Microencapsulation using Chitosan

8.1. Introduction

Chitosan [poly (1,4-beta-D-glucopyranosamine)] is a polysaccharide derived from naturally occurring chitin by alkaline deacetylation. This polymer has been investigated extensively for applications in various drug delivery systems. It has appealing intrinsic characteristics that include biodegradability, biocompatibility, and non-toxicity (Sinha et al., 2004; Souza et al., 2005; Ubaidulla et al., 2007).

In addition, chitosan is practically insoluble in neutral or alkali solutions at pH above approximately 6.5. The pH inside the oral cavity has been reported to be about 6.8 (Hashimoto et al., 2002). Thus chitosan 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.

No reference to any work on the use of ionotropically cross iinked chitosan microparticles to mask the bitterness could be found in the literature. Hence this was the objective of present study.

Various crosslinking agents such as tripolyphosphate (TPP), formaldehyde, glutaraldehyde, sodium citrate and sodium hydroxide have been reported for chitosan (Desai and Park, 2005; Eroglu et al., 2007; Lu et al., 2005). Among these, sodium hydroxide is a weak crosslinking agent (Eroglu et al., 2007). 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. The objective of the present study is to reduce the drug release at pH 6.8 for 5 min using sodium hydroxide as crosslinking agent.

The taste masked microparticles might be ruptured during compression and produce bitterness. In addition, it has been reported that chitosan is more swellable when cross linked with sodium hydroxide (Eroglu et al., 2007). 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.

8.2. Artemether (ARM)

8.2.1. Experimental

8.2.1.1. Materials

Chitosan was a gift from Primax Biopolymers, (Iceland). 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.

8.2.1.2. Preparation of Microparticles

Microparticles were prepared by coacervation phase separation method. A concentrated solution of chitosan (1%w/v) was prepared in 1%v/v acetic acid. The required quantity of ARM (38 mg of ARM in 5 mL of chitosan solution) was dispersed and mixed with concentrated chitosan solution. The microparticles were prepared by dropping chitosan containing ARM from the dropping device, glass syringe with a 18G×½" flat-cut hypodermic needle to a magnetically stirred (15 mL of 10%w/v) sodium hydroxide solution. The droplets were amputated at a flow rate of 2.5 ml/min into sodium hydroxide solution. Different amount of ARM, chitosan and sodium hydroxide were used as mentioned in Table 8.1. 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.

8.2.1.3. Response Surface methodology (RSM)

Response surface designs permit to define empirical models (usually quadratic polynomials) that describe accurately how responses behave at all values of the studied variables in the experimental region (Ficarra et al., 2002). The aim of RSM is to determine conditions that provide process improvement.

In order to calculate quadratic regression model coefficients, each design variable has to be studied at three distinct levels at least and, consequently, the central composite design (CCD) is often used to provide estimation of a second-order equation. Among the standard designs applied in RSM, the CCD represents a good choice because of its high efficiency with respect to the number of runs required. A CCD for k factors consists of 2k factorial points, 2k axial or 'star' points and $n_0 \geq 2$ center points. The axial points are located at a distance, α, from the design center with a choice of $\alpha = \sqrt[4]{NF}$, where NF represents the number of factorial runs (Rekhi et al., 1999).

The key factors, selected during the optimization process, were: incorporation efficiency, particle size, drug release at pH, 1.2 and 6.8 along with bitterness score. The coded and actual values of independent variables are reported in Table 8.1.

The experimental matrixes at three factors (CCD) consists of fourteen experiments, expressed in coded variables, gives the runs expressed for dependent variables, as shown in Table 8.2. The central point was repeated six times to estimate the experimental error variance. All experiments were performed in randomized order to minimize the effects of uncontrolled factors that may introduce a bias on the measurements.

Table 8.1. Process variables and their levels for Central Composite Design (CCD)

Coded values	Actual Values		
	Amount of ARM (A in g)	**Amount of Chitosan (B in g)**	**Amount of Sod. hydroxide (C in mL*)**
-1.63	0.011	0.011	3.15
-1	0.03	0.03	5
0	0.05	0.05	10
1	0.07	0.07	15
1.63	0.114	0.114	24.45

