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NANOPARTICLE FOR COLON CANCER: DESIGN, DEVELOPMENT AND EVALUATION

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Abstract:

The cornerstone of this investigation was to determine was to design, develop and optimize Nanoparticles loaded Capecitabine and to evaluate their antitumor activity in vitro in order to achieve controlled release of the drug, mean while avoiding adverse side effects by the conventional formulation. Eudragit RL100, Eudragit RS100, PVA, PVP and DMSO were used as polymer, emulsifier and permeation enhancer. Capecitabine nanoparticles were prepared by solvent evaporation method. The Optimization was done by using design expert 11.1.2.0. The physicochemical properties of Capecitabine nanoparticles were investigated by particle size analysis, zeta potential measurement, drug entrapment efficiency (EE), stability and in vitro release. The Capecitabine nanoparticle showed stable size distribution at 153.6 nm, ideal drug EE and relative long term physical stability after being stored for 3 months. The drug release of Capecitabine was up to 8 hours which exhibited a controlled release profile which made it a promising carrier for parenteral drug delivery. The drug release from optimized formulation was found to be zero order so it was found to be time independent. It also showed almost linear regression in Higuchi's plot which confirms that diffusion is one of the mechanism for drug release and n value of Korsmeyer- Peppas plot was found to be 0.788 so, it indicates the drug release followed non-Fickian diffusion controlled mechanism.

Key words: Capecitabine, nanoparticles, solvent evaporation, Eudragit RL & RS100.

Introduction: Cancer is one of the most terrible and most dangerous diseases in the world, causing more than 6 million deaths a year.[1] According to the US National Centre for Health statistics in 2014; 65,000 women and

71,830 men were diagnosed with colorectal cancer with more than 25,000 fatalities.[2] Colon cancer is considered another leading cause of death among people in developed and developing countries. Every year, the incidence of colon cancer worldwide is around one million.4 and has high morbidity and mortality compared to other types of cancer. Timely detection, diagnosis and use of the colon delivery system can improve the outcome of therapy.[3]Most of the CRCs occur due to lifestyle and increasing age with only a minority of cases associated with underlying genetic disorders. The CRC basically starts in the lining of the bowel and if not treated, can grow into the muscle layers underneath, and the bowel wall. There are environmental (chemicals, infectious agents, radiation) and genetic (mutations, immune system and hormone dysfunction) factors that can interact in a variety of ways to potentiate carcinogenesis.[4]

NPs have the potential to deliver drugs specifically to cancer cells and reduce their interaction with healthy cells or tissues to improve efficiency and safety. NP targeting mechanisms can be classified as passive or active-targeting. The passive-targeting mechanism is also called the 'Enhanced Permeability and Retention (EPR) effect', where the NPs that facilitate normal blood transfusions can choose the tumor by their leaky surrounding blood vessels and remain in tumor interstitial space. It is believed that the size of the NP 10 ~ 100 nm is best for the EPR effect.[5] Capecitabine is a fluoropyrimidine carbamate with antineoplastic activity for treating metastatic breast cancer and colon cancer. It is an oral systemic proliferation that has a small pharmacological activity until it is converted to fluorouracil by enzymes expressed in higher concentrations in many tumors. Fluorouracil then metabolizes normal and tumor cells to 5-fluoro-2'-deoxyuridine 5'-monophosphate (FdUMP) and 5-fluorouridine triphosphate (FUTP).[6] Eudragit RL and RS have high physico-chemical stability, high band width profiles, mucoadhesiveness, targeted drugs on the gastrointestinal tract, high retention time of the gastrointestinal tract and non-toxicity. Eudragit RL and RS are PH dependent which dissolve at pH 6.0 and / or 7.0.[7]

Materials: Capecitabine, Eudragit RL100 and RS 100, Polyvinyl alcohol(PVA), Polyvinyl Pyrrolidine(PVP), Dimethyl Sulphoxide(DMSO).

Equipment: UV-visible Spectrophotometer, Electronic Analytical balance, FTIR, P^H meter 7007, Magnetic stirrer, Dissolution Apparatus, Homogenizer.

Methods of preparation:

Capecitabine of calculated amount was dissolved in 5ml DMSO. Dissolve Eudragit RL100 and RS 100 in 10ml methanol. Mix both in 2ml centrifuge tube. PVA as stabilizer (2.5ml) in 50ml glass beaker keep in ice bath. Organic solution of drug and polymer was added drop wise to stabilizer solution using syringe drop wise with homogenization at 15,000 rpm for 10 min. Add this drop wise in PVP as co-stabilizer(2.5ml) ice bath kept in ice bath. Homogenize for 10 min. Immediately sonicated using 10s pulse. Subject to magnetic stirring (3hr) for evaporation of organic solvent.

