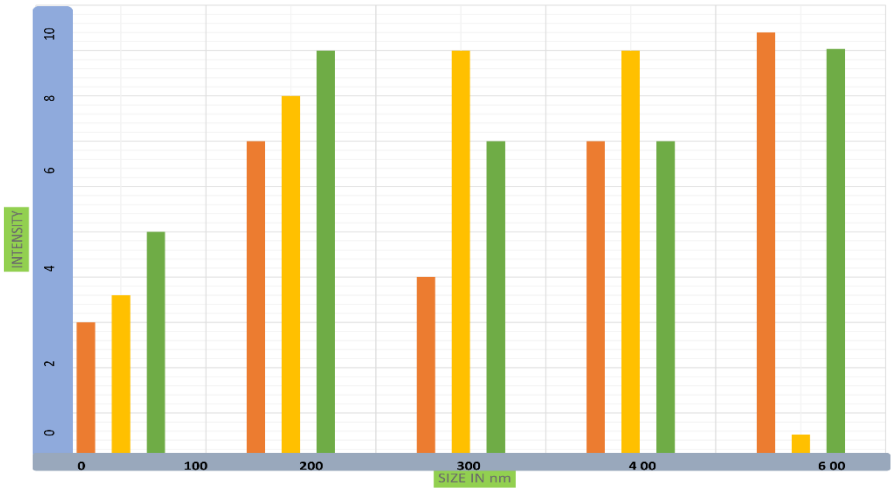


Figure 3:shown the formulations A3's size of particle dispersion.

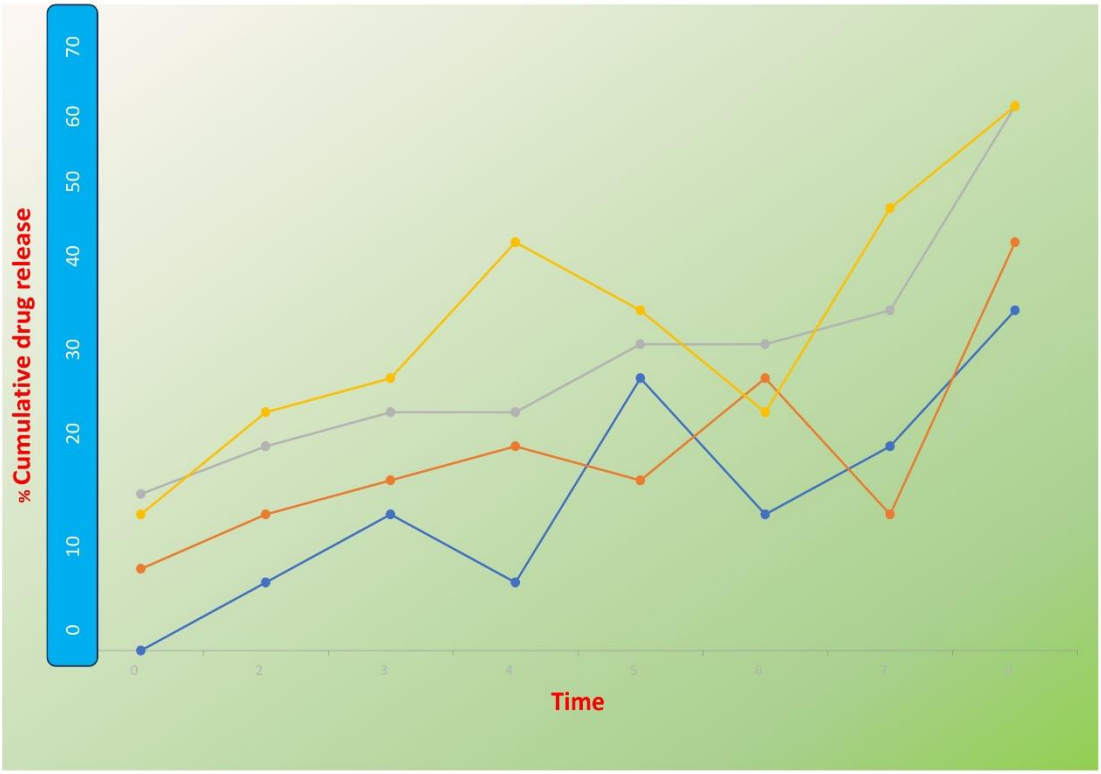


Figure 4: symbolize the drug distribution in vitro for each of formulations A1–A6.

