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Development of Temozolamide Solid Lipid Nanoparticles: Formulation, in vitro characterization and invivo pharmacokinetic studies in animals

Dr. K. Bhaskar Reddy
Sri Venkateswara College Of Pharmacy, RVS Nagar, Tirupati Road, CHITTOOR – 517127

N. Audinarayana
Sri Venkateswara College Of Pharmacy, RVS Nagar, Tirupati Road, CHITTOOR – 517127

G. Swapna
Sri Venkateswara College Of Pharmacy, RVS Nagar, Tirupati Road, CHITTOOR – 517127

M.D. Kusuma Priya
Sri Venkateswara College Of Pharmacy, RVS Nagar, Tirupati Road, CHITTOOR – 517127

Abstract: In this work, water-soluble prodrugs with reduced T1/2 were encapsulated into nanobeads via surfactant polymer ionic-gelation cross linking and targeted to the colon using Eudragit-S-100. FT-IR, DSC were used to characterise Capecitabine and nanobeads. Zeta Sizer, SEM, Drug loading, Entrapment efficiency, in-vitro and in-vivo pharmacokinetic investigations described Capecitabine nanobeads. The weight fluctuation, hardness, and friability of Capecitabine nanobead tablets were tested. The optimization of formulation and process variables by 23 factorial design using Expert-Design software shows that increasing the speed of the Hi-Speed Homogenizer (a process variable) decreases the size of the Capecitabine nanobeads. The concentration of Calcium chloride (Cacl2 percent, a formulation variable) also affects the size of the beads, and 3 percent Cacl2 is the best concentration for preparing Capecitabine nanobeads. Capecitabine and nanobeads' physicochemical properties were confirmed. DSC showed that Capecitabine and polymers were compatible, and the medication was somewhat amorphous and distributed in polymer matrix. Characterization of Capecitabine nanobeads characteristics showed that F2 is superior to F1 and F3.

INTRODUCTION

The use of herbal medicines has increased remarkably in line with the global trend of people returning to natural therapies. Herbal formulations have reached widespread acceptability as therapeutic agents for diabetics, arthritics, liver diseases, cough remedies, memory enhancers and adoptogens. Herbal medicine products are dietary supplements that people take to improve their health and are sold as tablets, capsules, powders, tea, extracts, fresh and dried plants. Though herbals are traditionally considered to be harmless, some may cause health problems, some are not effective or some may react with other drugs. Hence standardization of herbal formulations is essential in order to assess the quality of drugs based on the concentration of their active principles.

Various advanced methods such as chromatographic, spectrophotometric and combination of these methods,

electrophoresis, polarography and the use of molecular biomarkers are currently employed in standardization of herbal drugs. Chemical fingerprints obtained by these methods have become the powerful tools for the authenticity and quality control of traditional herbal medicine. However, there is a need for a rapid and specific method allowing the manufacturers to set quality standards and specifications so as to seek marketing approval from regulatory authorities for therapeutic efficacy, safety and shelf life of herbal drugs. Chemical markers play a pivotal role at various stages of the development and manufacturing of a herbal medicine such as authentication and differentiation of species, stability assessments and quality control of herbal medicines. An attempt is proposed to develop a method for simultaneous estimation of four chemical markers that are commonly present in many of the poly herbal formulations. The proposed methods offer rapid and cost-effective analysis of Gallic acid, Ascorbic acid, Embelin, and Piperine either individual or in combination from different ayurvedic formulations.

Approved Objectives of the Proposal :

The principal objectives of the present research investigation are as follows:

- To design and develop sustained release encapsulated Anti cancer drug containing Nanoparticle (Nanobeads) formulation by optimizing various process and formulation variables.
- Formulation of targeted encapsulated Nanobeads core tablet for treatment of colorectal cancer.
- To enhance the bioavailability of the Anticancer drug at the colorectal system.
- To evaluate the prepared Nanobeads and Nanobeads core tablet
- Characterization of Nanobeads with Drug Loading and Entrapment Efficiency
- Detection of Weight variation test, Hardness and Friability of the Nanobeads core tablet.
- In-vitro release kinetics of Anti Cancer drug containing Nanobeads core tablet.
- In-vivo pharmacokinetic studies of the Anti Cancer Nanobeads in Swiss Albino Mouse.

Methodology

Methodology**: BCS Class II Anticancer drug having low solubility so Surfactant are in need to enhance the BA, so Ionic-Gelation Cross Linking Method will be the Selected formulation for production of Nanobeads.**

Surfactant-Polymer Nanobeads of Anticancer drug were formulated by Ionic-gelation method by optimizing various process variables in following steps.

Step 1: Polymer solution of sodium alginate (2%) (dissolved in distilled water) was prepared and to this required drug amount i.e. 180mg (Anticancer drug, less than 40% than polymer concentration to have good nanobeads) and homogenized for 5min.

