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Development and evaluation of capecitabine loaded human serum albumin nanoparticles for breast cancer

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Abstract

Capecitabine is an orally-administered chemotherapeutic agent used in the treatment of metastatic breast and colorectal cancers. Albumin nanoparticles indicated high drug loading capacity in a composite with biodegradability and biocompatibility. The aim of the present study is to develop capecitabine loaded albumin nanoparticles and their evaluation. Capecitabine loaded albumin nanoparticles were prepared by desolvation method. The prepared nanoparticles were characterised for mean particle size, zeta potential, and drug loading capacity. The process yields and drug loading ranged between 66%w/w to 84.6%w/w and 5.06%w/w to 15.36%w/w respectively. The mean particle size of nanoparticle size of various batches F1 to F4 were found to be 151.18nm, 134.9nm, 178.8nm, and 151.3nm respectively. The zeta potential of the batch F2 was found to be -21.1mV. The drug release was ranged from 32.75% to 51.2% for 24h depending on drug polymer ratio. The *in vitro* release studies showed a biphasic release pattern with an initial burst effect followed by sustained release. Release kinetic model revealed that the mechanism of drug release from human serum albumin nanoparticles followed Fickian model.

Keywords: Capecitabine, Human Serum Albumin, desolvation method.

Introduction

Capecitabine is a potentially effective anticancer drug which can be administered orally. It is a prodrug of fluorouracil (1). The fluorinated analogue of uracil was first synthesized in 1957. Fluorouracil is used as a chemotherapeutic agent against various types of solid tumours those of the head and neck, prostate, breast, liver, pancreas, and genitourinary and gastrointestinal tracts (2). Capecitabine itself is not an active drug. It is converted to activated 5-flurouracil through a series of enzymatic steps. Selective conversion of its final metabolite 50-deoxy- 5-fluorouridine to 5-Flurouracil takes place within the human cancer cells and in healthy liver tissue also. The adverse effects associated with capecitabine include cardiotoxicity, bone-marrow depression, nausea and vomiting, stomatitis, diarrhoea, dermatitis, etc… Both the inclusion of the drug into replicating RNA and depletion of thymidine causes cytotoxicity (3). In order to reduce its toxicity and to enhance its bioavailability Capecitabine can be formulated to various formulations like extended-release tablets, controlled release tablets, microspheres, nanoparticles etc. (4) Capecitabine can be formulated as a controlled release dosage form which may provide greater *in vitro* and *in vivo* antitumor activity, and thus reduces its toxic side effects. The controlled release can be achieved using various biocompatible polymers (5).

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The adverse effects associated with capecitabine include cardiotoxicity, bone-marrow depression, nausea and vomiting, stomatitis, diarrhoea, dermatitis, etc. Both the inclusion of the drug into replicating RNA and depletion of thymidine causes cytotoxicity (6). In order to reduce its toxicity and to enhance its bioavailability Capecitabine can be formulated to various formulations like extended-release tablets, controlled release tablets, microspheres, nanoparticles etc. (7) Capecitabine can be formulated as a controlled release dosage form which may provide greater *in vitro* and *in vivo* antitumor activity, and thus reduces its toxic side effects. The controlled release can be achieved using various biocompatible polymers (8).

Capecitabine oral administration simplifies care and hence it is increasingly used for off-label indication including monotherapy in the advanced or metastatic cancers, combination therapy in conjunction with other drugs in the advanced or metastatic cancers, and with radiation therapy for the neoadjuvant treatment of rectal cancer (9). Capecitabine offers the patients more freedom from hospital visits as it can be taken by own and have less inconvenience.(10) Its toxicity profile is manageable, but it requires a strong recognition and prompt intervention by the clinicians (11).

Nanoparticles are defined as particulate dispersions or solid particles with a size in the range of 10-1000nm (12). The drug dissolved, entrapped, encapsulated or attached to nanoparticle matrix. Nanoparticles have been shown to increase bioavailability, decrease side effects of highly toxic drugs, and prolong drug release (13). The drug accumulation in cells is particle size dependent with an increasing effect for smaller particle size and highest efficiency for the nanoparticles of around 100 nm. Appropriate surface modification of NPs enhances their localisation and specific accumulation in cancer cells (14).