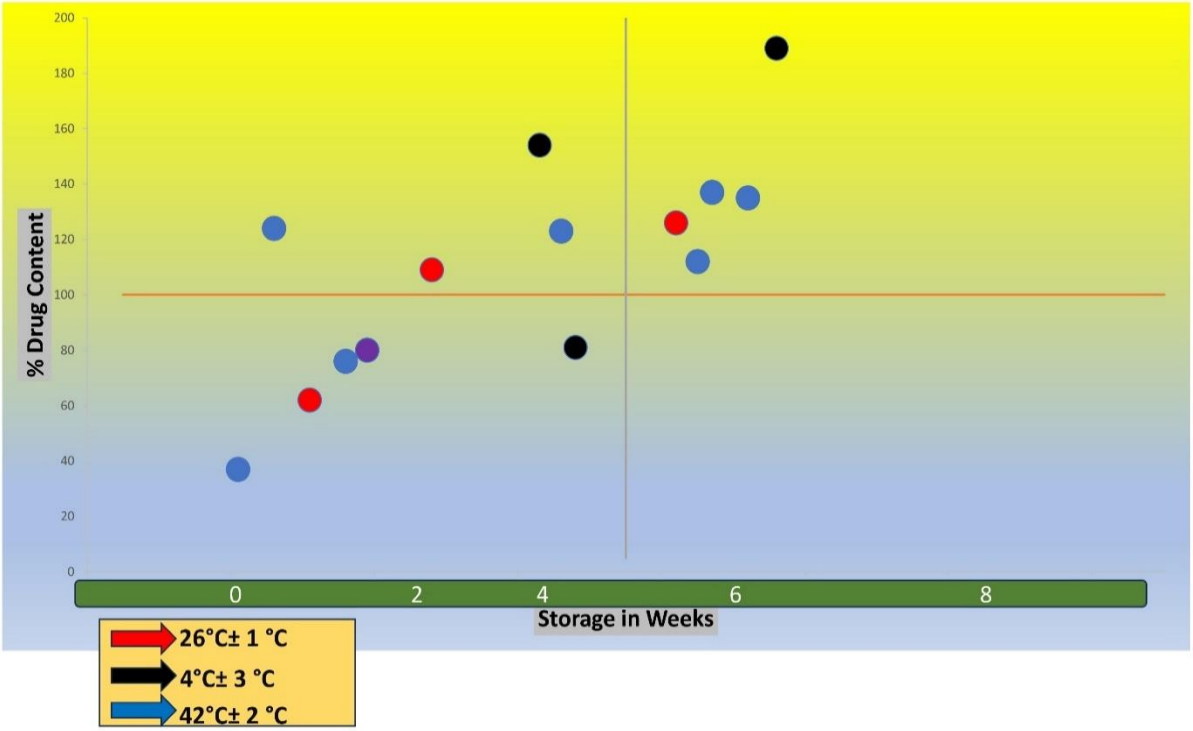


Figure 5:demonstrates how formulation A4 is stored at various temperatures

