WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.045
Volume 3, Issue 9, 815-828. Research Article ISSN 2277– 7105

PREPARATION AND CHARACTERISATION OF CAPECITABINE LOADED PLGA NANOPARTICLES FOR COLORECTAL CANCER

Prasanna S.Sutar*, Vijaya G. Joshi[1], Kishori P. Sutar[2], Kishore G.Bhat[3]

*Assistant Professor, Department of Pharmacognosy, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka, India.

[1]Professor and Head, Department of Pharmaceutics, Government College of Pharmacy, Bangalore, Karnataka, India.

[2]Lecturer, Department of Pharmaceutics, Rani Chennamma College of Pharmacy, Belgaum, Karnataka, India.

[3] Professor and Head, Department of Microbiology, Maratha Mandal's Nathajirao G.Halgekar Institute of Dental Sciences and Research Centre, Belgaum, Karnataka, India.

Article Received on
04 September 2014,

Revised on 29 Sept 2014,
Accepted on 23 Oct 2014

***Correspondence for Author**
Prasanna S. Sutar
Assistant Professor, Department of Pharmacognosy, Maratha Mandal's College of Pharmacy, Belgaum, Karnataka,India.

ABSTRACT

The main goal of the present investigation was to formulate and evaluate Capecitabine loaded PLGA nanoparticles for colon cancer treatment. Nanoparticles of Capecitabine were prepared by Emulsion droplet coalescence method using PLGA 50-50 as the polymer. The nanoparticles obtained were evaluated for morphology, loading efficiency, in vitro release study. C2 formulation showed drug release of 66.14 at 24 hours with smaller particle size of 141.66 ± 10.23 and 61% drug loading. Concentration of PLGA 50-50 and drug release was proportionate to each other. DSC and FTIR studies showed that the drug and polymers were compatible with each other and did not reveal any signs of interactions. SEM image of optimized formulation C2 showed spherical and smooth surfaced particles. Biological evaluation was carried by using HT29 (Human colorectal Adenocarcinoma) cell lines and formulation C2 showed 60% cell lysis. This drug delivery targeted the colon cancer cells and provided a better therapy to the patient by reducing toxicity to the normal cells. Kinetic study indicated that the formulations followed first order kinetics.

KEYWORDS: Nanoparticles, Capecitabine, Colorectal cancer, PLGA.

INTRODUCTION

Among various diseases, cancer has become a big threat to human beings globally. Cancer is the second most common disease in India responsible for maximum mortality with about 0.3 million deaths per year. This is owing to the poor availability of prevention, diagnosis and treatment of the disease. All types of cancers have been reported in Indian population including the cancers of skin, lungs, breast, rectum, stomach, prostate, liver, cervix, esophagus, bladder, blood, mouth etc [1]. Most frequently observed cancers in Indian population are of lungs, breast, colon, rectum, stomach and liver [2, 3, 4]. Colorectal cancer (CRC) is the third most common cancer in men (663,000 cases, 10.0% of the total cancers) and the second in women (570,000 cases, 9.4% of the total cases) worldwide [5]. The term nanotechnology was first defined by Tokyo science university Professor Norio Taniguchi in 1974.Nanoparticles have become one of the most active areas of research in the field of drug delivery due to their ability to deliver drugs to the right place, at appropriate times, and in the right dosage [6]. Nanoparticles can be defined as solid, sub-micron, colloidal particles ranging in size from 10 nm to 1000 nm in diameter, generally but not necessarily made of natural or synthetic polymers, in which drugs can be adsorbed, entrapped, encapsulated or covalently attached and are produced by mechanical or chemical means [7]. Due to their sub-cellular and sub-micron size, nanoparticles can penetrate deep into tissues through fine capillaries, cross the fenestration present in the epithelial lining (e.g., liver), and are generally taken up efficiently by the cells. This allows efficient delivery of therapeutic agents to target sites in the body. Also, by modulating polymer characteristics one can control the release of a therapeutic agent from nanoparticles to achieve desired therapeutic level in target tissue for required duration for optimal therapeutic efficacy [8, 9]. These are mainly used in the oncology of early detection of malignancy and precise location of cancer therapeutics without or with minimal adverse effects to the somatic tissues [10]. Capecitabine is universally used as an antineoplastic agent in gastrointestinal cancer. It gets distributed throughout the body tissue and fluids, crosses the blood brain barrier and appears in the cerebrospinal fluid and disappears from the plasma in about 3 hours [11].

