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Original Article

SCREENING OF FACTORS USING PLACKETT BURMAN DESIGN IN THE PREPARATION OF CAPECITABINE-LOADED NANO POLYMERIC MICELLES

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ABSTRACT

Objective: Quality is considered to be the major tool in pharmaceutical industry. Failures in the quality of upcoming new chemical entities have been seen in the recent years due to complications in the formulation process. Capecitabine (CTB), a anticancer pro-drug which orally shows fluctuation in plasma drug concentration and serious side effects. Purpose of this study is to understand the effect of variables on quality of the formulation. The aim of the present study was to develop CTB-loaded nano polymeric micelles (PMs) using Quality-by-Design (QbD) approach to screen the effect of eight formulation and process factors on the formulation.

Methods: CTB-loaded nano PMs were prepared by organic solvent/water (o/w) emulsion technique. Plackett-Burman (PB) design was used to screen the effect of eight formulation and process factors to improve anticancer efficacy. The nano PMs were characterized by drug content (% DC), entrapment efficiency (% EE), particle size, zeta potential, TEM, PXRD, SEM, and *in-vitro* drug release.

Results: Particle Size and zeta potential of nano PMs were found to be in the range of 178 – 1572 nm and -10.9 to -36.1 mV respectively. Analysis of the Pareto chart revealed that the HP β-CD and Eudragit S100 significantly influences drug content and entrapment efficiency respectively. Nano PMs demonstrated sustained release of CTB.

Conclusion: The study concludes that the statistical PB design could be useful to identify influencing variables such as HP β-CD and Eudragit S100 that can be used for further investigation.

Keywords: Anticancer; Nano polymeric micelles; Plackett-Burman design; Quality by Design

INTRODUCTION

Capecitabine (CTB), a pro-drug is used in the treatment of metastatic colorectal and breast cancer. Upon oral administration, it is readily absorbed from the gastrointestinal tract and converted into active metabolite 5-fluorouracil in cancer tissues [1,2]. It has a short biological half life (0.5-1 h), with a high dose of 2.5 g/m^2 per day. It often causes marked fluctuations in drug plasma concentration resulting in serious side effects such as bone-marrow depression, cardiotoxicity, nausea and vomiting, stomatitis, dermatitis, etc [3]. Furthermore, a sustained release formulation is another crucial factor in the effective management of colorectal and breast cancer. So, capecitabine loaded chitosan-poly (ethylene oxide-g-acrylamide) hydrogel microspheres and solid lipid nanoparticles have been developed to improve its therapeutic efficacy [4,5].

However, the particle growth, unpredictable gelation tendency, burst release and drug expulsion after polymeric transition process limits their practical usefulness. Hence, there is a need of alternative drug delivery system for CTB to improve its therapeutic efficacy. Recently, nano polymeric micelles (PMs) have drawn major attention in drug delivery due to its potential features such as smaller particle size, good thermodynamic stability, increase in solubility of hydrophobic drugs and prolong drug release and avoid recognition by the reticuloendothelial system (RES) [6-8].

The nano PMs comprise a drug-loading core and a hydrophilic shell. Amphiphilic block copolymer forms micelles when in contact with an aqueous vehicle by self assembly resulting in hydrophobic interactions wherein hydrophobic drugs can be encapsulated into the central core of micelles through hydrophobic interactions [9,10]. These novel carriers have been successfully investigated for delivery of various anticancer drugs such as paclitaxel [11], doxorubicin [12], methotrexate [13], sagopaline [14] etc to improve their therapeutic efficacy. Eudragit S100 is an anionic copolymerization product of methacrylic acid to methyl methacrylate in the ratio of 1:2. It does not degrade below pH 7 thus provide film coats that are resistant to gastric media but soluble in intestinal fluid [15]. It has been used in various pharmaceutical applications including enteric coating and controlled release oral as well as ophthalmic dosage forms [16,17]. HP β-Cyclodextrin (HP β-CD), a biocompatible polymer form an inclusion complex with insoluble drugs and improves its solubility and absorption process. A combination of Eudragit S100 and HP β-CD nano PMs can improve the drug loading capacity and therapeutic efficacy of poor water soluble drugs. According to the ICH Guideline Q8 (R2) (FDA/ICH, 2009), "Quality-by-Design (QbD) is a systematic approach to the development that starts with predefined objectives and emphasizes product and process understanding and process control, based on sound science and quality risk management." It identifies and understands the influence of input (formulation and process) parameters on the critical quality attributes (CQAs) [18]. Design of Experiment (DOE) provides a mean to determine the multi-factorial relationship among the input parameters that influence experimental output [19,20]. Plackett-Burman (PB), a statistical screening design has been used to study the main effect on formulation [21,22]. The present study was designed to investigate potential of CTB-loaded nano PMs. Literature survey reveals that no such formulation has been reported till date. The novelty of this study aims to investigate, statistically oriented, best influencing formulation parameters using QbD approach. A Plackett–Burman design was employed to screen various factors on drug content (%DC), entrapment efficiency (%EE), particle size and zeta potential for production of CTB-loaded nano PMs to improve anticancer efficacy.

