Microencapsulation using Eudragit E100

6.1. Introduction

Eudragit E 100 (EE), is a cationic copolymer based on dimethyl aminoethyl methacrylate and neutral methacrylic esters soluble up to pH 5 (Esposito et al., 2000; Sinha and Rachna, 2002). In addition the polymer retards the drug release above pH 5 due to its insolubility. The pH inside the oral cavity has been reported to be about 6.8 (Hashimoto et al., 2002). Thus EE retards drug release at pH 6.8 and acts as physical barrier between drug and taste buds. This results in taste masking of bitter drugs.

Various methods such as coating (Yu and Roche, 2003), dispersion coating (Ishikawa et al., 1999), spray drying (Cumming and Harris, 1990) and emulsion solvent diffusion (Gao et al., 2006) have been reported for microencapsulation using eudragits. In present study, microparticles were prepared by coacervation phase separation method. Sodium hydroxide was used as nonsolvent for the polymer. EE is dissolved in acetic acid (good solvent). Similarly drug is dispersed in polymer solution. To this solution, sodium hydroxide (poor solvent) is added drop wise with constant stirring. Sudden change in pH of polymer solution causes formation of microparticles. This alternative method has gained attention due to its simplicity, low cost and lack of need for special equipment. In addition this method avoids use of hazardous organic solvents.

The objective of present investigation was to completely disguise the bitter taste of drug by encapsulation in microparticles and to develop a palatable formulation. A 3^2 full factorial design was used for optimization of microparticles wherein the drug concentration (A) and polymer concentration (B) were selected as independent variables while the particle size, drug release at pH, 1.2 and 6.8 along with bitterness score were selected as the dependent variables (responses).

These taste masked microparticles might be ruptured when compressed in tablets and produce bitterness. In addition it has been reported that EE is swellable and permeable above pH 5 (Esposito et al., 2000; Sinha and Rachna, 2002). This may result in leaching of drug, in aqueous medium after

reconstitution on storage, and produce bitterness. To avoid this problem single dose of suspension powder (cachets) were prepared.

6.2. Artemether (ARM)

6.2.1. Experimental

6.2.1.1. Materials

Eudragit E 100 (EE) (Batch no. G041131159) was a gift from Degussa India Pvt. Ltd., (Mumbai, India). Methanol was purchased from Qualigens Fine Chemicals (Mumbai, India) and was used as received. Sodium hydroxide, hydrochloric acid, potassium dihydrogen phosphate, and acetic acid were purchased from S. D. Fine-Chem Ltd., (Mumbai, India) and were used as received.

6.2.1.2. Preparation of Microparticles

Microparticles were prepared by coacervation phase separation method. A concentrated solution of EE (1%w/v) was prepared in 1%v/v acetic acid. The required quantity of the ARM (0.04 g in 15 mL of 1%w/v EE solution) was mixed for 5 min. 10 mL of 10%w/v sodium hydroxide solution was introduced into a 10-ml of glass syringe with a 18G×½" flat-cut hypodermic needle and added drop wise into EE solution. Different concentrations of ARM and EE were used as mentioned in Table 6.1. The resulting microparticles were allowed to harden for 60 min under gentle stirring (Remi Equipments Pvt. Ltd., Mumbai, India) with small magnetic bar, decanted on Buckner funnel, rinsed with the deionized double-distilled water, and dried to a constant weight in hot air oven (Shree Kailash Industries, Baroda, India) at 70^0C for 24 hours, and then stored in the desiccator until use.

Table 6.1. Process variables and their levels for 3^2 full factorial design

Coded values	Actual values	
	Amount of Drug (A in g)	Amount of Polymer (B in mL)*
-1	0.01	5
0	0.03	10
1	0.05	15

**mL of 1%w/v EE solution*

6.2.1.3. Experimental design

A 3^2 full factorial design was employed to systematically study the joint influence of the effect of independent variables amount of drug (A) and polymer (B) on the dependent variables such as particle size, drug release at pH, 1.2 and 6.8 along with bitterness score. In this design, 2 factors were evaluated, each at 3 levels, and experimental runs were performed at all 9 possible combinations. The experimental runs along with their measured responses (dependent variables) are reported in Table 6.2.

Table 6.2. Experimental runs for 3^2 full factorial design with their measured responses

Batch no.	Factors and factor levels		Incorporation efficiency (%) ± SD*	Particle size (μm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug release at pH 6.8 (t5 in %) ± SD*	Bitterness score
	A	B					
ARM 1	-1	-1	83.63 ± 1.29	44.08 ± 4.29	62.57 ± 1.13	4.43 ± 0.79	1
ARM 2	0	-1	74.48 ± 1.74	45.17 ± 3.84	72.64 ± 1.04	4.24 ± 0.83	2
ARM 3	1	-1	79.52 ± 1.49	45.31 ± 3.16	85.70 ± 0.96	5.39 ± 0.64	3
ARM 4	-1	0	82.49 ± 1.21	142.58 ± 4.37	79.25 ± 1.27	5.71 ± 0.73	0
ARM 5	0	0	78.94 ± 1.16	92.46 ± 3.52	84.26 ± 0.86	5.62 ± 0.92	0
ARM 6	1	0	84.27 ± 1.37	64.49 ± 3.74	89.28 ± 1.18	6.47 ± 0.62	1
ARM 7	-1	1	77.86 ± 1.19	241.84 ± 3.93	93.39 ± 1.19	3.81 ± 0.84	0
ARM 8	0	1	82.78 ± 0.78	120.72 ± 3.24	90.35 ± 1.28	3.70 ± 0.81	0
ARM 9	1	1	83.44 ± 1.26	54.36 ± 4.63	87.32 ± 0.98	4.45 ± 0.93	0

*A – amount of ARM; B – amount of EE; t5 and t15 – percent drug released in 5 and 15 min, respectively; *Values represent the mean ± SD of 3 experiments.*

A statistical model incorporating interactive and polynomial terms was used to evaluate the response (Rane et al., 2007a).

$$Y = b0 + b1A + b2B + b11A^2 + b22B^2 + b12AB \qquad \text{(Equation 6.1)}$$

where, Y is the dependent variable, b0 is the arithmetic mean response of the nine runs while b1 and b2 are the estimated coefficient for the factors, A and B. The main effects (A and B) represent the average result of changing one factor at a time from its low to high value. The interaction terms (AB) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (A^2 and B^2) are included to investigate nonlinearity.

Further the model was evaluated for best fit using various statistical parameters such as PRESS (predicted residual error sum of squares), Adj-R^2, Pred- R^2 and Adeq Precision.

PRESS (predicted residual error sum of squares) indicates how well the model fits the data. The coefficients for the model were calculated without the first point. This new model was then used to estimate the first point and calculate the residual for point one. This was done for each data point and the squared residuals were summed.

Adj-R^2 measures variation around the mean explained by the model, adjusted for the number of terms in model.

$$AdjR^2 = 1 - \frac{\frac{SS_{residual}}{DF_{residual}}}{\frac{SS_{model} + SS_{residual}}{DF_{model} + DF_{residual}}} \qquad \text{(Equation 6.2)}$$

Pred-R^2 measures amount of variation in new data explained by model.

$$PredR^2 = 1 - \frac{PRESS}{SS_{total} - SS_{block}} \qquad \text{(Equation 6.3)}$$

Adequate precision (*Adeq Precision*) is a signal to noise ratio. It compares the range of predicted value at the design points to the average prediction error.

$$Adeq\,\mathrm{P}\,recision = \frac{pd^2}{n}$$ (Equation 6.4)

Where p is number of model parameters including intercept (b0), d is residual mean square (MS) from ANOVA table and n is number of experiments.

6.2.1.4. Incorporation efficiency

Microparticles containing 10 mg of drug were weighed accurately and dissolved in methanol. Drug concentration was determined by UV spectrophotometry (Shimadzu, UV visible spectrophotometer 1700). A calibration curve was used, based on standard solutions in methanol. To determine the incorporation efficiency, the following practical relationship was used:

$$(\%)Incorporation\ efficiency = \frac{Calculateddrug\ concentration}{Theoratical\ drug\ concentration} \cdot 100$$ (Equation 6.5)

6.2.1.5. Particle size analysis

The average particle diameter and size distribution of microparticles were determined by using Malvern particle size analyser (Mastersizer 2000 Malvern Instruments, UK). Approximately 10mg of microparticles were dispersed in 2–3 ml of filtered and degaussed phosphate buffer pH 6.8 containing 0.1% Tween 80 for one minute using an ultrasonic bath. An aliquot of the microparticle suspension was then added into the small volume recirculation unit and circulated 3500 times/min. Each sample was measured in triplicate in the analysis. Particle size was expressed as the weighted mean of the volume distribution.

6.2.1.6. *In vitro* drug release

In vitro drug release study was carried out as mentioned in 3.2.1.7.

6.2.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

6.2.1.8. Optimization of responses using desirability

The multiple response method makes use of an objective function called the desirability function. It reflects the desirable ranges for each response (di). Each response is associated with its own partial desirability function. If the value of the response is optimum, its desirability equals 1, and if it is totally unacceptable, its value is zero. Thus the desirability for each response can be calculated at a given point in the experimental domain. The optimum is the point with the highest value for the desirability (Rane et al., 2007b).

The percent drug release at pH 1.2 was targeted to maximum as higher value of this was desired. Greater percent drug release at pH 1.2 leads to greater availability of ARM in stomach. Moreover, microparticles showed complete release within few min. Hence percent drug release at 15 min (t15) was selected. The values of Ymin and Ymax of percent drug release at pH 1.2 in 15 min (t15) were 62.57 and 93.39, respectively. The desirability function of this parameter has been calculated by following equation.

$$di = \left(\frac{Yi - Y\min}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 6.6)}$$

where di is the individual desirability, Yi is the experimental result and s is used to change the shape of the desirability goal by weight field.

To avoid grittiness of microparticles after ingestion in oral cavity, minimum particles size was desired. The observed Ymin and Ymax values of particle size were 44.08 and 241.84, respectively. Further the problem of bitter taste of the drug, generally encountered due to dissolution of the active component in oral cavity. In addition the microparticles remain for maximum 5 min in oral cavity. To avoid this, minimum percent drug release at 5 min was desired. The values of Ymin and Ymax of percent drug release at pH 6.8 in 5 min (t5) were 3.7 and 6.47, respectively. Similarly the lowest value of bitterness score was desired for complete taste masking. Though the observed Ymax value of bitterness score was 3, it was selected as 0.5 because no bitterness was desired. The values of Ymax and Ymin of bitterness score were 0.5 and 0, respectively. So the desirability function of

particle size, drug release at pH 6.8 and bitterness score was calculated by using following equation.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 6.7)}$$

where di is the individual desirability, Yi is the experimental result and s is used to change the shape of the desirability goal by weight field. All the experiments were performed by choosing s = 1 in equations 6.8, 6.9 and 6.10.

$$di = 1 \qquad \text{if Yi < Ymin} \qquad \text{(Equation 6.8)}$$

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{if Ymin} \leq \text{Yi} \leq \text{Ymax} \qquad \text{(Equation 6.9)}$$

$$di = 0 \qquad \text{if Yi > Ymax} \qquad \text{(Equation 6.10)}$$

The overall desirability value was calculated from the individual values by using following equation:

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \qquad \text{(Equation 6.11)}$$

Where D is overall desirability and d1, d2, d3, d4 are individual desirability values of measured responses.

6.2.1.9. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

6.2.1.10. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

6.2.1.11. Preparation of single dose suspension powder (cachets)

There might be a chance of leaching of drug after reconstitution on storage. This may result in bitterness. To avoid this problem a single dose suspension powder (cachet) was prepared. Suspension powder containing equivalent of 50mg of ARM were prepared from ARM and optimized microparticles. Xanthan gum and Avicel CL611 were used as suspending agents. Citric acid monohydrate was used as pH modifier.

The suspension powder was prepared and reconstituted as mentioned in 5.2.1.8.

6.2.1.12. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9.

6.2.1.13. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

6.2.1.14. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

6.2.1.15. Investigation of chemical stability of ARM

Chemical stability of ARM was studied as mentioned in 5.2.1.12.