Step 2: Then this homogenized polymer solution was added drop wise to the external medium (which was prepared by various concentrations of surfactant and calcium chloride dissolved in distilled water as per 2^3 factorial design) through 32 gauge syringe.

Step 3: Both the external medium (Sodium Lauryl Sulphate and Calcium Chloride solution) and drop wise polymer solution with drug was homogenized with high speed homogenizer (at various speeds as per 2^3 factorial design) and alternate constant sonication for 30 pulse/2sec for 5min with

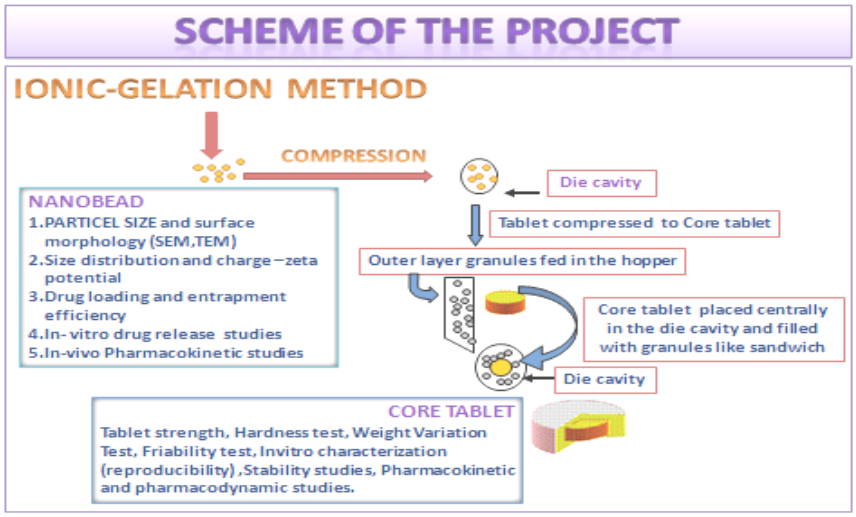
sonicator after every 30min of homogenization.

Step 4: The above procedure was continued for 3hours and remove the homogenized solution and air dried for 6hrs to remove water and ultracentrifuge (10,000×g/45min) to precipitate the encapsulated cross linked nanobeads then lyophilized to get solid (Anticancer drug) nanobeads.

- Characterization of physicochemical parameters: The polymers used for nanobeads should be characterized for not altering the chemical structure of drug and polymer was compatible with polymers without any decomposition of drug was studied by Fourier Transform Infra-Red Spectroscopy (FTIR) and determination of Drug-Excipients Compatibility, purity of drug and melting point of the standard drug studies done by Differential Scanning Calorimetry (DSC)
- Optimization of Methodology: The process and formulation variables are optimized by 2^3 factorial design using Expert-Design software for production of best formulations of Anticancer drug nanobeads.
- Characterization of nanobeads: The characterization of nanobeads is necessary to determine the size, shape, surface charge, average particle size distribution and in-vitro and in-vivo studies to determine the release studies of nanobeads and release kinetics to show which model of release taking place.
  - Particle Size and surface morphology of formed nanobeads is needed for confirmation of size and shape to determine nanobeads or not studies done by Scanning Electron Microscopy (SEM).
  - Particle size distribution of the nanobeads, average nanosize of the nanobeads studies and Zeta potential (Particle charge) done by Zeta Sizer.
  - Drug content in nanobeads is determined studies by Drug Loading and Entrapment Efficiency using direct comparison method.
  - In- vitro studies needed for determination of release kinetics and which order the formulation follows using Dissolution Apparatus of USP 1.
  - In-vivo Pharmacokinetic studies to determine $T_{max,}$ $C_{max,}$ $T_{1/2,}$ MRT, AUC and AUMC by using Swiss albino mouse with help of HPLC

Step 5: Formulation of Core tablet containing Nanobead

Step 6: Evaluation of Anticancer drug nanobeads Core Tablets for Post formulation tests needed for determination of Tablet strength, Hardness test, Weight Variation Test, Friability test, In vitro characterization and Stability studies.



EXPERIMENTAL RESULTS

Drug-Excipient Compatibility By Fourier Transform Infra- Red Spectroscopy (FTIR):