MATERIAL AND METHODS

Materials

CTB was obtained as a gift sample from Neon laboratories Limited (Mumbai, India). Eudragit S100 was a gift sample from Evonik (Mumbai, India). HP β-Cyclodextrin was a generous gift from Cadila Pharmaceuticals Limited (Ahmedabad, India). Methanol (AR grade) was purchased from S. D. Fine Chemicals Limited (Mumbai, India).

Preparation of CTB-loaded nano polymeric micelles

CTB-loaded nano PMs were prepared using organic solvent/water (o/w) emulsion technique [23]. Specific amount of CTB was dissolved in methanol containing Eudragit S100 (Figure 1). The resulting organic solution was added drop wise to 30 ml of distilled water containing HP β-CD under vigorous stirring at 2000 rpm (Remi magnetic stirrer). Magnesium chloride ($MgCl_2$) was added into the formed emulsion and ultrasonicated for 2 mins (30 sec. on and 5 sec. off, 45°C 70% amplitude) followed by overnight stirring to get CTB-loaded nano PMs [24]. Further the final formulation was subjected to lyophilization (2.5 Freezone, lab PONCO Equipment ltd.) at – 55°C for 24 hr.

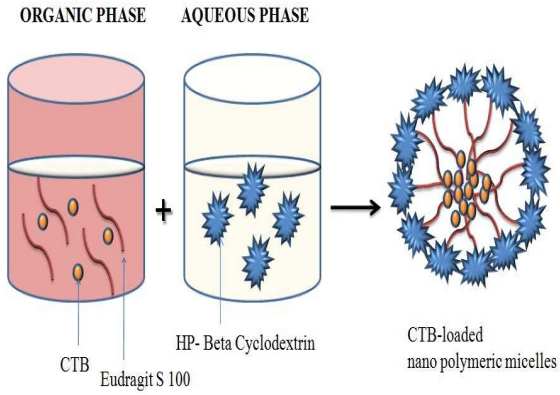

Fig.1: Schematic representation of developing capecitabine-loaded nano polymeric micelles

Plackett Burman screening design

A set of experiments using the PB screening design was adopted to prepare CTB-loaded nano PMs. This design investigates every input factor and arranges them on the Pareto chart based on the magnitude of its influence with positive or negative sign respectively (colour and colourless) [25]. PB design screens large number of input factors and at the same time reduces the number of runs [26, 27]. 't' statistic is determined by estimating the standard effect of each input factor. The factors with bar extending beyond the vertical line on the pareto chart shows significant influence at 95% confidence level [28].

The factors show positive or negative sign on the pareto chart reflecting increased or decreased effect respectively when moving from lowest to the highest level for the specific factor. The ANOVA results are used to determine the most influencing effect. Total twelve experimental trials involving eight independent variables were generated using STATGRAPHICS XVI. The amount of Eudragit S100 (A), HP β-CD (B), drug concentration (C), organic solvent (D), aqueous vehicle (E), ultrasonication time (F), ultrasonication amplitude (G) and amount of divalent cation ($MgCl_2$) (H) were selected as independent variables and the drug content, entrapment efficiency, particle size and zeta potential were set as response variables. The variables were correlated using the following polynomial equation with PB design.

$$Y = A_0 + A_1X_1 + A_2X_2 + A_3X_3 + A_4X_4 + \ldots\ldots\ldots\ldots + AnXn \quad \textbf{(1)}$$

Where, Y is the response, A_0 is the constant, and A_1 is the coefficients of the response [26].

Characterization of CTB-Loaded Polymeric Micelles

Determination of drug content and entrapment efficiency

The drug content of CTB-loaded nano PMs were determined by dissolving formulation in methanol and the absorbance was measured at 240 nm using UV spectrophotometer (Jasco-V-530, Japan). The drug content was calculated using the equation (2), Drug content (%) = CA × (VA /WA) × 100 **(2)** Where, CA is the total concentration of CTB-loaded nano PMs; WA is the theoretical amount of CTB added; VA is the volume of nano PMs.

The entrapment efficiency was determined by separating CTB from the nano PMs by centrifugation (Beckman Coulter, Allegra 64R centrifuge) at 20,000 rpm for 20 min at 4°C. The supernatant was assayed spectrophotometrically at 240 nm for free drug content and entrapment efficiency was calculated using the equation (3),

% E.E = (Total drug content - Free dissolved drug) / Drug amount used × 100 **(3)**

Determination of particle size

Particle size was determined using laser diffraction technique (Malvern 2000 SM, Instruments, UK). The particle size measurements were carried out at a 90° scattering angle. The samples were dispersed in distilled water. The average particle size was determined and expressed in terms of d(0.9) nm.

Zeta potential analysis

The zeta potential was measured using the laser Doppler electrophoretic mobility measurement technique (Zeta Potential Measurement ZS 90, Malvern Instruments, UK) at a temperature of 25°C.