Chronotherapeutics consists of delivery of treatments based on the dynamic changes both in drug pharmacology and in disease-related processes. A first approach to chronotherapeutics is the determination of an optimal dosing time of a drug so that toxicities are reduced and/or efficacy is improved. In certain instances, chronotherapeutics may be achieved by unequal morning and evening dosing schedules of sustained release 12 hours medication systems,

better timing of conventional once a day medication/delivery systems, or application of special tablet and capsule formulations dosed at designated times to proportion medications over the 24 hours in synchrony with rhythm determined requirements. The goal of chronotherapeutics is the management or reversal of existing acute or chronic medical conditions that is delivery of drugs to the body to the right site, at the right time, at optimal dose [12]. A Pulsatile drug delivery system delivers drug in rapid and burst manner within a short time period immediately after a programmable lag phase. A delivery system with a release profile that is characterized by a time period of no release (lag time) followed by a rapid and complete drug release (pulse release) can be called as an ideal pulsatile drug delivery system. In other words, it is required that a drug should not be released at all during the initial phase of dosage form administration. Lag time is defined as the time between when a dosage form is placed into an aqueous environment and the time at which the active ingredient begins to get released from the dosage form. A pulsatile drug delivery system is characterized by a lag time that is an interval of no drug release followed by rapid drug release [13]. A timed, pulsatile delivery system provides one or more rapid release pulses at predetermined lag times or at specific sites resulting in better absorption of the drug, and thereby providing more effective plasma concentration time profile [14].

MATERIALS AND METHODS

Materials

Capecitabine was obtained as a gift sample from Naprod Life Sciences Pvt. Ltd Mumbai. PLGA polymer with ratio 50-50 was purchased from Sigma-Aldrich (Steinheim, Germany); Eudragit S 100 was obtained as a gift sample from Evonik Pvt. Ltd., (Mumbai, India). PVA was procured from Sigma Aldrich (St. Louis, MO, USA) and all organic solvents were of analytical grade.

Method- Modified Emulsification Solvent Evaporation Technique

Capecitabine loaded PLGA polymer nanoparticles were prepared by Modified emulsification solvent evaporation technique. An attempt has been made to optimize the nanoparticle formulation using PLGA 50:50. Different drug polymer ratios were used. Dichloromethane was used as organic solvent and PVA as surfactant in a fixed concentration of (0.5%, w/v). Weighed quantity of Drug (20mg) was dissolved in 40ml water. Varying polymer (PLGA 50:50) concentration was dissolved in 20 ml of dichloromethane. Specific quantity of Eudragit S 100 (50mg) was added to 20ml acetone. Firstly PLGA solution and Eudragit

solution was mixed together with slow stirring and drug solution was added to it slowly and stirred for ten minutes. This homogeneous solution was added slowly to 100 ml of 0.5% (w/v) aqueous surfactant (PVA) solution using high speed emulsifier (ULTRA-TURRAX, Germany) and stirred continuously for 4 hrs at 18,000 rpm to prepare the emulsion. The emulsion formed was stirred on laboratory magnetic stirrer for 2 hrs at 25°C followed by centrifugation (SIGMA, Germany) for 30 minutes at 15,000 x g. After centrifugation the supernatant was discharged and the pallets obtained were washed by using the same volume of distilled water as of the supernatant and again centrifuged at 15,000 x g for 10 minutes. The washing process was repeated three times and the washed nanoparticles were subjected to freeze drying using 5% of sucrose as a cryoprotectant [15] (Christ Alpha 2-4 LD, Freeze Drying Solutions, UK). Composition of nanoparticles is depicted in (Table 1).

Characterisation of Capecitabine Loaded Plga Nanoparticles

FT- IR Studies

FTIR studies between Capecitabine and the excipients were carried out to find interactions among the drug and polymers. Peaks of pure drug and combination with the polymer were obtained and were further checked for compatibility between them. FTIR spectra of pure drug and the drug-polymer mixture were obtained in KBR pellets using IR- affinity-I Shimadzu Auto 00518 spectrometer [16].

Drug Scanning Colorimetry

The thermogram of pure Capecitabine drug and physical mixture with PLGA and pure drug was obtained. 10 mg of sample was sealed in aluminum micropan and introduced in analytical system (DSC-50 Tokyo, Japan).

Solubility Study

Capecitabine is soluble in water, ethanol, methanol, DMSO and DMF. Solubility analysis is important because the drug has to be soluble in the solvents and also in the dissolution medium.

Melting Point Determination

The melting point of, Capecitabine was found to be 110-121°C. This complied with IP and BP standards thus indicating the purity of the drug sample.

Surface Morphology

The particle shape and morphology of the prepared Capecitabine loaded PLGA nanoparticles were determined by SEM analysis. The nanoparticles were viewed using a Jeol-5610 LV (Tokyo, Japan) for morphological examination. Powder samples of dried nanoparticles were mounted on to aluminum stubs using double side adhesive tape and then sputter coated with a thin layer of gold at 10 Torr for vacuum before examination. The specimens were scanned with an electron beam of 1.2 kv acceleration potential and images were corrected in secondary electron mode [17].