**mL of 10%w/v sodium hydroxide solution*

Table 8.2. Central Composite Design (CCD) with the measured responses

ES	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	C					
1	1	1	-1	67.98 ± 2.83	282.33 ± 5.38	83.91 ± 2.37	6.98 ± 1.26	3
2	-1	1	1	64.15 ± 1.39	322.89 ± 4.69	79.17 ± 2.65	3.92 ± 1.74	0
3	0	0	0	82.32 ± 1.87	233.49 ± 3.29	88.64 ± 2.94	3.74 ± 1.13	1
4	0	0	0	82.83 ± 1.93	231.62 ± 4.47	88.98 ± 1.76	3.87 ± 0.72	1
5	-1	-1	-1	67.27 ± 2.66	264.43 ± 4.78	67.56 ± 2.74	4.67 ± 1.41	2
6	1	-1	1	90.65 ± 2.54	104.39 ± 3.49	73.38 ± 2.37	4.33 ± 1.53	2.5
7	-1	1	-1	52.50 ± 1.48	337.48 ± 5.13	78.79 ± 2.19	4.23 ± 1.78	1
8	0	0	0	85.32 ± 1.67	242.61 ± 3.21	87.18 ± 1.78	3.69 ± 0.64	1
9	1	1	1	82.38 ± 1.79	253.96 ± 3.29	90.57 ± 2.39	6.76 ± 1.36	2.5
10	-1	-1	1	81.81 ± 2.27	169.57 ± 4.70	63.26 ± 1.84	3.54 ± 1.32	0
11	0	0	0	85.32 ± 1.38	239.62 ± 3.16	87.73 ± 1.68	3.92 ± 0.97	1
12	1	-1	-1	74.17 ± 1.68	217.28 ± 3.19	71.21 ± 1.72	5.97 ± 1.74	3
13	-1.63	0	0	54.22 ± 1.53	303.49 ± 4.23	70.48 ± 1.84	3.79 ± 1.19	0.5
14	0	0	0	80.87 ± 2.18	239.18 ± 3.26	87.39 ± 1.59	3.91 ± 1.26	1
15	0	0	-1.63	67.59 ± 1.54	307.71 ± 3.14	74.36 ± 1.83	5.83 ± 1.29	3
16	1.63	0	0	81.34 ± 1.62	219.02 ± 4.27	82.26 ± 1.48	6.81 ± 1.54	3+
17	0	-1.63	0	85.85 ± 1.44	148.47 ± 3.56	68.66 ± 2.54	3.87 ± 1.12	2
18	0	1.63	0	66.21 ± 1.36	296.22 ± 3.53	92.35 ± 2.74	5.59 ± 1.43	1
19	0	0	1.63	91.40 ± 1.82	192.26 ± 3.61	75.38 ± 1.44	3.82 ± 0.96	0.5
20	0	0	0	82.32 ± 1.76	233.41 ± 3.74	86.59 ± 1.83	3.69 ± 0.89	1

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

Five experimental responses were studied: Y1= incorporation efficiency, Y2= particle size; Y3= drug release at pH 1.2; Y4= drug release at pH 6.8; Y5= bitterness score. A classical second-degree model was postulated for each experimental response Yi, as follows:

$$Yi = b0 + b1A + b2B + b3C + b11A^2 + b22B^2 + b33C^2 + b12AB + b23BC + B13AC$$

(Equation 8.1)

where b0 is the arithmetic mean response of the twenty runs while b1, b2 and b3 are the estimated coefficient for the factors, A, B and C. The main effects (A, B and C) represent the average result of changing one factor at a time from its low to high value. The interaction terms (AB, BC and AC) show how the response changes when 2 factors are simultaneously changed. The polynomial terms (A^2, B^2 and C^2) are included to investigate nonlinearity. All experimental results were computed by statistical software, DOE v6.0.5 (Stat-Ease, Inc.).

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*.

8.2.1.4. Incorporation efficiency

The incorporation efficiency was determined by dissolving the microparticles in magnetically stirred (500 rpm) mixed phosphate buffer (India Pharmacopoeia III, 5.04 g of disodium hydrogen phosphate and 3.01 g of potassium dihydrogen phosphate in sufficient water to produce 1000mL, pH adjusted to about 4) at room temperature for about 90 min. An aliquot of 2 mL was mixed with methanol. The resulting solution was centrifuged at 2500 rpm for 10 min (Remi Instruments Ltd, India) and the supernatant was assayed for drug using UV spectrophotometry (Shimadzu UV visible spectrophotometer 1700). To determine the incorporation efficiency, the following practical relationship was used:

$$Incorporation\ efficiency(\%) = \frac{Drug\ content}{Theoretical\ drug\ content} \times 100$$

(Equation 8.2)

8.2.1.5. Particle size analysis

The average particle diameter and size distribution of microparticles were determined by using Malvern particle size analyzer (Mastersizer 2000 Malvern Instruments, UK). Approximately 10mg of microparticles were dispersed in 2–3 ml of filtered and degassed petroleum ether for one minute using an ultrasonic bath. An aliquot of the microparticles 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.

8.2.1.6. *In vitro* drug release

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

8.2.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

8.2.1.8. 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., 2007).

The percent incorporation efficiency value was targeted to maximize in the procedure, as higher values of this was desired to reduce total weight of microparticles, equivalent to 50 mg ARM. The values of Ymin and Ymax of percent incorporation efficiency were 52.5 and 91.4, respectively. 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 pH 1.2 in 15 min (t15) was selected. The values of Ymin and Ymax of percent drug release at pH 1.2 in 15 min

(t15) were 63.26 and 92.35, respectively. The desirability function of incorporation efficiency and drug release at pH 1.2 has been calculated by following equation.

$$di = \left(\frac{Yi - Ymin}{Ymax - Ymin} \right)^{s} \qquad \text{(Equation 8.3)}$$

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 104.39 and 337.48, 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 pH 6.8 in 5 min was desired. The values of Ymin and Ymax of percent drug release at pH 6.8 in 5 min (t5) were 3.54 and 6.98, 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.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 8.4)}$$

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 7.5, 7.6 and 7.7.

$$di = 1 \qquad \text{if Yi < Ymin} \qquad \text{(Equation 8.5)}$$

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{if Ymin} \leq \text{Yi} \leq \text{Ymax} \qquad \text{(Equation 8.6)}$$

$di = 0$ if Yi > Ymax (Equation 8.7)

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

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \quad \text{(Equation 8.8)}$$

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

8.2.1.9. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

8.2.1.10. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

8.2.1.11. 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 was used as suspending agents. Citric acid monohydrate was used as pH modifier.