Pre-formulation studies:

Drug excipient interaction studies by Fourier Transform Infrared Spectroscopy

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.

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.

Particle size analysis:

The average particle size of the nanoparticles was determined by using a Zetasizer DTS ver. 5.03 (Malvern Instrument, Worcestershire, England). The samples of nanoparticles dispersions were diluted to ten times their volume with distilled water.

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.

$$\text{E.E (\%)} = \frac{Wt - Wu}{Wt} \times 100\%$$

Where, Wt is the weight of initial drug and Wu is the weight of un-encapsulated drug.

Differential Scanning Calorimetry (DSC) [8]:

Differential scanning Calorimetry (DSC) was employed as a tool to investigate the physico-chemical compatibility between the drug and the number of commonly used excipients which can affect the stability of the drug by chemical and physical interactions thus posing a threat to the stability or bioavailability. DSC is a fast and reliable method to screen any drug excipient interaction as compared to the time consuming method of accelerated stability studies.

Stability study[9]:

The stability study was carried out for Capecitabine loaded Eudragit NPs as per ICH guidelines. Nanoparticles of the optimized formulation were placed in screw capped glass container and stored at various ICH storage condition which are 25°C ± 20°C (60%± 5%RH) and 40° C ± 20°C (75%± 5%RH) for a period of 90 days. The samples were analyzed for physical appearance and for the entrapment efficiency at regular interval of 15 days. Drug release was performed after 90 days.

In vitro Drug Release[10]:

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 nanoparticles 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 5 ml of the samples

were withdrawn and replaced with fresh buffer. The sample was filtered & analyzed by UV-spectrophotometer at 304 nm.

Discussion:

Drug excipients interaction studies by Fourier Transform Infrared Spectroscopy:

The FT-IR spectroscopy study was carried out to check out the compatibility between the drug Capecitabine and excipients Eudragit RL 100 and RS 100 which are used for the preparation of Capecitabine nanoparticles. Characteristic peaks in the region of 3000 cm^{-1}, 1716.60 cm^{-1}, 1239.42 cm^{-1}, 1255.20 cm^{-1}, 1606.35cm^{-1} were found to be observed in physical mixture which was identical to that of the pure drug; this confirmed the intactness of the drug in the physical mixture.

Differential Scanning Calorimetry (DSC)

DSC is the one of the techniques used to determine the drug, polymer and other solvents interaction during the formulation development. Further, also to confirm the crystalline changes of the drug loaded into the nanoparticle formulation.

The DSC thermogram of Capecitabine showed a sharp endothermic peak at 121.61^{0}C and it was corresponding to reporting melting point temperature range (110-132°C). In case of DSC thermogram of physical mixture at 1:1 ratio, the drug and polymer showed endothermic peaks at 192.60°c and 180.6°C. The DSC curve of physical mixture of drug with formula at IVE ingredients showed no shift in the endothermic peak of pure drug. The DSC curve of Capecitabine showed a single peak at 121.64°C which is the melting point of the pure drug.

% Entrapment efficiency:

The entrapment efficiencies for formulation were found to be between 52.013 ± 0.98 % and 79.023 ± 1.50 %. A close look of the results has revealed that the encapsulation efficiency increased with increase of polymer amount and concentration of Solubilizers as well as rpm.

Particle size analysis:

The particle sizes of the formulations were found between 153.6nm.

***In-vitro* release:** The *in vitro* release of Capecitabine nanoparticle formulations was studied in pH 1.2, 6.8 and 7.4 phosphate buffers which is the intestinal pH. The drug release was measured for 8 hr. In all formulations

there was a burst drug release initially and sustained release later. The burst release may be due to the drug which is present on the surface of nanoparticles. The nanoparticles produced with longer time of homogenization showed drug release higher than the drug release of nanoparticles produced with short time of homogenization. Formulation F5 showed high percentage of drug release i.e. 90.8313 ± 1.134 for a period of 8hr and F4 showed less % drug release i.e. 73.75 ± 0.921 for 8 hr when compared with other formulations.

Drug release at 1hr:

The amount of Capecitabine released from nanoparticles in 1 hr ranges from 12.60% to 20.69%. As the concentration of Eudragit increased the drug release increased. With the increase in rpm the release of drug from the formulation was higher.

Drug release at 8 hr:

The amount of Capecitabine released from nanoparticles in 8 hr ranges from 73.75% to 90.83%. (Table no: 10 and 11). The concentration of surfactant does not affect the release at 8hrs. However, drug to lipid ratio appears to have a significant impact on drug release. As the drug to lipid ratio decreases, the drug release also gets decreased.