6.2.1.16. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

6.2.2. Results and discussion

6.2.2.1. Experimental design

Preliminary investigations of the process parameters revealed that factors amount of drug (A) and polymer (B) highly influenced the bitterness in human volunteers, particle size and drug release at pH, 1.2 and 6.8. Hence A and B were used for further systematic studies. The dependent and

independent variables were related using mathematical relationships obtained with the statistical package, DOE v6.0.5 (Stat-Ease, Inc.). The fitted polynomial equations (full and reduced model) relating the response to the transformed factors are shown in Table 6.3. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e., positive or negative. F-value compares the variance with the residual (error) variance. The terms having *Prob > F value* more than 0.05 were omitted in reduced model (Gohel and Panchal, 2002; Shah et al., 2007). The surface plots are shown in Figure 6.1.

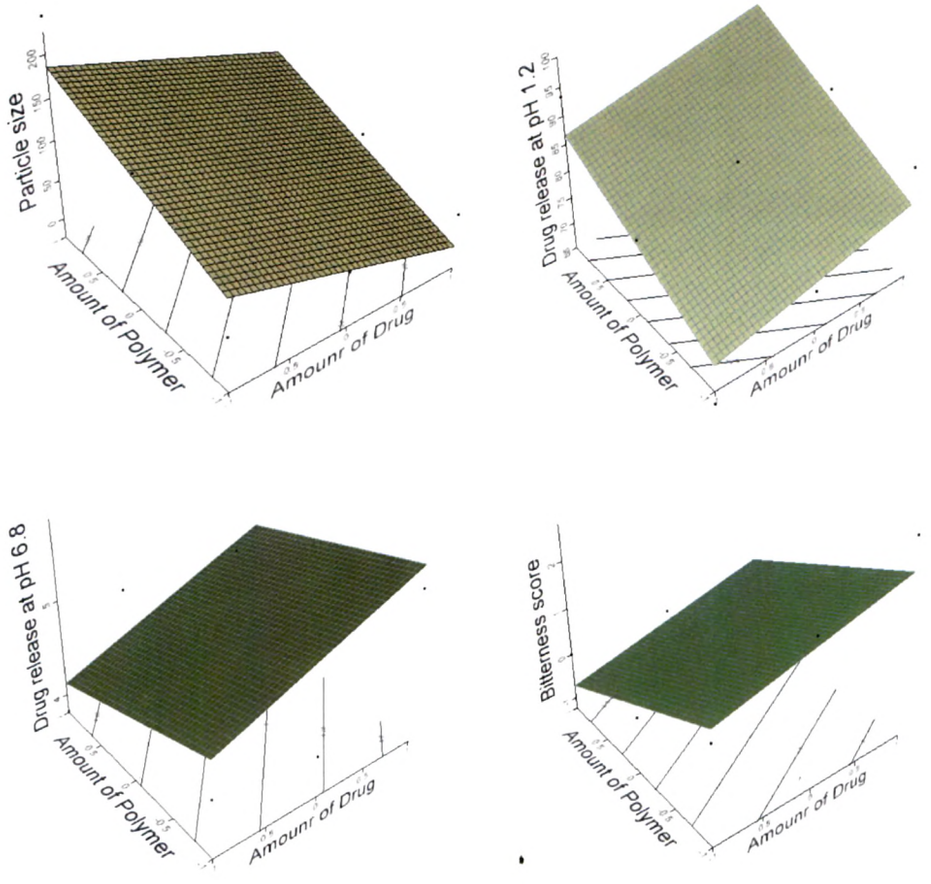

Figure 6.1. Surface plots showing the effect of amount of drug and polymer on particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 6.3. Results of regression analysis

Terms	Particle size (μm)				Drug release at pH 1.2 (t15 in %)				Drug release at pH 6.8 (t5 in %)				Bitterness Score			
	FM		RM		FM		RM		FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	91.40	NA	94.56	NA	83.93	NA	84.27	NA	5.58	NA	5.58	NA	0.22	NA	0.33	NA
A	-44.06	0.0020	-44.06	0.0003	4.51	0.0007	4.51	0.0001	0.39	0.0016	0.39	0.0009	0.50	0.0079	0.50	0.0039
B	47.06	0.0017	47.06	0.0003	8.36	0.0001	8.36	< 0.0001*	-0.52	0.0022	-0.35	0.0014	-1.00	0.0010	-1.00	0.0003
A^2	12.66	0.1695	–	–	0.50	0.4228	–	–	0.52	0.0034	0.52	0.0025	0.17	0.3081	–	–
B^2	-7.93	0.3674	–	–	-2.27	0.0246	-2.27	0.0129	-1.60	0.0001	-1.60	< 0.0001*	0.67	0.0163	0.67	0.0099
AB	-47.18	0.0030	-47.18	0.0005	-7.30	0.0003	-7.30	< 0.0001*	-0.08	0.1623	–	–	-0.50	0.0138	-0.50	0.0080

*EC, Estimated coefficient; NA, Not applicable; – indicates term is omitted in reduced model; FM, full model; RM, reduced model; t5 and t15, percent drug released at 5 and 15 min respectively; *Statistically significant ($p < 0.05$).*

Table 6.4 ANOVA results showing effect of independent variables on the measured responses

Measured Responses	Model	Sum of square (SS)	DF	Mean Square (MS)	F value	Prob > F	PRESS	R^2	Adj-R^2	Pred-R^2	Adeq Precision
Particle Size (µm)	FM	34282.99	5	6856.60	61.13	0.0032	4090.40	0.99	0.97	0.88	22.89
	RM	33836.67	3	11278.89	72.04	0.0002	2596.09	0.97	0.96	0.92	22.59
Drug release at pH 1.2 (t15 in %)	FM	765.34	5	153.07	262.40	0.0004	20.15	0.99	0.99	0.97	50.21
	RM	764.84	4	191.21	339.92	< 0.0001	16.25	0.99	0.99	0.97	56.02
Drug release at pH 6.8 (t5 in %)	FM	7.34	5	1.47	194.93	0.0006	0.26	0.99	0.99	0.96	40.42
	RM	7.31	4	1.83	151.72	0.0001	0.24	0.99	0.98	0.96	35.00
Bitterness Score	FM	9.44	5	1.89	51.00	0.0042	0.83	0.98	0.96	0.91	20.15
	RM	9.39	4	2.35	56.33	0.0009	0.47	0.98	0.96	0.95	20.81

**ANOVA indicates analysis of variance; *statistically significant ($p < 0.05$); df - degrees of freedom; SS - sum of squares; MS - mean of squares; F - Fischer's ratio; R^2 - regression coefficient; FM - full model; RM - reduced model; t5 and t15 – percent drug released at 5 and 15 min respectively.*

Multiple linear regression analysis (Table 6.3) revealed that A^2 and B^2 terms were insignificant for particle size while A^2 term was insignificant for bitterness score and dissolution at pH 1.2. The term AB was insignificant for drug release at pH 6.8.

Table 6.4 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors (Bolton and Charles, 2004). High values of correlation coefficient (R^2) for all dependent variables indicate a good fit.

PRESS values for all formulation showed good fit of model. Adj-R^2 and Pred-R^2 values were in reasonable agreement, signifying good model fit. Further models, full model (FM) and reduced model (RM), showed *Adeq precision value* greater than 4, indicating adequate model discrimination.

6.2.2.2. Incorporation efficiency

Incorporation efficiency is important factor in the evaluation of the quality of the microparticles. The incorporation efficiency varied for all batches, showed in Table 6.2. The high content of ARM in the microparticles was believed to be due to poor solubility of ARM in EE solution. Incorporation efficacy improves with increase in polymer (Satturwar et al., 2002). This suggests that the present method is suitable for the preparation of microparticles of a poorly water-soluble drug, such as ARM.

6.2.2.3. Particle size

For particle size, the amount of ARM (A) is negative while amount of EE (B) is positive. This indicates that on increasing the amount of EE, particle size increases. It was observed that the polymer viscosity influenced particle size (Satturwar et al., 2002). Increasing the amount of EE has led to an increase in its viscosity and consequently a decrease in the frequency of dissociation or separation of the particles with the addition of sodium hydroxide. This resulted in an increase in the overall size of the microparticles.

6.2.2.4. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of ARM (A) and EE (B) are positive. This indicates additive effect of amount of ARM and EE. This suggests that the ARM release would be improved at acidic pH, resulting in improved availability of ARM in stomach. ARM release from microparticles was completed within few minute, followed by a plateau. This may be because of the high porosity of the microparticles, the hydrophilic nature of EE, and improved wettability, provided by the dissolved EE (Mingshi et al., 2004; Rao et al., 2003; Valizadeh et al., 2004).

For *in vitro* drug release at pH 6.8, the amount of ARM (A) is positive while amount of EE (B) is negative. This indicates that on increasing the amount of EE, drug release from microparticles decreases. As the amount of EE increased, thicker film was formed around the ARM particles, which retarded the ARM release, because of being insoluble at salivary pH (Sinha and Rachna, 2002). EE is expected to behave as insoluble and inert material at pH 6.8 and showed decreased drug release. This is due to the decrease in drug diffusion and/or membrane infiltration (Rao et al., 2003; Valizadeh et al., 2004). Figure 6.2 shows dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8.

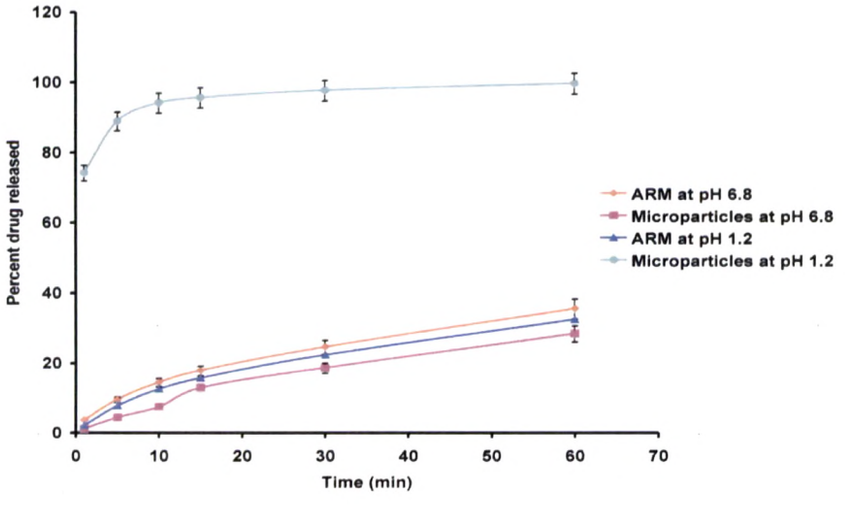

Figure 6.2. Dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8

6.2.2.5. Gustatory sensation test

For bitterness score, the amount of ARM (A) is positive while amount of EE (B) is negative. This indicates that on increasing amount of EE, bitterness score of microparticles decreases. This finding is in agreement with *in vitro* drug release study carried out at pH 6.8, because the pH of the saliva is 6.8 (Hashimoto et al., 2002). It has been reported that the bitter drug like ARM seems to bind G-protein coupled receptors, present on the apical taste cell membrane and produce bitterness (Yamamoto et al., 1998). EE is expected to behave as insoluble at pH 6.8 and showed decreased drug release in microparticles. Thus EE forms physical barrier between ARM and G-protein coupled receptors present on the apical taste cell membrane and reduce bitterness score of ARM in microparticles. Bitterness score of optimized microparticles is shown in Table 6.5.

6.2.2.6. Optimization using desirability function

Any process can only be authenticated when optimum level of its variables (affecting the process) for a product of good quality characteristics is recognized. Desirability function is one excellent tool for identifying the optimum levels of variables. In this procedure, all the measured responses for independent variables which are supposed to affect the quality of the product are taken into consideration. Particle size, drug release at pH 6.8 and bitterness score had to be minimized while drug release at pH 1.2 had to be maximized in order to pour desired characteristics in the product.

Table 6.5. Optimum levels of independent variables and their responses

Actual values of independent variables for optimized batch		Incorporation efficiency (%) ± SD*	Particle size (μm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug release at pH 6.8 (t5 in %) ± SD*	Bitterness score	Overall Desirability
A in g	B in mL#						
0.04	15	82.93 ± 1.32	85.89 ± 1.53	89.42 ± 1.27	4.17 ± 0.72	0	0.88

*A - amount of ARM, B - amount of EE, #mL of 1%w/v EE solution, *Values represent the mean ± SD of 3 experiments, t5 and t15 - percent drug dissolved in 5 and 15 min, respectively.*

Using the desirability function, all the measured responses were combined to get one overall response i.e., the overall desirability. The overall desirability response was calculated from the individual desirability of each of the responses using DOE v6.0.5 (Stat-Ease, Inc.). The optimized batch was identified with a desirability value of 0.88. Table 6.5 enlists the optimized values for independent variables and their responses.