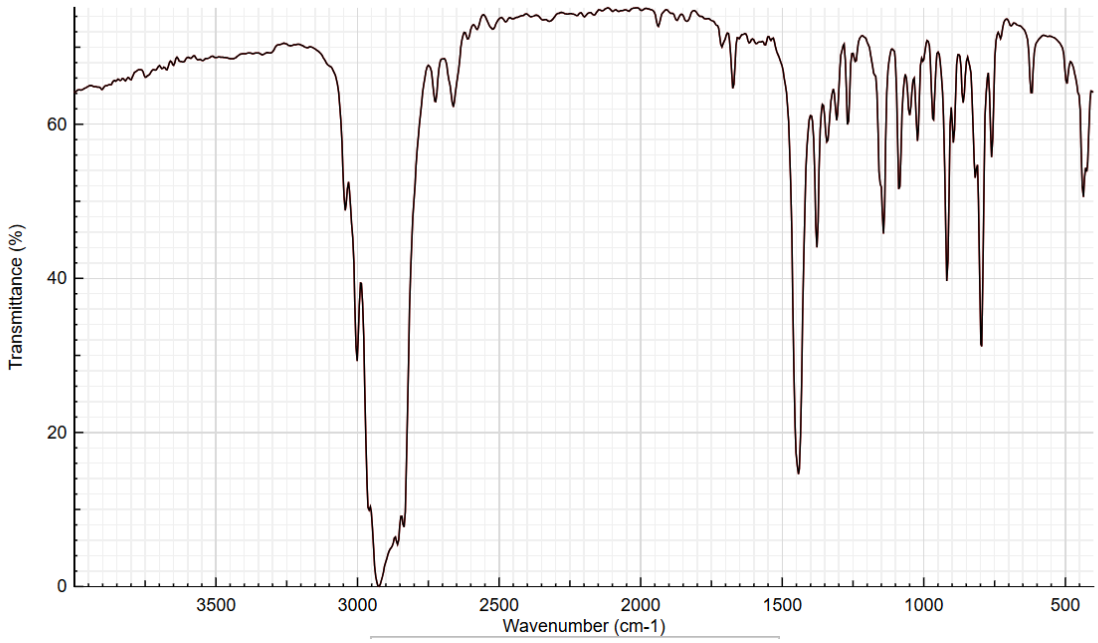
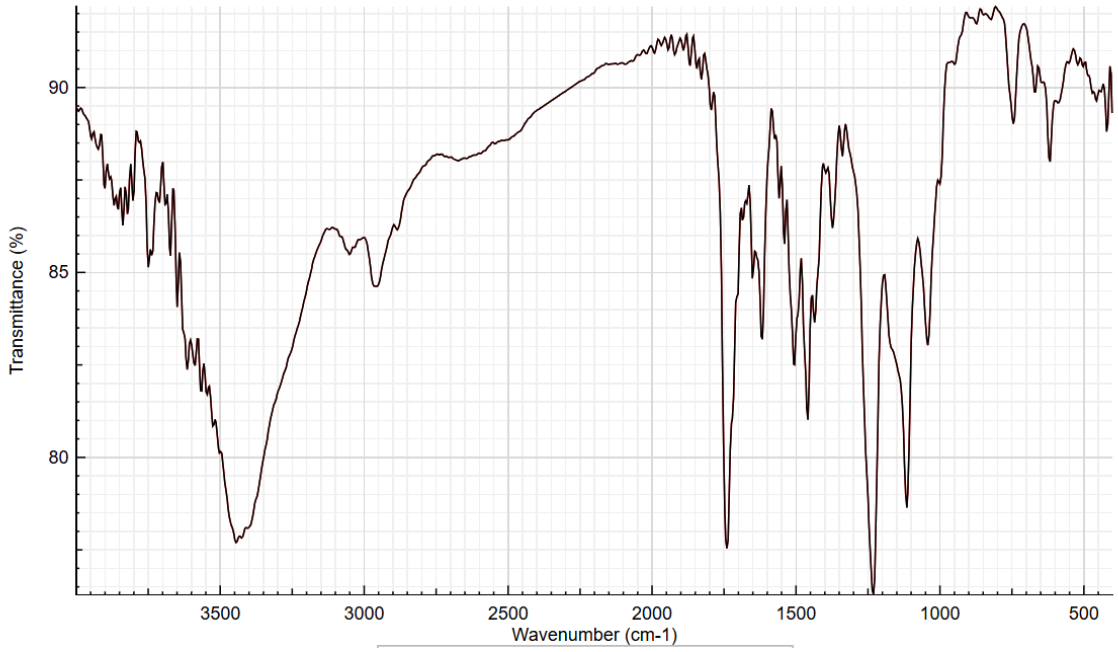
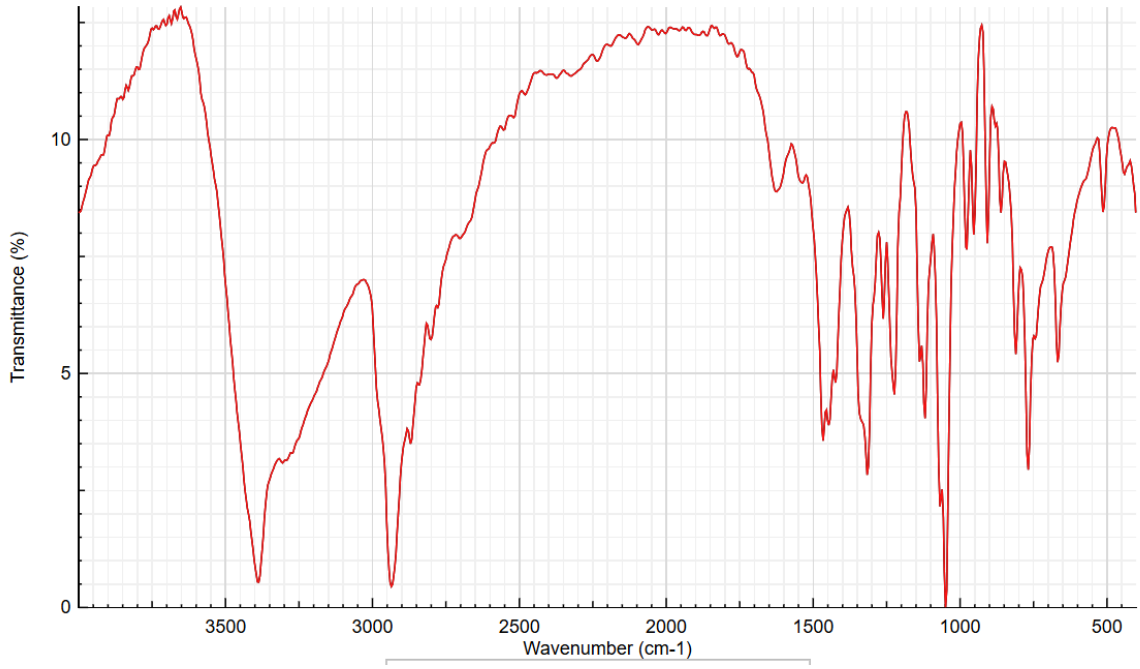