Transmission electron microscopy (TEM)

The morphology of CTB-loaded nano PMs was performed using transmission electron microscopy (Tecnai G^2 Ultra twin FEI, Netherland). A drop of the sample was placed onto a carbon coated grid to form a thin liquid film. The excess solution was removed and sample was examined and photographed at an accelerating voltage of 120 KV.

Powder X-ray diffraction (XRD)

Powder X-ray diffraction patterns were recorded by X-ray diffractometer (x-Pert, Philips, UK) using Cu-Ka radiation (1.542A) with a voltage of 40 kV and a current of 35 mA. Samples were scanned from 2° to 50° 2θ.

Scanning electron microscopy (SEM)

The external morphology was determined by scanning electron microscopy (Oxford Instruments, INCA X Sight, UK) Samples were mounted on double-faced adhesive tape and coated with a thin gold–palladium layer by sputter-coated unit and surface topography was analyzed.

***In-vitro* drug release**

CTB release from nano PMs was studied using a dialysis bag diffusion technique [29]. 2ml of CTB-loaded nano PMs solution equivalent to 0.2 mg CTB was introduced into the dialysis bag (cellulose membrane, mw cut off 12,000 Da). The bag was hermetically sealed and immersed in 50 ml of 0.1N hydrochloric acid. The entire arrangement was maintained at 37 ± 0.5°C with continuous magnetic stirring at 50 rpm for 2 h. After 2 h, the dissolution medium was replaced by 50 ml of acetate buffer pH 4.5 and the study was extended for further 2 h. Uninterruptedly dissolution was continued with phosphate buffer pH 7.4 for 24 h. At selected time interval, samples were removed and replaced with fresh medium in order to maintain sink conditions. The samples were analyzed using UV spectrophotometer for CTB content.

RESULTS

Screening design

PB design was applied as a screening method for identifying the most influencing significant factors. Prediction of the main effect of formulation and process parameters on the responses is a crucial requirement in the development of CTB-loaded nano PMs by o/w emulsion technique. Eight factors that may affect the experimental responses were selected as independent variables at two levels for the study as shown in Table 1. Table 2 shows the outline and observed responses of PB formulation (PBF) on two levels. Polynomial equations for individual response reflect the relationship between dependent and independent factors.

Effect of organic solvent, aqueous system and stabilizer concentration

Nano PMs were formulated by screening the best suitable organic and aqueous vehicles. Methanol and distilled water were selected as suitable organic and aqueous system for preparation of CTB-loaded nano PMs. Addition of divalent cation ($MgCl_2$) into the formulation can stabilize the system, preventing the aggregation and Ostwald ripening [23]. As $MgCl_2$ showed positive influence on drug content (Figure 2a) and negative influence on entrapment efficiency (Figure 3a), particle size (Figure 4a) and zeta potential (Figure 5a) $MgCl_2$ was excluded.

Effect of independent factors on:

a) Drug Content

Percent drug content of CTB-loaded nano PMs was found to be in the range of 26.85 to 106.74% w/v depending upon the polymer concentration (Table 2). The Pareto chart indicates that the factors HP β-CD, ultrasonication time and drug concentration possess significant influence on the drug content (Figure 2a). The HP-β-CD had pronounced positive effect as confirmed by least p value of 0.0036 denoted in Table 3. The ultrasonication time significantly influenced the drug content with negative impact as depicted by negative sign in the chart Figure 2a and Eq. 4. The ANOVA results confirm that all the three factors, HP β-CD, ultrasonication time and drug concentration exhibit p-values less than 0.05 indicating that the factors are significantly different from zero at 95.0% confidence level (Table 3). The regression coefficient for drug content indicates 97.42 % of variability around the mean. Correlation between the study factors on the response is shown in the equation. (4)

% DC = -4.06+0.0723A+0.351B+2.182C-0.818D-0.103E-6.425F+0.245G+104.78H **(4)**

Response surface plot (Figure 2b) also confirms the direct relationship between amount of HP β-CD and drug content. Based on the above findings the input factor HP β-CD should be fixed at appropriate values for further optimization studies.

b) Entrapment Efficiency

%EE of CTB-loaded nano PMs ranged from 25.95 to 85.52% w/v, depending upon the concentration of polymers used (Table 2). Among all the input factors, Eudragit S100 was found to possess significant influence on the entrapment efficiency with a positive effect as shown in Pareto chart (Figure 3a). ANOVA results (Table 3) depict p-value 0.0232 for Eudragit S100 indicating that the factor is significantly different from zero at the 95.0% confidence level.

HP β-CD was found to possess statistically insignificant but positive influence on the entrapment efficiency (Figure 3a). The regression coefficient of the entrapment efficiency indicates 92.07 % of variability around the mean. The correlation between the study factors on the response is as shown in the equation (5)

%EE = 74.43+0.087A+0.048B-0.751C+0.085D-0.095E-0.31F-0.15G-10.05H **(5)**

High ultrasonication amplitude and time could result in lower %EE as indicated by negative effect in Figure 3a. Response surface plot (Figure 3b) confirms Eudragit S100 and HP β-CD together exhibit direct relationship with the % EE. Based on the above findings the input factor Eudragit S100 should be fixed at appropriate values for further optimization studies.