Materials and Methods

Materials

Capecitabine was a kind gift from Shilpa Medicare Ltd. Raichur (India); Human serum albumin and Glutaraldehyde were purchased from SD Fine Chemical Ltd, Mumbai, India; Ethanol was supplied by Shri Maruti enterprises Pvt. Ltd. Mumbai; and all other chemicals were used of analytical grade.

Method

Preparation of Capecitabine Loaded Albumin Nanoparticles

The desolvation method was used for the preparation of capecitabine loaded human serum albumin nanoparticles (15). 10mM NaCl solution was taken and accurately weighed quantity of human serum albumin and the drug capecitabine was dissolved in this solution. Using 1M NaOH solution pH was adjusted to 7 to 8. Ethanol was added at a rate of 1ml/min dropwise to the drug polymer solution under magnetic stirring at the speed of 500 rpm until the turbidity appears. After 30 min 25µl of 4% v/v of glutaraldehyde was added as a cross linking agent to this turbid solution and stirred continuously for 8h at room temperature. The nanosuspensions were purified by centrifugation at 10,000 rpm for 10min. The settled down nanoparticle mass was subjected to freeze drying. The dried nanoparticles were then stored in vials at 4°C.

Characterisation of Capecitabine Loaded Albumin Nanoparticles

Drug - Polymer Compatibility Study

Any possible interaction between drug capecitabine and polymer human serum albumin were determined by drug polymer compatibility studies. Required amount of sample was taken in a motor and gently triturated. The physical mixture of drug capecitabine and polymer human serum albumin is taken as sample. A little quantity of this sample mixture was taken and placed in a sample holder and scanned at 4000 cm^{-1} to 400 cm^{-1} in Bruker alpha 2 analyzer. This study was carried out for drug, polymer and physical mixture of drug and polymer and also for nanoparticles. The spectra obtained were

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compared and interpreted for functional group peaks.

The physical state of capecitabine in the nanoparticles was analysed by differential scanning calorimeter (DSC). The thermogram of the drug and nanoparticles gives information regarding the physical properties and melting point of the drug.

Determination of % Process Yield

To determine % process yield of capecitabine loaded albumin nanoparticles the final dried nanoparticles were weighed and the yield was calculated with respect to initial weight of capecitabine and human serum albumin used for preparation of nanoparticles (16).

$$\text{Process Yield (\%)} = \frac{\text{Practical Yield}}{\text{Theoretical Yield}} \times 100$$

Determination of % Drug Loading

The % drug loading was determined by completely extracting the capecitabine from known weight of capecitabine loaded albumin nanoparticles in pH 7.4 phosphate buffer. Weighed quantity of nanoparticles were taken from each batch and the drug was completely extracted using pH 7.4 phosphate buffer. The drug concentration was determined by using a UV spectrophotometer (Shimadzu 160A, Japan) at a wavelength of 295nm against blank and % drug loading was calculated using the formula.

$$\text{\% Drug Loading} = \frac{\text{Actual drug content}}{\text{Weight of NPs taken}} \times 100$$

Determination of Particle Size Distribution and Zeta Potential

The nanoparticles size and polydispersity were determined by Malvern zetasizer (17). It is the routine method to determine the mean hydrodynamic diameter and the particle size distribution of nanoparticles. The dynamic light scattering measurement were done at 25°C with an angle detection of 90° and 173°. The zeta potential of nanoparticles was measured from the mobility of the electrons of nanoparticles using laser doppler electrophoresis via Malvern zetasizer. The measurement was carried out at 25°C in a carbon electrode cell.

Determination of Surface Morphology

The Transmission electron microscope (TEM) is a type of electron microscope that gives images of the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. Transmission electron microscopy was performed to characterize the surface morphology of the formed nanoparticles at 20 kV. Prior to examination, samples were gold-coated to render them electrically conductive and then examined under the microscope.