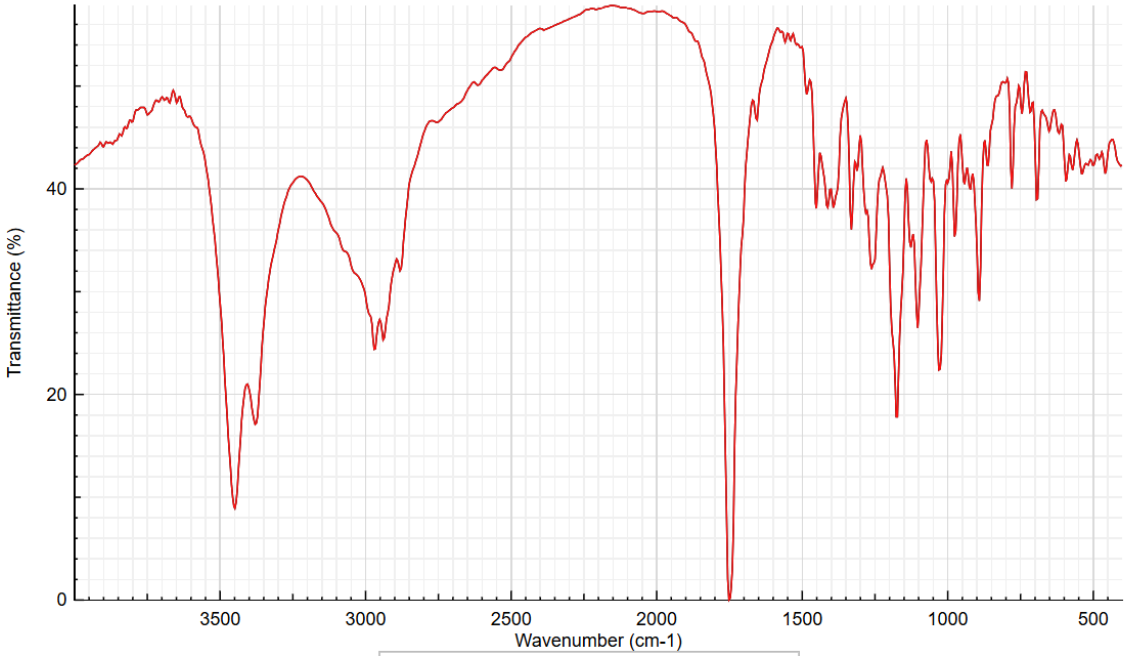
Figure 6: illustrates the storage of formulation A4 at various degrees.



(b)Figure 7: display the chitosan's FTIR spectrum.



(c)Figure 8: display the Fe3O4 nanomaterials' FTIR spectra.



(d) Figure 9: display the sodium tripolyphosphate FTIR spectrum.

