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Polymeric Micelles of Capecitabine: Design, Characterization & Cytotoxic Study

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Abstract : The present study was aimed to prepare polymeric micelles of capecitabine: design, characterization & cytotoxic study. Capecitabine is a prodrug used in the treatment of metastatic colorectal cancer and breast cancer. Polymeric micelles formulations are being formulated to achieve smaller particle size, good stability, increase the solubility, and prolong release of the drug. The micelles have been prepared by using organic solvent/water emulsion technique. Then the prepared formulation has been undergone for drug content, entrapment efficiency, particles size, zeta potential. From this best formulation is converted into tablets by using the excipients. Preformulation study was carried out by using with and without excipients. Then tablets were made using direct compression method by using 9mm concave punches in rotary tablet press. Then the tablet is dip coated using ethyl cellulose of different concentrations. The formulations were evaluated for thickness, hardness, disintegration, dissolution. The tablets shows release formulation upto 12 h.Cytotoxic study has been done in human colon cancer (HT-29) cells by using MTT assay.
Keywords : Capecitabine, polymeric micelles, entrapment efficiency, dip coating, ethyl cellulose.

Introduction & Experimental

Capecitabine a prodrug is used in the treatment of metastatic colorectal cancer and breast cancer. This compound belongs to the class of organic compounds known as glycosylamines. As it is a prodrug once it gets absorbed it is converted into active metabolite 5-Fluorouracil in cancer tissues. It has a very short half life of about 45-60 mins. It shows fluctuations in drug plasma concentration[1,2]. And so a sustained release formulation is another factor for effective management of colorectal cancer. So capecitabine loaded hydrogel microspheres have been formulated to improve its therapeutic efficacy[3].

The main goal for developing this formulation to enhance the therapeutic efficacy, minimum side effects, improve the stability of the product, less plasma fluctuations and so that nano-polymeric micelles have shown good drug delivery of the drug[4].

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The nano-polymeric micelles comprise the drug loading core and a hydrophilic shell. Micelles would come in contact with the aqueous vehicle were hydrophobic drugs is made to encapsulated in to central core[5, 6]. These types has been made to improve the therapeutic efficacy of some of the anticancer drugs such as cisplatin[7], doxorubicin, paclitaxeletc., Cellulose acetate phthalate is a polymer[8] and it does not degrade below pH 7 and it provides coating to the gastric media. Disodium EDTA is a biocompatible polymer and also a complexing agent. The combination of both the polymers would improve the therapeutic efficacy of the drug and it gets directly targeted to the colon[9]. Optimization technique[10]i.e., factorial design has been used to only for the polymers in order to get the best formulation. Various factors such as drug loading capacity, entrapment efficiency, particle size, zeta potentialhave been evaluated.

Then the prepared product is lyophilized and it is converted to tablet by direct compression method[11,12,13]. Then the prepared tablet has been coated with ethyl cellulose[14] using dip coating method and it is made targeted directly to the colon tissue. And then dissolution studies have been carried out upto 12 hrs to achieve the target product profile. The prepared formulation has been undergone for cytotoxic studies by using capecitabine as the standard. And it has been done under human colon cancer (HT 29) cells using MTT assay.

Materials and Methods

Materials

Pure drug capecitabine was obtained gift sample, cellulose acetate phthalate, disodium EDTA was obtained from Sigma Aldrich Chemic USA., Magnesium stearate, talc, and ethyl cellulose was obtained from LobaChemie Ltd.

Methods

Preformulation studies

Solubility

Solubility of the drug was measured by dissolving a suitable quantity in water, organic solvents such as methanol, ethanol, etc., and solubility range was found.

Melting point

Melting point was measured by using capillary method in melting point apparatus.

Determination of lambda max

The lambdamax was measured by UV- spectrophotometer in the range from 200-400nm

Determination of the standard graph

The drug is taken in 100ml phosphate buffer. i.e.1000mcg/ml. Then from the above 1ml is taken and made up to 100ml i.e. 10mcg/ml. From the above serial dilutions were made from 1-10 mcg/ml and it is viewed under UV spectrophotometer at 239nm.

Drug excipient compatibility studies

It is done inorder to find out the compatibilities between the drug substance and the different excipients and it has been done for all the solid dosage forms. The study was performed by means of potassium bromide pellet technique was done in IR spectroscopy in the range of 400-4000 cm^{-1}

Preparation of capecitabine loaded nano-polymeric micelles

It is prepared by using organic solvent/water emulsion technique. Capecitabine is made to dissolve in the organic solvent containing cellulose acetate phthalate. Then this organic solution is made to add dropwise to the water containing disodium EDTA under vigorous stirring so that it is formed into emulsion. Then it is

sonicatedfor 2mins then it is followed by overnight stirring in magnetic stirrer. So the drug gets loaded in the polymeric micelles. The formulation is then subjected to lyophilization to get the freeze dried product (Table 1).