6.2.2.7. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of ARM, EE, blank microparticles and optimized microparticles are shown in Figure 6.3. The characteristic peaks of ARM at 2873 cm^{-1} are assigned to C-H stretching vibration in CH_3, CH_2. In addition, the absorption peak at 2844 cm^{-1} can be assigned to C-H stretching vibration in C-O-CH_3. The peak at 1137 cm^{-1} can be assigned to C-O stretching vibration in C-O-C. The peaks at 2953 and 2916 cm^{-1} are assigned to C-H stretching in -CH_3. The spectrum of EE is dominated by the carbonyl (C=O) stretching vibration at 1735 cm^{-1} and the ester C-O stretching vibrations at 1148 and 1188 cm^{-1}. In addition, C-H vibrations can be discerned at 1389, 1450-1490 and 2962 cm^{-1}. The absorptions at 2772 and 2822 cm^{-1} can be assigned to the dimethyl-amino groups.

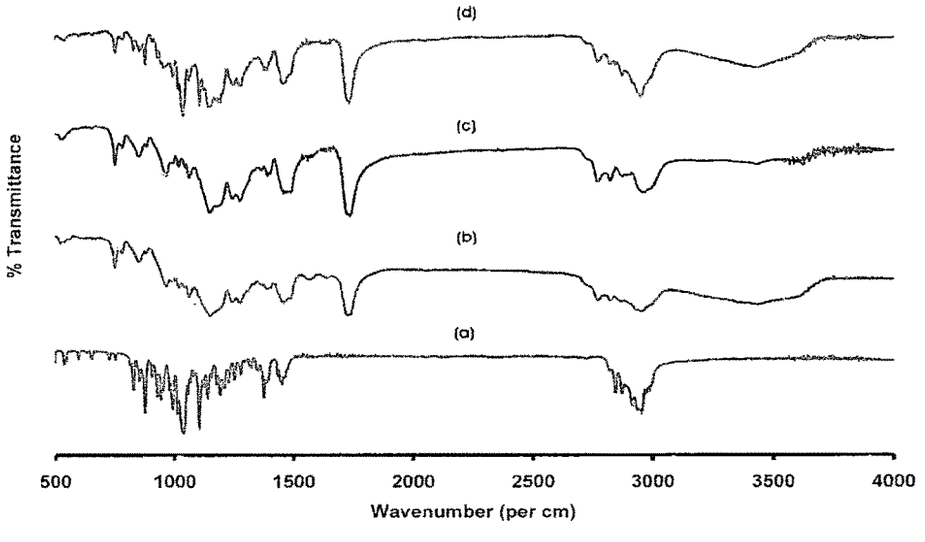

Figure 6.3. FT-IR spectra of (a) ARM, (b) EE, (c) blank microparticles and (d) optimized microparticles

The spectrum of microparticles corresponds to the superimposition of ARM and EE with no significant shift in the major peaks. This confirms presence of ARM in microparticles.

6.2.2.8. Differential scanning calorimeter (DSC)

Figure 6.4 shows the DSC curve of ARM, EE, blank microparticles and optimized microparticles. The pure ARM shows an endothermic peak at 87.94^{0}C, followed by exothermic peak at 180.28^{0}C. The endothermic peak corresponding to melting peak of ARM was broadened and shifted towards lower temperature, with reduced intensity in the microparticles. This could be attributed to higher polymer concentration and uniform distribution of drug in crust of polymer, resulting complete miscibility of molten drug in polymer.

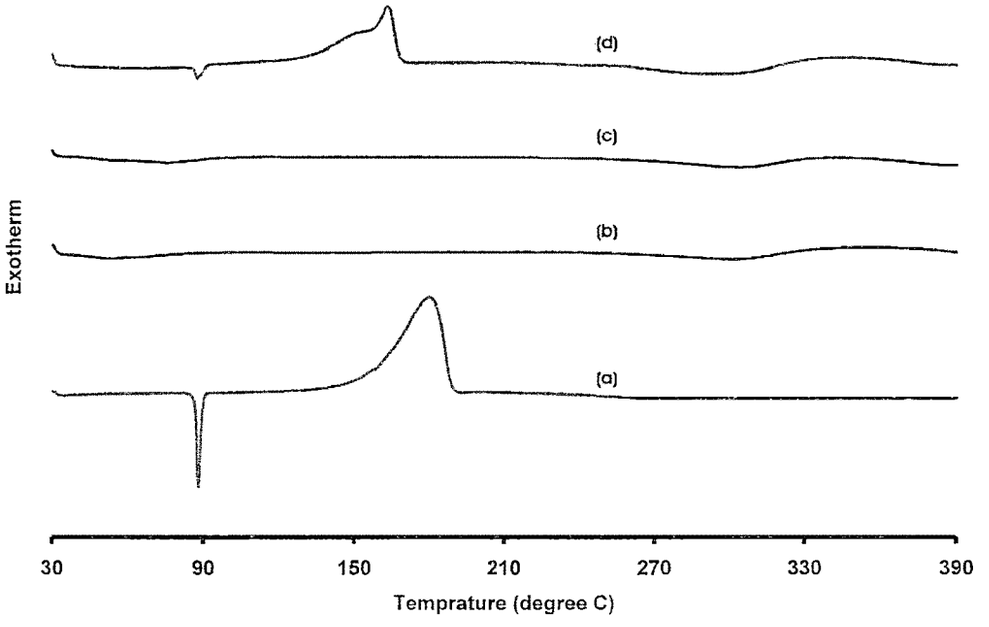

Figure 6.4. DSC curve of (a) ARM, (b) EE, (c) blank microparticles and (d) optimized microparticles

6.2.2.9. Preparation of single dose suspension powder (cachets)

Optimized microparticles batch, based on bitterness score, was selected to formulate single dose suspension powder (cachets). The formula of different

suspension powders prepared is summarized in Table 6.6. The formula of optimized suspension powder (DS34) was further used to prepare suspension powder of plain ARM (DS35). The characteristics of suspension powder are summarized in Table 6.7.

Table 6.6. Formulation of suspension powder

Drug/Excipients	For 6 cachets				
	DS31	DS32	DS33	DS34	DS35
ARM (g)	-	-	-	-	0.300
Microparticles eq. to 0.050 g ARM (g)	1.718	1.718	1.718	1.718	-
Xanthan gum (g)	0.004	0.006	0.009	0.011	0.011
Microcrystalline cellulose (Avicel PH 302) (g)	0.357	0.355	0.352	0.350	1.768
Citric acid (g)	0.015	0.015	0.015	0.015	0.015
Methyl paraben (g)	0.004	0.004	0.004	0.004	0.004
Propyl paraben (g)	0.001	0.001	0.001	0.001	0.001
Sunset yellow FCF (g)	0.001	0.001	0.001	0.001	0.001
Total filled weight per 6 cachets (g)	2.100	2.100	2.100	2.100	2.100

Table 6.7. Physical properties of suspension powder

Parameters	DS31	DS32	DS33	DS34	DS35
Angle of repose (^{0}) ± SD*	30.25 ± 0.53	29.80 ± 0.48	29.40 ± 0.62	31.69 ± 0.41	29.25 ± 0.37
F value (after reconstitution) ± SD*	0.22 ± 0.09	0.47 ± 0.07	0.79 ± 0.08	0.94 ± 0.04	0.95 ± 0.04
pH (after reconstitution)	4.6-4.7	4.6-4.7	4.5-4.6	4.6-4.7	4.5-4.7

**Values represent the mean ± SD of 3 experiments.*

6.2.2.10. Preliminary stability test on suspension powder after reconstitution

As ARM is known to be sensitive to chemical instability, a stability test was performed with reconstituted suspension at room temperature for the whole period of administration, which is at least 6 days, but a longer period is preferable. Practically, stability was followed over a period of 8 days. Figure 6.5 represents the chemical stability results of ARM in reconstituted suspension. The degradation curves of ARM in reconstituted suspension (Figure 6.5) at room temperature, suggest a 'lag'-time of 4 days. After that period, ARM is degrading.

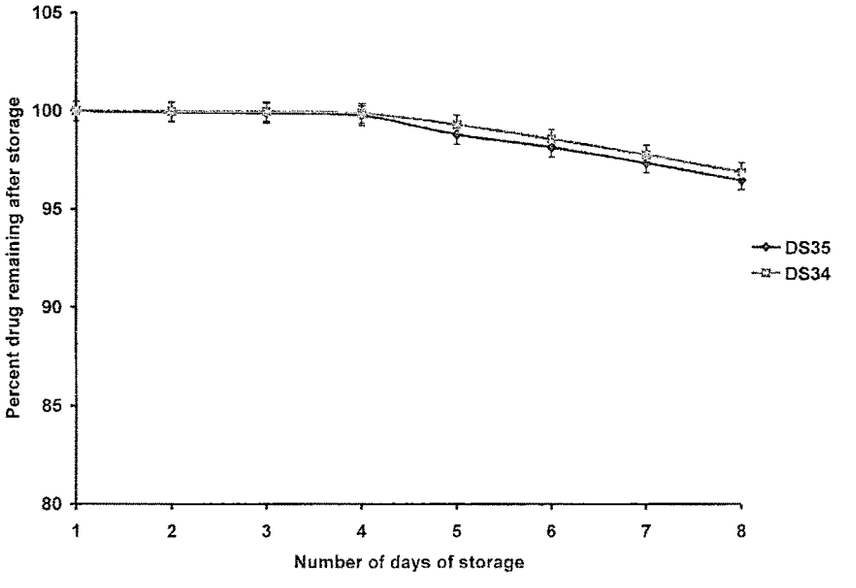

Figure 6.5. Degradation of ARM in DS34 and DS35 after reconstitution as a function of time

6.2.2.11. Gustatory sensation test for suspension powder

The cachets prepared using ARM and the taste masked microparticles of ARM were subjected to taste evaluation by the same panel of twenty selected volunteers. For DS35, the 5% of panel rated it as very strongly bitter, 85% strongly bitter and 10% moderate to strong bitter. DS34 was rated as tasteless by 100% of volunteers of panel (Table 6.8).

Table 6.8. Bitterness score evaluation by a panel of twenty human volunteers

Formulation	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
Optimized Taste masked microparticles	19	1						
DS35						2	17	1
DS34	20							

6.2.2.12. Stability studies

Evaluation of the shelf life was carried out as per ICH Q1E, step 4 (Evaluation of stability data) guidelines for drug substances intended for room temperature storage. The accelerated stability data of DS34 showed little change over time, and so a shelf life up to 1540.87 days (51.36 months) can be proposed. The extrapolation to change with time is to determine the time at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion (not more than 5% change in assay from initial value).

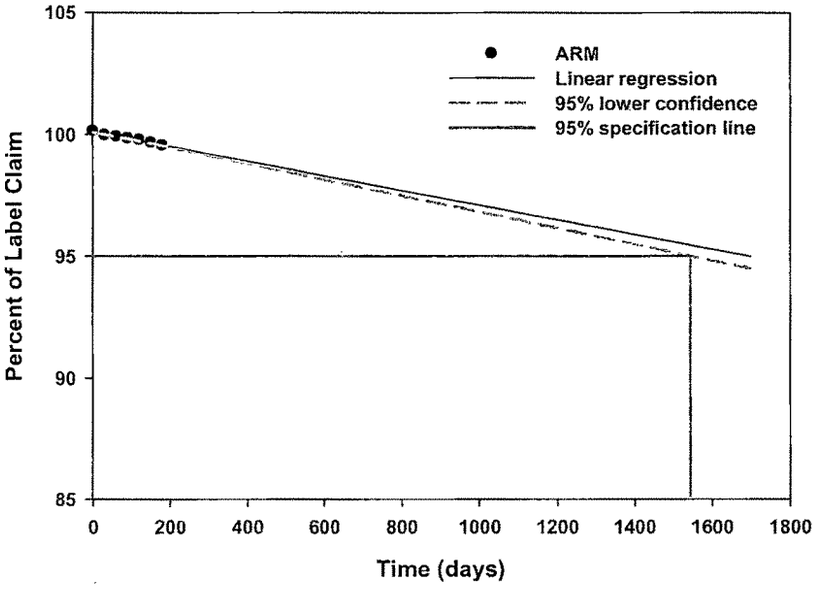

Figure 6.6. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of ARM in microparticles using EE as polymer. Present work suggests that both variables have its own significant complimentary role in enhancement of the process rather than having exclusive effect. Application of experimental design along with desirability function can be proved as an ideal tool to optimize independent variables like amount of ARM and EE, which have significant effect on microparticles's desired properties. The FTIR and DSC studies indicated uniform dispersion of ARM, at the molecular level, in EE microparticles. These taste masked microparticles were further incorporated into cachets to avoid rupturing of microparticles on compression and leaching of drug after reconstitution on storage.