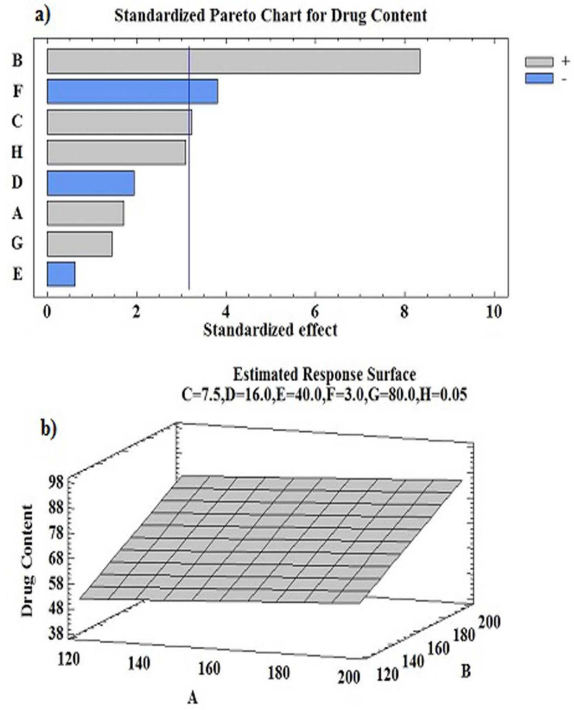

Fig. 2: (a) Pareto chart of the standardized effects of independent factors on drug content and (b) Response surface plot for drug content

***c)* Particle Size**

The mean particle size was in the range of 178-1572 nm and it was strongly affected by the selected variables (Table 2). The Pareto chart (Figure 4a) depicts that all the studied factors have shown statistically insignificant effect on particle size however HP β-CD has shown more influential effect on particle size**.**

The ANOVA results showed none effects have p-values less than 0.05, which indicates that factors are insignificantly different from zero at the 95.0% confidence level (Table 3). The regression coefficient of particle size indicates 67.49 % of variability around the mean. The correlations between the factors on the response are as shown in the equation (6).

Particle size = 5370.0-1.47A-5.49B-19.9C-75.94D-11.23E-108.08F-10.97G-1888.33H **(6)**

ANOVA confirmed that the model was not significant and independent variables had no relationship with the response ($p > 0.05$) as shown in Table 3.

Table 1: The experimental variables and levels of PB design

Independent Variables	Low	High	Units
Eudragit S100 (A)	120	200	mg
HP β-CD (B)	120	200	mg
Drug Concentration (C)	5	10	mg
Organic solvent (D)	12	20	mL
Aqueous vehicle (E)	30	50	mL
Ultrasonication Time (F)	2	4	mins
Ultrasonication Amplitude (G)	70	90	%
$MgCl_2$ (H)	0	0.1	%

Table 2: Outline and observed responses of PBF

PBF No.	A	B	C	D	E	F	G	H	Drug Content (%DC)	Entrapment Efficiency (%EE)	Particle Size (nm)	Zeta Potential(mV)
1	+	+	+	-	+	+	-	+	88.40±0.14	77.21±1.04	557±2.29	-29.4±7.23
2	-	-	-	+	+	+	-	+	42.98±0.89	69.67±2.08	370±1.34	-17.2±6.21
3	+	+	-	+	+	-	+	-	86.07±1.24	71.78±1.39	590±0.81	-32.4±3.98
4	-	+	-	-	-	+	+	+	79.20±1.06	75.52±1.09	178±3.17	-34.9±5.42
5	+	-	-	-	+	+	+	-	41.90±0.71	74.80±2.21	693±0.97	-36.1±2.51
6	-	+	+	-	+	-	-	-	87.30±0.78	72.45±0.56	481±1.18	-10.3±7.09
7	+	+	-	+	-	-	-	+	84.00±1.68	83.89±1.89	606±1.82	-28.1±3.43
8	-	-	-	-	-	-	-	-	82.40±2.09	81.20±2.87	261±2.20	-31.9±4.21
9	+	-	+	+	-	+	-	-	45.80±0.14	79.60±1.21	460±1.86	-32.8±3.85
10	-	+	+	+	-	+	+	-	74.38±0.56	68.78±2.85	481±4.76	-10.9±4.49
11	-	-	+	+	+	-	+	+	62.00±2.88	66.40±0.21	520±3.72	-17.1±2.64
12	+	-	+	-	-	-	+	+	48.00±2.12	73.67±1.27	1572±6.6	-30.2±5.82

Table 3: Summary of analysis of variance

	Drug Content (%DC)		Entrapment Efficiency (%EE)		Particle Size (nm)		Zeta Potential (mV)	
	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value	F-Ratio	P-Value
A	2.94	0.1851	18.45	0.0232	0.11	0.760	5.56	0.099
B	69.54	0.0036	5.60	0.0988	1.56	0.301	0.58	0.500
C	10.44	0.0481	5.31	0.1045	0.20	0.682	3.35	0.164
D	3.77	0.1476	0.18	0.7024	1.07	0.377	1.69	0.284
E	0.38	0.5817	1.39	0.3239	0.59	0.500	0.81	0.433
F	14.49	0.0319	0.15	0.7227	0.97	0.398	0.30	0.622
G	2.12	0.2417	3.40	0.1626	1.00	0.392	0.07	0.803
H	9.63	0.0531	0.38	0.5809	0.74	0.453	0.03	0.870