Cachets were prepared and reconstituted as mentioned in 5.2.1.8.

8.2.1.12. Angle of repose

Angle of repose was carried out as mentioned in 5.2.1.9.

8.2.1.13. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

8.2.1.14. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

8.2.1.15. Investigation of chemical stability of ARM

Chemical stability of ARM was studied as mentioned in 5.2.1.12.

8.2.1.16. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

8.2.2. Results and discussion

8.2.2.1. Experimental design

Preliminary investigations of the process parameters revealed that factors, amount of drug (A), polymer (B) and crosslinking agent (C), highly influenced the bitterness in human volunteers, incorporation efficiency, particle size, drug release at pH, 1.2 and 6.8. Hence A, B and C 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 8.3. 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).

Multiple linear regression analysis (Table 8.3) revealed that AC and BC terms were insignificant for incorporation efficiency, AB and AC terms were insignificant for particle size, AB term was insignificant for drug release at pH 1.2 and AC term was insignificant for drug release at pH 6.8 while AB and BC terms were insignificant for bitterness score. The surface plots are shown in Figure 8.1.

Table 8.4 shows the results of analysis of variance (ANOVA), which was performed to identify insignificant factors (Bolton and Charles, 2004).

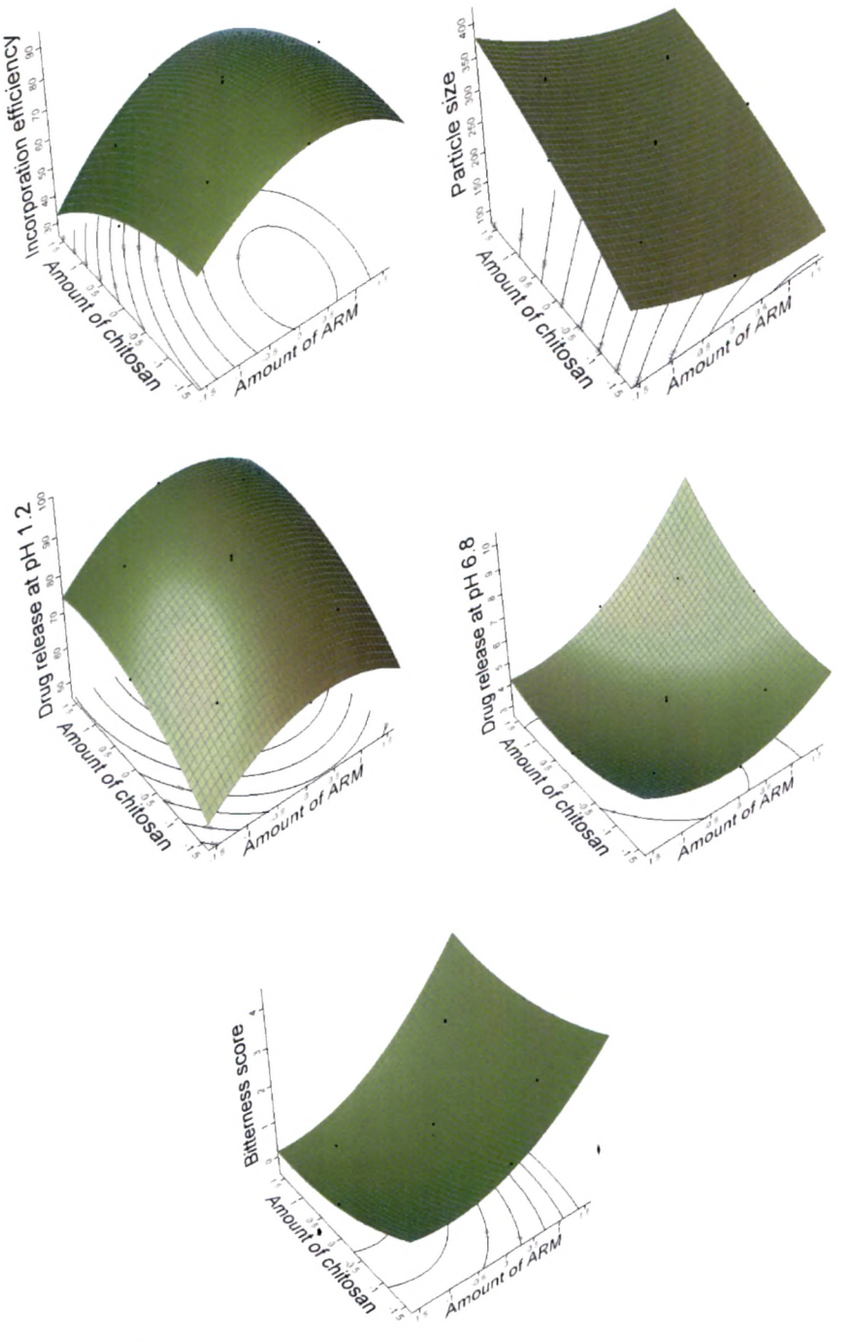

Figure 8.1. Surface plots showing the effect of amount of drug, polymer and crosslinking agent on incorporation efficiency, particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 8.3. Results of regression analysis