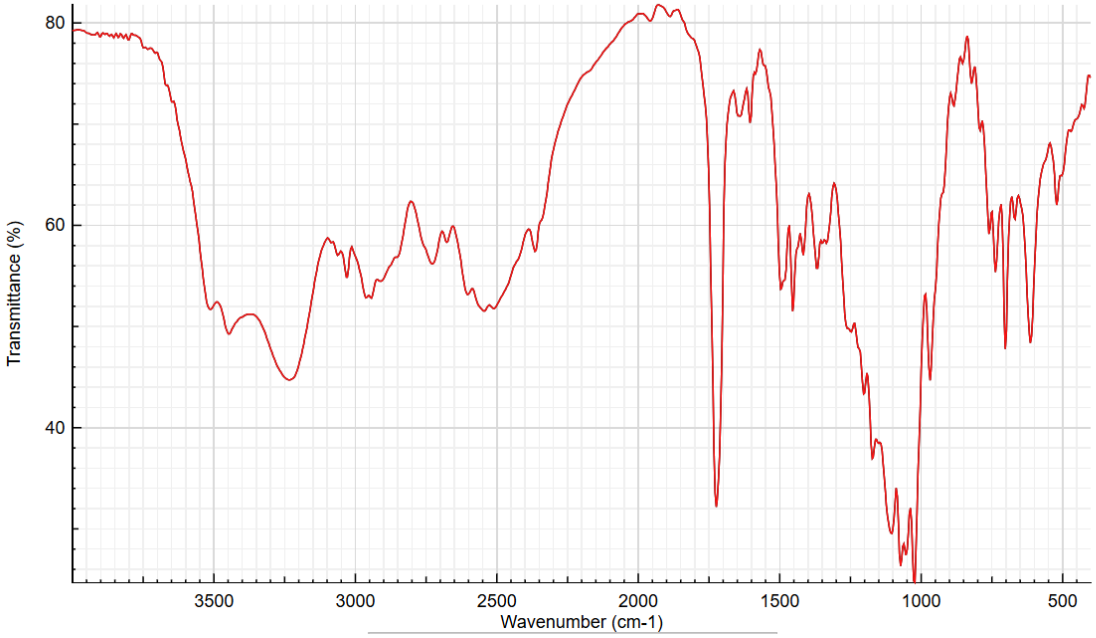


Figure 10: (e) display the Formulation A3 FTIR spectrum.