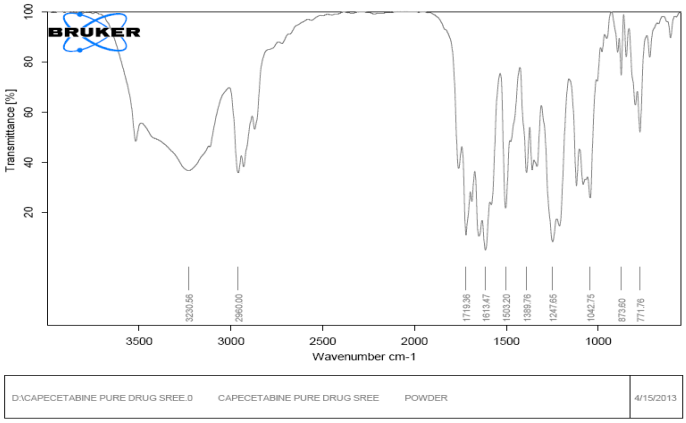


Figure 1: Pure Capecitabine FT-IR Spectra

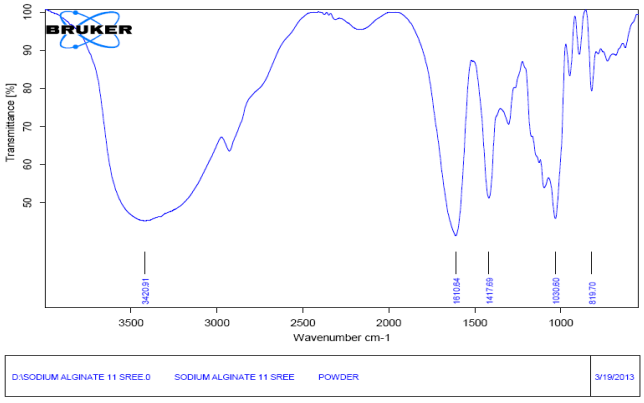


Figure 2: Sodium Alginate FT-IR Spectra

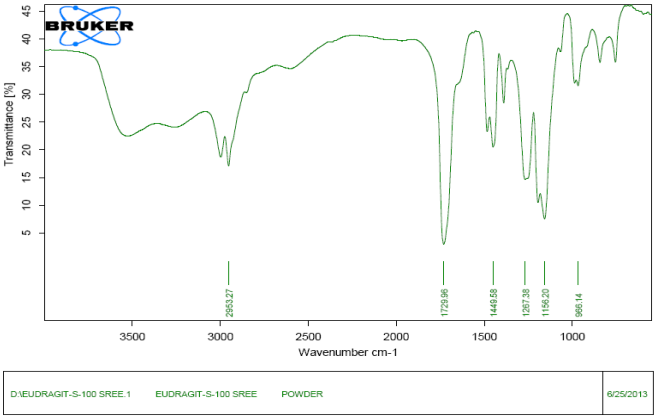


Figure 3: Eudragit-S-100 FT-IR Spectra

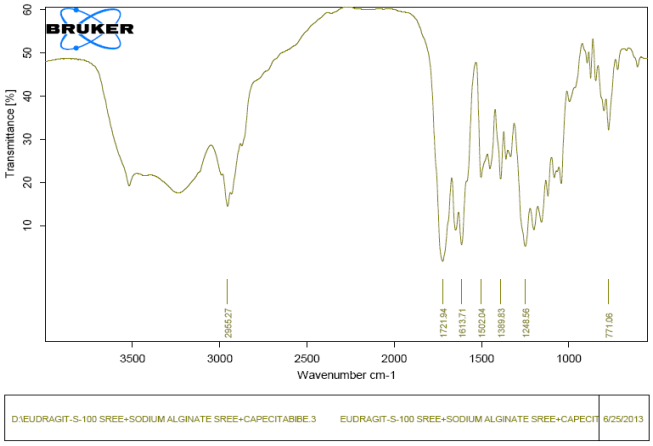


Figure 4: Capecitabine + Sodium Alginate + Eudragit-S-100 FT-IR Spectra

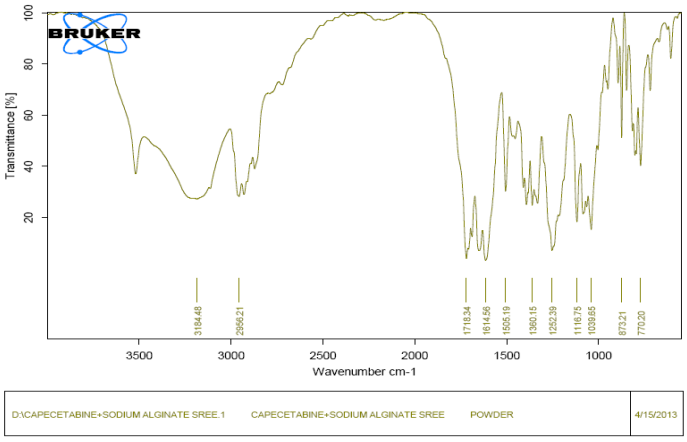


Figure 5: Capecitabine Nanobeads Formulation FT-IR Spectra.

Pure drug Capecitabine shown FT-IR spectra as following.

Table 1: Capecitabine pure drug shown following FT-IR spectra peak.