Kinetics of drug release:

The drug release data were fitted into the different models like zero order, first order, Higuchi equation and Korsmeyer-Peppas. (Table no12). The results have shown very close and above 0.94 R^2 values for first order. It indicates that the drug release is directly proportional to amount of drug remained to be released.

The results have also shown very close and above 0.9 R^2 values for higuchi and Korsmeyer Peppas model. The n values lie between 0.723 – 0.879, which indicates non Fickian diffusion and therefore suggested that the release mechanism is by both diffusion and relaxion.

Table no: 1: Formulation of Capecitabine by using factorial design (Design expert).

RUN	EUDRAGIT	PVA	RPM
1	100	3	20000
2	300	3	20000
3	100	1	20000

4	300	1	10000
5	300	1	20000
6	300	3	10000
7	100	3	10000
8	100	1	10000

Table no: 2 Interpretation for FT-IR spectra of Capecitabine and excipients.

Name of pure drug	Standard value of drug (cm^{-1})	Observed value with Eudragit RL100 (cm^{-1})	Observed value with Eudragit RS100(cm^{-1})
Capecitabine	3300 - 2500	2930.08	2931.56
	1710-1665	1685.21	1687.88
	1400-1000	1238.33	1465.71
	1335-1250	1329.98	1320.19
	1650-1580	1646.24	1606.24

Table no 3: Data showing evaluation of % entrapment efficiency

Formulation code	% Entrapment efficiency
F1	74.321
F2	54.122
F3	79.023
F4	67.213
F5	58.34
F6	52.013
F7	67.541
F8	72.021

Table no 4: Data showing comparison of kinetics for formulations F1-F8

Formulation code	F1	F2	F3	F4	F5	F6	F7	F8
Zero order R^2	0.999	0.993	0.997	0.997	0.996	0.994	0.996	0.998
First order R^2	0.946	0.940	0.961	0.953	0.925	0.934	0.950	0.948
Higuchi model R^2	0.973	0.953	0.971	0.965	0.971	0.958	0.974	0.972
Korsmeyer-peppas model R^2	0.984	0.979	0.979	0.989	0.98	0.987	0.979	0.995
n-value	0.723	0.84	0.744	0.852	0.747	0.844	0.719	0.879

Response variable

Response 1: 1 hr release

Table no 5: ANOVA for selected factorial model for 1hr release.

Source	Sum of Squares	Df	Mean Square	F-value	p-value	
Model	79.87	3	26.62	46.59	0.0014	significant
A-eudragit	75.94	1	75.94	132.90	0.0003	
B-pva	2.07	1	2.07	3.63	0.1294	
C-rpm	1.85	1	1.85	3.24	0.1460	
Residual	2.29	4	0.5714			
Cor Total	82.15	7				

Table no 6: Estimated regression coefficients.

Factor	Coefficient Estimate	Df
Intercept	16.37	1
A-eudragit	-3.08	1
B-pva	-0.5092	1
C-rpm	0.4814	1

Response 2: 8 hr release

Table no 7: ANOVA for selected factorial model for 8 hr release.

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	200.38	3	66.79	21.31	0.0064	Significant
A-eudragit	119.69	1	119.69	38.19	0.0035	
B-pva	23.72	1	23.72	7.57	0.0513	
C-rpm	56.97	1	56.97	18.18	0.0130	
Residual	12.54	4	3.13			
Cor Total	212.92	7				

Table no 8: Estimated regression coefficients

Factor	Coefficient Estimate	Df
Intercept	80.65	1
A-eudragit	-3.87	1
B-pva	-1.72	1
C-rpm	2.67	1

Response 3: entrapment efficiency

Table no 9: ANOVA for selected factorial model for entrapment efficiency

Source	**Sum of Squares**	**df**	**Mean Square**	**F-value**	**p-value**	
Model	576.86	3	192.29	8.01	0.0363	significant
A-eudragit	468.46	1	468.46	19.51	0.0115	
B-pva	102.25	1	102.25	4.26	0.1080	
C-rpm	6.16	1	6.16	0.2564	0.6392	
Residual	96.03	4	24.01			
Cor Total	672.88	7				

Table no 10: Estimated regression coefficients

Factor	Coefficient Estimate	Df
Intercept	65.57	1
A-eudragit	-7.65	1
B-pva	-3.58	1
C-rpm	0.8772	1

Optimized formula

Table no 11: Composition of optimized formula.

Ingredients	Quantity taken
CAPECITABINE	1g
EUDRAGIT RL 100: EUDRAGIT RS100	200 mg
POLYVINYL ALCOHOL	2 mL
RPM	15000

Table no 12: Data showing *in vitro* drug release for formulation F9.