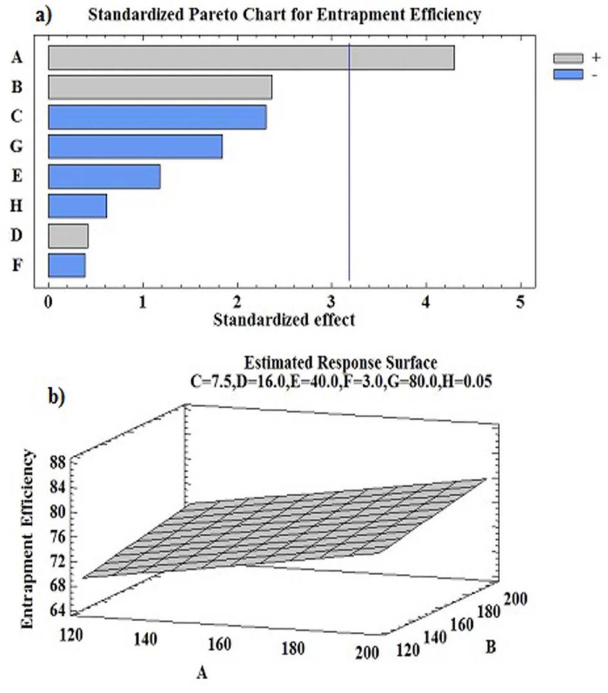

Fig. 3: (a) Pareto chart of the standardized effects of independent factors on entrapment efficiency and (b) Response surface plot for entrapment efficiency

c) Zeta potential

The zeta potential of prepared formulations was found to be in the range of -10.3 mV to -36.1 mV (Table 2). The influence of factors on zeta potential is shown in Pareto chart (Figure 5a). Eudragit S100 showed statistically insignificant negative effect on zeta potential.

The ANOVA results for zeta potential have shown that none of the effects have p value less than 0.05 which indicates that they are insignificantly different from zero at 95.0% confidence level (Table 3). The regression coefficient indicates variability of 80.52% in zeta potential. Equation (7) describes the effects of factors on the zeta potential.

Zeta potential = -26.23-0.21A+0.070B+1.68C+1.99D+0.276E-1.25F-0.062G-8.16H **(7).**

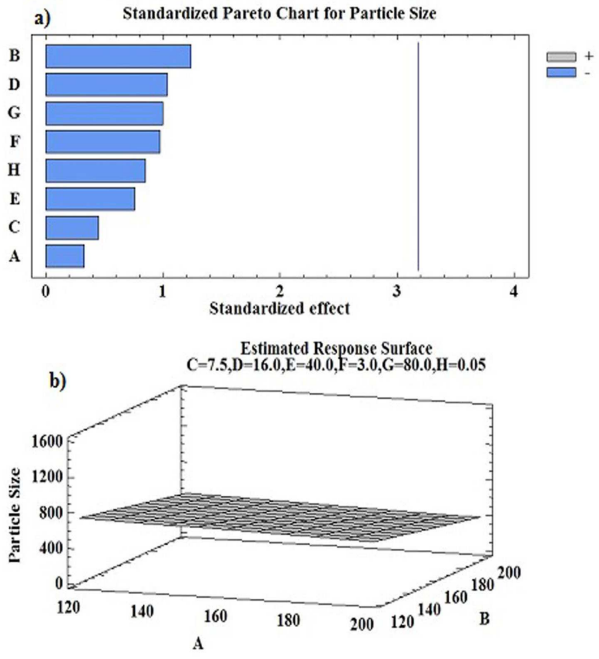

Fig. 4: (a) Pareto chart of the standardized effects of independent factors on particle size and (b) Response surface plot for particle size

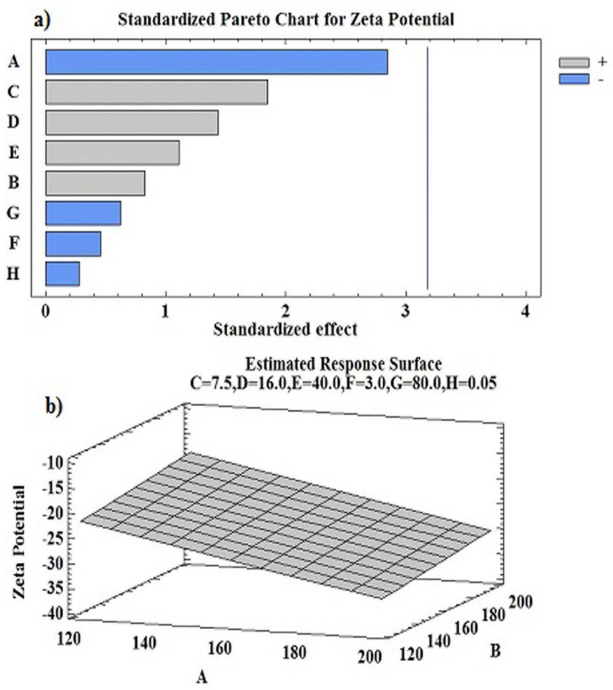

Fig. 5: (a) Pareto chart of the standardized effects of independent factors on zeta potential and (b) Response surface plot for zeta potential

Transmission electron microscopy

The spherical micelles and their self-organized aggregates were clearly visible in the TEM image (Figure 6).