Determination of In Vitro Drug Release Studies

The *in vitro* release studies of capecitabine loaded albumin nanoparticles were performed using dialysis membrane method (18). The prepared nanoparticles equivalent to 5mg of drug were placed in a dialysis membrane and 5ml of pH 7.4 phosphate buffer. The membrane was knotted at both ends and immersed in 50mL of pH 7.4 phosphate buffer, ensuring that it was thoroughly immersed in the dissolution fluid. It was stirred at 100rpm at 37 ^{o}C temperature and the receptor compartment was closed to prevent dissolution media from evaporating. At predetermined time intervals 5ml of the dissolution medium was withdrawn by replacing fresh dissolution medium. The amount of capecitabine released was determined by measuring the absorbance at 295 nm using UV- Visible spectrophotometer.

Determination of Release Kinetics

Release kinetics studies was carried out to understand the mechanism behind the

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release and to figure out the best fit plot with the kinetic model (19). The data obtained from the *in vitro* release studies were fitted to various kinetic model such as Zero order, First order, Higuchi model and Korsmeyer-Peppas model.

Results and Discussion

The drug capecitabine was subjected to various preformulation studies namely solubility, melting point and drug-polymer compatibility. The solubility of capecitabine in 10 mg/10 ml of solvent was carried out and it reveals that it is soluble in water, phosphate buffer pH7.4 and sparingly soluble in ethanol. The melting point of capecitabine was found to be 113^{o}C and it was within the range, which supports the drug to formulate into nanoparticles.

Drug - Polymer Compatibility Study

Drug-polymer interaction was studied using FT-IR analysis and it showed that there were no changes in the IR spectra of pure drug capecitabine in presence of albumin. Figure 1 which showed that the polymer did not alter the performance characteristics of drug, thus revealing compatibility of the selected drug with the polymer.

Preparation of Nanoparticles

The preparation of capecitabine loaded HSA nanoparticles was performed by desolvation method using ethanol as a desolvating agent. Four different batches were prepared by varying the concentration of the polymer HSA and keeping the drug concentration constant Table 1.

The % process yield for capecitabine loaded HSA nanoparticles was in the range 66%w/w to 84.6%w/w depending on the drug polymer ratio. Due to the presence of many binding sites in their molecules, protein nanoparticles have a high drug loading capacity. Covalent bonding, electrostatic

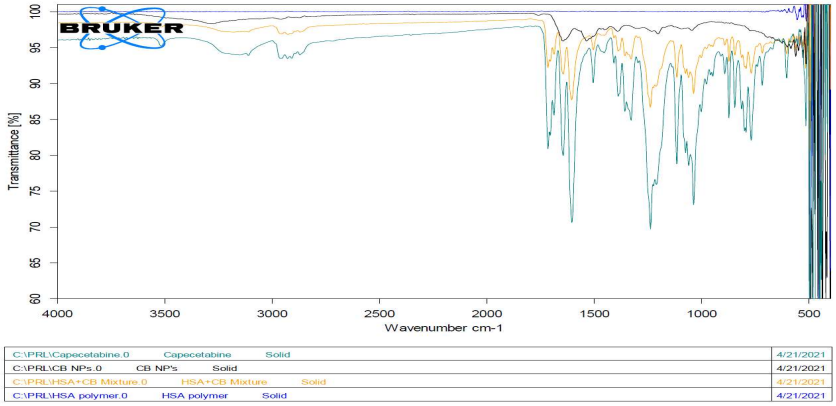

Fig 1. FT-IR Spectrum of drug, polymer, Drug-polymer mixture and nanoparticles

Table 1. Formula for Preparation Capecitabine Loaded Albumin Nanoparticles

S No	Ingredients	F1	F2	F3	F4
1	Capecitabine	10 mg	10 mg	10 mg	10 mg
2	Human Serum Albumin	10 mg	20 mg	30 mg	40 mg
3	10 mM Sodium Chloride solution	5 ml	5 ml	5 ml	5 ml
4	1% Glutaraldehyde	25 ul	25 ul	25 ul	25 ul

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attraction, and hydrophobic interactions are all plausible drug loading methods. All carriers should have a high drug loading capacity in order to reduce the quantity of solid materials required per millilitre of injection (20). Albumin nanoparticles have showed a strong affinity for a wide range of pharmaceuticals (21). The drug loading into the albumin nanoparticles can be achieved in two ways; pre-loading and post-loading. We have employed the later technique, in which the medication is entrapped before the glutaraldehyde solution is used to crosslink the molecules. The prepared HSA nanoparticles showed a drug loading capacity in the range of 5.06%w/w to 15.36%w/w. The table 2 represents the process yield and the percentage drug loading of different formulation of nanoparticles.