6.3. Mefloquine hydrochloride (MFL)

6.3.1. Experimental

6.3.1.1. Preparation of Microparticles

A concentrated solution of EE (1%w/v) was prepared in 1%v/v acetic acid. The required quantity of the MFL (0.6 g in 50 mL of 1%w/v EE solution) was mixed for 5 min. 10 mL of 10%w/v sodium hydroxide solution was introduced into a 10-ml of glass syringe with a 18G×½" flat-cut hypodermic needle and added drop wise into EE solution. Different concentrations of MFL and EE were used as mentioned in Table 6.9. The resulting microparticles were allowed to harden for 60 min under gentle stirring (Remi Equipments Pvt. Ltd., Mumbai, India) with small magnetic bar, decanted on Buckner funnel, rinsed with the deionized double-distilled water, and dried to a constant weight in hot air oven (Shree Kailash Industries, Baroda, India) at 100^0C for 24 hours, and then stored in the desiccator until use.

Table 6.9. Process variables and their levels for 3^2 full factorial design

Coded values	Actual values	
	Amount of Drug (A in g)	Amount of Polymer (B in mL)*
-1	0.3	10
0	0.5	30
1	0.7	50

**mL of 1%w/v EE solution*

6.3.1.2. Experimental design

Experimental design was carried out as per 6.2.1.3.

The experimental runs along with their measured responses (dependent variables) are reported in Table 6.10.

Table 6.10. Experimental runs for 3^2 full factorial design with their measured responses

Batch no.	Factors and factor levels		Incorporation efficiency (%) ± SD*	Particle size (μm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug release at pH 6.8 (t5 in %) ± SD*	Bitterness score
	A	B					
MFL1	-1	1	31.17 ± 1.51	236.78 ± 2.98	99.59 ± 0.48	2.45 ± 0.52	0
MFL2	0	0	94.48 ± 1.17	81.96 ± 3.14	81.29 ± 0.37	4.46 ± 0.53	1
MFL3	0	1	59.97 ± 1.74	120.56 ± 2.72	93.11 ± 1.32	2.57 ± 0.39	0
MFL4	-1	-1	91.81 ± 0.48	32.08 ± 1.86	59.83 ± 1.41	3.84 ± 0.79	1
MFL5	1	-1	94.52 ± 0.89	34.63 ± 1.33	77.42 ± 1.68	4.36 ± 0.56	3
MFL6	-1	0	66.33 ± 0.72	131.38 ± 2.41	80.44 ± 1.39	4.35 ± 0.49	0
MFL7	1	1	57.93 ± 1.39	44.51 ± 2.51	85.15 ± 0.62	3.35 ± 1.17	0
MFL8	1	0	82.60 ± 0.94	52.24 ± 1.93	80.04 ± 1.82	5.25 ± 1.09	1
MFL9	0	-1	83.28 ± 1.73	40.17 ± 1.78	77.84 ± 0.91	3.84 ± 0.86	2

*A – amount of MFL, B – amount of EE, t5 and t15 – percent drug released in 5 and 15 min, respectively; *Values represent the mean ± SD of 3 experiments.*

6.3.1.3. Incorporation efficiency

Incorporation efficiency was carried out as mentioned in 6.2.1.4.

6.3.1.4. Particle size analysis

Particle size analysis was carried out as mentioned in 6.2.1.5

6.3.1.5. *In vitro* drug release

In vitro drug release study was carried out as mentioned in 3.2.1.7.

6.3.1.6. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

6.3.1.7. Optimization of responses using desirability

The percent drug release at pH 1.2 was targeted to maximum as higher value of this was desired. Greater percent drug release at pH 1.2 leads to greater availability of MFL in stomach. Moreover microparticles showed complete release within few min. Hence percent drug release at 15 min (t15) was selected. The values of Ymin and Ymax of percent drug release at pH 1.2 in 15 min (t15) were 59.83 and 99.59, respectively. The desirability function of this parameter has been calculated by following equation.

$$di = \left(\frac{Yi - Y\min}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 6.12)}$$

where di is the individual desirability, Yi is the experimental result and s is used to change the shape of the desirability goal by weight field.

To avoid grittiness of microparticles after ingestion in oral cavity, minimum particles size was desired. The observed Ymin and Ymax values of particle size were 32.08 and 236.78, respectively. Further the problem of bitter taste of the drug, generally encountered due to dissolution of the active component in oral cavity. In addition the microparticles remain for maximum 5 min in oral cavity. To avoid this, minimum percent drug release at 5 min was

desired. The values of Ymin and Ymax of percent drug release at pH 6.8 in 5 min (t5) were 2.45 and 5.25, respectively. Similarly the lowest value of bitterness score was desired for complete taste masking. Though the observed Ymax value of bitterness score was 3, it was selected as 0.5 because no bitterness was desired. The values of Ymax and Ymin of bitterness score were 0.5 and 0, respectively. So the desirability function of particle size, drug release at pH 6.8 and bitterness score was calculated by using following equation.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 6.13)}$$

where di is the individual desirability, Yi is the experimental result and s is used to change the shape of the desirability goal by weight field. All the experiments were performed by choosing s = 1 in equations 6.14, 6.15 and 6.16.

$$di = 1 \qquad \text{if Yi} < \text{Ymin} \qquad \text{(Equation 6.14)}$$

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{if Ymin} \leq \text{Yi} \leq \text{Ymax} \qquad \text{(Equation 6.15)}$$

$$di = 0 \qquad \text{if Yi} > \text{Ymax} \qquad \text{(Equation 6.16)}$$

The overall desirability value was calculated from the individual values by using following equation:

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \qquad \text{(Equation 6.17)}$$

Where D is overall desirability and d1, d2, d3, d4 are individual desirability values of measured responses.

6.3.1.8. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

6.3.1.9. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

6.3.1.10. Preparation of single dose suspension powder (cachets)

Suspension powder containing equivalent of 100mg of MFL were prepared from MFL and optimized microparticles. Sodium carboxy methyl cellulose (HVP) was used as suspending agent. Citric acid monohydrate was used as pH modifier.

The suspension powder was prepared and reconstituted as mentioned in 5.2.1.8.

6.3.1.11. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9.

6.3.1.12. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

6.3.1.13. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

6.3.1.14. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

6.3.2. Results and discussion

6.3.2.1. Experimental design

Preliminary investigations of the process parameters revealed that factors, amount of drug (A) and polymer (B), highly influenced the bitterness in human volunteers, particle size and drug release at pH, 1.2 and 6.8. Hence A and B were used for further systematic studies. The dependent and independent variables were related using mathematical relationships obtained with the statistical package, DOE v6.0.5 (Stat-Ease, Inc.). The fitted

polynomial equations (full and reduced model) relating the response to the transformed factors are shown in Table 6.11. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e., positive or negative. F-value compares the variance with the residual (error) variance. The terms having *Prob > F value* more than 0.05 were omitted in reduced model (Gohel and Panchal, 2002; Shah et al., 2007). The surface plots are shown in Figure 6.7.

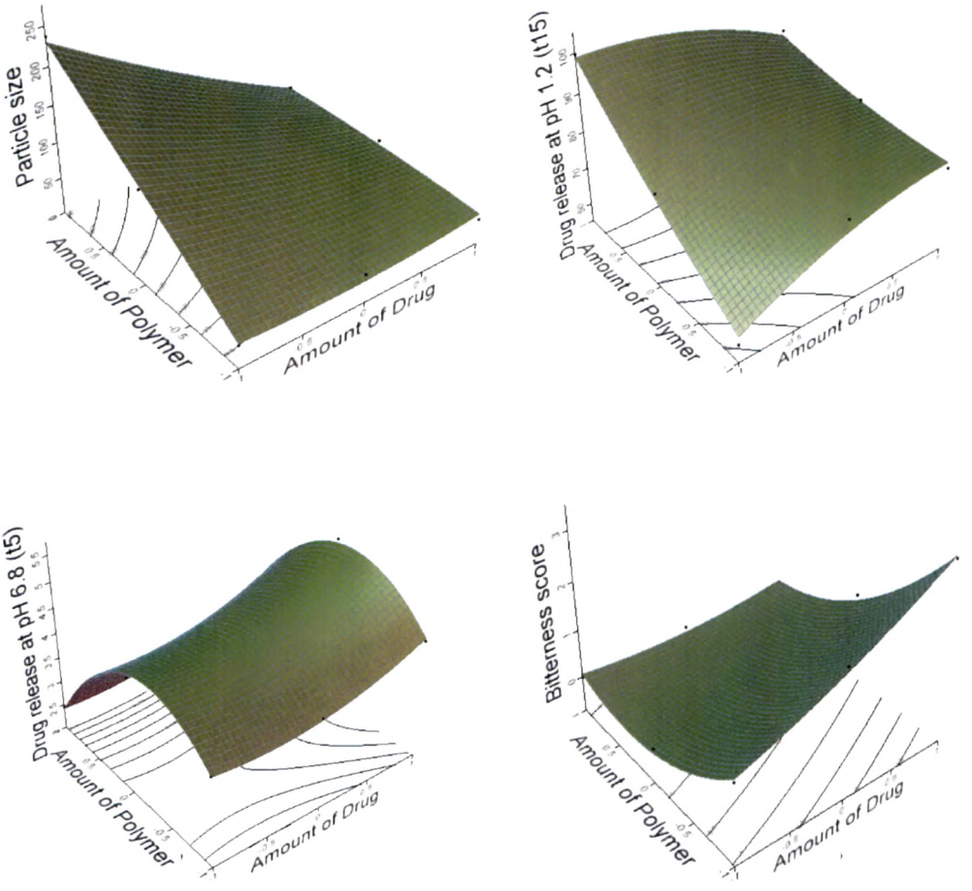

Figure 6.7. Surface plots showing the effect of amount of drug and polymer on particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 6.11. Results of regression analysis

Terms	Particle size (μm)				Drug release at pH 1.2 (t15 in %)				Drug release at pH 6.8 (t5 in %)				Bitterness Score			
	FM		RM		FM		RM		FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	83.39	NA	83.39	NA	83.04	NA	81.63	NA	4.48	NA	4.48	NA	0.22	NA	0.33	NA
A	-44.81	0.0018	-44.81	< 0.0001*	0.46	0.7541	0.46	0.7662	0.39	0.0009	0.39	0.0011	0.50	0.0079	0.50	0.0039
B	49.16	0.0014	49.16	< 0.0001*	10.46	0.0043	10.46	0.0008	-0.61	0.0002	-0.61	0.0002	-1.00	0.0010	-1.00	0.0003
A^2	7.71	0.3740	–	–	-3.67	0.2109	–	–	0.31	0.0081	0.31	0.00176	0.17	0.3081	–	–
B^2	-3.74	0.6480	–	–	1.57	0.5467	–	–	-1.28	0.0001	-1.28	< 0.0001	0.67	0.0163	0.67	0.0099
AB	-48.71	0.0030	-48.71	0.0002	-8.01	0.0163	-8.01	0.0065	0.095	0.0721	–	–	-0.50	0.0138	-0.50	0.0080

*EC, Estimated coefficient; NA, Not applicable; – indicates term is omitted in reduced model; FM, full model; RM, reduced model; t5 and t15, percent drug released at 5 and 15 min, respectively; *Statistically significant ($p < 0.05$).*

Table 6.12 ANOVA results showing effect of independent variables on the measured responses

Measured Responses	Model	Sum of square (SS)	DF	Mean Square (MS)	F value	Prob > F	PRESS	R^2	Adj-R^2	Pred-R^2	Adeq Precision
Particle Size (μm)	FM	36184.28	5	7236.86	66.16	0.0029	3979.0	0.99	0.97	0.89	23.36
	RM	36037.54	3	12012.51	126.47	0.0001	1880.91	0.98	0.97	0.94	30.12
Drug release at pH 1.2 (t15 in %)	FM	1032.07	5	206.41	99.38	0.0016	71.66	0.99	0.98	0.93	32.80
	RM	1022.98	3	340.99	111.29	0.0001	56.84	0.98	0.97	0.94	33.07
Drug release at pH 6.8 (t5 in %)	FM	6.67	5	1.33	274.85	0.0003	0.17	0.99	0.99	0.97	48.59
	RM	6.64	3	1.66	130.98	0.0002	0.26	0.99	0.98	0.96	31.82
Bitterness Score	FM	9.44	5	1.89	51.00	0.0042	0.83	0.98	0.96	0.91	20.15
	RM	9.39	4	2.35	56.33	0.0009	0.47	0.98	0.96	0.95	20.81

**ANOVA indicates analysis of variance; Statistically significant ($p < 0.05$); df - degrees of freedom; SS - sum of squares; MS - mean of squares; F - Fischer's ratio; R^2 - regression coefficient; FM - full model; RM - reduced model; t5 and t15 – percent drug released at 5 and 15 min respectively.*

Multiple linear regression analysis (Table 6.11) revealed that A^2 and B^2 terms were insignificant for particle size and drug release at pH 1.2 while AB term was insignificant for drug release at pH 6.8. A^2 term was insignificant for bitterness score.