Table 1: Compositions of different formulation of capecitabine

Formulation code	Drug (mg)	Cellulose acetate phthalate (mg)	Disodium EDTA (mg)	Organic solvent (ml)	Aqueous solvent (ml)
F1	50	25	25	10	30
F2	50	50	50	10	30
F3	50	25	50	10	30
F4	50	50	25	10	30

Characterization studies

Determination of the particle size

Particle size determination was done by using Malvern zeta sizer. The measurements were carried out at a 90° scattering angle

Zeta potential

The zeta potential was measured using laser Doppler electrophoretic mobility measurement technique using Malvern instruments at a temperature of 25°C

Lyophilized product

The above prepared best formulation is subjected to lyophilization by using lyedolfreeze drier for 3 days and then the product is compressed into tablets.

Determination of drug content

The drug content of the polymeric micelles was determined by dissolving the formulation in water and the absorbance is measured at 239 nm using UV-spectrophotometer. It is calculated by using the formula,

Amount of drug = Concentration x Dilution factor x Correction factor x Volume of medium

Entrapment efficiency

2 ml of the formulation F2 was taken based on drug content study and centrifuged at 13,000 rpm for 30 mins and the free drug was analyzed by UV-spectrophotometer at 239 nm. Based on this best formulation F2 was taken for further studies.

Pre-compression evaluation of formulation-F2[15]

Angle of repose[16]

Angle of repose was measured by using fixed funnel method. The funnel is made to keep in the height of 2cm and it is made to flow and radius is measured. It is calculated by using the formula:

$\Theta = \tan^{-1}(h/r)$ where, h= height of the pile
r= radius of the pile

Bulk density and tapped density

Bulk density is measured by measuring the volume of weighed quantity of the product and tapped density is measured by continuous tapping of about 100 times and it is calculated.

Carr's index and hausner's ratio

It is calculated by using the formula;

Carr's index=[(tapped density-bulk density)/tapped density]×100
Hausner'sratio= Tapped density/ Bulk density

Preparation of the tablets

Lyophilized product is subjected to tablet preparation by means of direct compression method by using the excipients magnesium stearate and talc. The tablets were punched using 9mm concave punches using rotary tablet press (Table 2).

Table 2: Composition of the formulation F2 tablet

S.No	Ingredients	Amount of the tablet(mg)
1	Formulation containing drug	577
2	Magnesium stearate	11.56
3	Talc	5.78
4	Total weight	594.34

Coating of prepared F2 tablets

The prepared tablet is dip coated using ethyl cellulose 0.5% was used. And it is dipped for 3 times in the interval of 5 sec then it allowed for drying. Evaluations were carried out for these tablets.

Evaluation of formulated F2 tablets

The above designed coated tablets were studied for the physicochemical properties like weight variation, thickness, hardness.

Weight variation

Weight variation is done by taking the tablets and weighing individually as well as collectively on a digital weighing balance. Here not more than two of the individual weights should deviate from the percentage as shown in the table as per Pharmacopoeial limits.

Hardness

Hardness test is done by applying the force on the tablet and hardness of the tablet was measured by using Pfizer hardness tester and the average is calculated and it is presented with the standard deviation.

Thickness

The thickness of the tablet was measured by using vernier caliper apparatus.

Disintegration test

Disintegration was carried out in two medium pH1.2 and 6.8 and the time at which drug dissolves and time is noted.

In-vitro drug release studies of formulation-F2

Dissolution was carried out in USP type-II paddle apparatus at 50 rpm. It is performed using 900 ml of pH1.2acidic buffer for first 2 h then pH7.4 phosphate buffer for next 3 h then it is continued with pH6.8 phosphate buffers. Bath temperature was found to be 37°C. Samples were taken periodically and it is replaced with fresh buffer in order to maintain sink conditions. The samples were filtered through a membrane filter and diluted to a suitable concentration. Then the samples were analyzed using UV spectrophotometer at 239nm. The cumulative percentage drug release was plotted against time to find out the release profile of the drug**.**

Drug release kinetics study of formulation-F2

Mathematical model plays important role in the prediction of mechanism of drug release and also provides more general guidelines for development of other system. To describe the drug releaserate from different drug delivery system a large numbers of model were developed. Some of the important models are,

- Zero order kinetic model
- First order kinetic model
- Higuchi model
- Korsmeyer-peppas model

The model that best fits the release data is selected based on the correlation coefficient value in various data. The data were processed for regression analysis using MS-Excel.The model that gives high R^2 value is considered as the best fit of the release data.