Zeta Potential

Zeta potential was measured by using zeta potentiometer (Zeta 3.0+ meter, USA). Sample was filled into the cell; electrodes are inserted, placed under the microscope, and connected them to the Zeta-Meter 3.0+ unit. Electrodes energized and the colloids watched to move across a grid in the microscope eyepiece. Track one by simply pressing a "track" button and holding it down while the colloid traverses the grid. When the "track" button released, the Zeta-Meter 3.0+ instantly calculates and displays the colloid's zeta potential (or electrophoretic mobility) [18].

Determination of Entrapment Efficiency

The amount of drug entrapped in the nanoparticles was calculated from the difference between the total amount of drug added to the nanoparticles and the amount of drug remained in supernatant. The latter was determined by separating drug loaded nanoparticles from aqueous medium by centrifugation at 5000 rpm for 30 minutes. Supernatant was collected and nanoparticles were washed with water, and again subjected for centrifugation. The amount of free drug was determined by UV-spectrophotometer at 304 nm [19].

$$E.E.(\%)=\frac{W_t - W_u}{W_t} \times 100$$

Where, W_t is the weight of initial drug and W_u is the weight of un-encapsulated drug.

In vitro Drug Release

The in-vitro release characterization of Capecitabine from the prepared nanoparticles was evaluated in pH medium gradually changing from (pH 1.2, 6.8 and 7.4). The pH of the dissolution medium was kept 1.2 for 2 hour using 0.1 HCl. Then KH_2PO_4 and $Na_2HPO_4.2H_2O$ were added to the dissolution medium, adjusting the pH to 6.8 with 1M

NaOH, and then the release study was continued for an additional 4 hours. After 4 hours, the pH of the dissolution medium was adjusted to 7.4 with 0.1 N NaOH and maintained up to 24 hours. Capecitabine loaded PLGA; Eudragit S-100 nanoparticles (equivalent to 20 mg of Capecitabine) were suspended in the dissolution medium. The dissolution studies were carried out in 900 ml of pH medium with 37ºC ±0.5ºC at 60 rpm. The simulation of GI transit condition was achieved by altering the pH of dissolution medium at different time interval. At selected time interval 2.0 ml of the samples were withdrawn and replaced with fresh buffer. The sample was filtered & analyzed by UV-spectrophotometer at 304 nm [20].

In vitro kinetic Studies of Capecitabine Nanoparticles

The results of In vitro release profile obtained from all the formulations were plotted to know the mechanism of drug release. The data were treated according to zero order release, first order release, higuchi's and korsemeyer peppa's model equation. The release rate kinetics data for all the other equation can be seen in (Table 3).

In Vitro Cytotoxicity Assay

Preparation of Cell Culture

The cell lines were maintained in 96 wells micro titer plate containing MEM media supplemented with 10% heat inactivated fetal calf serum (FCS), containing 5% of mixture of Gentamycin, Penicillin (100 Units/ml) and Streptomycin (100µg/ml) in presence of 5% CO_2 at 37ºC for 3-4 days. After 3-4 days, the supernatant was removed and MEM media was replaced with Hank's balanced solution supplemented with Gentamycin, Penicillin and Streptomycin. Incubate this preparation overnight. HT-29 - Human colorectal adenocarcinoma cell lines were used for the study.

Cytotoxicity Assay

In-vitro growth inhibition effect of test compound was assessed by calorimetric or spectrophotometric determination of conversion of MTT into "Formazan blue" by living cells. The supernatant was removed from the plate and fresh Hank's balanced salt solution was added and finally treated with different concentration of extract or compound appropriately diluted with DMSO. Control group contains only DMSO. After 24 hours incubation at 37ºC in a humidified atmosphere of 5% CO_2, the medium was replaced with MTT solution (100µl, 1mg per ml in sterile Hank's balanced solution) for further 4 hour incubation. The supernatant was carefully aspirated, the precipitated crystals of "Formazan

blue' were solublised by adding DMSO (200µl) and optical density was measured at wavelength of 570nm. The results were represented as the mean of three readings.

Principle of Assay

This is a colorimetric assay that measures the reduction of yellow 3-(4, 5-dimethythiazol- 2-yl) - 2, 5- diphenyl tetrazolium bromide (MTT) by mitochondrial succinate dehydrogenase. The MTT enters the cells and passes into the mitochondria where it is reduced to an insoluble colored (dark purple) formazan product. The cells are then solubilised with an organic solvent (e.g. DMSO, Isopropanol) and the released, solubilised formazan reagent is measured spectrophotometrically. Since reduction of MTT can only occur in metabolically active cells the level of activity is a measure of the viability of the cells [21].