Table 6.12 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors (Bolton and Charles, 2004). High values of correlation coefficient (R^2) for all dependent variables indicate a good fit.

PRESS values for all formulation showed good fit of model. Adj-R^2 and Pred-R^2 values were in reasonable agreement, signifying good model fit. Further models, full model (FM) and reduced model (RM), showed *Adeq precision value* greater than 4, indicating adequate model discrimination.

6.3.2.2. Incorporation efficiency

Incorporation efficiency is important factor in the evaluation of the quality of the microparticles. The incorporation efficiency varied for all batches, showed in Table 6.10. Incorporation efficiency improves with increase in polymer (Satturwar et al., 2002). However higher quantity of EE solution, prepared in 1% acetic acid, showed solubilization of MFL. This resulted in decreased incorporation efficiency (Rama Rao et al., 2005). This finding suggests that the present method is suitable for the preparation of microparticles of a slightly water-soluble drug, such as MFL.

6.3.2.3. Particle size

For particle size, the amount of MFL (A) is negative while amount of EE (B) is positive. This indicates that on increasing the amount of EE, particle size increases. It was observed that the polymer viscosity influenced particle size (Satturwar et al., 2002). Increasing the amount of EE has led to an increase in its viscosity and consequently a decrease in the frequency of dissociation or separation of the particles with the addition of sodium hydroxide. This resulted in an increase in the overall size of the microparticles.

6.3.2.4. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of MFL (A) and EE (B) are positive. This indicates additive effect of amount of MFL and EE. This suggests that the MFL release would be improved at acidic pH, resulting in improved availability of MFL in stomach. MFL release from microparticles was completed within few minute, followed by a plateau. This may be because of the high porosity of the microparticles, the hydrophilic nature of EE, and improved wettability, provided by the dissolved EE (Mingshi et al., 2004; Rao et al., 2003; Valizadeh et al., 2004).

For *in vitro* drug release at pH 6.8, the amount of MFL (A) is positive while amount of EE (B) is negative. This indicates that on increasing the amount of EE, drug release from microparticles decreases. As the amount of EE increased, thicker film was formed around the MFL particles, which retarded the MFL release, because of being insoluble at salivary pH (Sinha and Rachna, 2002). EE is expected to behave as insoluble and inert material at pH 6.8 and showed decreased drug release. This is due to the decrease in drug diffusion and/or membrane infiltration (Rao et al., 2003; Valizadeh et al., 2004). Figure 6.8 shows dissolution profile of MFL and optimized microparticles at pH, 1.2 and 6.8.

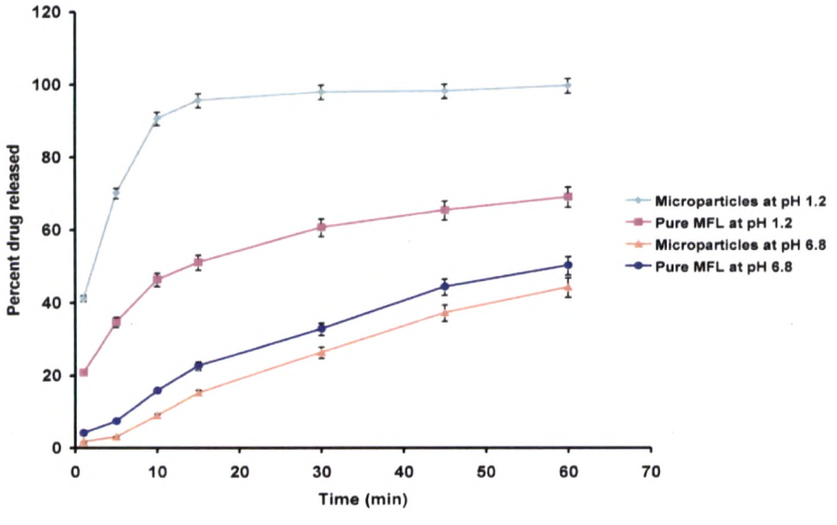

Figure 6.8. Dissolution profile of MFL and optimized microparticles at pH, 1.2 and 6.8

6.3.2.5. Gustatory sensation test

For bitterness score, the amount of MFL is positive (A) while amount of EE (B) is negative. This indicates that on increasing amount of EE, bitterness score of microparticles decreases. This finding is in agreement with *in vitro* drug release study carried out at pH 6.8, because the pH of the saliva is 6.8 (Hashimoto et al., 2002). It has been reported that the quinine moiety of MFL is responsible for the higher bitterness score. In addition MFL produces bitterness by depolarizing taste cells though K^+ channels (Yamamoto et al., 1998). The microparticles are insoluble at salivary pH and forms physical barrier between the MFL and K^+ channel present in the cell membrane of taste buds. Thus reducing the bitterness score of MFL in microparticles. Bitterness score of optimized microparticles is shown in Table 6.13.

6.3.2.6. Optimization using desirability function

Any process can only be authenticated when optimum level of its variables (affecting the process) for microparticles of best quality characteristics is recognized. Desirability function is one excellent tool for identifying the optimum levels of variables. In this procedure, all the measured responses for independent variables which are supposed to affect the quality of the microparticles are taken into consideration. Particle size, drug release at pH 6.8 and bitterness score have to be minimized while drug release at pH 1.2 has to be maximized in order to pour desired characteristics in the product.

Table 6.13. Optimum levels of independent variables and their responses

Actual values of independent variables for optimized batch		Incorporation efficiency (%)± SD*	Particle size (μm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug release at pH 6.8 (t5 in %) ± SD*	Bitterness score	Overall Desirability
A in g	B in mL#						
0.6	50	40.73 ± 1.37	81.61 ± 1.29	90.05 ± 0.78	2.92 ± 0.53	0	0.83

*A – amount of MFL, B – amount of EE, #mL of 1%w/v EE solution, *Values represent the mean ± SD of 3 experiments, t5 and t15 – percent drug dissolved in 5 and 15 min, respectively.*

Using the desirability function, all the dependant variables were combined to get one overall response i.e., the overall desirability. The overall desirability response was calculated from the individual desirability of each of the responses using DOE v6.0.5 (Stat-Ease, Inc.). The optimized batch was identified with a desirability value of 0.83. Table 6.13 enlists the optimized values for independent variables and their responses.

6.3.2.7. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of MFL, EE, blank microparticles and optimized microparticles are shown in Figure 6.9. The characteristic peaks of MFL at 3110 cm^{-1} are assigned to N-H stretching vibration. In addition, the absorption peaks at 1603, 1363, 1111, and 1069 cm^{-1} can be assigned to quinine ring stretching vibration.

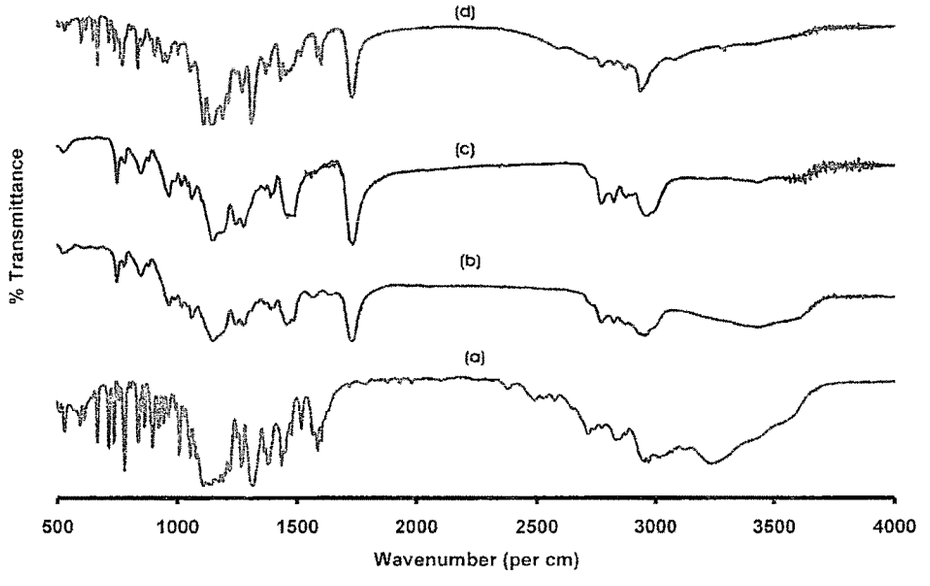

Figure 6.9. FT-IR spectra of (a) MFL, (b) EE, (c) blank microparticles and (d) optimized microparticles

The peak at 1316 cm^{-1} can be assigned to CF_3 stretching vibration. The peaks at 2875 and 2918 cm^{-1} are assigned to C-H bridge and CH_2 respectively. The peak at 1555 cm^{-1} is assigned to C=N/C=C. The peaks at 1288 and 1055 cm^{-1} are assigned to C-N and piperidine ring respectively. The peak at 1174 cm^{-1} is

due to the C-C/N-H stretching vibration. The spectrum of EE is dominated by the carbonyl (C=O) stretching vibration at 1735 cm^{-1} and the ester C-O stretching vibrations at 1148 and 1188 cm^{-1}. In addition, C-H vibrations can be discerned at 1389, 1450 - 1490 and 2962 cm^{-1}. The absorptions at 2772 and 2822 cm^{-1} can be assigned to the dimethyl-amino groups. The spectrum of microparticles corresponds to the superimposition of MFL and EE with no significant shift in the major peaks. This confirms presence of MFL in microparticles.

6.3.2.8. Differential scanning calorimeter (DSC)

Figure 6.10 shows the DSC curve of MFL, EE, blank microparticles and optimized microparticles. The pure MFL shows an endothermic peak at 271.38^{0}C, followed by exothermic peak at 308.36^{0}C. The characteristic endothermic peak corresponding to melting peak of MFL was broadened and shifted towards lower temperature (164.53^{0}C), with reduced intensity in the microparticles, suggesting phase transition during microencapsulation process.

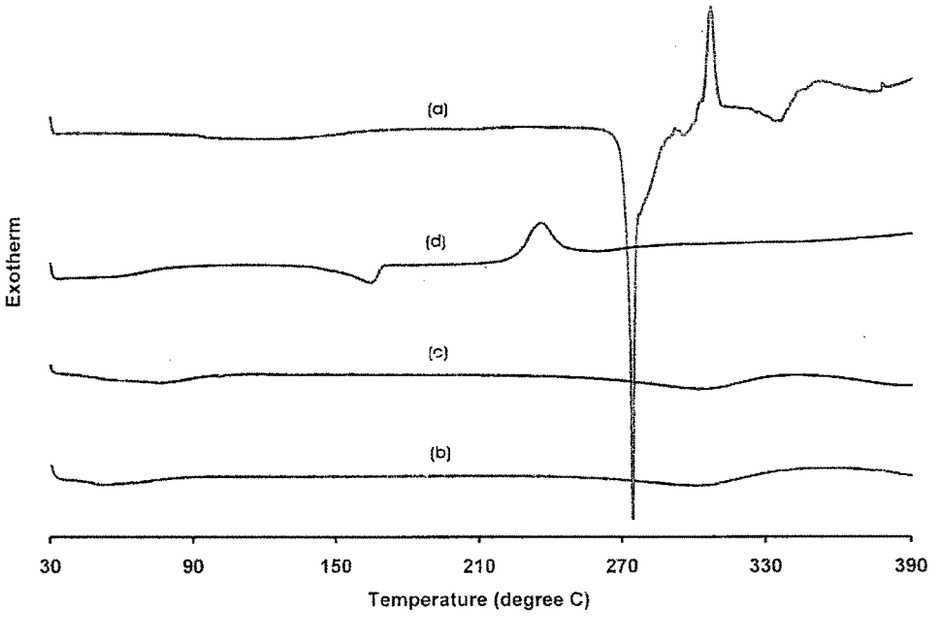

Figure 6.10. DSC curve of (a) MFL, (b) EE, (c) blank microparticles and (d) optimized microparticles

6.3.2.9. Preparation of single dose suspension powder (cachets)

Optimized microparticles batch, based on bitterness score, was selected to formulate single dose suspension powder (cachets). The formula of different suspension powders prepared is summarized in Table 6.14. The formula of optimized suspension powder (DS44) was further used to prepare suspension powder of plain MFL (DS45). The characteristics of suspension powder are summarized in Table 6.15.