Terms	Incorporation efficiency (%)				Particle size (μm)				Drug release at pH 1.2 (t15 in %)			
	FM		RM		FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	83.21	NA	83.21	NA	236.75	NA	236.75	NA	87.75	NA	87.75	NA
A	7.03	< 0.0001*	7.03	< 0.0001*	-28.08	< 0.0001*	-28.08	< 0.0001*	3.71	< 0.0001*	3.71	< 0.0001*
B	-5.92	< 0.0001*	-5.92	< 0.0001*	51.17	< 0.0001*	51.17	< 0.0001*	7.18	< 0.0001*	7.18	< 0.0001*
C	7.20	< 0.0001*	7.20	< 0.0001*	-32.94	< 0.0001*	-32.94	< 0.0001*	0.49	0.0236	0.49	0.0272
A^2	-5.94	< 0.0001*	-5.94	< 0.0001*	8.89	0.0011	8.89	0.0009	-4.26	< 0.0001*	-4.26	< 0.0001*
B^2	-2.84	0.0002	-2.84	< 0.0001*	-5.71	0.0160	-5.71	0.0159	-2.71	< 0.0001*	-2.71	< 0.0001*
C^2	-1.54	0.0109	-1.54	0.0084	4.66	0.0397	4.66	0.0410	-4.82	< 0.0001*	-4.82	< 0.0001*
AB	2.25	0.0054	2.25	0.0039	-1.47	0.5747	–	–	0.34	0.1805	–	–
AC	0.59	0.3781	–	–	-3.98	0.1474	–	–	1.59	< 0.0001*	1.59	< 0.0001*
BC	-0.62	0.3514	–	–	20.60	< 0.0001*	20.60	< 0.0001*	1.15	0.0007	1.15	0.0008

*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 8.3. ***(Continued)***

Terms	Drug release at pH 6.8 (t5 in %)				Bitterness score			
	FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	3.81	NA	3.81	NA	1.01	NA	1.01	NA
A	0.95	< 0.0001*	0.95	< 0.0001*	0.97	< 0.0001*	0.97	< 0.0001*
B	0.46	< 0.0001*	0.46	< 0.0001*	-0.20	0.0092	-0.20	0.0139
C	-0.49	< 0.0001*	-0.49	< 0.0001*	-0.61	< 0.0001*	-0.61	< 0.0001*
A^2	0.55	< 0.0001*	0.55	< 0.0001*	0.35	0.0002	0.35	0.0003
B^2	0.34	< 0.0001*	0.34	< 0.0001*	0.17	0.0228	0.17	0.0331
C^2	0.37	< 0.0001*	0.37	< 0.0001*	0.26	0.0018	0.26	0.0027
AB	0.44	< 0.0001*	0.44	< 0.0001*	0.13	0.1459	–	–
AC	-0.05	0.3962	–	–	0.25	0.0103	0.25	0.0154
BC	0.28	0.0008	0.28	0.0006	0.12	0.1459	–	–

*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 8.4. 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) 100	PRESS	R^2	Adj-R^2	Pred-R^2	Adeq Precision
Incorporation efficiency (%)	FM	2418.82	9	268.76	83.16	0.01*	145.67	0.98	0.97	0.94	31.69
	RM	2412.98	7	344.71	108.40	0.01*	120.53	0.98	0.97	0.95	35.78
Particle Size (µm)	FM	65269.71	9	7252.19	141.42	0.01*	3288.53	0.99	0.98	0.95	44.31
	RM	65125.97	7	9303.71	170.04	0.01*	2617.43	0.99	0.98	0.96	47.96
Drug release at pH 1.2 (t15 in %)	FM	1475.09	9	163.90	359.45	0.01*	9.34	0.99	0.99	0.99	60.91
	RM	1474.15	8	184.27	368.20	0.01*	13.22	0.99	0.99	0.99	62.01
Drug release at pH 6.8 (t5 in %)	FM	26.73	9	2.97	105.81	0.01*	1.85	0.98	0.98	0.93	32.141
	RM	26.70	8	3.34	121.30	0.01*	1.43	0.98	0.98	0.94	34.21
Bitterness Score	FM	21.23	9	2.36	46.93	0.01*	4.06	0.97	0.95	0.81	23.74
	RM	20.98	7	3.00	47.79	0.01*	3.00	0.96	0.94	0.86	23.76

**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.*

High values of correlation coefficient (R^2) for all dependent variables indicate a good fit.

PRESS values for all batches 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.