Peak (cm^{-1})	**Characterization**
3290.56cm^{-1}	O–H stretching
2960.00cm^{-1}	C–H stretching (aromatic)
1719.36cm^{-1}	C=O stretching (amide)
1613.47cm^{-1}	C=C stretching
1503.20cm^{-1}	N–H bending
1389.76cm^{-1}	C–N stretching (amide)
1247.65cm^{-1}	C–H bending
1042.75cm^{-1}	C–O bending vibrations
873.60cm^{-1}	C–F stretching
771.76cm^{-1}	C–H bending

The Capecitabine nanobead core tablet formulation shown same spectra as pure drug which demonstrates that the chemical structure of the drug doesn't change after converting to nanobeads and shows there is no interactions between the drug and polymers.

Drug-Excipient Compatibility by Differential Scanning Calorimetry (DSC):

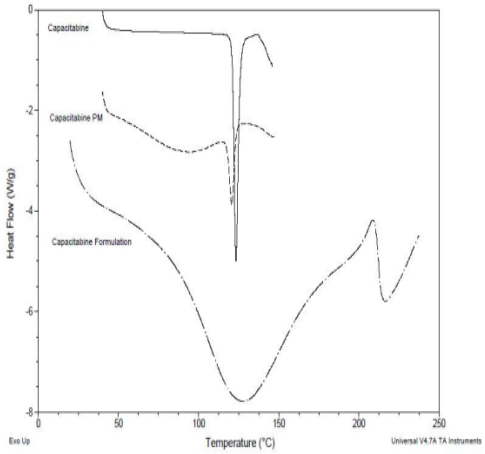


Figure 6: DSC of Capecitabine, Physical Mixture and Formulation over Lay

DSC studies gives information about the physical state properties like crystalline or amorphous nature of the sample. The DSC thermogram of pure drug Capecitbine given an endothermic peak at 123.26^{0}C corresponding to its melting point. The physical mixture (Capecitabine, Sodium alginate and Eudragit-S-100) given an endothermic peak at 120.74^{0}c and overlay of pure drug and physical mixture peaks, which proves that there is no interaction between drug and excipients. The DSC peak for Capecitabine nanobead core tablet formulation was given an endothermic peak at 126.64^{0}C without any sharp peak. This shows that crystallinity of the Capecitabine has been reduced significantly in the nanobeads and it could be concluded that Capecitabine was present in amorphous nature and may have been homogeneously dispersed in the sodium alginate nanobead matrix. The results are shown in figure 6.

Optimization of methodology using 2^3 factorial designs by using Design-Expert Software

Table 2: Original results data given to 2^3 factorial designs using Design-Expert.

Response	Name	Units	Obs Analysis	Mini-mum	Maxi-mum	Mean	Std. Dev	Ratio	Trans	Model
Y1	**ZETA SIZER**	d.nm	8 Factorial	235.0	1544.0	970.3	527.38	6.570	None	RMain effects
Y2	**POTE-NTIAL**	mV	8 Factorial	7.000	±37.9	±22.3	10.855	5.414	None	RMain effects
Y3	**PDI**		8 Factorial	0.323	0.950	0.547	0.254	2.941	None	R2FI

Response 1: ZETASIZER

ANOVA for selected factorial model

Table 3: Analysis of variance table of Zeta Sizer [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square Value	F	p-value Prob > F	
Model	1.884E+006	1	1.884E+006	33.11	0.0012	**significant**
A-RPM	1.884E+006	1	1.884E+006	33.11	0.0012	
Residual	3.413E+005	6	56891.50			
Cor Total	2.225E+006	7				

The Model F-value of 33.11 implies the model is significant. There is only a 0.12% chance that a "Model F-Value" this large could occur due to noise. Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case A are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 4: Predicted R-Squared with Adj R-Squared of Zeta Sizer

Std. Dev.	238.52	**R-Squared**	0.8466
Mean	970.25	**Adj R-Squared**	0.8210
C.V. %	24.58	**Pred R-Squared**	0.7273
PRESS	6.068E+005	**Adeq Precision**	8.138

The "Pred R-Squared" of 0.7273 is in reasonable agreement with the "Adj R-Squared" of 0.8210. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. Your ratio of 8.138 indicates an adequate signal. This model can be used to navigate the design space.