Table 6.14. Formulation of suspension powder

Drug/Excipients	For 6 cachets				
	DS41	DS42	DS43	DS44	DS45
MFL (g)	-	-	-	-	0.600
Microparticles eq. to 100 mg MFL (g)	2.700	2.700	2.700	2.700	-
Sodium carboxy methyl cellulose (g)	0.003	0.006	0.009	0.012	0.012
Polyethylene glycol 6000 (g)	0.03	0.03	0.03	0.03	0.03
Microcrystalline cellulose (Avicel PH 302) (g)	0.239	0.236	0.233	0.230	2.330
Citric acid (g)	0.021	0.021	0.021	0.021	0.021
Methyl paraben (g)	0.006	0.006	0.006	0.006	0.006
Propyl paraben (g)	0.001	0.001	0.001	0.001	0.001
Total filled weight per 6 cachets (g)	3.000	3.000	3.000	3.000	3.000

Table 6.15. Physical properties of suspension powder

Parameters	DS41	DS42	DS43	DS44	DS45
Angle of repose (^{0}) ± SD*	32.47 ± 0.24	33.12 ± 0.34	32.61 ± 0.39	32.29 ± 0.71	34.76 ± 0.53
F value (after reconstitution) ± SD*	0.26 ± 0.05	0.39 ± 0.03	0.78 ± 0.04	0.97 ± 0.03	0.98 ± 0.01
pH (after reconstitution)	4.4-4.5	4.5-4.6	4.5-4.6	4.4-4.5	4.4-4.5

**Values represent the mean ± SD of 3 experiments.*

6.3.2.10. Gustatory sensation test for suspension powder

The cachets prepared using MFL and the taste masked microparticles of MFL was subjected to taste evaluation by the same panel of twenty selected volunteers. For DS45, the 10% of panel rated it as very strongly bitter, 90% strongly bitter. DS44 was rated as tasteless by 100% of volunteers of panel (Table 6.16).

Table 6.16. Bitterness score evaluation by a panel of twenty human volunteers

Formulation	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
Optimized Taste masked microparticles	19	1						
DS45							18	2
DS44	20							

6.3.2.11. Stability studies

Evaluation of the shelf life was carried out as per ICH Q1E, step 4 (Evaluation of stability data) guidelines for drug substances intended for room temperature storage. The accelerated stability data of DS44 showed little change over time, and so a shelf life up to 1399.75 days (46.65 months) can be proposed. The extrapolation to change with time is to determine the time at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion (not more than 5% change in assay from initial value).

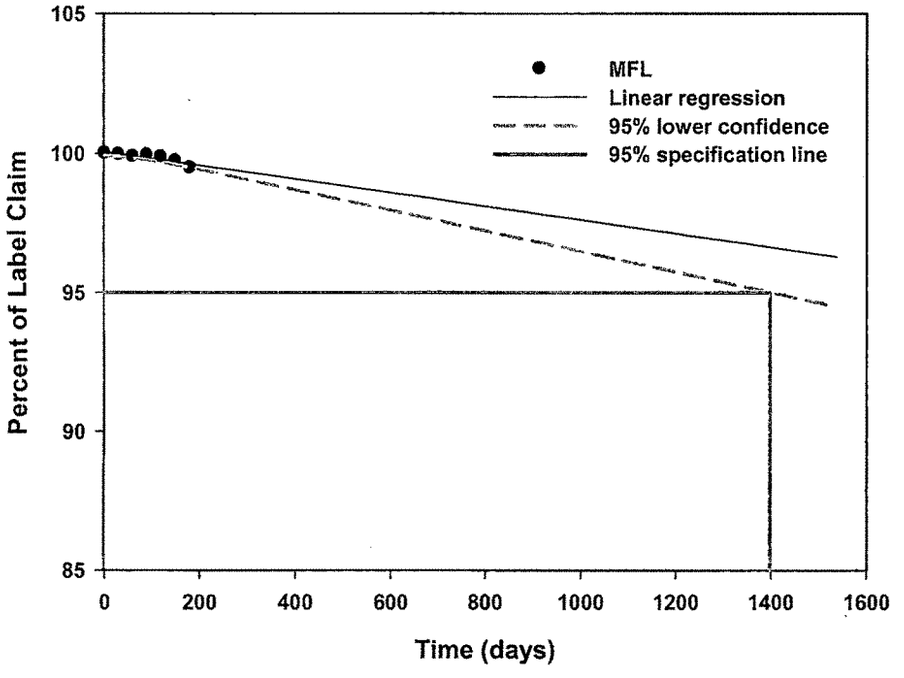

Figure 6.11. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of MFL in microparticles using EE as polymer. Present work suggests that both independent variables have its own significant complimentary role in enhancement of the process rather than having exclusive effect. Application of experimental design along with desirability function can be proved as an ideal tool to optimize independent variables like amount of MFL and EE, which have significant effect on microparticles's desired properties. The FTIR and DSC studies indicated uniform dispersion of MFL, at the molecular level, in EE microparticles. These taste masked microparticles were further incorporated into cachets to avoid rupturing of microparticles on compression and leaching of drug after reconstitution on storage.

6.4. Primaquine Phosphate (PRM)

6.4.1. Experimental

6.4.1.1. Preparation of Microparticles

A concentrated solution of EE (1%w/v) was prepared in 1%v/v acetic acid. The required quantity of the PRM (0.2 g in 50 mL of 1%w/v EE solution) was mixed for 5 min. 10 mL of 10%w/v sodium hydroxide solution was introduced into a 10-ml of glass syringe with a 18G×½" flat-cut hypodermic needle and added drop wise into EE solution. Different concentrations of PRM and EE were used as mentioned in Table 6.17. The resulting microparticles were allowed to harden for 60 min under gentle stirring (Remi Equipments Pvt. Ltd., Mumbai, India) with small magnetic bar, decanted on Buckner funnel, rinsed with the deionized double-distilled water, and dried to a constant weight in hot air oven (Shree Kailash Industries, Baroda, India) at 100^0C for 24 hours, and then stored in the desiccator until use.

Table 6.17. Process variables and their levels for 3^2 full factorial design

Coded values	Actual values	
	Amount of Drug (A in g)	Amount of Polymer (B in mL)*
-1	0.3	10
0	0.5	30
1	0.7	50

**mL of 1%w/v EE solution*

6.4.1.2. Experimental design

Experimental design was carried out as per 6.2.1.3.

The experimental runs along with their measured responses (dependent variables) are reported in Table 6.18.

Table 6.18. Experimental runs for 3^2 full factorial design with their measured responses

Batch no.	Factors and factor levels		Incorporation efficiency (%) ± SD*	Particle size (μm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug release at pH 6.8 (t5 in %) ± SD*	Bitterness score
	A	B					
PRM1	-1	1	29.16 ± 1.24	181.49 ±1.37	93.87 ± 1.81	5.53 ± 1.23	0
PRM2	0	0	25.33 ± 1.53	64.92 ± 1.56	91.64 ± 1.72	7.19 ± 1.15	1.00
PRM3	0	1	18.53 ± 1.32	98.32 ± 1.18	90.29 ± 1.39	5.22 ± 1.62	0
PRM4	-1	-1	30.42 ± 0.97	23.89 ± 1.63	81.63 ± 1.45	6.79 ± 1.27	1.00
PRM5	1	-1	34.17 ± 1.28	34.82 ± 1.14	91.83 ± 1.69	7.61 ± 1.34	3.00
PRM6	-1	0	33.26 ± 1.36	104.46 ± 1.08	90.45 ± 1.92	7.43 ± 1.46	0
PRM7	1	1	30.93 ± 1.21	36.41 ± 1.59	89.71 ± 1.19	6.78 ± 1.52	0
PRM8	1	0	36.79 ± 1.39	41.38 ± 1.47	95.15 ± 1.61	8.69 ± 1.26	1.00
PRM9	0	-1	22.83 ± 1.32	22.36 ± 1.28	85.61 ± 1.52	6.42 ± 1.64	2.00

*A – amount of PRM, B – amount of EE; t5 and t15 – percent drug released in 5 and 15 min, respectively; *Values represent the mean ± SD of 3 experiments.*

6.4.1.3. Incorporation efficiency

Incorporation efficiency was carried out as mentioned in 6.2.1.4.

6.4.1.4. Particle size analysis

Particle size analysis was carried out as mentioned in 6.2.1.5

6.4.1.5. *In vitro* drug release

In vitro drug release study was carried out as mentioned in 3.2.1.7.

6.4.1.6. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

6.4.1.7. Optimization of responses using desirability

The percent drug release at pH 1.2 was targeted to maximum as higher value of this was desired. Greater percent drug release at pH 1.2 leads to greater availability of PRM in stomach. Moreover microparticles showed maximum drug release within 15. Hence percent drug release at 15 min (t15) was selected. The values of Ymin and Ymax of percent drug release at pH 1.2 in 15 min (t15) were 81.63 and 95.15, respectively. The desirability function of this parameter has been calculated by following equation.

$$di = \left(\frac{Yi - Y\min}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 6.18)}$$

where di is the individual desirability, Yi is the experimental result and s is used to change the shape of the desirability goal by weight field.

To avoid grittiness of microparticles after ingestion in oral cavity, minimum particles size was desired. The observed Ymin and Ymax values of particle size were 22.36 and 181.49, respectively. However to have good flow property, Ymin was consider as 80. Further the problem of bitter taste of the drug, generally encountered due to dissolution of the active component in oral cavity. In addition the microparticles remain for maximum 5 min in oral cavity. To avoid this, minimum percent drug release at 5 min was desired. The values of Ymin and Ymax of percent drug release at pH 6.8 in 5 min (t5) were 5.22 and 8.69, respectively. Similarly the lowest value of bitterness score was desired for complete taste masking. Though the observed Ymax value of bitterness score was 3, it was selected as 0.5 because no bitterness to very slightly bitterness was desired. The values of Ymax and Ymin of bitterness score were 0.5 and 0, respectively. So the desirability function of

particle size, drug release at pH 6.8 and bitterness score was calculated by using following equation.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 6.19)}$$

where di is the individual desirability, Yi is the experimental result and s is used to change the shape of the desirability goal by weight field. All the experiments were performed by choosing s = 1 in equations 6.20, 6.21 and 6.22.

$$di = 1 \qquad \text{if Yi < Ymin} \qquad \text{(Equation 6.20)}$$

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{if Ymin} \leq \text{Yi} \leq \text{Ymax} \qquad \text{(Equation 6.21)}$$

$$di = 0 \qquad \text{if Yi > Ymax} \qquad \text{(Equation 6.22)}$$

The overall desirability value was calculated from the individual values by using following equation:

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \qquad \text{(Equation 6.23)}$$

Where D is overall desirability and d1, d2, d3, d4 are individual desirability values of measured responses.

6.4.1.8. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

6.4.1.9. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

6.4.1.10. Preparation of single dose suspension powder (cachets)

Suspension powder containing equivalent of 13.16 mg of PRM (equivalent to 7.5 mg primaquine base) were prepared from PRM and optimized microparticles. Sodium carboxy methyl cellulose (HVP) was used as suspending agent. Citric acid monohydrate was used as pH modifier.

The suspension powder was prepared and reconstituted as mentioned in 5.2.1.8.

6.4.1.11. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9.

6.4.1.12. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

6.4.1.13. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

6.4.1.14. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

6.4.2. Results and discussion

6.4.2.1. Experimental design

Preliminary investigations of the process parameters revealed that factors amount of drug (A) and polymer (B), highly influenced the bitterness in human volunteers, particle size, drug release at pH, 1.2 and 6.8. Hence A and B were used for further systematic studies. The dependent and independent variables were related using mathematical relationships obtained with the statistical package, DOE v6.0.5 (Stat-Ease, Inc.). The fitted polynomial equations (full and reduced model) relating the response to the transformed factors are shown in Table 6.19. The polynomial equations can be used to draw conclusions after considering the magnitude of coefficient and the mathematical sign it carries, i.e., positive or negative. F-value compares the

variance with the residual (error) variance. The terms having *Prob > F value* more than 0.05 were omitted in reduced model (Gohel and Panchal, 2002; Shah et al., 2007).