8.2.2.2. Incorporation efficiency

As shown in Table 8.3, the amount of chitosan (B) had a negative coefficient, while the amount of ARM (A) and sodium hydroxide (C) had positive coefficients. This indicates that on increasing the amount of chitosan, incorporation efficiency decreases. The percent incorporation efficiency was found to be in the range between 52 and 91. The percent incorporation efficiency showed a dependence on the extent of crosslinking and amount of drug added. The microparticles with higher amount of drug added exhibited higher incorporation efficiencies. This is due to the accumulation of more drug molecules. The extent of crosslinking has a significant effect on percent incorporation efficiency. As the amount of crosslinking agent increased, an increase in percent incorporation efficiency was observed. This is because at higher extent of crosslinking, there will be a formation of more rigid network structure, which would cause the retention of more of drug molecules during the microsphere preparation (Rokhade et al., 2007).

8.2.2.3. Particle size

As shown in Table 8.3, the amount of chitosan (B) had a positive coefficient, while the amount of ARM (A) and sodium hydroxide (C) had negative coefficients. This indicates that on increasing the amount of chitosan, particle size increases. The particle size is influenced by the orifice of the needle and the viscosity of the chitosan solution. The increased viscosity at higher amount of chitosan resulted in larger particles. A high amount of sodium hydroxide resulted in smaller particle size due to high degree of crosslinking. Similar results were observed for glutaraldehyde (Kumbar et al., 2002; Rokhade et al., 2007).

8.2.2.4. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of ARM (A), chitosan (B) and sodium hydroxide (C) had positive coefficient. This indicates additive effect of amount of ARM, chitosan and sodium hydroxide. 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 improved wettability, provided by the dissolved chitosan (Rane et al., 2007). Figure 8.2 shows dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8.

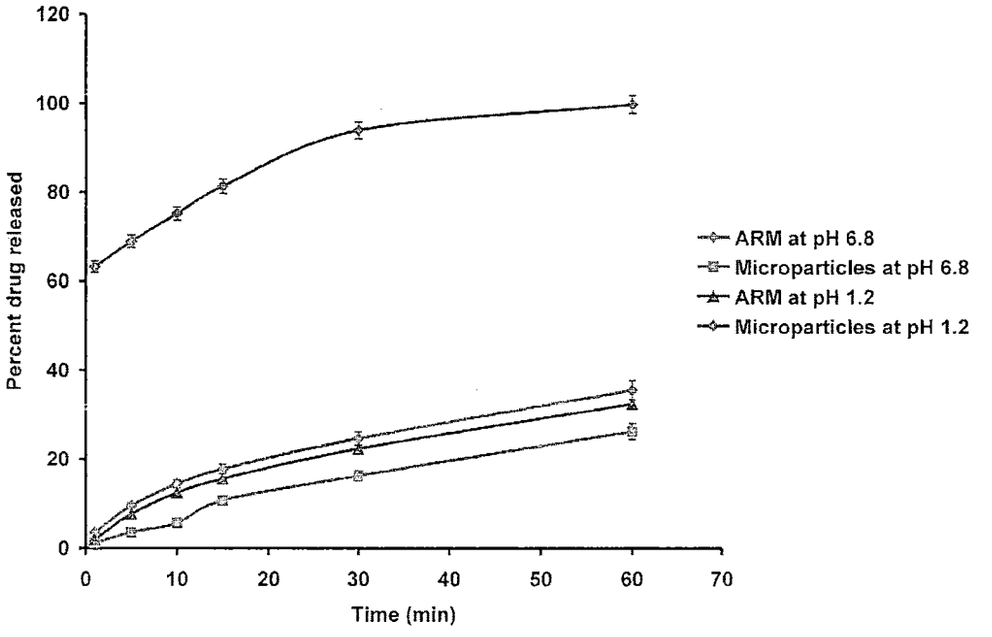

Figure 8.2. Dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8

For *in vitro* drug release at pH 6.8, the amount of ARM (A) and chitosan (B) had positive coefficient while the amount of sodium hydroxide (C) had negative coefficient. This indicates that on increasing the amount of sodium hydroxide, drug release of microparticles at pH 6.8 decreases. The effect of amount of chitosan on the release of drug was found to be meager. The drug release after 5 min was decreased, when the amount of sodium hydroxide

was increased. This may be due to the fact that as the amount of sodium hydroxide was increased, it produced microspheres with pronounced cross-linking between polymer chains that retarded the release of drug (Ko et al., 2002; Remunan-Lopez and Bodmeier, 1997). Similar results were observed with glutaraldehyde (Chourasia and Jain, 2004). In addition, the drug release from chitosan microparticles increases with increase in drug content (Bayomi, 2004).

8.2.2.5. Gustatory sensation test

As shown in Table 8.3, amount of ARM (A) had positive coefficient, while amount of chitosan (B) and sodium hydroxide (C) had negative coefficient. This indicates that on increasing the amount of chitosan and sodium hydroxide, 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 seem to bind G-protein coupled receptors, present on the apical taste cell membrane and produce bitterness (Yamamoto et al., 1998). Chitosan is expected to behave as insoluble at pH 6.8 and showed decreased drug release in microparticles with increase in crosslinking. Thus chitosan acts as physical barrier between ARM and G-protein coupled receptors present on the apical taste cell membrane and thus reduces bitterness score of ARM in microparticles. Bitterness score of optimized microparticles is shown in Table 8.5.

8.2.2.6. 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 incorporation efficiency and drug release at pH 1.2 had to be maximized in order to pour desired characteristics in the product. 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 0.70. Table 8.5 enlists the optimized values for independent variables and their responses.