Particle Size and Surface Charge

The particle size was determined by dynamic light scattering, using Malvern system. The mean particle size of HSA nanoparticles containing capecitabine was found to be 151.18nm, 134.9nm, 178.8nm, and 151.3nm respectively (Fig. 2). The ability of nanoparticles to alter the biodistribution and pharmacokinetics of drugs, have important in vivo therapeutic applications. In this respect, the size and surface characteristics of nanoparticles are of prime importance.

Table 2. Data of Process Yield and Percentage Drug Loading of F1 to F4

Batch Code	Process Yield (w/w)	Drug Loading (%w/w)
F1	73.1±0.786	5.06±1.234
F2	80.2±0.924	15.36±0.456
F3	84.6±0.916	7.97±1.098
F4	66±0.745	5.82±0.976

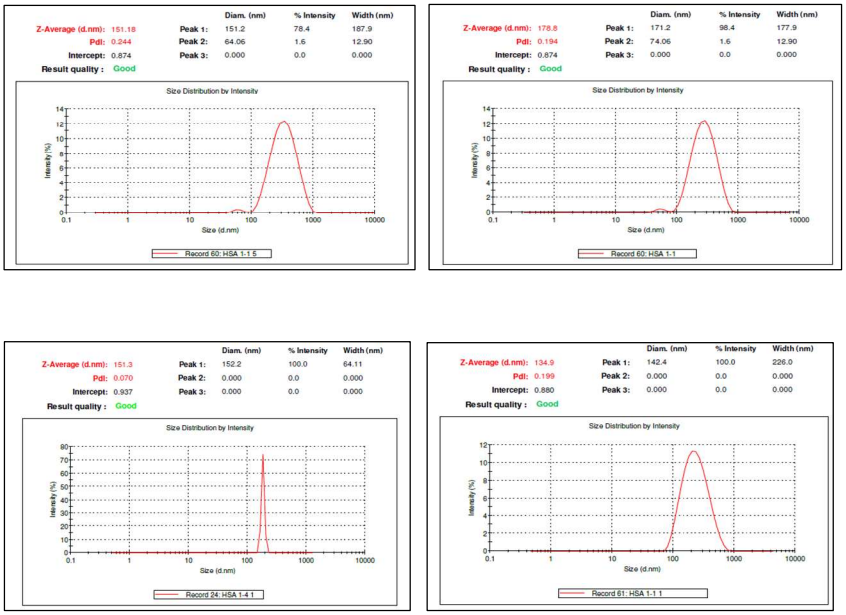

Fig 2. Particle size distribution of capecitabine loaded human serum albumin nanoparticles (F1- F4)

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Nanoparticles of fewer diameters with hydrophilic surface have longer circulation in blood. Such systems prolong the duration of drug activity and also increase the targeting efficiencies to specific sites. Smaller the size of the particle better is the chance for permeation. In addition to the albumin concentration, pH also affects the coagulation of albumin molecules (22). It was also reported that pH also plays a vital role in formation of particle. In this study, the pH of solution mixture containing drug-polymer was adjusted to 8 to enhance the electrostatic repulsion which in turn minimizes the chances of coagulation among albumin molecules which otherwise may lead to the formation of larger particles. Small particles, due to their Brownian motion, can get adequate energy through to keep them agitated preventing the precipitation of nanosuspension and hence enhances the stability. Polydispersity index talks about the homogeneity of the particles. Closer the value of polydispersity to zero better is the homogeneity of the nanoparticles. The zeta potential of the nanoparticles was measured in 0.1 mM NaCl using Malvern Zetasizer. Surface charge plays a vital role in the stability of nanoparticle suspension. Larger the value of zeta potential more is the number of charged particles and in turn more is the repulsion between the particles, leading to the enhancement in the stability of nanoparticles. Ideally the nanoparticles with the zeta potential values above 30 mV and below -30 mV are considered to have better stability. Zeta potential of HSA nanoparticles it was found to be -21.1 mV (Fig. 3).

Surface Morphology

The surface morphology of capecitabine loaded albumin nanoparticles was studied by Transmission electron microscopy and the study revealed that the nanoparticles were spherical and uniform in size (Fig.4).