Formula

$$\text{Surviving cells (\%)} = \frac{\text{Mean OD of test compound}}{\text{Mean OD at control}} \times 100$$

RESULTS AND DISCUSSION

Drug polymer compatibility studies were carried by FTIR spectroscopy and results are depicted in Fig. 1 and Fig. 2 and the frequencies of the functional groups are represented in (Table 2). Results indicated that there was no interaction between the drug and polymers. The thermograms of Capecitabine and physical mixture of drug with formulative ingredients were obtained. The DSC curve of physical mixture of drug with formulative ingredients showed no shift in the endothermic peak of pure drug. Thus it indicated absence of strong interaction between drug and polymer. The DSC curve of Capecitabine showed a single peak at 122.64°C which is the melting point of the pure drug. The DSC Thermograms are depicted in Fig.3 and Fig. 4. SEM image of the optimized formulation is depicted in Fig.5. The nanoparticles appeared spherical; good dispersed and were uniform size. Zeta potential values are in negative and they are depicted in (Table 3). Nanoparticles showed a biphasic release pattern: firstly a lag period of about 3-4 hrs in order to deliver the drug to the colon. Increase in the concentration of PLGA lead to an increase in drug entrapment. This was probably caused by the increasing viscosity of organic phase (polymer solution), which increases the diffusional resistance to drug molecules from organic phase to the aqueous phase, thereby entrapping more drug in the polymer nanoparticles. Better entrapment efficiency was obtained due to non- ionic surfactant (polyvinyl alcohol 0.5%, w/v) which stabilizes the solution and prevents agglomeration of the nanoparticles. Formulation C2

showed 61.36 ± 1.34 drug entrapments as depicted in (Table 3). Increasing polymer concentration also increases particle size due to poorer dispersability of the PLGA solution into the aqueous phase. Moreover, an increase in the viscous forces resist the droplet breakdown by opposing the shear stresses in the organic phase and the final size and size distribution of particles depends on the net shear stress available for droplet breakdown. PVA has a greater tendency to migrate to the surface of PLGA nanoparticles. It stabilizes the nanoparticles surface more effectively and hence a lower particle size is obtained. The mean diameter of all the nanoparticles formulations are determined and are listed in (Table 3). Particle size of nanoparticles was found in the range of 120.67 ± 17.75 to 162.55 ± 12.65 nm which depends on concentration of PLGA. The zeta potential of various formulations ranges between -14.6 ± 3.3 to -28.5 ± 2.3 and is depicted in table 3. The in vitro release profiles of Capecitabine in Fig.6 from the prepared particulate system were studied in gradually pH changing buffers. All the formulations showed a lag phase of 4 hours. The nanoparticles show a biphasic release pattern: one initial burst release followed by a second slow-release phase (extended release). Formulation C2 showed a first burst release of 15.77%, which may be probably due to the drug that was adsorbed or close to the surface of the nanoparticles. Moreover, the smaller particle size of nanoparticles is associated with smaller diffusion path, so drug accessible to the solid/dissolution medium interface can diffuse easily to the medium Thereafter, the release rate decreased that reflects the release of drug entrapped in the polymer and would mainly depend on the drug diffusion and the degradation of the bulk polymer. The initial burst release can be helpful to improve the penetration of drug whereas controlled release delivers the drug over a prolonged period of time. Drug loaded PLGA nanoparticles released 75-80% of the drug in 24 hours. Lag phase is achieved due the enteric polymer Eudragit S 100 which is insoluble in acidic pH and starts dissolving at pH 7 and above. Once the formulation reaches ileocecal region of the GI tract, drug was released from the PLGA matrix by slow diffusion in the sustained manner. The in vitro release data was applied to various kinetic models to predict the drug release kinetic mechanism. The release constant was calculated from the slope (n) of appropriate plots and the regression coefficient (R2). Based on regression values (r), all the formulations followed first order drug release kinetics. From Peppas model, it was found that all the formulations showed anomalous transport kinetics i.e., a combined mechanism of pure diffusion and Case II transport and the results are given in (Table 4). Cytotoxicity study of pure drug showed that at 30 µl dilution of stock solution (10 mg/ml) cell lysis was observed. 60% of the cell lysis took place after

subjecting formulation C2 to the cell lines and the details are depicted in table 5, 6, 7 and in Fig. 7.

Table 1- Composition of Capecitabine loaded PLGA nanoparticles.