Table 8.5. 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 g	C in mL#						
0.038	0.05	15	83.43 ± 2.42	234.87 ± 2.71	80.29 ± 1.36	3.57 ± 1.18	0	0.70

*A – amount of ARM, B – amount of chitosan, C – amount of NaOH, #mL of 10%w/v sodium hydroxide solution, *Values represent the mean ± SD of 3 experiments, t5 and t15 – percent drug dissolved in 5 and 15 min, respectively.*

8.2.2.7. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of ARM, chitosan, blank microparticles and optimized microparticles are shown in Figure 8.3. Blank microparticles showed shifting of absorption peak of –OH from 3430 to 3461 cm^{-1}, compared to chitosan. In addition, the absorption peak of amide band, a C-O stretching mode together with an N-H deformation mode, located at 1658 cm^{-1} shifted to 1666 cm^{-1}, compared to chitosan. The absorption bands of blank microparticles are again shifted to 3448 cm^{-1} and 1654 cm^{-1}, for –OH stretching and amide band, respectively in optimized microparticles. This finding confirms the crosslinking of chitosan in presence of sodium hydroxide.

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

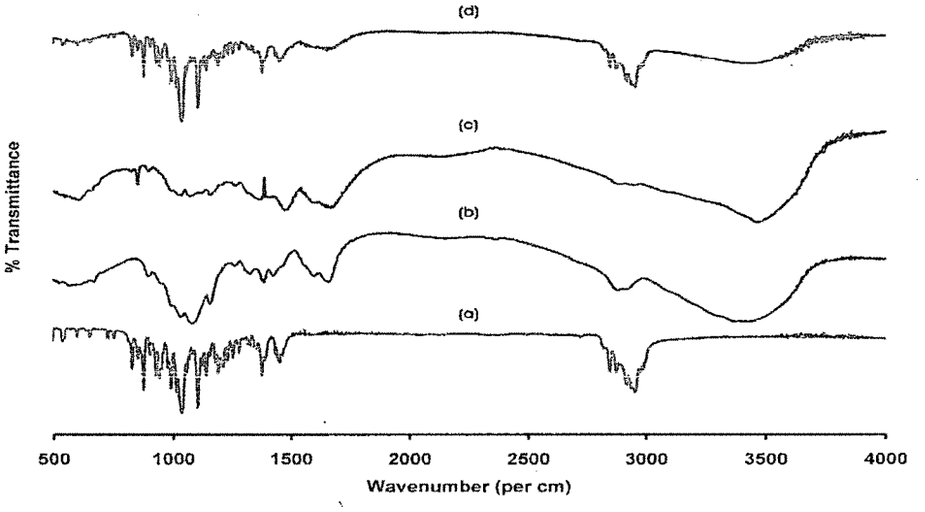

Figure 8.3. FT-IR spectra of (a) ARM, (b) chitosan, (c) blank microparticles and (d) optimized microparticles

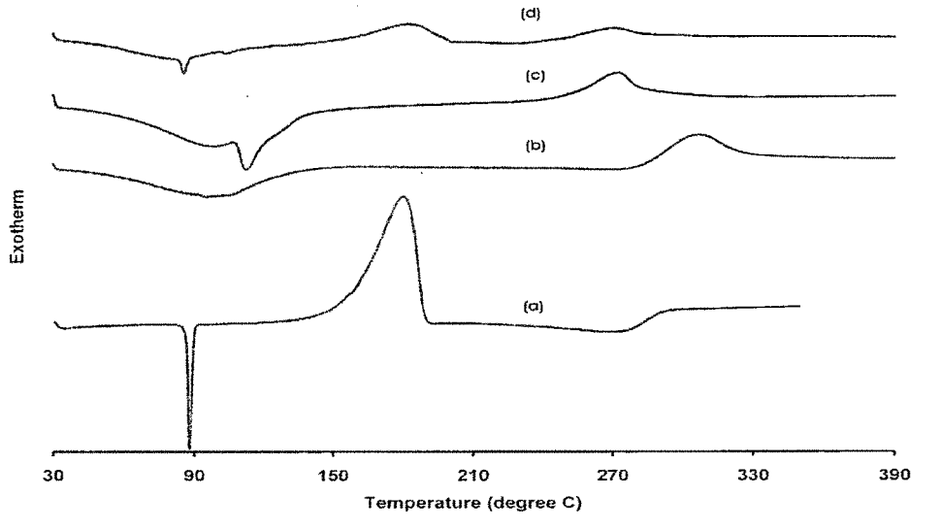

Figure 8.4. DSC curve of (a) ARM, (b) chitosan, (c) blank microparticles and (d) optimized microparticles

8.2.2.8. Differential scanning calorimeter (DSC)

Figure 8.4 shows the DSC curve of ARM, chitosan, blank microparticles and optimized microparticles. The pure ARM showed 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 (85.62^{0}C), 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.

8.2.2.9. 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 8.6. The formula of optimized suspension powder (DS75) was further used to prepare suspension powder of plain ARM (DS76). The characteristics of suspension powder are summarized in Table 8.7.