Time(hr)	% Cumulative drug release
0	0
1	16.02± 1.63
2	24.37 ± 1.41
3	32.92± 1.13
4	42.78 ± 1.17
5	52.11 ± 1.36
6	61.65 ± 1.57
7	70.65 ± 1.40
8	80.29 ± 0.96

Table no 13: Comparison of drug release kinetics of formulation F9.

Formulation code	Zero order	First order	Higuchi model	Korsmeyer-peppas model
F9	y = 9.247x + 5.989 R^2 = 0.999	y = -0.086+ 2.066 R^2 = 0.954	y =35.62x– 24.99 R^2 = 0.976	y = 0.788x + 1.171 R^2 = 0.991

Table no 14: Comparison between the experimental (E) and predicted (P) values for the most probable optimal formulation (F9).

Optimized formulation(F9)	Dependable variables		
	Drug release		
	Drug release at 1hr (%)	Drug release at 8hr (%)	Entrapment efficiency
Pred.	16.3742	80.6504	65.5743
Exp.	16.0215	80.2989	65.1102

Stability Study

Table no 15: Physicochemical properties of optimized formulation(After stability)

Time	Drug content(%)	E.E(%)	Appearance
Initial	96.87	92.12	Whitish
After 3 months	95.94	92.01	No change

Figures:

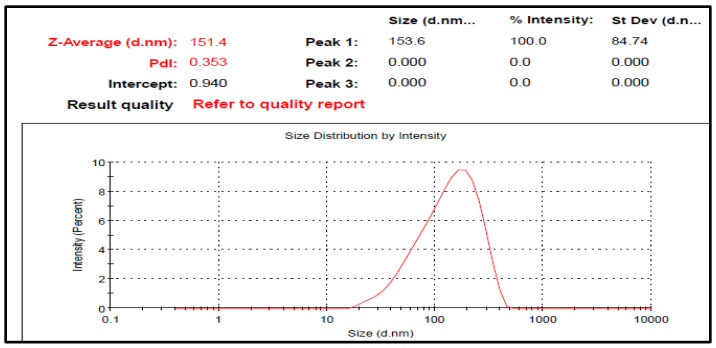


Fig.no: 1: Particle size analysis.

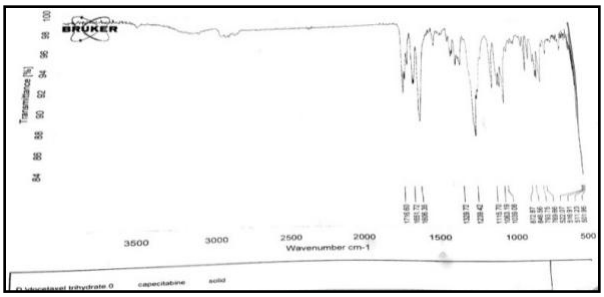


Fig. no: 2: FTIR OF Capecitabine.

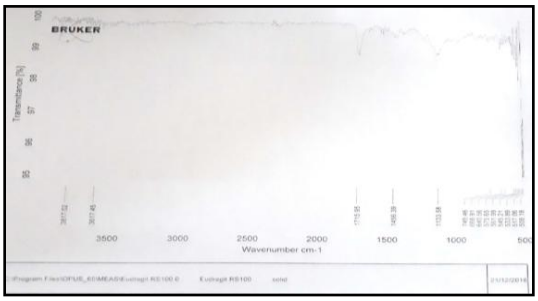


Fig.no: 3: FTIR of Eudragit RS100.

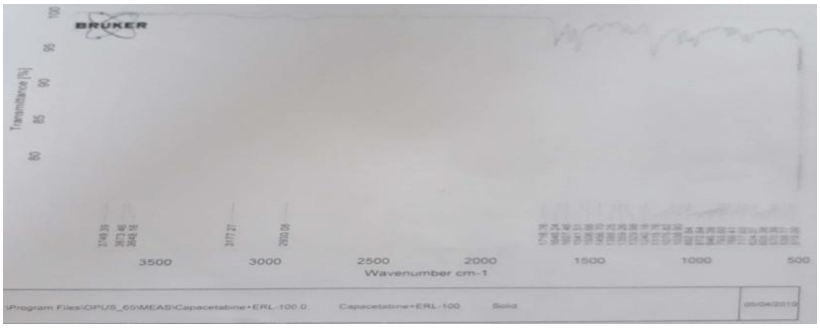


Fig. no: 4: FTIR SPECTRA OF CAPECITABINE+ EUDRAGIT RS 100.