Cytotoxic study of formulation-F2

The inhibitory concentration (IC_{50}) value was evaluated using an MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cancer cells were grown (1×10^4 cells/well) in a 96-well plate for 48 h in to 75% confluence. The medium was replaced with fresh medium containing serially diluted synthesized compounds, and the cells were further incubated for 48 h. The culture medium was removed, and 100µL of the MTT [3-(4,5-dimethylthiozol-2-yl)-3,5-diphenyl tetrazolium bromide] (Hi-Media) solution was added to each well and incubated at 37^0 C for 4 h. After removal of the supernatant, 50 µL of DMSO was added to each of the wells and incubated for 10 min to solubilize the formazan crystals. The optical density was measured at 620 nm in an ELISA multiwell plate reader. The OD value was used to calculate the percentage of viability using the following formula.

% of viability = OD value of experimental sample/OD value of experimental control ×100

Results and discussion

Preformulation studies

Drug was found to be soluble in water, slightly soluble in ethanol.Melting point was found to be 121°C and lambda max was found to be 239 nm. Standard curve was obtained with R^2 value of 0.997 for 1 mcg / ml to 10 mcg / ml concentration of drug at 239 nm in UV spectrophotometer and IR study also confirms that there was no interaction between drug and excipients.

Characterization studies

Zeta potential of the prepared formulations was found to be in the range from -14 to -22.3Mv with PDI of less than 0.4and the Particle size was found to be in the range from 1155 – 2752 nm. The best formulation F2 has been selected with size of 1754 nm.

Drug content

The drug content of all the above formulation was carried out and it was found to be in the range of 5-5.6 mg. From the above formulations, Formulation-2 was 5.6 mg of drug content and taken as the best, because the drug present in it was found to be more when compared to other formulations.

Entrapement efficiency

The formulation F2 was having 66.8 % of entrapement efficiency.

Precompression evaluation of formulations-F2

Flow properties of formulation F2

Precompressionparameters of the formulation was done for angle of repose, bulk density, tapped density, carr's index, hausner's ratio. The angle of repose for the best formulation with excipients has shown good flow property. The bulk density, tapped density was found to be within the range. Carr's index and hausner's ratio indicates the good flow and compressibility of the blends (Table 3).

Table 3: Pre-compression evaluation of formulation-F2

	Bulk density (gm/cm^3)	**Tapped density (gm/cm^3)**	**Carr's index (%)**	**Hausner's ratio (H_R)**	**Angle of repose (Θ)**	
					With out excipients	**With excipients**
Trial 1	0.28	0.32	12.84	1.14		
Trial 2	0.27	0.31	12.1	1.14	59.35° (Poor)	35.53° (Good)
Trial 3	0.25	0.30	16.9	1.16		
Average	0.266±0.015	0.31±0.01	13.946±2.58	1.156±0.028		

Results of post-compression parameters of formulation F2 tablets

The weight variation of the tablets was found to be within the pharmacopoeial limits. The hardness and thickness was given below (Table 4).

Table 4: Post-compression evaluation of formulation-F2 tablets

S.No	**Weight variation (mg)***	**Hardness (kg/cm)#**	**Thickness (cm)#**
1	594.18±5%	5.67±0.025	0.65±0.025

*****Average weight of 8 tablets.
Average of 3 tablets.

Disintegration of formulation F2 tablets

Disintegration was carried out using pH 1.2 and 6.8 medium and it was found that the tablet disintegrates in 6.8 medium after 30mins. The drug did not release in 1.2 medium because of the coating of the tablet.

In-vitro drug release of formulation F2 tablets[17, 18]

From the in-vitro release studies, coated tablets show better release. It has released 55.2% of the drug was released at the end of 12 h (Figure 1).