In Vitro Drug Release Studies

One of the main criteria to examine the performance of a nanosystem before it is utilized on a biological system is to assess its *in vitro* drug release profile using a suitable release media at physiological pH. This study helps in understanding the release patterns of the drug and thus give us an idea of what modification might be required to achieve the objective of the research. The *in vitro* release of drug capecitabine from the various nanoparticle formulations was carried out by using dialysis method in 7.4 pH phosphate buffer for 24 h. The slow sustained release is highly desirable as it minimizes the drug release from the nanoparticles before they

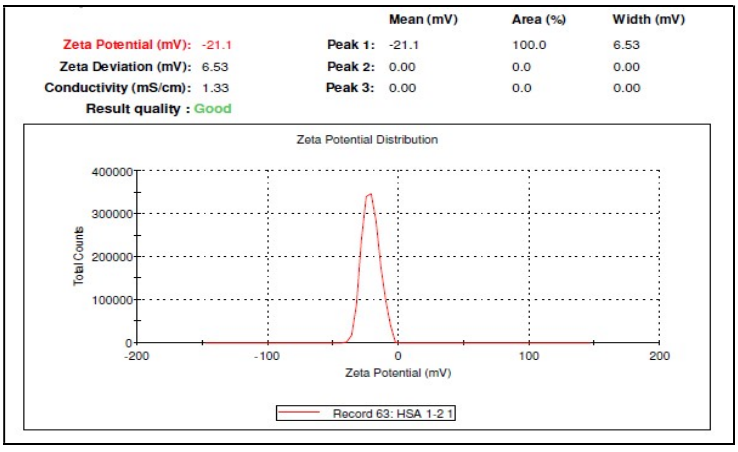

Fig 3. Zeta potential of capecitabine loaded human serum albumin nanoparticles (F2)

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reach the target organ/tissue. The results shown in Fig. 5 indicated that the formulations showed a sustained release of drug over a period of 24 h. The cumulative percentage release of capecitabine from prepared HSA nanoparticles varied from 46.2%, 51.2 %, 47.55 %, 32.75 %. These results demonstrated the sustained release of drug from various formulations

Release Kinetics

The *in vitro* release data obtained from formulations were fitted to various kinetic models to reveal the drug release mechanism from nanoparticles (Table 3 & Fig. 6). Diffusion controlled drug release was observed with higher r2 value in Higuchi model for all the formulations. The diffusion exponent (n) value is used to characterize different release mechanism in

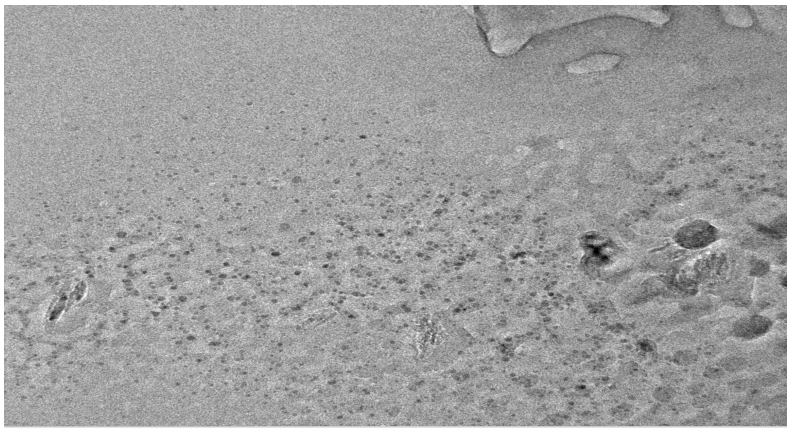

Fig 4. Transmission electron microscopic image of capecitabine loaded human serum albumin nanoparticles (F2)