Table 8.6. Formulation of suspension powder

Drug/Excipients	For 6 cachets					
	DS71	DS72	DS73	DS74	DS75	DS76
ARM (g)	-	-	-	-	-	0.300
Microparticles eq. to 0.050 g ARM (g)	0.832	0.832	0.832	0.832	0.832	-
Xanthan gum (g)	0.004	0.005	0.006	0.007	0.008	0.008
Microcrystalline cellulose (Avicel PH 302) (g)	0.351	0.350	0.349	0.348	0.347	0.879
Citric acid (g)	0.009	0.009	0.009	0.009	0.009	0.009
Methyl paraben (g)	0.002	0.002	0.002	0.002	0.002	0.002
Propyl paraben (g)	0.001	0.001	0.001	0.001	0.001	0.001
Sunset yellow FCF (g)	0.001	0.001	0.001	0.001	0.001	0.001
Total filled weight per 6 cachets (g)	1.200	1.200	1.200	1.200	1.200	1.200

Table 8.7. Physical properties of suspension powder

Parameters	DS71	DS72	DS73	DS74	DS75	DS76
Angle of repose (^{0}) ± SD*	36.18 ± 0.49	34.56 ± 0.58	34.78 ± 0.74	35.39 ± 0.56	34.43 ± 0.59	35.75 ± 0.67
F value (after reconstitution) ± SD*	0.14 ± 0.07	0.27 ± 0.09	0.42 ± 0.08	0.63 ± 0.08	0.96 ± 0.02	0.95 ± 0.03
pH (after reconstitution)	4.6-4.7	4.6-4.7	4.6-4.7	4.5-4.6	4.5-4.6	4.5-4.7

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

8.2.2.10. 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 8.5 represents the chemical stability results of ARM in reconstituted suspension. The degradation curves of ARM in reconstituted suspension (Figure 8.5) at room temperature, suggest a 'lag'-time of 5 days. After that period, ARM is degrading.

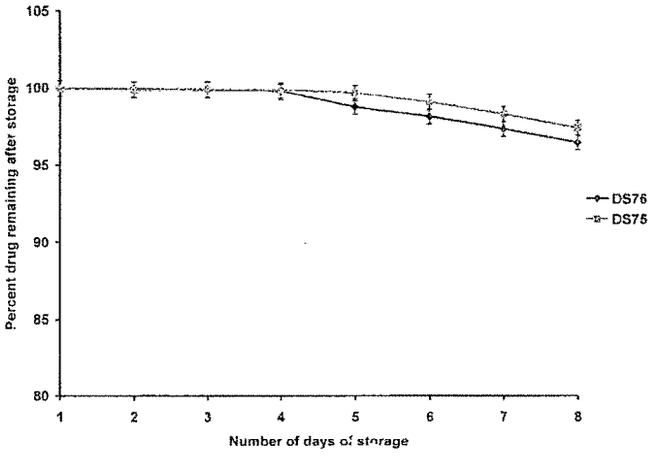

Figure 8.5. Degradation of ARM in DS75 and DS76 after reconstitution as a function of time

8.2.2.11. 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 DS76, the 5% of panel rated it as very strongly bitter, 90% strongly bitter and 5% moderate to strong bitter. DS75 was rated as tasteless by 100% of volunteers of panel (Table 8.8).

Table 8.8. 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	20							
DS76						1	18	1
DS75	20							

8.2.2.12. 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 DS74 showed little change over time, and so a shelf life up to 1396.23 days (46.54 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).

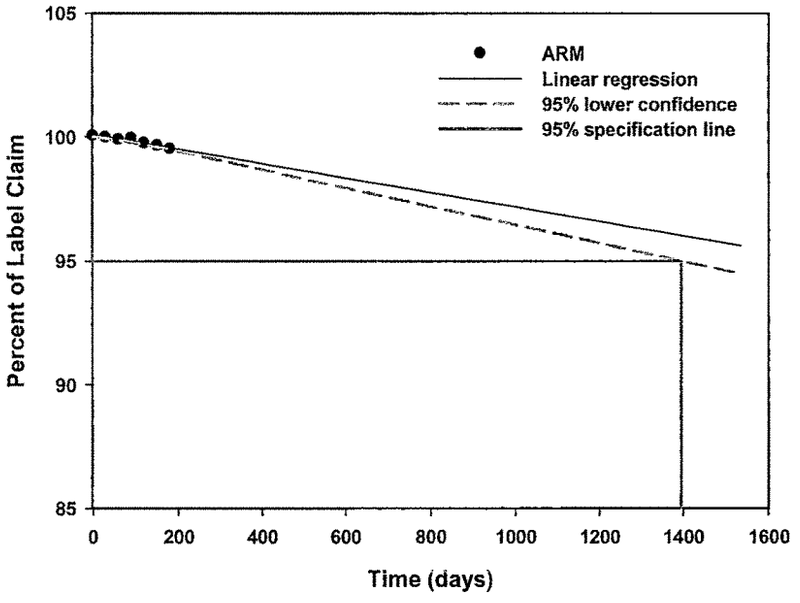

Figure 8.6. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of ARM in microparticles using chitosan as polymer. Present work suggests that all three 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, chitosan and sodium hydroxide, which have significant effect on microparticle's desired properties. The FTIR and DSC studies indicated uniform dispersion of ARM, at the molecular level, in chitosan 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.