Ingredients	C1	C2	C3	C4	C5
Capecitabine	20 mg	20 mg	20 mg	20 mg	20 mg
PLGA	20 mg	40 mg	60 mg	80 mg	100 mg
Eudragit S-100	50 mg	50 mg	50 mg	50 mg	50 mg
PVA solution	0.5%w/v	0.5%w/v	0.5%w/v	0.5%w/v	0.5%w/v
Dichloromethane	20 ml	20 ml	20 ml	20 ml	20 ml
Acetone	20 ml	20 ml	20 ml	20 ml	20 ml

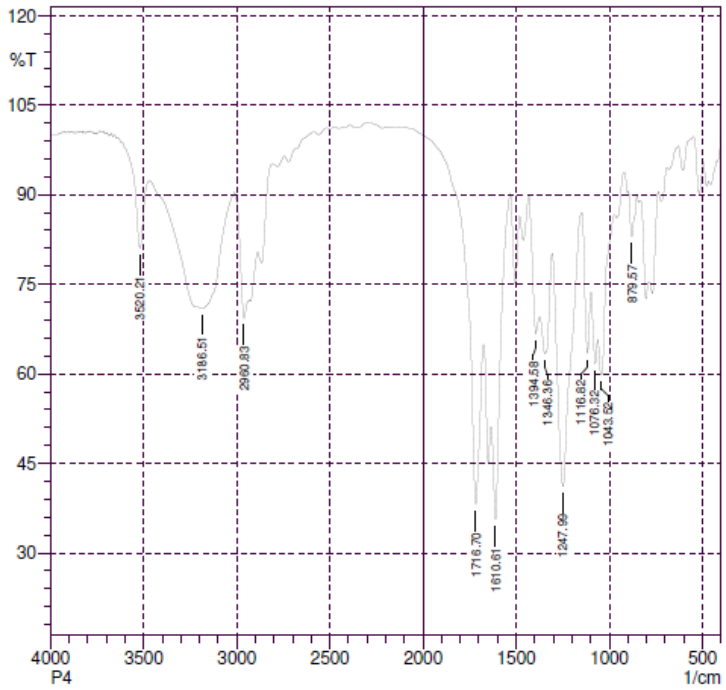


Figure 1: IR spectrum of Capecitabine.

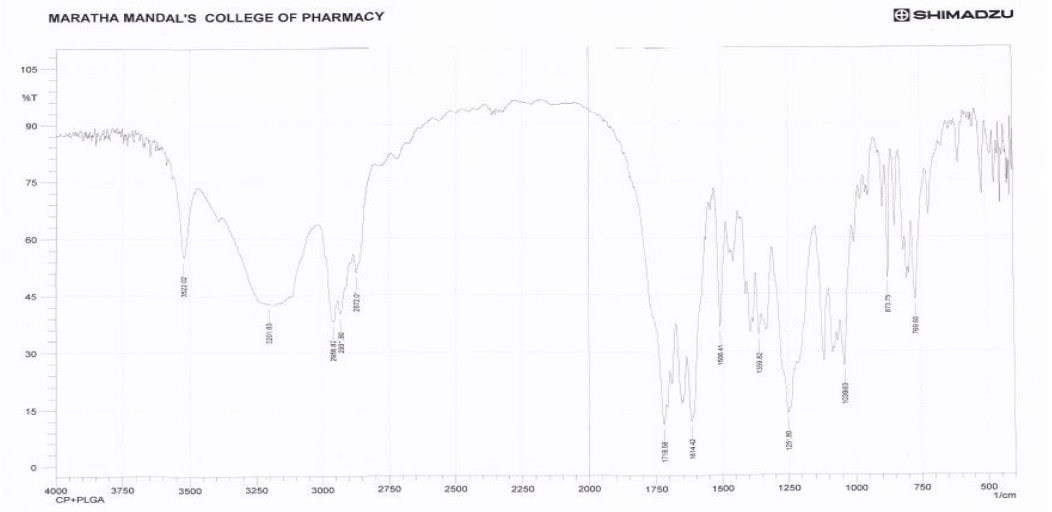


Figure 2: IR Spectrum of Capecitabine and PLGA

Table 2: Frequencies of respective functional groups of Capecitabine.

Sr. No	Frequencies (cm^{-1})	Functional group
1	3520.21 cm^{-1}	NH
2	1716.70 cm^{-1}	C=O
3	1247.99 cm^{-1}	C-F
4	2960.83 cm^{-1}	CH Aliphatic
5	1076.32 cm^{-1}	C-N
6	1610.61 cm^{-1}	C=N
7	1116.82 cm^{-1}	C-O-C
8	3186.51 cm^{-1}	OH