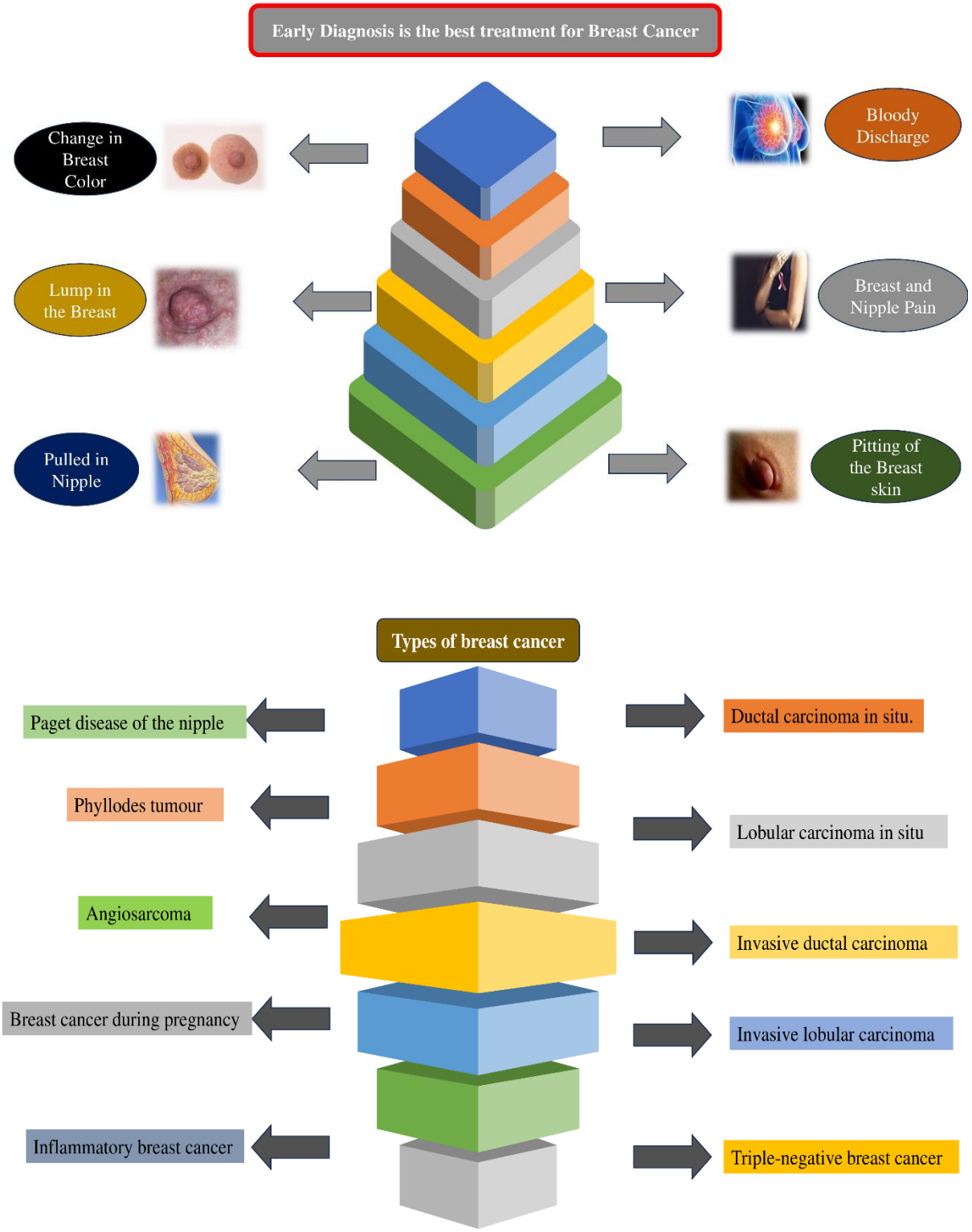
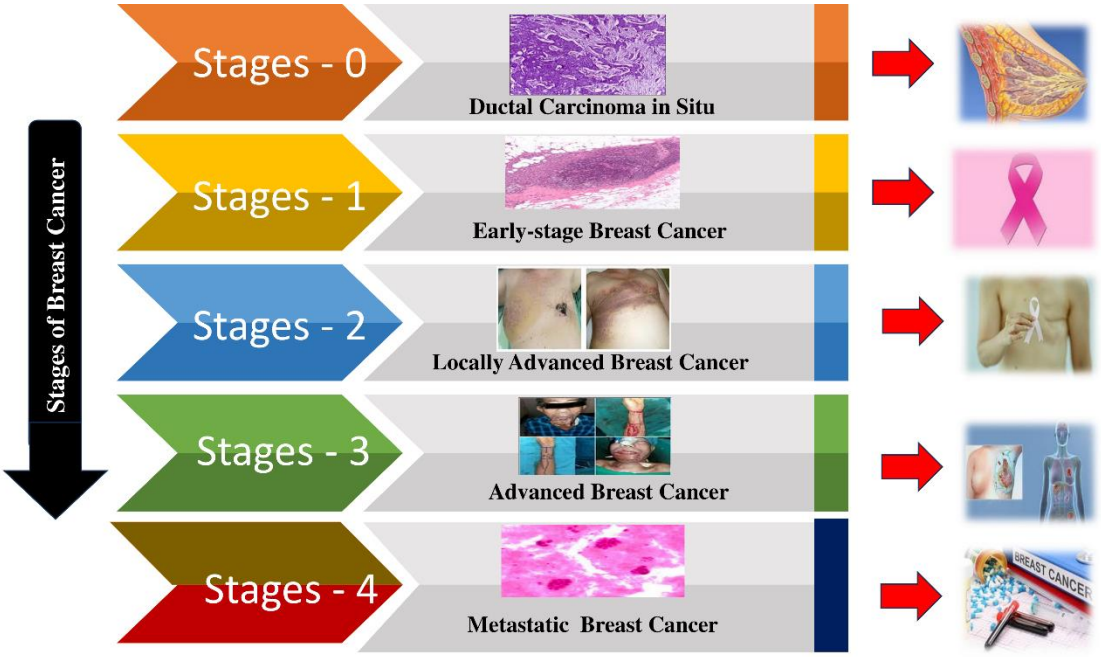


Figure 10: Type of breast cancer



The formulation improvement at different drug polymer ratios is displayed in Table 1.

Code forcompounding	Quantity of medication (mg)	The cellulose content (mg)	TPP quantity (%)	Small particles of Fe3O4 concentration (mg)	Numbers of drugs to polymers
A1	10	10	0.25	5	1:1
A2	10	20	0.25	5	1:2
A3	10	30	0.25	5	1:3
A4	10	40	0.25	5	1:4
A5	10	50	0.25	5	1:5
A6	10	60	0.25	5	1:6

Fe3O4 Small particles Manufacture

The most widely used technique for creating Fe3O4 nanoparticles was dissolving 100 milliliters of deionized water containing 6.1 grams of FeCl3.6H2O and 4.2 gram of FeSO4.7 hydrogen. It was also placed in a flask with three necks, and 10 milliliters of a solution containing 25% ammonium hydroxide was added. while being constantly stirred at 50 to 55 °C in a N2 environment. Use ammonium hydroxide to get the pH between 11.0 and 12.0. After raising the temperature to 65°C, the mixture was kept at that temperature for almost two hours. After adding a diluted HCl solution to bring the pH down to 6-7, the temperature was progressively increased to 80°C. about 1⁄2 hour, stirring constantly. At last, the pH gradually dropped to 3.4–4.09. Additionally, the black precipitate was repeatedly cleaned with DI water, filtered, allowed to air dry, and the nanoparticles were collected. The nanoparticles of Fe3O4 were produced and kept for use in further formulation preparation.