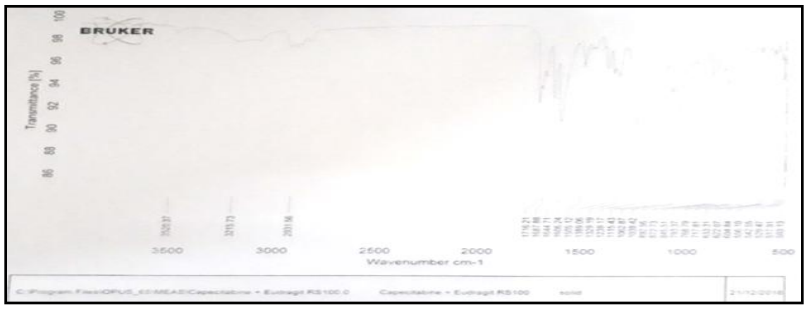


Fig. no: 5: FTIR SPECTRA OF CAPECITABINE+ EUDRAGIT RL100.

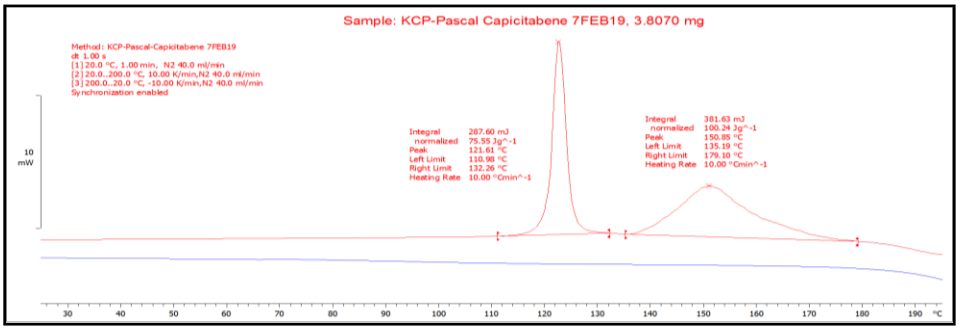


Fig. no: 6: DSC OF CAPECITABINE.

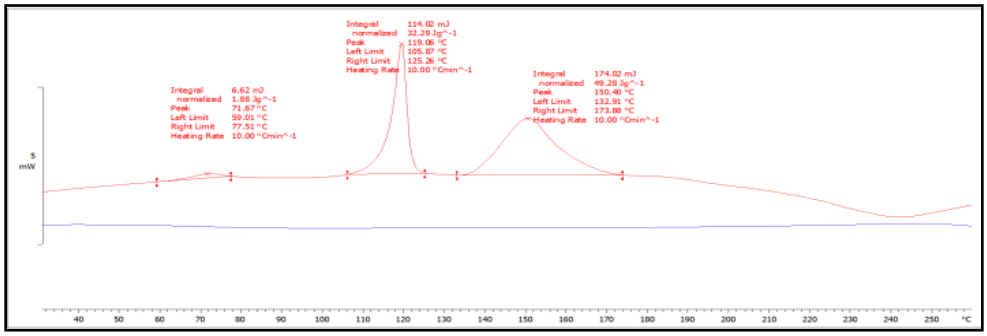


Fig. no:7: DSC OF CAPECITABINE+ EUDRAGIT RL 100+ EUDRAGIT RS100.

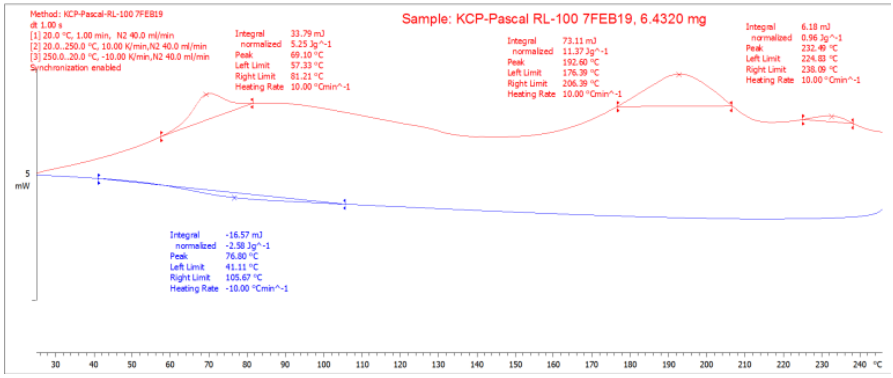


Fig. no: 8: EUDRAGIT RL 100.