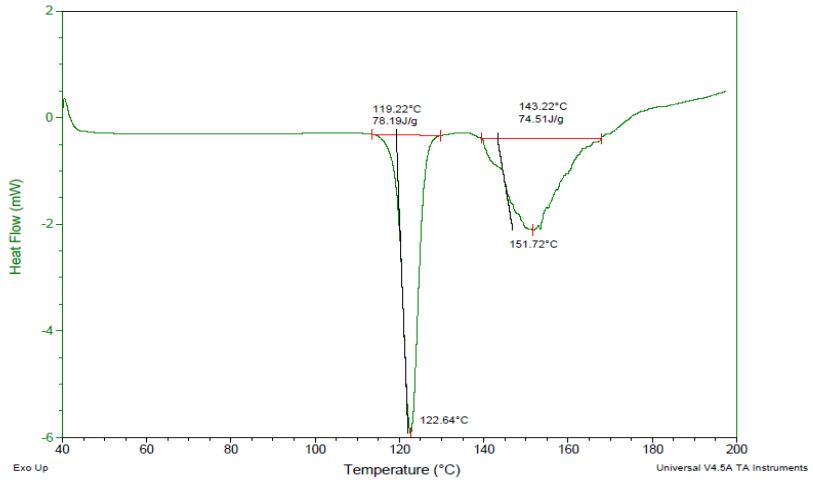


Figure 3: DSC Thermogram of Capecitabine pure drug.

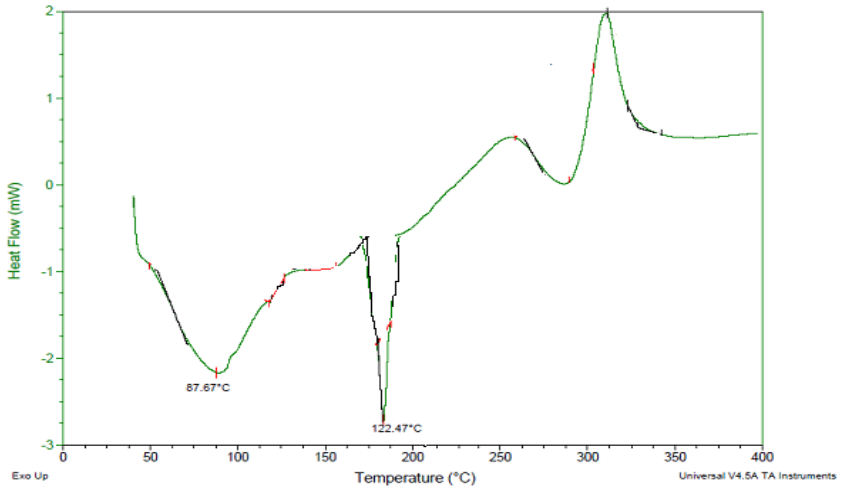


Figure 4: DSC Thermogram of Capecitabine and PLGA 50-50

Table 3: Characterization of Capecitabine Nanoparticles.

Formulation	Drug entrapment (%)	Particle size (nm)	Drug content (%)	Zeta potential (mV)
C1	58.24 ± 1.22	130.34 ± 12.14	15.73 ± 1.24	-18.3 ± 2.2
C2	61.36 ± 1.34	141.66 ± 10.23	22.44 ± 1.56	-19.5 ± 5.4
C3	69.13± 0.25	149.37 ± 11.25	30.67 ± 1.27	-21.2 ± 4.1
C4	72.25± 1.33	157.43± 15.33	36.23 ± 1.33	-23.1 ± 3.6
C5	76.14 ± 0.25	162.55 ± 12.65	45.67 ± 1.29	-25.3 ± 4.7