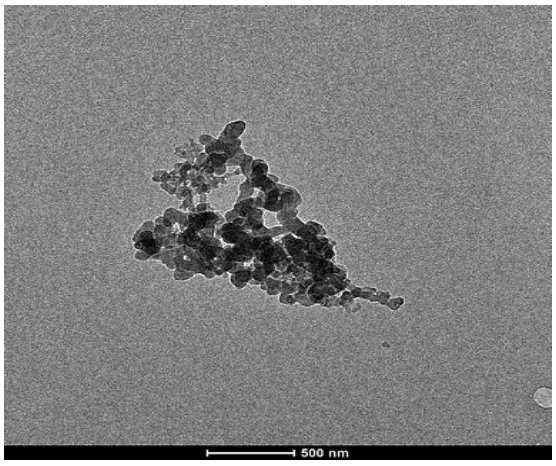

Fig. 6: TEM image of CTB-loaded polymeric micelles

X-ray diffraction

X-ray diffraction spectra of CTB, placebo and lyophilized CTB-loaded nano PMs with different intense peaks are observed in Figure 7.

Scanning Electron Microscopy

The microphotographs showed that pure CTB showed oblong crystals with fully developed corners (Figure 8). The lyophilized CTB-loaded nano PMs consisted of smooth surfaced spherical-shaped smaller particles with a narrow particle size distribution and smooth surface

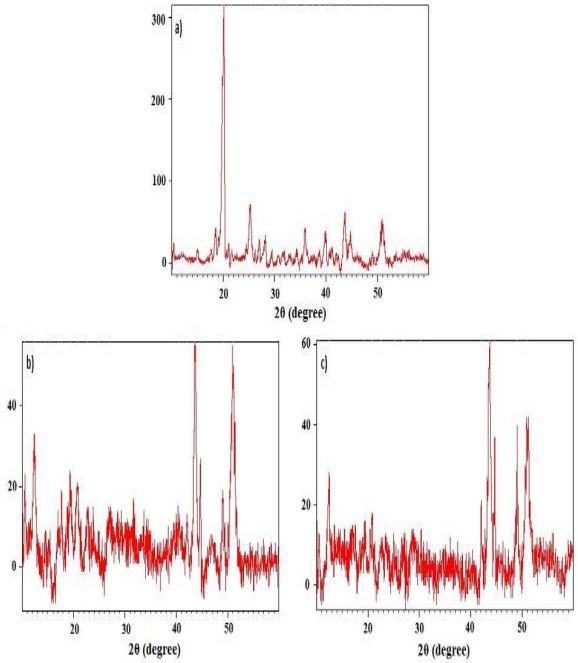

Fig. 7: XRD diffractograms of: (a) CTB, (b) Placebo and (c) Lyophilized CTB-loaded nano PMs

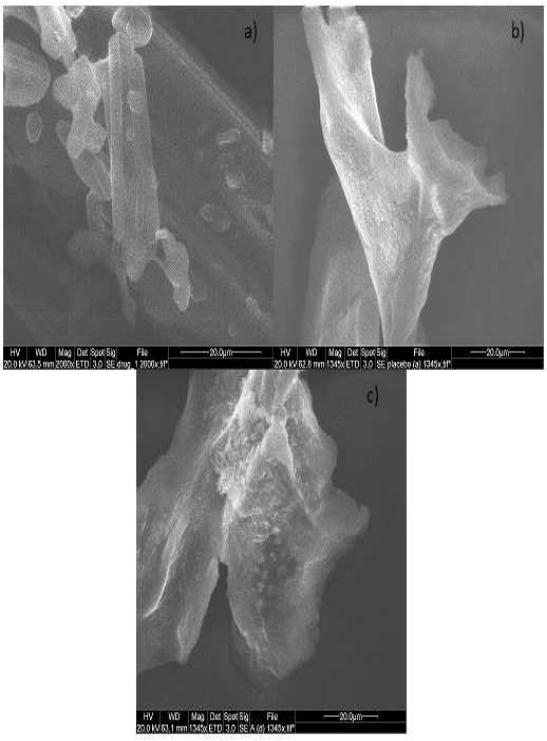

Fig. 8: SEM image of (a) CTB, (b) placebo and (c) Lyophilized CTB-loaded nano PMs

In-vitro drug release

In-vitro CTB release was studied at pH 1.2, 4.5 and 7.4 which represent the approximate pH values of the stomach, intestine and colon respectively. Initial burst release at pH 1.2 for 2h was observed as shown in Figure 9a and b followed by the sustained manner with 6.83 % of cumulative drug release observed for 24 h at pH 7.4.