Table 5: model used to navigate the design space of Zeta Sizer

Coefficient Factor	Standard Estimate	df	Error	95%CI Low	95%CI High	VIF
Intercept	970.25	1	84.33	763.90	1176.60	
A-RPM	-485.25	1	84.33	-691.60	-278.90	1.00

Final Equation in Terms of Coded Factors: ZETA SIZE =+970.25 -485.25 * A
Final Equation in Terms of Actual Factors: ZETA SIZER =+2823.03 -0.1103 * RPM

Response 2: POTENTIAL ANOVA for selected factorial model

Table 6: Analysis of variance table of potential [Partial sum of squares - Type III]

Source	Sum of Squares	Df	Mean Square Value	F	p-value Prob > F	
Model	633.68	1	633.68	12.30	0.0127	**Significant**
B-SLS	633.68	1	633.68	12.30	0.0127	
Residual	309.00	6	51.50			
Cor Total	942.67	7				

The Model F-value of 12.30 implies the model is significant. There is onlya1.27% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case B are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 7: Predicted R-Squared with Adj R-Squared of potential

Std. Dev.	7.18	**R-Squared**	0.6722
Mean	22.28	**Adj R-Squared**	0.6176
C.V. %	32.22	**Pred R-Squared**	0.4173
PRESS	549.32	**Adeq Precision**	4.961

The "Pred R-Squared" of 0.4173 is not as close to the "Adj R-Squared" of 0.6176 as one might normally expect.This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable.Your ratio of 4.961 indicates an adequate signal. This model can be used to navigate the design space.

Table 8: model used to navigate the design space of potential

Coefficient Factor	Standard Estimate	df	Error	95%CI Low	95%CI High	VIF
Intercept	22.28	1	2.54	±16.07	±28.48	
B-SLS	8.90	1	2.54	±2.69	±15.11	1.00

Final Equation in Terms of Coded Factors: POTENTIAL = +22.28 +8.90 * B
Final Equation in Terms of Actual Factors:POTENTIA = -4.42500 +11.8666 * SLS

Respons 3:PDI ANOVA for selected factorial model

Table 9: Analysis of variance table of PDI [Partial sum of squares - Type III]

Source	Sum of Squares	df	Mean Square Value	F	p-value Prob > F	
Model	0.28	1	0.28	7.31	0.0354	**significant**
AC	0.28	1	0.28	7.31	0.0354	
Residual	0.23	6	0.039			
Cor Total	0.52	7				

The Model F-value of 7.31 implies the model is significant. There is onlya 3.54% chance that a "Model F-Value" this large could occur due to noise.Values of "Prob > F" less than 0.0500 indicate model terms are significant. In this case AC are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve your model.

Table 10: Predicted R-Squared with Adj R-Squared of PDI

Std. Dev.	0.20	**R-Squared**	0.5493
Mean	0.55	**Adj R-Squared**	0.4741
C.V. %	36.05	**Pred R-Squared**	0.1987
PRESS	0.41	**Adeq Precision**	3.824

The "Pred R-Squared" of 0.1987 is not as close to the "Adj R-Squared" of 0.4741 as one might normally expect. This may indicate a large block effect or a possible problem with your model and/or data. Things to consider are model reduction, response tranformation, outliers, etc. "Adeq Precision" measures the signal to noise ratio. A ratio of 3.82 indicates an inadequate signal and we should not use this model to navigate the design space.

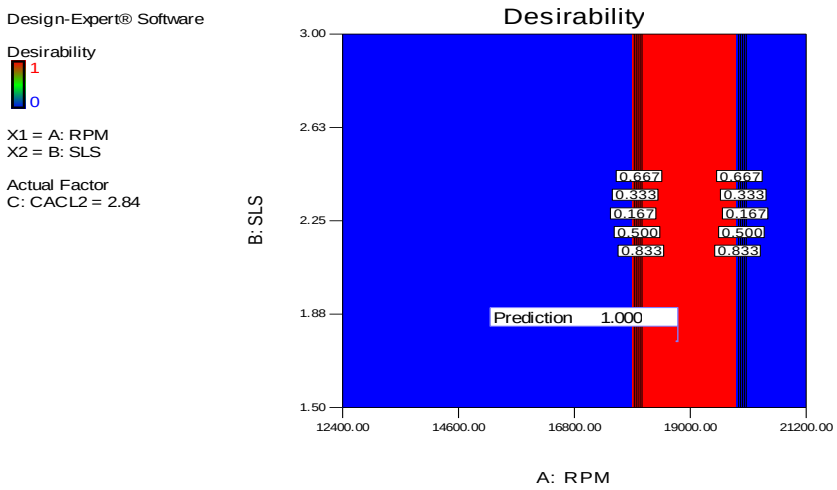
Table 11: model used to navigate the design space of PDI

Coefficient Factor	Standard Estimate	df	Error	95%CI Low	95%CI High	VIF
Intercept	0.55	1	0.070	0.38	0.72	
AC0.19	1	0.070	0.018	0.36	1.00	