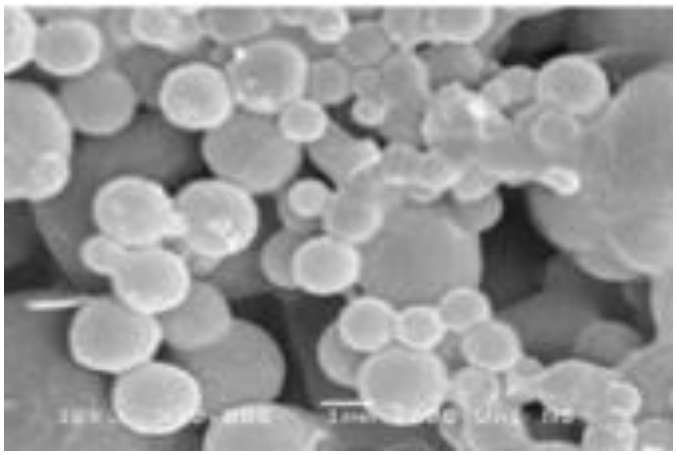


Figure 5: SEM image of Capecitabine loaded PLGA (50-50) nanoparticles

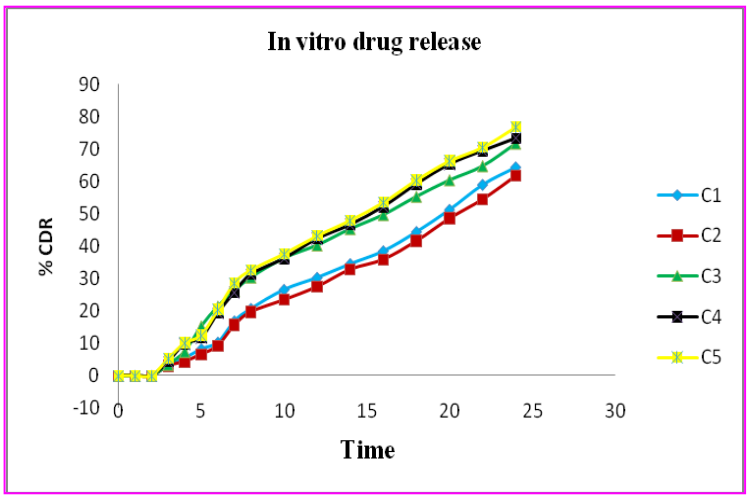


Figure 6: In vitro drug release of Capecitabine nanoparticles

Table 4: Kinetic data for zero and first order analysis

Formulation code	Zero order plot (R^2)	First order plot (R^2)	Higuchi plots (R^2)	Korsmeyer Peppas plot (n)
C1	0.993	0.969	0.919	1.312
C2	0.988	0.965	0.897	1.323
C3	0.983	0.962	0.884	1.344
C4	0.976	0.958	0.879	1.356
C5	0.971	0.953	0.866	1.387

Table-5: Cytotoxicity studies of Capecitabine on HT29 (Human colorectal Adenocarcinoma) Cell lines.

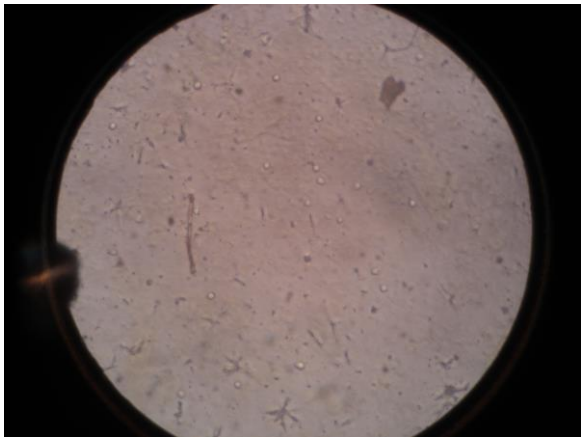
Sr. No	Compound	Concentration (10mg/ml) Control OD- 2.35		
		10µl	20 µl	30 µl
1.	Capecitabine	O.D-1.98 MTT-84.25%	O.D-1.96 MTT-83.40%	O.D-1.92 MTT-81.70%

Table 6: Cytotoxicity Assay

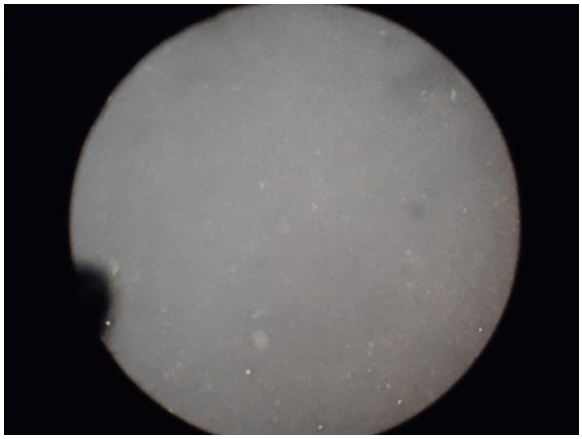
S. No	Compound	10µl (Concentration)	20 µl (Concentration)	30 µl (Concentration)
1.	Capecitabine	No Lysis	No Lysis	No Lysis

Table 7: Results of Capecitabine Nanoparticles on HT29 (Human colorectal Adenocarcinoma) Cell lines.

S. No	Compound	Concentration (µg)	OD at 492 nm	% cell lysis	IC 50
1.	Capecitabine	10	0.520	No lysis	30 µG
2.	Capecitabine	20	0.849	55%	
3.	Capecitabine	30	0.986	60%	
4.	Control	-	0.253	No lysis	-



Control HT-29



C2 Nanoparticle formulation

Figure 7: Showing the cell lysis in Control and C2 Nanoparticle formulation

CONCLUSION

Capecitabine loaded PLGA nanoparticles were prepared efficiently by modified emulsion solvent evaporation method. Since the nanoparticles are formulated with Eudragit S-100 there will be targeted drug delivery to the tumorous colon cells, and hence gives lesser toxicity to the host cells. The side effects related to chemotherapy of Capecitabine such as high dose

dumping can be reduced by site specificity which delivers appropriate amount of drug in correct time and at a proper site.