Multiple linear regression analysis (Table 6.19) revealed that B^2 term was insignificant for particle size. A^2 term was insignificant for drug release at pH 1.2 while AB term was insignificant for drug release at pH 6.8. A^2 and B^2 terms were insignificant for bitterness score. The surface plots are shown in Figure 6.12.

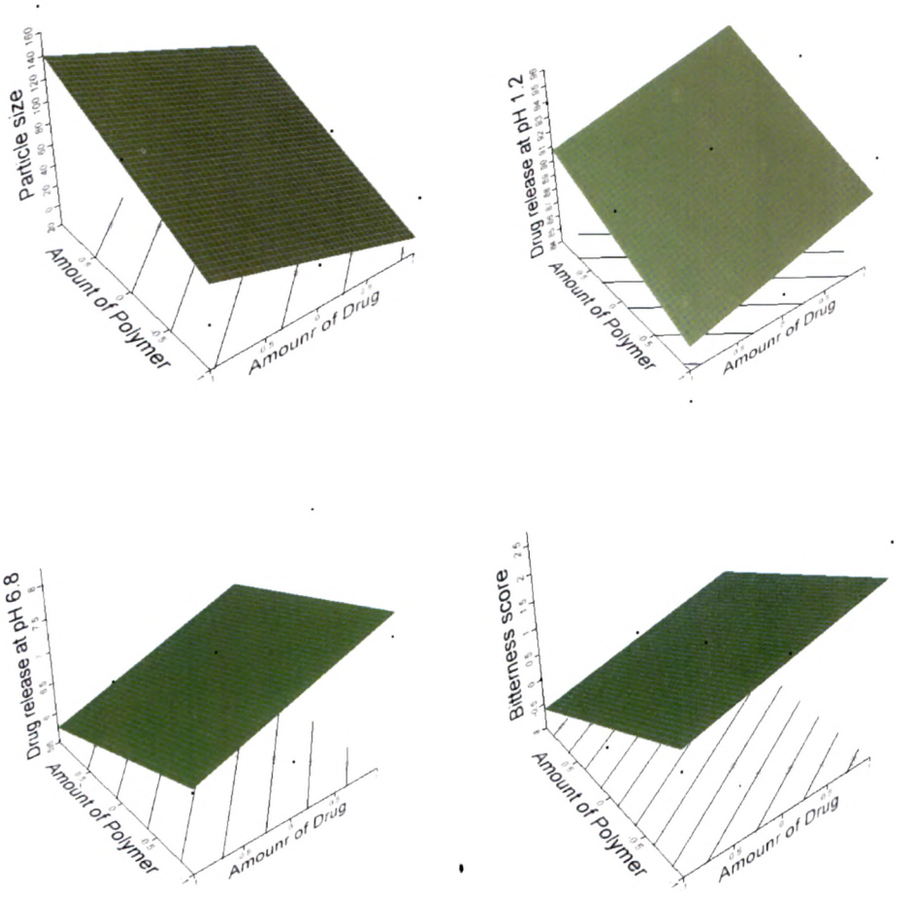

Figure 6.12. Surface plots showing the effect of amount of drug and polymer on particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 6.19. Results of regression analysis

Terms	Particle size (μm)				Drug release at pH 1.2 (t15 in %)				Drug release at pH 6.8 (t5 in %)				Bitterness Score			
	FM		RM		FM		RM		FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	64.56	NA	61.87	NA	91.57	NA	92.41	NA	7.20	NA	7.20	NA	0.78	NA	0.89	NA
A	-32.87	< 0.0001*	-32.87	< 0.0001*	1.79	0.0047	1.79	0.0127	0.56	0.0007	0.56	0.0005	0.50	0.0079	0.50	0.0071
B	39.19	< 0.0001*	39.19	< 0.0001*	2.47	0.0019	2.47	0.0041	-0.55	0.0007	-0.55	0.0005	-1.00	0.0010	-1.00	0.0003
A^2	8.54	0.0070	8.54	0.0070	1.26	0.0538	–	–	0.86	0.0009	0.86	0.0008	-0.17	0.3081	–	–
B^2	-4.04	0.0523	–	–	-3.59	0.0031	-3.59	0.0077	-1.38	0.0002	-1.38	0.0001	0.33	0.0917	–	–
AB	-39.00	< 0.0001*	-39.00	< 0.0001*	-3.59	0.0011	-3.59	0.0022	0.11	0.0996	–	–	-0.50	0.0138	-0.50	0.00158

*EC, Estimated coefficient; NA, Not applicable; – indicates term is omitted in reduced model; FM, full model; RM, reduced model; t5 and t15, percent drug released at 5 and 15 min, respectively; *Statistically significant ($p < 0.05$).*

Table 6.20 ANOVA results showing effect of independent variables on the measured responses

Measured Responses	Model	Sum of square (SS)	DF	Mean Square (MS)	F value	Prob > F	PRESS	R^2	Adj-R^2	Pred-R^2	Adeq Precision
Particle Size (µm)	FM	21962.52	5	4392.50	1315.41	< 0.0001	120.74	0.99	0.99	0.99	106.43
	RM	21929.90	4	5482.47	514.37	< 0.0001	240.53	0.99	0.99	0.98	65.25
Drug release at pH 1.2 (t15 in %)	FM	136.24	5	27.25	81.83	0.0021	12.13	0.99	0.98	0.91	28.07
	RM	133.06	4	33.26	31.88	0.0027	26.03	0.96	0.93	0.81	17.37
Drug release at pH 6.8 (t5 in %)	FM	8.98	5	1.80	216.00	0.0005	0.30	0.99	0.99	0.96	44.89
	RM	8.94	4	2.23	125.55	0.0002	0.36	0.99	0.98	0.96	33.62
Bitterness Score	FM	8.78	5	1.76	47.40	0.0047	0.83	0.98	0.96	0.90	19.09
	RM	8.50	3	2.83	36.43	0.0008	1.17	0.95	0.93	0.86	16.13

**ANOVA indicates analysis of variance; Statistically significant ($p < 0.05$); df - degrees of freedom; SS - sum of squares; MS - mean of squares; F - Fischer's ratio; R^2 - regression coefficient; FM - full model; RM - reduced model; t5 and t15 – percent drug released at 5 and 15 min respectively.*

Table 6.20 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors (Bolton and Charles, 2004). High values of correlation coefficient (R^2) for all dependent variables indicate a good fit.

PRESS values for all formulation showed good fit of model. Adj-R^2 and Pred-R^2 values were in reasonable agreement, signifying good model fit. Further models, full model (FM) and reduced model (RM), showed *Adeq precision value* greater than 4, indicating adequate model discrimination.

6.4.2.2. Incorporation efficiency

Incorporation efficiency is important factor in the evaluation of the quality of the microparticles. The incorporation efficiency varied for all batches, showed in Table 6.18. Incorporation efficiency improves with increase in polymer (Satturwar et al., 2002). However higher quantity of EE solution showed solubilization of PRM. This resulted in decreased incorporation efficiency (Rama Rao et al., 2005). This finding suggests that the present method is suitable for the preparation of microparticles of a water-soluble drug, such as PRM.

6.4.2.3. Particle size

For particle size, the amount of PRM (A) is negative while amount of EE (B) is positive. This indicates that on increasing the amount of EE, particle size increases. It was observed that the polymer viscosity influenced particle size (Satturwar et al., 2002). Increasing the amount of EE has led to an increase in its viscosity and consequently a decrease in the frequency of dissociation or separation of the particles with the addition of sodium hydroxide. This resulted in an increase in the overall size of the microparticles.

6.4.2.4. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of PRM (A) and EE (B) are positive. This indicates additive effect of amount of PRM and EE. This suggests that the PRM release would be improved at acidic pH, resulting in improved availability of PRM in stomach. PRM release from microparticles was

completed within few minute, followed by a plateau. This may be because of the high porosity of the microparticles, the hydrophilic nature of EE, and improved wettability, provided by the dissolved EE (Mingshi et al., 2004; Rao et al., 2003; Valizadeh et al., 2004).

For *in vitro* drug release at pH 6.8, the amount of PRM (A) is positive while amount of EE (B) is negative. This indicates that on increasing the amount of EE, drug release from microparticles decreases. As the amount of EE increased, thicker film was formed around the PRM particles, which retarded the PRM release, because of being insoluble at salivary pH (Sinha and Rachna, 2002). EE is expected to behave as insoluble and inert material at pH 6.8 and showed decreased drug release. This is due to the decrease in drug diffusion and/or membrane infiltration (Rao et al., 2003; Valizadeh et al., 2004). Figure 6.13 shows dissolution profile of PRM and optimized microparticles at pH, 1.2 and 6.8.

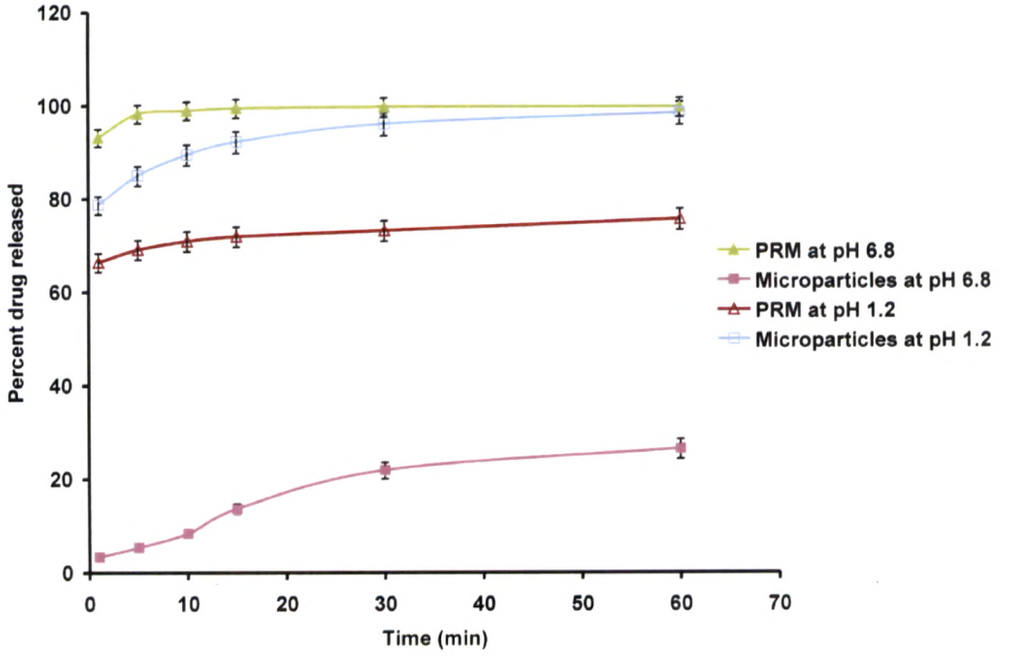

Figure 6.13. Dissolution profile of PRM and optimized microparticles at pH, 1.2 and 6.8

6.4.2.5. Gustatory sensation test

For bitterness score, the amount of PRM (A) is positive while amount of EE (B) is negative. This indicates that on increasing amount of EE, bitterness score of microparticles decreases. This finding is in agreement with *in vitro* drug release study carried out at pH 6.8, because the pH of the saliva is 6.8 (Hashimoto et al., 2002). It has been reported that the quinine moiety of PRM is responsible for the higher bitterness score. In addition PRM produces bitterness by depolarizing taste cells through K^+ channels (Yamamoto et al., 1998). The microparticles are insoluble at salivary pH and forms physical barrier between the PRM and K^+ channel present in the cell membrane of taste buds. Thus reducing the bitterness score of PRM in microparticles. Bitterness score of optimized microparticles is shown in Table 6.21.

6.4.2.6. Optimization using desirability function

Any process can only be authenticated when optimum level of its variables (affecting the process) for microparticles of best quality characteristics is recognized. Desirability function is one excellent tool for identifying the optimum levels of variables. In this procedure, all the measured responses for independent variables which are supposed to affect the quality of the microparticles are taken into consideration. Particle size, drug release at pH 6.8 and bitterness score have to be minimized while drug release at pH 1.2 has to be maximized in order to pour desired characteristics in the product. Using the desirability function, all the dependant variables were combined to get one overall response i.e., the overall desirability.