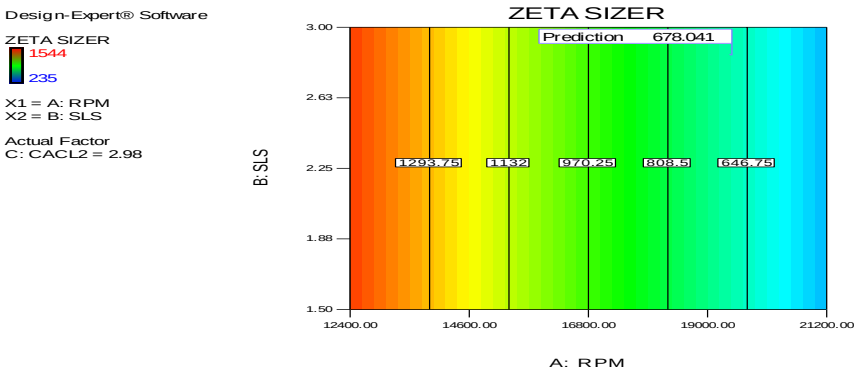
Final Equation in Terms of Coded Factors: PDI = +0.55 +0.19 * A * C

Design-Expert 2^3 Factorial Design Graphs Of Process And Formulation Variables Of Optimized Capecitabine Methodology.

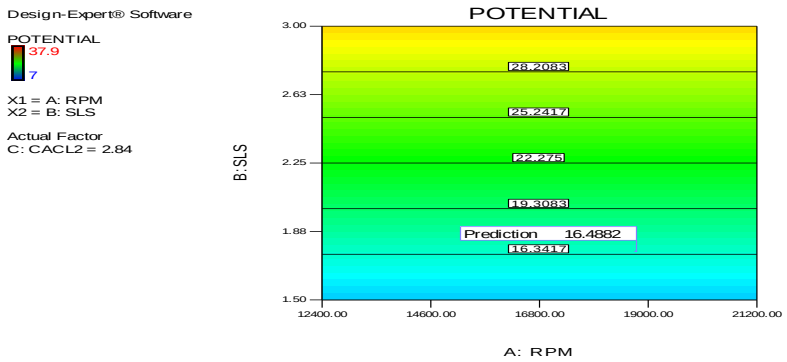
Graph 1: Desirability Graph Of Process And Formulation Variables



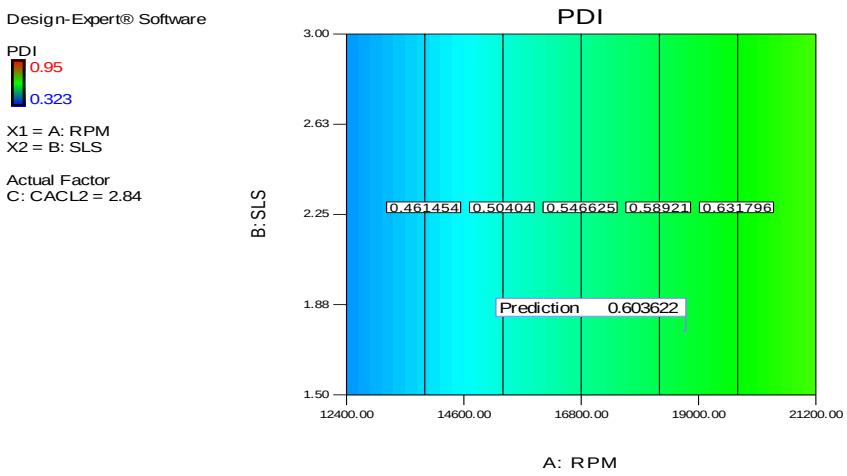
Graph 2: Graph of predicted values of zeta sizer



Graph 3: Graph of predicted values of zeta potential



Graph 4: Graph of predicted values of polydispersity index (PDI)



FIRST SOLUTION:

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	**RPM**	18763.00	12400.00	21200.00	0.000	Actual
B	**SLS**	1.76	1.50	3.00	0.000	Actual
C	**CACL2**	2.84	2.00	3.00	0.000	Actual

Table 12: First preticted solution of Design-Expert 2^3 Factorial Design

Response	Prediction	SE Mean	95%CI low	95%CI high	SE Pred	95% PI low	95% PI high
Zeta sizer	753.762	92.34	527.81	979.71	255.77	127.92	1379.61
Potential	16.4882	3.03	9.08	23.89	7.79	-2.57	35.55
PDI	0.60362	0.073	0.43	0.78	0.21	0.090	1.12

Table 13: Second preticted solution of Design-Expert 23 Factorial Design

Factor	Name	Level	Low Level	High Level	Std. Dev.	Coding
A	**RPM**	19449.60	12400.00	21200.00	0.000	Actual
B	**SLS**	2.85	1.50	3.00	0.000	Actual
C	**$CaCl_2$**	2.98	2.00	3.00	0.000	Actual

Particle Size Distribution and Zeta Potential Measurements by Zeta Sizer and Zeta Potential:

Formulation 1:

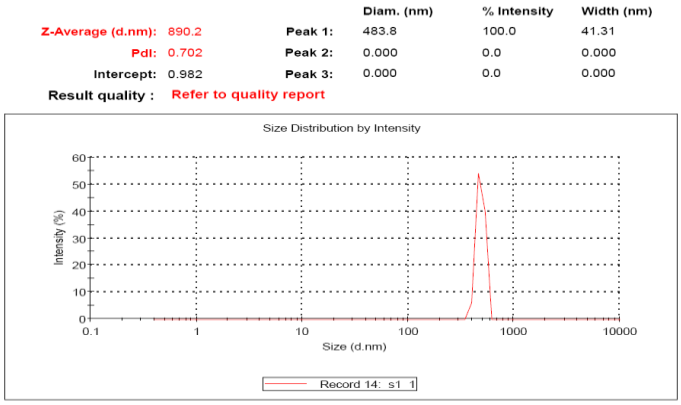


Figure 7: Particle size distribution report for formulation F1 by Zeta Sizer

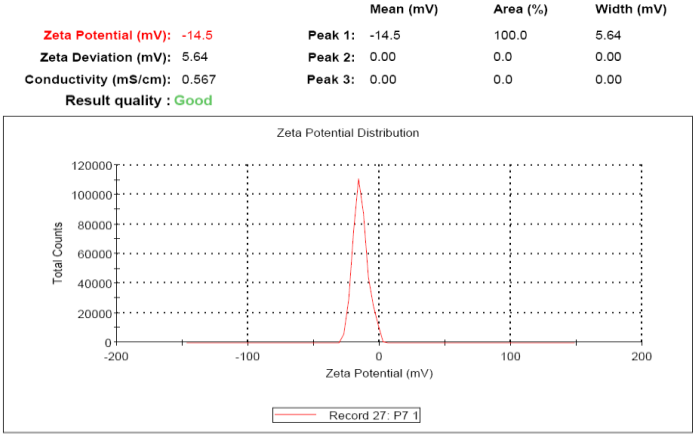


Figure 8: Zeta potential for formulation F1 by Zeta Sizer

Formulation 2:

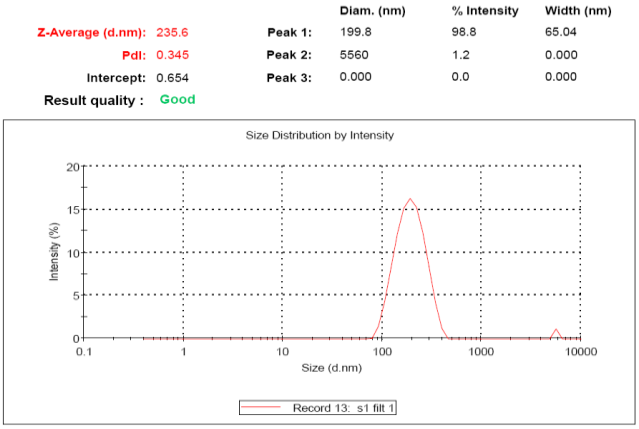


Figure 9: Particle size distribution report for formulation F2 by Zeta Sizer.

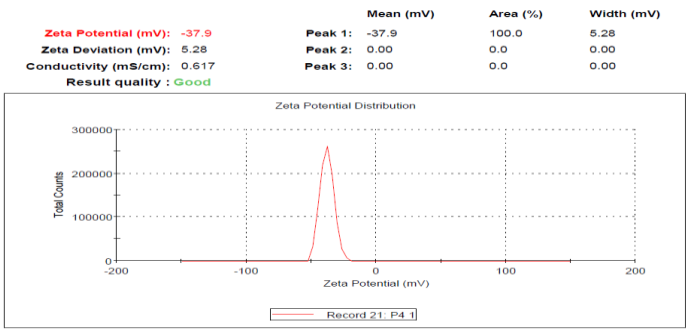


Figure 10: Zeta potential report for formulation F2 by Zeta Sizer

Optimization of process variable: The table 15 shows that by increasing speed of Hi-Speed Homogenizer (a process variable) the size of the Capecitabine nanobead decreases.

Optimization of formulation variables: The concentration of Calcium chloride ($Cacl_2$ % a formulation variable) also affect the size of beads and from the data it shows that approximately 3% $Cacl_2$ is best concentration for preparation of Capecitabine nanobeads and concentration of surfactant (SLS % a process variable) also important to optimize has it also affecting the size and potential of the bead and from the table 15 shown that around 1.5% SLS is the best for producing Capecitabine nanobeads.

In particular, it was interesting to produce nanosize beads or particles, because they possess the potential to accumulate at inflammatory sites such as tumor, with a so-called enhanced permeability and retention (EPR) effect.

The formulations F1, F2 and F3 given results as shown in figure 7,9,11 with zeta sizes (average particle distribution) of 890.2 d.nm, 235.6 d.nm and 446.6 d.nm with polydispersity index of 0.7, 0.35 and 0.46 and also figure 8, 10 and 12 shown zeta potential of -14.5mV, -37.9 mV and -22.1mV respectively. The formulation F2 is best as it shown average particle size distribution of 235.6d.nm and zeta potential value is -37.9 mV with PDI 0.35 which is lower than -30.0 mV the typical threshold value for flocculation and good to achieve EPR effect.