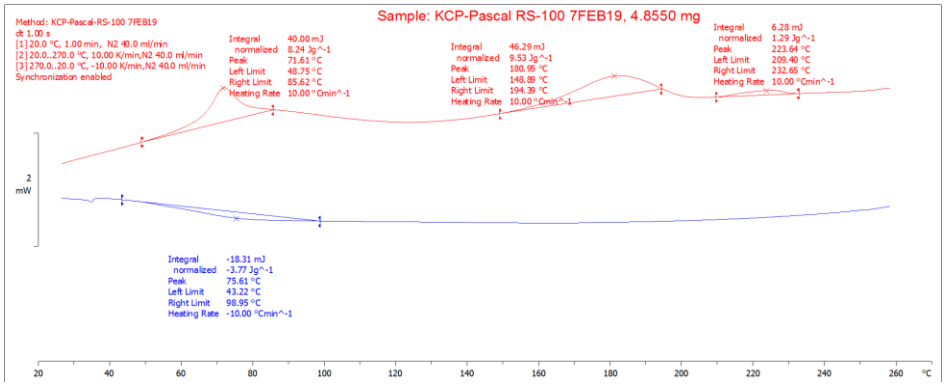


Fig.no:9: EUDRAGIT RS 100.

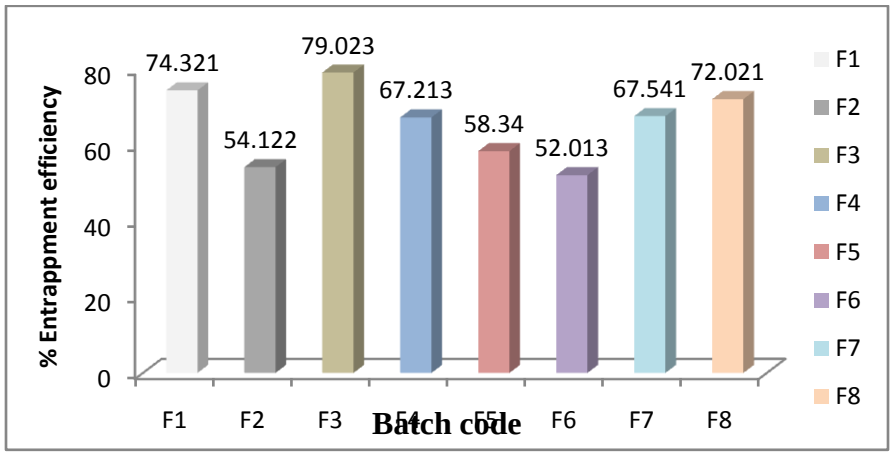
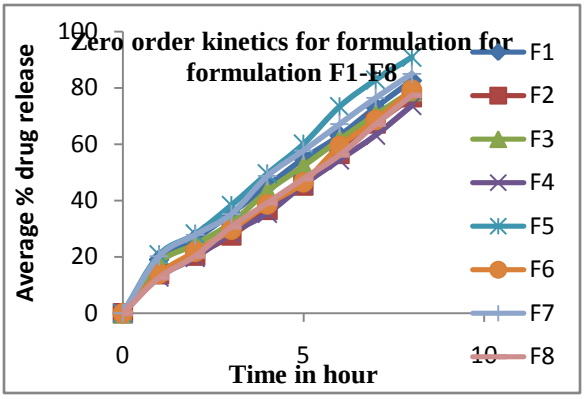


Fig no 10: Comparison of % Entrapment efficiency.



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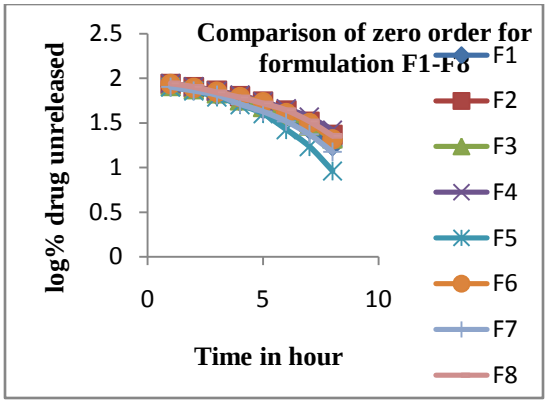

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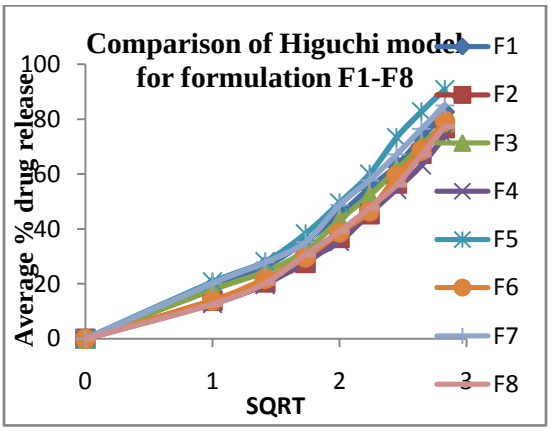