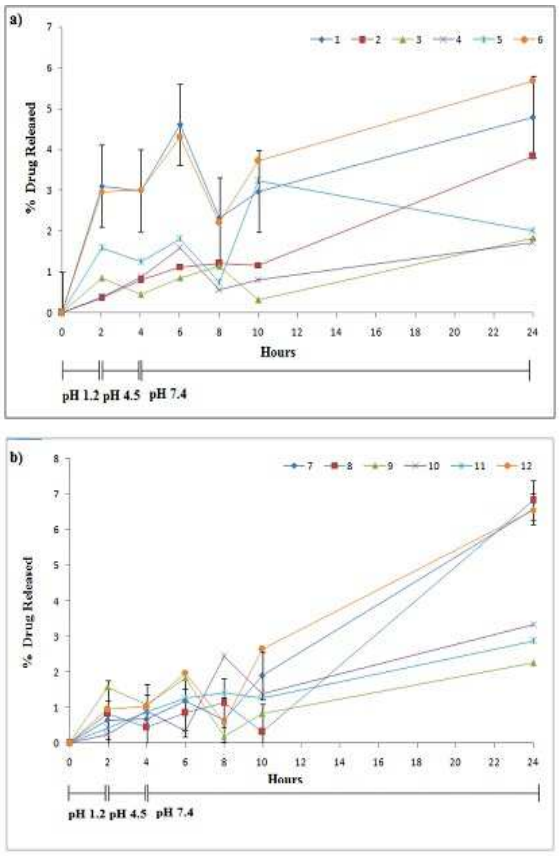

Fig. 9: *In vitro* drug release study (a) PBF No. 1–6, (b) PBF No. 7–12

DISCUSSION

Capecitabine has been reported to be soluble in organic solvents (Ethanol- 207mg/ml, Methanol- >40%, Dimethyl Formamide- ~14mg/ml and Dimethyl Sulfoxide- 72mg/ml at 25°C) [30]. In house analysis confirmed the highest solubility of CTB in methanol and its miscibility with aqueous phase (26mg/ml) hence, methanol was selected as the choice of solvent for preparation of nano PMs. In the present study, $MgCl_2$ was selected as a stabilizer on account of its strong bonding with carboxyl terminal group. Positive influence of $MgCl_2$ on drug content explains the ion-dipole interaction, where cation (Mg^{++}) creates local bridges with divalent ions of interacting oxygen atoms (Figure 2a). This interaction increases the residence time of the host drug molecule inside the micelles. After overnight stirring, aggregated structures with an unstable system of nano PMs were observed which may be ascribed to strong interactions between the divalent cations and oxygen atoms of cyclodextrin that lead to reinforce intermicellar bridges resulting in precipitation. So $MgCl_2$ was removed from the final formulation. Influence of independent factors on %DC, %EE, particle size and zeta potential are explained according to their rank order significance. The pronounced positive effect of HP-β-CD on drug content can be attributed to the composition of branched copolymer HP-β-CD which comprises a cyclic oligosaccharide with seven D-glucose units linked by α-1,4-glucose bonds.

This composition provides unique rigid architecture wherein the primary hydroxyl groups are directed to the narrow side and the secondary hydroxyl groups are on the wide side of the torus. Due to this arrangement of the functional groups, HP-β-CD forms hydrophilic outer surface and hydrophobic inner cavity which encapsulates drug molecules by providing a molecular shield, thus avoiding the leakage of drug molecules through inner cavity [31,32]. This study indicated increase in HP-β-CD and drug concentration together contributed to high drug content in nano PMs. The variable ultrasonication time showed negative significant impact on drug content depicted negative sign in the chart Figure 2a and Eq. 4, that might be ascribed to the precipitates observed in formulation when subjected to high levels of ultrasonication time (>4 mins) resulted in lower CTB loading, as shown in Figure 2a. Eudragit S100 showed the prominent positive influence on %EE which can be attributed to its hydrophobic methacrylate composition. Eudragit S100 comprises anionic copolymerization product of methacrylic acid and methyl methacrylate which provide thick polymeric surfaces. Further the lower percentage of quaternary ammonium groups in Eudragit S100 restricts the diffusion of the drug particles to the surrounding medium [33]. Figure 3a shows statistically insignificant but positive influence of HP β-CD on the %EE. Cyclodextrins (CDs) on account of their inclusion complex entraps the drug molecules into hydrophobic cavity. The complexation process involves replacement of water molecules by drug molecules thereby improving the %EE [34]. The precipitates were observed due to high ultrasonication amplitude and time in formulation when subjected to high levels of ultrasonication time (> 4 mins) and amplitude (90%) that resulted in lower %EE as indicated by negative effect in Figure 3a. Further, increase in the drug concentration resulted in reduced %EE due to fact that the amount of polymer was not sufficient to effectively encapsulate the drug [33, 35].