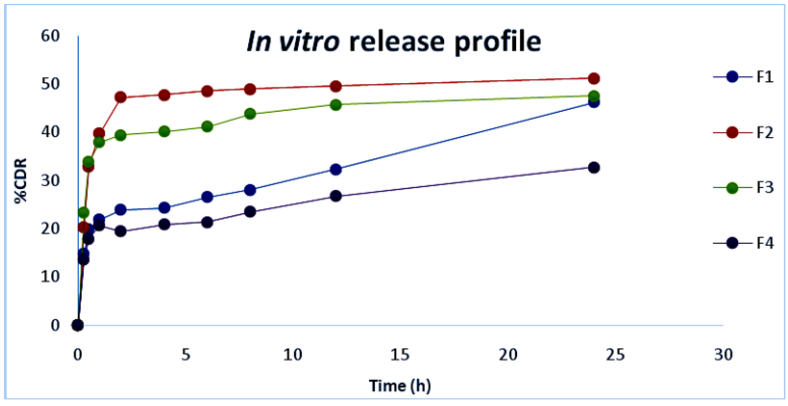

Fig 5. *In vitro* drug release profile of capecitabine loaded human serum albumin nanoparticles

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Fig 6. Release kinetics of capecitabine loaded human serum albumin nanoparticles

Table 3. Data of release kinetics of capecitabine loaded human serum albumin nanoparticles

S No	Formulation Code	Release kinetics							
		Zero order		First order		Higuchi		Korsmeyer-peppas	
		r^2	n	r^2	n	r^2	n	r^2	n
1	F 1	0.4449	2.703	0.4808	-0.0141	0.7021	8.8662	0.8782	0.1589
2	F 2	0.5099	5.7315	0.5745	-0.0368	0.7704	18.395	08288	0.2518
3	F 3	0.3759	4.0654	0.4203	-0.0241	0.6398	13.849	0.7407	0.1518
4	F 4	0.3371	1.9783	0.3551	-0.0099	0.593	6.8509	0.727	0.1171

Korsmeyer-Peppas model. The n value was found to be in the range of 0.1171 and 0.2518 for all the formulations indicating that drug release was controlled by anomalous diffusion, i.e. the mechanism of drug release is controlled simultaneously by diffusion and erosion of the matrix type formulations.

Conclusion

Anticancer drug - capecitabine loaded HSA nanoparticles were developed by desolvation method. This method was able to produce desired size and uniformly shaped nanoparticles. All the formulations showed good process yield and % drug loading. Particle size analysis showed that the formed particles were in nano size and the mean zeta potential studies demonstrated that the nanoparticles possess a negative surface charge which indicates high degrees of stability due to inter particle repulsions. The *in vitro* drug release profile of all formulation was able to sustain the release of drug for 24h. The

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release kinetics data showed that capecitabine release from nanoparticles was diffusion controlled and the n value of Korsmeyer- Peppas model indicates the release mechanism followed Fickian model. Based on this observation, it can be concluded that the formulated nanoparticle drug delivery system containing drug capecitabine is safe and able to sustain the release of drug for 24h. Overall it can be concluded that the prepared nanoparticles can be an efficient platform for the treatment of breast cancer by animal studies furtherly.

Conflict of Interests

The authors that there is no conflict of interests regarding the publication of this paper.

References

1. Ceylan Hepokura Ishak Afşin Kariper, Sema Mısır, Ebrunur Ay, Servet Tunoglu et al., Silver nanoparticle/capecitabine for breast cancer cell treatment. Toxicol *In Vitro*. 2019; 61:104600.

2. Wagstaff AJ, Ibbotson T, Goa KL. Capecitabine A Review of its Pharmacology and Therapeutic Efficacy in the Management of Advanced Breast Cancer. Adis International Limited. Drugs. 2003; 63 (2): 217-236

3. Muthadi Radhika Reddy, Hrushitha Reddy M. Preparation and Development of Capecitabine Microspheres for Colorectal Cancer. J. Pharm. Sci. & Res. 2017; 9(1): 37-43.

4. Narendar Dudhipala, Goverdhan Puchchakayala. Capecitabine lipid nanoparticles for anti-colon cancer activity in 1,2-dimethylhydrazine-induced colon cancer: preparation, cytotoxic, pharmacokinetic, and pathological evaluation, Drug Development and Industrial Pharmacy. 2018; 44 :10,1572-1582.

5. Suzuki M, Hori K, Abe I, Saito S, Sato H. A new approach to cancer chemotherapy: selective enhancement of tumor blood flow with angiotensin II. The Journal of the National Cancer Institute. 1981; 67(3):663-9.