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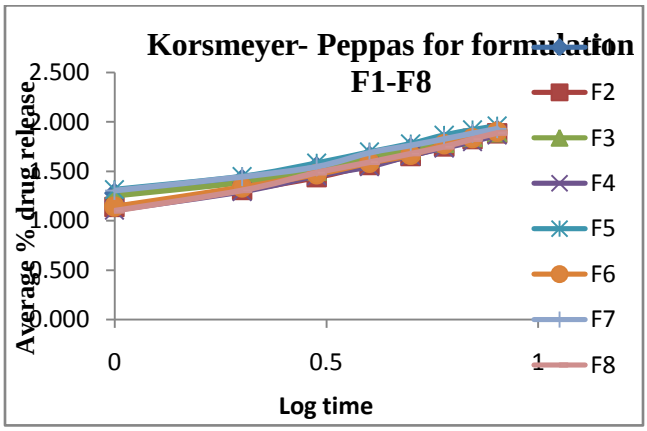


Fig. no:14

Fig no 11: Comparison of zero order kinetics for formulation F1-F8

Fig no 12 : Comparison of zero order kinetics for formulation F1-F8

Fig13: Comparison of Higuchi model for formulation F1-F8

Fig 14: Comparison of Korsmeyer- Peppas for formulation F1-F8

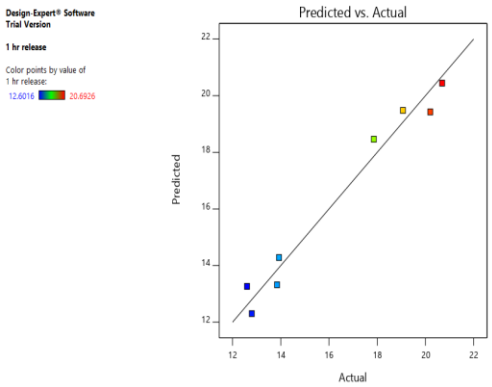


Fig no 15: Correlation between predicted and actual values for 1 hr release

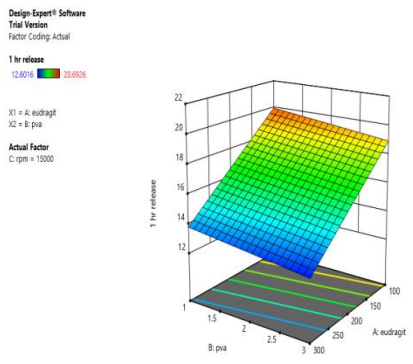


Fig no.16 : 3-D graph showing release for 1 hr

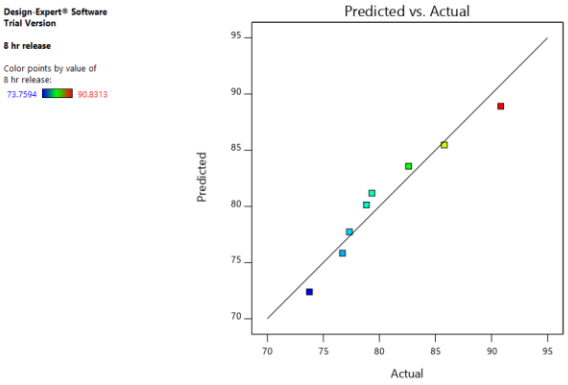


Fig no 17: Correlation between predicted and actual values for 8 hr release

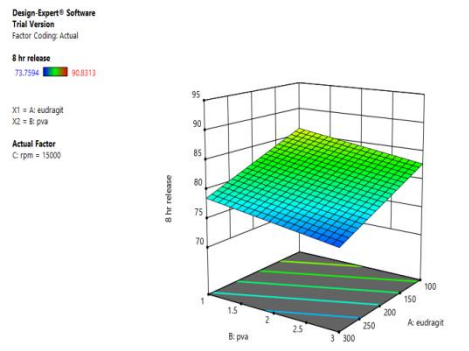


Fig. no 18: 3-D graph showing drug release at 8hr

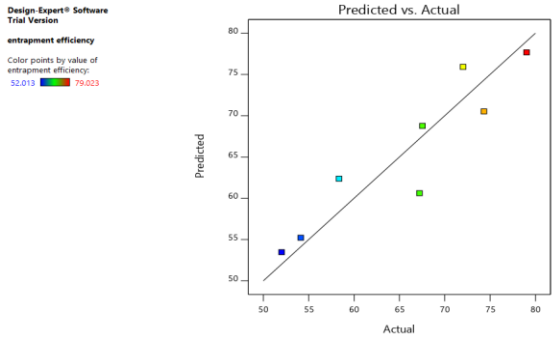


Fig no 19: Correlation between predicted and actual values for entrapment efficiency