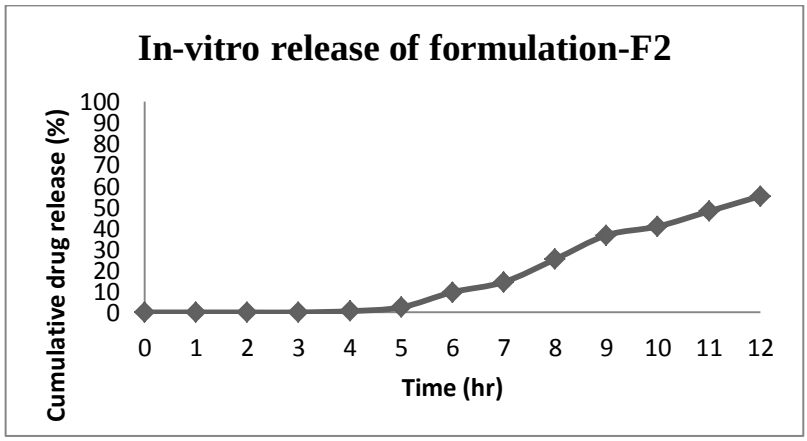

Figure 1: In-vitro drug release of formulation-F2 tablets

Drug-release kinetics of formulation F2 tablets

The release date of capecitabine were fitted to the models such as zero order, first order, higuchi model, korsmeyerpeppas model to find out the release mechanisms. So the data wereanalysed using MS excel and results are shown in the (Table 5) and it has shown that drugs gets released through diffusion mechanism. In this study by comparing the R^2 values, First order gives high R^2value(0.882). So, this model first order release kinetics has been considered as the best release kinetics.

Table 5:Kinetic Drug Release data of formulation-F2 tablets

Model	**R^2**
Zero order	0.878
First order	0.882
Higuchi model	0.671
Korsmeyer-peppas model	0.680 n= 1.322

Results of cytotoxic studies[19]

We examined the effect of formulation F2 on the cell response of the human colon cancer (HT-29) cells by using the MTT assay. In vitro cytotoxicity activity of compounds (10-100µg concentrations) against selected cancer cells. The experimental results demonstrate that the formulation has the ability to inhibited cell proliferation in a dose dependent manner. From the figure 9 (b-d) the IC_{50} values of formulation obtained for against HT-29 cancer cells was 37± 1.5 against 29±1.0 of standard capecitabine. It can perceive from the results that the observed IC_{50} values of formulation effective against colon cancer cells (Figure 2).

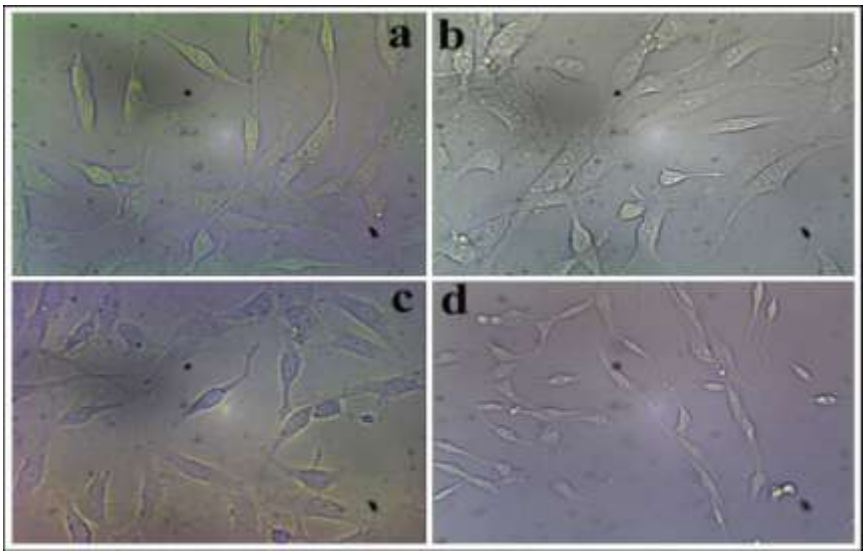

Figure 2: Image of cytotoxic study of formulation F2

This study discuss about preparing the drug by nano-polymeric micelles technique. It has been done in order to achieve the targeted drug release to the colon. The drug release shows the sustained release pattern. The two formulation factors are cellulose acetate phthalate and disodium EDTA has shown good entrapment efficiency. From this best formulation F2 has been taken and it is converted into tablets by direct compression method. Then it is made dipcoating by using ethyl cellulose. And the physical parameters were evaluated. The in-vitro drug release of ethyl cellulose (0.5%) has shown good release pattern of the drug with 55 % drug release at 12 h. The release data were fitted to models such as zero order, first order, higuchi model, korsmeyerpeppas model and from this R^2 values has been calculated. From this first order shows highest R^2 value of 0.882. Cytotoxic study has been undergone in human colon cancer (HT-29) cells by using MTT assay. This study confirmed the formulation F2 with better cytotoxic activity.

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