Making Fe3O4 Encapsulated in Capecitabine Nanoparticles are Loaded with Chitosan

The ionic gelation process was used to create chitosan nanoparticles. Positively charged amino groups are found in chitosan, a polymer, while negatively charged molecules of sodium TPP and TPP join to generate gelation. TPP is multivalent, nontoxic, and can gel when oppositely charged particles or molecules contact ionotically. Using the aforementioned techniques, batches of capecitabine-loaded

chitosan-Fe3O4 nanoparticles with varying ratios of medication to polymer (1:1, 1:2, 1:3, 1:4, 1:5, 1:6) were made. The charge density of TPP and chitosan may be regulated based on the formulation's pH.10. The weighted chitosan was dissolved and agitated with 0.1% Glacial acetic acid.Dissolve TPP separately in DI water. Additionally, Tween 20 was added to the top of solutions after being dissolved in D.I. water. Moreover, the produced formulations were gradually supplemented with Fe3O4 nanoparticles (5 mg/5 ml). Then, under continuous magnetic stirring, the TPP and Tween20 solutions were added drop by drop to the top of the solutions. For three to five hours, the stirring operation is continued. Capecitabine is given to the chitosan nanoparticles drop by drop after being dissolved in phosphate buffer (pH-6.7).

Infrared Spectroscopy using Fourier Transforms (FTIR)

The spectrum derived from Fe3O4, sodium tripolyphosphate, chitosan, and capecitabine FTIR analysis Shielded with Chitosan Capecitabine loaded to verify the drug-polymer interaction, sodium tripolyphosphate (TPP) and capecitabine (pure drug) were triturated using 1:3 proportions of KBr, and particle forms were documented. as well as the physiochemical characteristics of chitosan. For IR analysis, each sample was compressed using a pellet11,12. These samples' spectra, which covered a range of around 450 to 4000 cm 1 on a Perkin Elmer FT-IR Spectrometer in the USA, were examined and interpreted13. Calculation of Entrapment Effectiveness The produced formulations were collected and centrifuged at 12,000 rpm for approximately 30 minutes. The sample was extracted from the solution of supernatant because it possesses an unentrapped medication. Following centrifugation and re-dispersion of the leftover solution trapped in the buffer, the process was repeated two or three times before being further examined using UV spectrophotometry at 240 nm. The concentration of capecitabine is measured and computed14. The following formulas were used to determine the entrapment efficiency (EE%):

Calculating the Size of the Particle

The Malvern analyzer was used to determine the formulation's particle size distribution. After diluting the sample drop with 1 milliliter of DI water, it was put into the sample cuvette. Particle size analysis of the produced formulation F3 revealed that its average particle size is around 308 nm.

Finding the Surface Topology

Fe3O4 was the formulation's particle size. Scanning Electron Microscopy (SEM) was used to observe and take pictures of the Chitosan-loaded Capecitabine Nanoparticles (QUANTA 3D FE-SEM). On a double-sided carbon tape, the produced nanoparticles are dropped. At room temperature, the solution gradually evaporated. SEM mode was used to obtain the image at the specified magnification.Chitosan nanoparticles made from Fe3O4 coated with capecitabine have a spherical shape and size, measuring between 100 and 500 nm, according to the SEM picture.

Investigations of In-Vitro Emission

The dialysis bag membrane technique was used to conduct the release tests of the produced formulations. The receptor compartment's phosphate buffer and chitosan-loaded capecitabine nanoparticles encapsulate the Fe3O4 in the donor compartment. For 12 hours, the experiment is conducted at room temperature, with 2/3 ml of sample being removed every hour. Each withdrawal is followed by a fresh medium. As seen in figure [4], The total percentage of release for the generated formulations A1–A6 was calculated after the sample was examined spectrophotometrically at 240 nm. Studies on Stability To ensure the long-term stability of Fe3O4 encased in capecitabine nanoparticles loaded with chitosan, the medication's composition was investigated15. After eight weeks, the optimal formulation A3 was kept at 4℃, 25℃, and 40℃.It stayed constant for the exact amount of time.