Surface Morphology and Particle Size by Scanning Electron Microscopy (SEM):

Formulation 1:

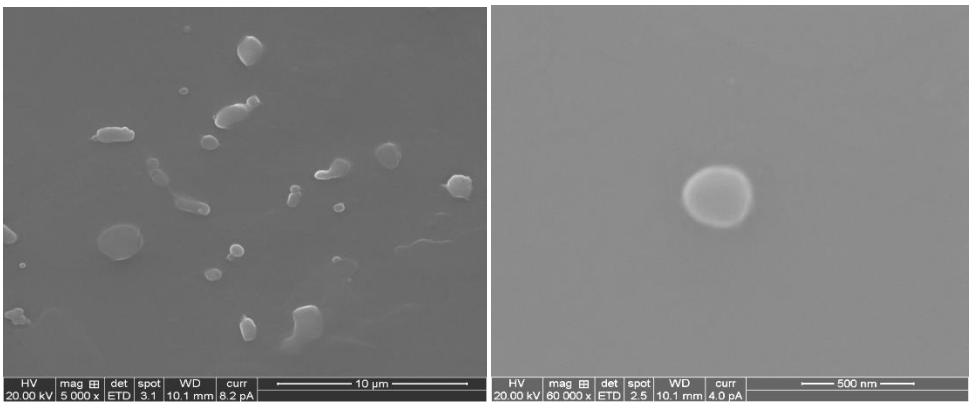
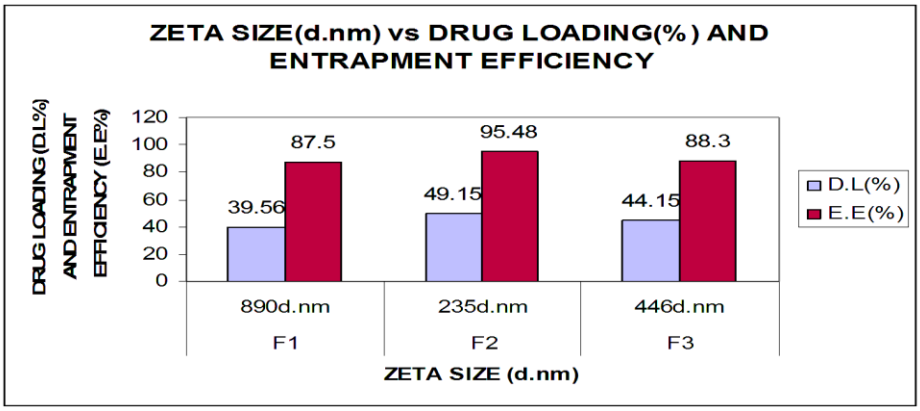


Figure 13: SEM image of Nanobead in 5000× resolutions showing many Capecitabine nanobeads at 10µm and 500nm Capecitabine nanobead.

The morphology and size of the Capecitabine nanobeads were determined by Scanning electron microscopy (SEM). The SEM results shown that the morphology of Capecitabine nanobeads were round and spherical. The SEM image of formulations as in figure 13 was shown between 300nm-500nm.

Entrapment Efficiency and Drug Loading:

Graph 5: Graph showing best formulation by determining Drug Loading and Entrapment Efficiency with zeta size



As shown in graph 5 the second formulation (F2) having with 235.6d.nm particle size distribution had good drug loading and entrapment efficiency than first and third formulations (F1 and F3).

Conclusion

In the current research, water-soluble prodrugs with a shorter T1/2 were effectively encapsulated into nanobeads using a surfactant polymer ionic-gelation cross linking approach. Additionally, a tablet containing Capecitabine nanobead core was targeted to the colon by covering it with Eudragit-S-100.

By using FT-IR and DSC, the researchers were able to characterize the physicochemical properties of capecitabine and capecitabine nanobeads. In order to characterise the capecitabine nanobeads, we used the Zeta Sizer, the scanning electron microscope, the drug loading, the entrapment efficiency, and in-vitro and in-vivo pharmacokinetic tests. The capecitabine nanobeads core tablets were characterized by means of a test for weight fluctuation, as well as tests for hardness and friability. The optimization of formulation and process variables by 23 factorial design utilizing Expert-Design software gives results such as by increasing the speed of the Hi-Speed Homogenizer (a process variable), the size of the Capecitabine nanobead decreases. Next, the concentration of Calcium chloride (Cacl2 percent, a formulation variable) also affects the size of beads, and it was shown that approximately 3 percent Cacl2 is the best concentration for the preparation of Capecitabine nanobeads and concentration.

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