6. J K McGavin, KL Goa. Capecitabine A Review of its Use in the Treatment of Advanced or Metastatic Colorectal Cancer. Adis International Limited. Drugs. 2001; 61 (15): 2309-2326.

7. Storp Bv, Engel A, Boeker A, Ploeger M, Langer K. Albumin nanoparticles with predictable size by desolvation procedure. J Microencapsul. 2012; 29(2):138-46.

8. Ghadiria M, Farahania EV, Atyabib F, Kobarfardc F, Hosseinkhani H. *In-Vitro* Assessment of Magnetic Dextran-Spermine Nanoparticles for Capecitabine Delivery to Cancerous Cells. Iranian Journal of Pharmaceutical Research. 2017; 16 (4): 1320-1334.

9. Kimura K, Yamasaki K, Nakamura H, Haratake M, Taguchi K, Otagiri M. Preparation and in Vitro Analysis of Human Serum Albumin Nanoparticles Loaded with Anthracycline Derivatives. Chem Pharm Bull. 2018; 66(4):382-390.

10. Zhao S, Wang W, Huang Y, Yuhang Fu and Yi Cheng. Paclitaxel loaded human serum albumin nanoparticles stabilized with intermolecular disulfide bonds, Medicinal Chemistry Communication. 2014;5(11):1658-1663.

11. Sebak S, Maryam S, Meenakshi M, Arun M, Satya Prakash K. Human serum albumin nanoparticles as an efficient noscapine drug delivery system for potential use in breast cancer: preparation and in vitro analysis. Int J Nanomedicine. 2010; 5(1):525-32.

12. Jenita JL, Chocalingam V, Wilson B. Albumin nanoparticles coated with polysorbate 80 as a novel drug carrier for the delivery of antiretroviral drug—Efavirenz. International journal of pharmaceutical investigation. 2014; 4(3):142-148.

13. Lu B, Xiong SB, Yang H, Yin XD, Chao RB. Solid lipid nanoparticles of mitoxantrone for local injection against breast cancer and its lymph node metastases. Eur J Pharm Sci. 2006;28(1-2):86-95.

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10.5530/ctbp.2022.3s.61

14. Shenoy DB, Amiji MM. Poly(ethylene oxide)-modified poly(epsilon-caprolactone) nanoparticles for targeted delivery of tamoxifen in breast cancer. Int J Pharm. 2005; 11;293(1-2):261-70.

15. Lopes T, Cuevas JL, Jardon G, Gomez E, Ramirez P, Navaro O. Preparation and characterization of antiepileptic drugs encapsulated in sol gel titania nanoparticles as controlled release system. Med. Chem. 2015; S2: 003.

16. Rohiwal SS, Pawar SH. Synthesis and characterization of bovine serum albumin nanoparticles as a drug delivery vehicle. Int. J. Pharm. Bio. Sci. 2014; 5(4): 51-57.

17. Kumar PV and Jain NK. Suppression of agglomeration of ciprofloxacin loaded human serum albumin nanoparticles. AAPS. Pharm. Sci. Tech. 2007; 8(1): E1-E6.

18. S.R. Mudshinge, A.B. Deore, S. Patil, C.M. Bhalgat. Nanoparticles. Emerging carriers for drug delivery, Saudi Pharm J. 2011; 19: 129–141.

19. M.C. Roco, C.A. Mirkin, M.C. Hersam. Nanotechnology research directions for societal needs in 2020: summary of international study. J. Nanoparticle Res. 2011; 13: 897–919.

20. J. Jeevanandam, A. Barhoum, Y.S. Chan, A. Dufresne, M.K. Danquah. Review on nanoparticles and nanostructured materials: history, sources, toxicity and regulations. Beilstein J. Nanotechnol. 2018; 9 1050–1074.

21. Wilson, T.V. Ambika, R.D.K. Patel, J.L. Jenita, S.R.B. Priyadarshini. Nanoparticles based on albumin: preparation, characterization and the use for 5-flurouracil delivery. Int. J. Biol. Macromol. 2012; 51: 84–88.

22. Rohiwal SS, Pawar SH. Synthesis and characterization of bovine serum albumin nanoparticles as a drug delivery vehicle. Int. J. Pharm. Bio. Sci. 2014; 5(4): 51-57.