Outcomes And Conversations

The chemical precipitation approach was similarly successful in producing the Fe3O4 nanoparticles. The formulations of Fe3O4 encapsulated by capecitabine nanoparticles loaded with chitosan were successfully made. Figure 1 displays the Capecitabine's linearity curve plots wavelength (nm) versus dosage (µg/ml). Beer's Lambert rules are followed by the curve. In accordance with FTIR spectrum analysis When the distinctive peak of the pure medication capecitabine exhibits O-H stretching when the free hydroxyl group is present, N-H stretching vibrations when wave number 3248 cm-1, C-H stretching when wave number 2968 cm-1, and O-H stretching when wave number 2855 cm-1.

Vibrations at 1770 and 1629 cm-1, N=O bending vibrations at 1507 cm-1, and C-N bending vibrations at 1247 cm-1 all point to the presence of the aldehyde group group (C-H=O). The characteristic peaks are displayed in Figure (7) 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 vibrationat the number of waves 3441 cm-1 of chitosan. The N-H vibrations that stretch are shown by the distinctive peak of the nanoparticles made from Fe3O4 in Figure (8) at 3417 cm-1, followed by the group consisting of aldehyde (C-H=O) at 2851 cm-1, the C=O carbonyl atom at 1757 cm-1, and the (N=O) at fourteenth century cm-1. Nitro groups are found with bending vibrations, and the existence of a C-N group with bending vibrations is shown at 1042 cm-1. Figure (9) shows the sodium tripolyphosphate FTIR spectra for PO4 group ions at wave number 1074 cm-1. According to Figure (10)'s A3 Formulation, the OH stretching vibrations are indicated by the distinctive peak at wave number 3254 cm-1, followed by the alkenes/alkynes stretching vibrations at 2115 cm-1 C=C, the C-N stretching vibrations at 1636 cm-1, and the carbonyl group stretching vibrations at 1076 cm-1 C=O. Overall, there is no evidence of molecular interaction in the FTIR spectra of capecitabine, Fe3O4 nanoparticles, and A3 formulation. The drug's and polymer's functional groups also existing inside the formulation, but they don't interact with one another. As seen in figure 3, the produced formulations' entrapment efficiencies were determined to be A1-50.7%, A2-58.80%, A3-60.17%, A4 58.70%, A5-57.52%, and A6-59.25%, in that order. Figure 3 illustrates the developed formulation A3's particle size distribution, which ranges from 80 to 530 nm. Additionally, it was discovered that the A3 formulations had an average particle size of 296 nm. Figure 11's Scanning Electron Microscope (SEM) pictures verified that formulation A3 was nanoscale. Capecitabine derived from Fe3O4 nanoparticles and chitosan The SEM picture shows distinct spherical structures and particles ranging in size from 100–500 nm. Figure 4 displays the cumulative percentage of drug release from in-vitro drug release experiments of variousFe3O4 preparations enclosed in capecitabine for nanoparticle loaded with chitosan. Figure 6 illustrates how different ratios of the formulations 1:1, 1:2, 1:3, 1:4, 1:5, and 1:6 showed different drug outputs of 56.35%, 63.90%, 65.20%, 57.20%, 63.20%, and 62.50%, correspondingly, over a period of about 12 hours. An initial quick release indicates that a medicine was present on the nanoparticles' shell. Compared to other formulations, Formulation A3 (1:3) demonstrated a better regulated release. formulas and provides the best confirmation. Figure 5 shows the stability studies for formulation A3. The drug content did not significantly alter when refrigerated at 4ºC±3ºC as opposed to room temperature at 26ºC±1ºC and at higher temperatures of 42ºC±2ºC. In comparison to room and refrigerator temperature circumstances, the formulations stored at the higher temperature of 42ºC±2ºC gradually deteriorate. Overall, the formulation A3 was best stable at 4ºC±3ºC when refrigerated.

FINAL RESULTS

Using the ionic gelation process, Capecitabine, which nanoparticles loaded with chitosan were able to effectively encapsulate Fe3O4. The effectiveness of drug entrapment in many Fe3O4 formulations encapsulated by capecitabine as nanocrystals loaded with mucin was assessed using varying drug:polymer ratios; formulation A3 ratio demonstrates the highest drug entrapment efficiency. iron oxide enclosed in capecitabine in nanoparticle loaded with chitosan was able to release the medication continuously with 65.20% for 12 hours, according to the in-vitro release profile. The nanoparticles' distinct spherical and round form is revealed by the SEM picture. The current study's findings indicate that Fe3O4 enclosed by Capecitabine, which nanoparticles loaded with chitosan are effective delivery systems for the development of tailored medication delivery in order to treat breast cancer. In vitro cell lines, in vivo studies, and cellular toxicity were envisagedfor future research.

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