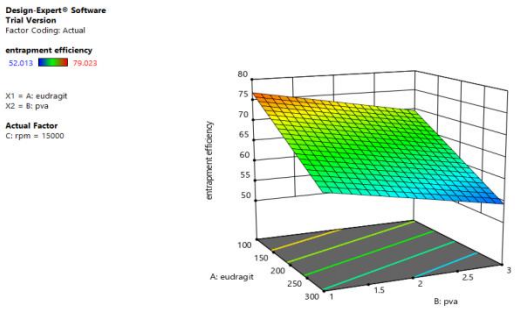


Fig no 20: 3-D graph showing drug release for entrapment efficiency

Drug release kinetics for optimized formula:

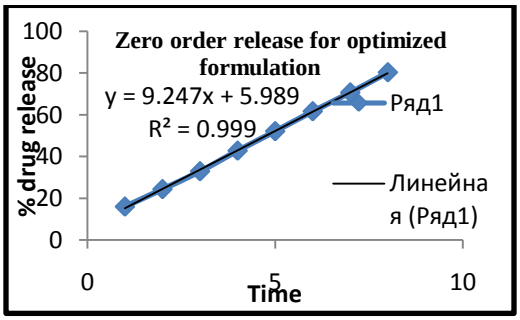
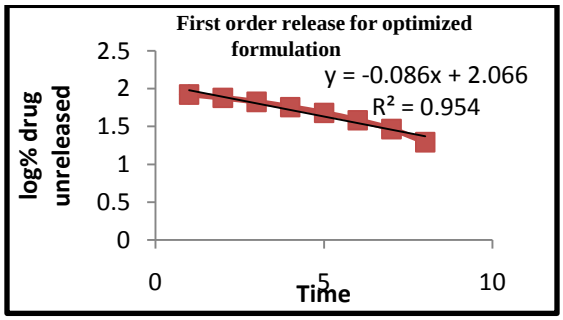


Fig.no:21



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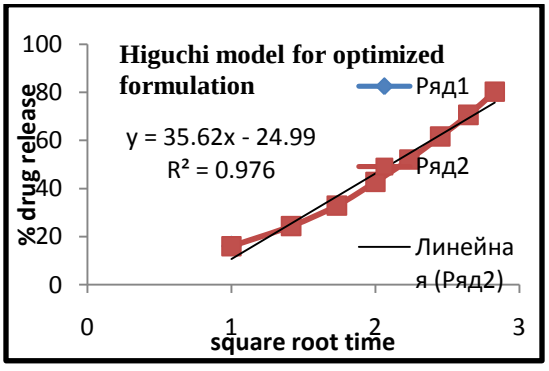

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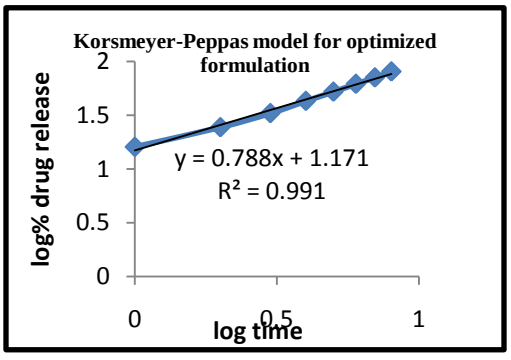

Fig. no: 24

Fig no 21: Zero order kinetic plot of optimized formulation

Fig no 22: First order kinetics plot for optimized formulation

Fig no 23: Higuchi model kinetics for formulation

Fig no 24: Korsmeyer-Peppas model for formulation

Conclusion:

In this present work an attempt was made to formulate nanoparticles loading Capecitabine.The study led to following conclusions. The FT –IR and DSC spectra revealed that there was no interaction between drug and excipients used, hence they are compatible. Solvent evaporation technique can be used to prepare nanoparticles of Capecitabine using DMSO as surfactant, PVA and PVP as emulsifier. The particle size was influenced by drug, polymer concentration, PVA, PVP and rpm. With increase in the rpm the particle size decreased and with increase in the polymer content the particle size increased. The *in-vitro* drug release for optimized formulation was found to be 80.298±1.54 in 8 hrs and encapsulation efficiency of 65.1102 ± 1.98 %. As the time of homogenization increases, the drug release gets higher. With increase in the particle size the drug release was less. Particles with less size offered more surface area for the drug to release so the drug release was more. Results from the stability studies at 25°C/60 ± 5% RH and 40°C/70 ± 5% RH indicated good stability of the optimized formulation as there was no significant change in the physical appearance, drug content and drug release. For the mechanism of drug release, all the formulations showed first order release. Drug release was by non- Fickian diffusion and follows Higuchi equation.

Conflicts of Interest: NONE

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