HP β-CD plays an important role in bringing down the particle size in comparison with the other variables. Figure 4a confirms the influenced effect of HP β-CD on particle size. The ability of CDs to form inclusion complex by electrostatic, Van der Waal, hydrophobic-hydrophobic interactions and hydrogen bonding enhances the solubility, permeability, stability and decreases the particle size [36,37]. Zeta potential is the charge acquired by the particles in a dispersed system that supports the potential physical stability of the formulation. It is reported that the value of ± 30 mV assures the good stability of dispersed systems [38]. Micellar system exhibited negative zeta potential values owing to surface availability of polyanionic Eudragit S100 consisting of various carboxylic end groups. According to Figure 5a Eudragit S100 showed marked influence with negative effect on zeta potential. The increased zeta potential values may be due to the high viscosity of external aqueous vehicle that increase the hydrophilicity of particles. These might result in stronger repulsive interactions among the particle, and hence, higher stability of the particles in formulation. At high concentration of drug, the zeta potential was decreased which may be due to the loading of drug into micellar cavities resulting into destabilization of the system [39].

The powder X-ray diffractograms of CTB showed a characteristic intense peak at 2θ of 20° that indicated the crystalline nature of the drug, however in case of both the placebo and CTB-loaded nano PMs, no intense drug peaks was observed at 2θ of 20° indicating existence of the amorphous phase. TEM image of freshly prepared CTB-loaded nano polymeric micelles revealed the presence of spherical particles with the tendency to agglomerate with each other. Figure 8 shows SEM images of pure CTB, placebo and lyophilized CTB-loaded nano PMs. The crystals of CTB were dispersed between the nano PMs in the formulation, which demonstrated that the drug was thoroughly mixed in the carrier with the loss of crystallinity. This change in particle morphology indicates formation of a new solid phase [40].

Formulation with good drug content and entrapment efficiency should provide the control release of drug from the cargo. Nano PMs provide a platform for the sustained release of drugs. The initial burst release may be due to free drug molecules associated with the interface of the micelles hydrophobic core and hydrophilic corona, or even within the micelle corona compartment facilitating the passive diffusion of drug [35,41]. The drug release was controlled at pH 4.5 which can be attributed to linkage of drug molecules with cyclodextrin units by inclusion interactions resulting in slower dissociation and diffusion. However, the sustained manner with 6.83 % of cumulative drug release was observed for 24 h at pH 7.4, which may be due to deprotonation of Eudragit carboxylic group causing swelling of the polymer and prolonged drug release (Figure 10). Release profile of the formulation (PBF No.8) supported the above findings and hence considered as an optimum batch for controlling the drug release among all the PB runs.

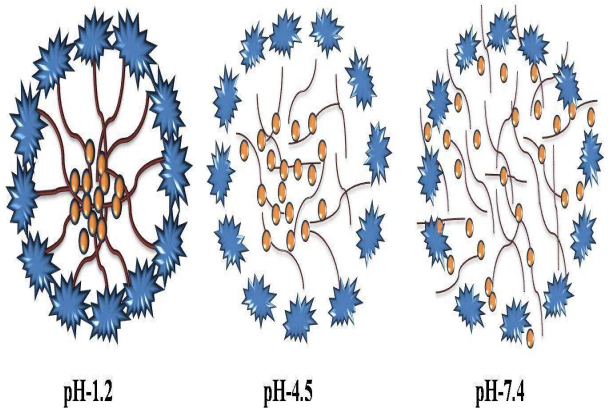

Fig. 10: Representation of drug release mechanism at different pH from pH-sensitive nano polymeric micelles

CONCLUSIONS

CTB-loaded nano PMs were successfully prepared by the solvent displacement method. Formulation and process variables were screened by a PB-QbD approach to understand the most important factors influencing the responses of CTB-loaded nano PMs. The *in-vitro* release study of CTB-loaded nano PMs has showed sustained release pattern. Within the formulation and process factors studied, two formulation factors HP β-CD and Eudragit S100 were found to have significant effect on %DC and %EE. The study concludes that the statistical PB design could be useful to identify influencing significant variables, HP β-CD and Eudragit S100 that can be used for further investigation. PB design was proved to be efficient tool to understand the parameters affecting the response variables and to recognize the most influencing factor.

CONFLICT OF INTEREST

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

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Abbreviations

PB design: Plackett-Burman screening design; **PBF:** Plackett-Burman formulations; **QbD:** Quality by Design; **CTB:** Capecitabine, **PMs:** Polymeric Micelles; **RES:** Reticuloendothelial system; **DOE:** Design of experiments; **CQAs:** Critical quality attributes; **HP β-CD:** Hydroxyl propyl beta cyclodextrin; **o/w technique:** organic solvent/water technique; **%DC:** percent drug content; **%EE:** percent entrapment efficiency; **ANOVA:** Analysis of Variance; **TEM:** Transmission electron microscopy; **SEM:** Scanning electron microscopy; **XRD:** Powder X-ray diffraction.

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