Table 6.21. Optimum levels of independent variables and their responses

Actual values of independent variables for optimized batch		Incorporation efficiency (%)± SD*	Particle size (µm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug release at pH 6.8 (t5 in %) ± SD*	Bitterness score	Overall Desirability
A in g	B in mL#						
0.2	50	24.49 ± 1.34	81.76 ± 1.64	92.16 ± 1.23	5.31 ± 0.16	0	0.82

*A – amount of PRM, B – amount of EE, #mL of 1%w/v EE solution, *Values represent the mean ± SD of 3 experiments, t5 and t15 – percent drug dissolved in 5 and 15 min, respectively.*

The overall desirability response was calculated from the individual desirability of each of the responses using DOE v6.0.5 (Stat-Ease, Inc.). The optimized batch was identified with a desirability value of 0.82. Table 6.21 enlists the optimized values for independent variables and their responses.

6.4.2.7. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of PRM, EE, blank microparticles and optimized microparticles are shown in Figure 6.14. The characteristic peaks of PRM at 2968 and 2878 cm^{-1} are assigned to C-H stretching vibration in CH_3, CH_2. In addition, the absorption peak at 2844 cm^{-1} can be assigned to C-H stretching vibration in C-O-CH_3. The peak at 1119 cm^{-1} can be assigned to C-O stretching vibration in C-O-C. The peak at 3305 cm^{-1} can be assigned to N-H stretching in primary amines. The spectrum of microparticles corresponds to the superimposition of PRM and EE with no significant shift in the major peaks. This confirms presence of PRM in microparticles.

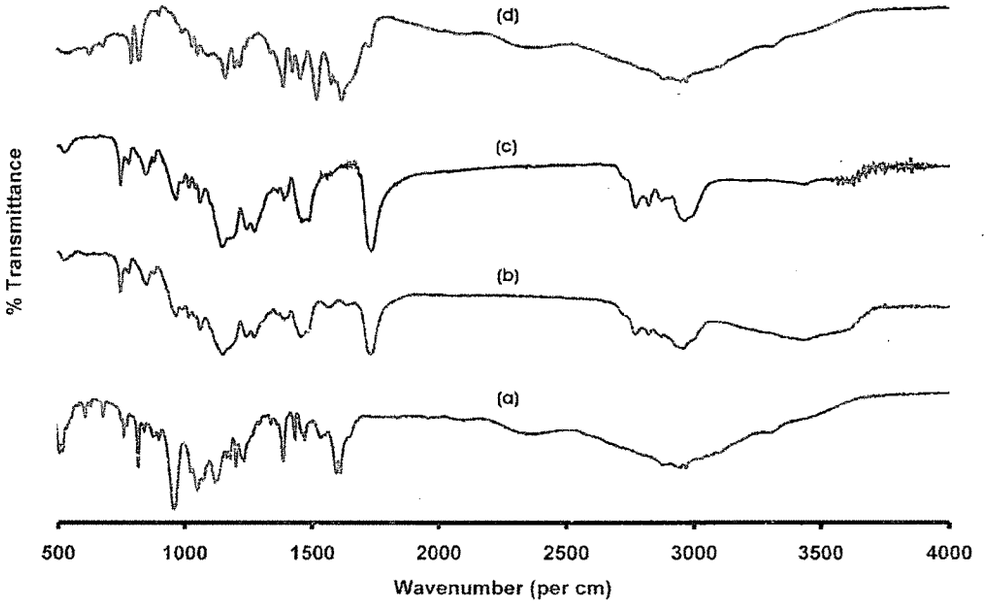

Figure 6.14. FT-IR spectra of (a) PRM, (b) EE, (c) blank microparticles and (d) optimized microparticles

6.4.2.8. Differential scanning calorimeter (DSC)

Figure 6.15 shows the DSC curve of PRM, EE, blank microparticles and optimized microparticles. The pure PRM shows an endothermic peak at 202.68^{0}C. The endothermic peak corresponding to melting peak of PRM was not observed in the microparticles. This could be attributed to higher polymer concentration and uniform distribution of drug in crust of polymer, resulting complete miscibility of molten drug in polymer.

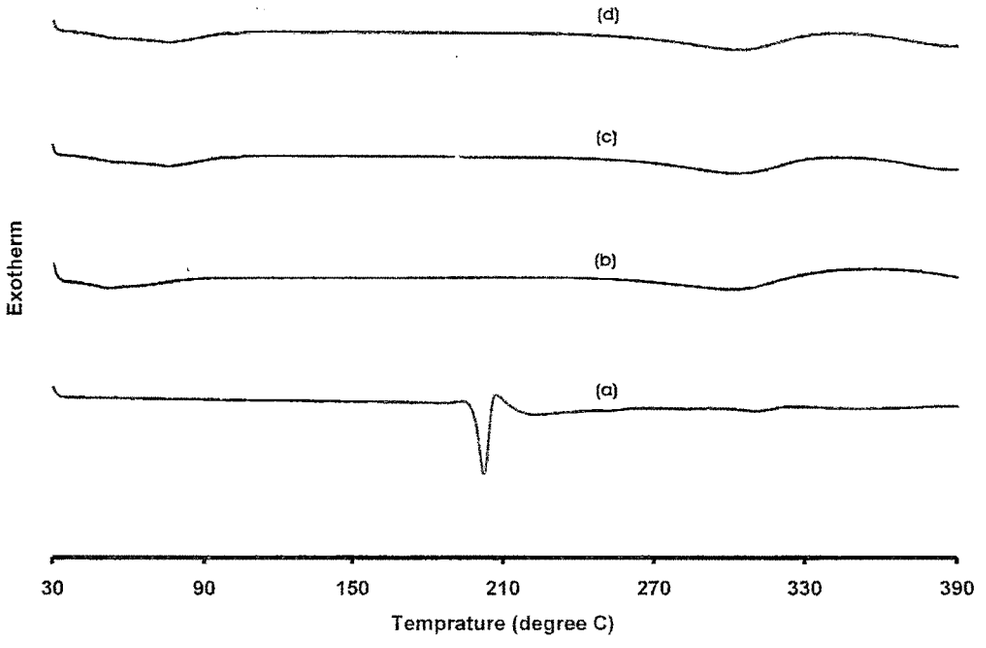

Figure 6.15. DSC curve of (a) PRM, (b) EE, (c) blank microparticles and (d) optimized microparticles

6.4.2.9. Preparation of single dose suspension powder (cachet)

Optimized microparticles batch, based on bitterness score, was selected to formulate single dose suspension powder (cachets). The formula of different suspension powders prepared is summarized in Table 6.22. The formula of optimized suspension powder (DS53) was further used to prepare suspension powder of plain PRM (DS54). The characteristics of suspension powder are summarized in Table 6.23.

Table 6.22. Formulation of suspension powder

Drug/Excipients	For 6 cachets				
	DS51	DS52	DS53	DS54	DS55
PRM (g)	-	-	-	-	0.078
Microparticles eq. to 13.16 mg PRM (g)	0.608	0.608	0.608	0.608	-
Sodium carboxy methyl cellulose (g)	0.002	0.004	0.006	0.008	0.006
Microcrystalline cellulose (Avicel PH 302) (g)	0.282	0.280	0.278	0.276	0.809
Citric acid (g)	0.005	0.005	0.005	0.005	0.005
Methyl paraben (g)	0.002	0.002	0.002	0.002	0.002
Propyl paraben (g)	0.001	0.001	0.001	0.001	0.001
Total filled weight per 6 cachets (g)	0.900	0.900	0.900	0.900	0.900

Table 6.23. Physical properties of suspension powder

Parameters	DS51	DS52	DS53	DS54	DS55
Angle of repose (^{0}) ± SD*	29.92 ± 0.24	29.18 ± 0.34	29.42 ± 0.39	29.56 ± 0.71	31.83 ± 0.53
F value (after reconstitution) ± SD*	0.21 ± 0.07	0.42 ± 0.08	0.96 ± 0.03	Highly viscous	0.97 ± 0.02
pH (after reconstitution)	4.6-4.7	4.6-4.7	4.6-4.7	4.6-4.7	4.5-4.6

**Values represent the mean ± SD of 3 experiments.*

6.4.2.10. Gustatory sensation test for suspension powder

The cachets prepared using PRM and the taste masked microparticles of PRM was subjected to taste evaluation by the same panel of twenty selected volunteers. For DS55, the 15% of panel rated it as very strongly bitter, 85% strongly bitter. DS53 was rated as tasteless by 100% of volunteers of panel (Table 6.24).

Table 6.24. Bitterness score evaluation by a panel of twenty human volunteers

Formulation	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
Optimized Taste masked microparticles	20							
DS55							17	3
DS53	20							

6.4.2.11. Stability studies

Evaluation of the shelf life was carried out as per ICH Q1E, step 4 (Evaluation of stability data) guidelines for drug substances intended for room temperature storage. The accelerated stability data of DS53 showed little change over time, and so a shelf life up to 1416.11 days (47.20 months) can be proposed. The extrapolation to change with time is to determine the time at which 95% one-sided confidence limit for the mean curve intersects the acceptance criterion (not more than 5% change in assay from initial value).

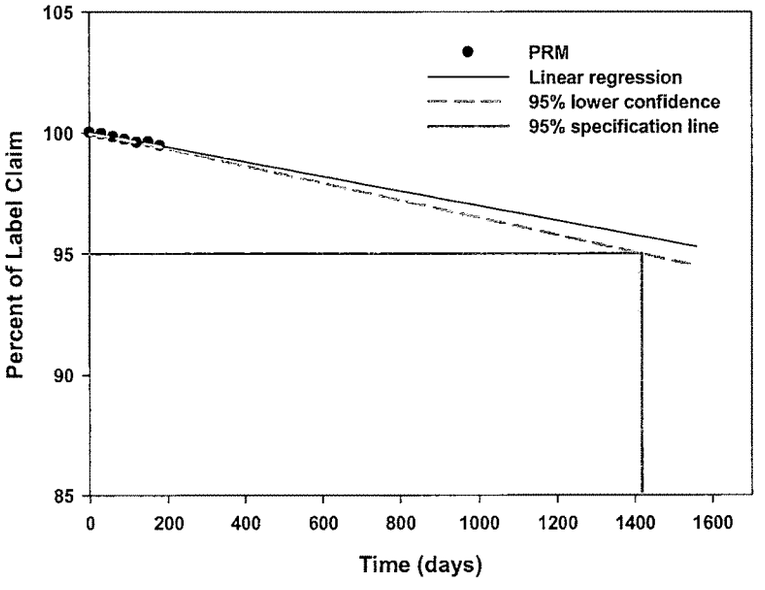

Figure 6.16. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of PRM in microparticles using EE as polymer. Present work suggests that both independent variables have its own significant complimentary role in enhancement of the process rather than having exclusive effect. Application of experimental design along with desirability function can be proved as an ideal tool to optimize independent variables like amount of PRM and EE, which have significant effect on microparticles's desired properties. The FTIR and DSC studies indicated uniform dispersion of PRM, at the molecular level, in EE microparticles. These taste masked microparticles further incorporated into cachets to avoid rupturing of microparticles on compression and leaching of drug after reconstitution on storage.

6.5. Summary

	ARM-EE	MFL-EE	PRM-EE
EE required per cachet	50 mg ARM + 236.40 mg EE	100 mg MFL + 350 mg EE	13.16 mg PRM + 88.17 mg EE
Incorporation efficiency (%) ± SD*	82.93 ± 1.32	40.73 ± 1.37	24.49 ± 1.34
Particle size (μm) ± SD*	85.89 ± 1.53	81.61 ± 1.29	81.76 ± 1.64
Drug release at pH 1.2 (t15 in %) ± SD*	89.42 ± 1.27	90.05 ± 0.78	92.16 ± 1.23
Drug release at pH 6.8 (t5 in %) ± SD*	4.17 ± 0.72	2.92 ± 0.53	5.31 ± 0.16
Bitterness score (for optimized microparticles)	0	0	0
Overall desirability	0.88	0.83	0.82
Total filled weight per cachet (g)	0.350	0.500	0.150
Angle of repose (^{0}) ± SD*	31.69 ± 0.41	32.29 ± 0.71	29.42 ± 0.39
F value (after reconstitution) ± SD*	0.94 ± 0.04	0.97 ± 0.03	0.96 ± 0.03
pH (after reconstitution)	4.6-4.7	4.4-4.5	4.6-4.7
Bitterness score (for dry suspension)	0	0	0
Shelf life (months)	51.36	46.65	47.20

** Values represent mean ± SD of 3 experiments, t5 and t15 – percent drug dissolved in 5 and 15 min, respectively.*

6.6. References

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