ACKNOWLEDGEMENT

Authors are greatly thankful to the Chairperson of Maratha Mandal's, Principal of Maratha Mandal's College of Pharmacy, Principal of Maratha Mandal's Dental College, Belgaum for providing the required facilities to carry out this research work.

REFERENCES

1. Imran Ali, Waseem A. Wani and Kishwar Saleem. Cancer Scenario in India with Future Perspectives. Cancer Therapy, 2011; 8: 56-70.
2. Nandakumar A. Consolidated report of the population based cancer registries. National Cancer Registry Programme. Indian Council of Medical Research. New Delhi, India, 1990-96.
3. Rao DN, Ganesh B. Estimate of cancer incidence in India in 1991. Indian J Cancer. 1998; 35: 10-8.
4. Murthy NS, Mathew A. Cancer epidemiology, prevention and control. 2004; 4-25.
5. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN. 2008. Int J Cancer. 2010; 127:2893–917.
6. Kreuter J. Nanoparticles. In Colloidal drug delivery systems. J. K. Ed. Marcel Dekker: New York, 1994; 219-342.
7. Baratt GM. Therapeutic application of colloidal drug carriers. Pharma Sci Technol. 2000; 3:163-71.
8. Dinarvand R, Sepehri N, Manoochehri H Rouhani F Atyabi Polylactide-co-glycolide nanoparticles for controlled delivery of anticancer agents. International Journal of Nanomedicine. 2011; 6: 877–895.
9. Panyam J, Labhasetwar J. Biodegradable nanoparticles for drug and gene delivery to cells and tissues. Advanced drug delivery rev. 2003; 55: 329-47.
10. M Sivabalan, Anto Shering, Phaneendhar Reddy, Vasudevaiah, Anup Jose and G Nigila. Formulation and evaluation of 5-fluorouracil loaded chitosan and eudragit nanoparticles for cancer therapy. Pharmacie globale (ijcp). 2011; 1(07): 1-4.
11. Joshi VG, Sutar PS, Sutar KP, Patil Prakash, Karigar Asif. Formulation and evaluation of pulsed drug delivery of 5 fluorouracil in treating colo rectal cancer. IRJP. 2012; 3(9): 106-09.

12. Belgamwar VS, Gaikwad MV, Patil GB, Surana S. Pulsatile drug delivery system.
13. Asian journal of pharmaceutics, 2008; 141-145.
14. Saigal N, Baboota S, Ahuja A, Ali J. Site Specific Chronotherapeutic Drug Delivery Systems: A Patent Review. Recent Patents on Drug Delivery & Formulation. 2009; 3: 70.
15. Dr. Venkatesh G. New Tool for Timed, Pulsatile Drug Delivery. Pharmaceutical formulation and quality. 2005.
16. Fonseca C, Simoes S, Gaspar R. Paclitaxel loaded PLGA nanoparticles: preparation, physicochemical characterization and in vitro anti-tumoral activity. Journal of Controlled Release. 2002; 83: 273-286.
17. Sutar PS, Sutar KP, Sambrekar AS, Patil VS, Kudalagi CR. Formulation and evaluation of Metformin hydrochloride loaded chitosan microspheres. JPSI. 2012; 1(2): 12-16.
18. Sivabalan M, Reddy P, Vasudevaiah, Jose A, Nigila G. Formulation and evaluation of 5-fluorouracil loaded chitosan and Eudragit nanoparticles for cancer therapy. Pharmacie globale. IJCP. 2011; 1(07):1-4.
19. Pahal S, Daisy A, Murugesan SK, Nanjaian M. Development of Paclitaxel loaded Immunonanoparticles for HER-2 Positive Breast Cancer Cells. International Journal of Recent Advances in Pharmaceutical Research. 2011; 1: 25-39.
20. Naveen K, Abhishek C, Jeffrey W, Sunil P. Nanoparticulate delivery of novel drug combination regimens for the chemoprevention of colon cancer. International journal oncology. 2010; 37: 177–185.
21. Bigucci F, Luppi B, Cerchiara T, Sorrenti M, Bettinetti G, Rodriguez L. Chitosan /pectin polyelectrolyte complexes: Selection of suitable preparative conditions for colon specific delivery of vancomycin. European Journal of Pharmaceutical Sciences. 2008; 35: 435–441.
22. Alan D, Brayan G. Cell and tissue culture for medical research. Cell Quantification- An Overview –MTT Assay. John Wiley and Sons Ltd. England. 2000; 49.