8.3. Mefloquine hydrochloride (MFL)

8.3.1. Experimental

8.3.1.1. Preparation of Microparticles

A concentrated solution of chitosan (1%w/v) was prepared in 1%v/v acetic

acid. The required quantity of MFL (36 mg of MFL in 4.6 mL of chitosan solution) was dispersed and mixed with concentrated chitosan solution. The microparticles were prepared by dropping chitosan containing MFL from the dropping device, glass syringe with a 18G×½" flat-cut hypodermic needle to a magnetically stirred (15 mL of 10%w/v) sodium hydroxide solution. The droplets were amputated at a flow rate of 2.5 ml/min into sodium hydroxide solution. Different concentrations of MFL and chitosan were used as mentioned in Table 8.9. 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 8.9. Process variables and their levels for Central Composite Design (CCD)

Coded values	Actual Values		
	Amount of MFL (A in g)	Amount of Chitosan (B in g)	Amount of Sod. hydroxide (C in mL*)
-1.63	0.011	0.011	3.15
-1	0.03	0.03	5
0	0.05	0.05	10
1	0.07	0.07	15
1.63	0.114	0.114	24.45

**mL of 10%w/v sodium hydroxide solution*

8.3.1.2. Response surface methodology (RSM)

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 8.10.

Table 8.10. Central Composite Design (CCD) with the measured responses

ES	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	C					
1	1	1	-1	57.05 ± 2.8	252.05 ± 4.32	90.85 ± 1.7	8.35 ± 0.48	3+
2	-1	1	1	60.92 ± 2.76	292.73 ± 3.58	83.67 ± 1.37	4.18 ± 0.24	0
3	0	0	0	78.12 ± 1.93	207.49 ± 2.27	91.81 ± 1.56	4.65 ± 0.31	1
4	0	0	0	76.67 ± 2.79	201.92 ± 3.43	91.98 ± 1.92	4.60 ± 0.36	1
5	-1	-1	-1	64.49 ± 2.58	235.20 ± 2.64	74.72 ± 1.78	5.02 ± 0.34	1.5
6	1	-1	1	87.03 ± 2.34	64.03 ± 3.24	79.24 ± 1.49	5.58 ± 0.39	2
7	-1	1	-1	43.09 ± 1.87	274.27 ± 4.28	83.5 ± 1.68	5.06 ± 0.43	1.5
8	0	0	0	79.24 ± 2.67	212.53 ± 4.68	92.81 ± 1.79	4.69 ± 0.38	1
9	1	1	1	80.11 ± 2.96	223.38 ± 3.29	96.98 ± 1.57	7.87 ± 0.29	3
10	-1	-1	1	75.71 ± 2.39	139.50 ± 4.76	72.85 ± 1.91	4.20 ± 0.29	0
11	0	0	0	75.96 ± 2.16	209.70 ± 5.18	92.81 ± 1.34	4.70 ± 0.51	1
12	1	-1	-1	67.58 ±2.52	187.90 ± 2.39	76.61 ± 1.44	6.33 ± 0.29	2.5
13	-1.63	0	0	47.61 ±2.48	273.16 ± 3.36	74.89 ± 1.85	4.29 ± 0.31	0
14	0	0	0	74.87 ±2.17	199.71 ± 2.53	91.38 ± 1.53	4.60 ± 0.42	1
15	0	0	-1.63	68.51 ± 1.83	287.00 ± 3.82	79.54 ± 1.43	5.87 ± 0.26	2
16	1.63	0	0	76.52 ± 2.16	158.97 ± 2.96	87.57 ± 1.54	8.04 ± 0.28	3
17	0	-1.63	0	76.12 ± 2.23	118.29 ± 3.64	72.18 ± 1.68	4.79 ± 0.40	1
18	0	1.63	0	57.46 ± 2.43	266.84 ± 3.83	96.28 ± 1.39	7.57 ± 0.53	2
19	0	0	1.63	89.05 ± 2.54	162.75 ± 2.93	83.59 ± 1.89	4.53 ± 0.28	1
20	0	0	0	77.59 ± 2.68	266.31 ± 3.19	92.81 ± 1.93	4.69 ± 0.54	1

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

8.3.1.3. Incorporation efficiency

Incorporation efficiency was carried out as mentioned in 6.2.1.4.

8.3.1.4. Particle size analysis

Particle size analysis was carried out as mentioned in 7.2.1.5

8.3.1.5. *In vitro* drug release

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

8.3.1.6. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

8.3.1.7. Optimization of responses using desirability

The percent incorporation efficiency value was targeted to maximize in the procedure, as higher values of this was desired to reduce total weight of microparticles, equivalent to 250 mg MFL. The values of Ymin and Ymax of percent incorporation efficiency were 43.09 and 89.05, respectively. 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 pH 1.2 in 15 min (t15) was selected. The values of Ymin and Ymax of percent drug release at pH 1.2 in 15 min (t15) were 72.18 and 96.98%, respectively. The desirability function of incorporation efficiency and drug release at pH 1.2 has been calculated by following equation.

$$di = \left(\frac{Yi - Ymin}{Ymax - Ymin} \right)^{s} \qquad \text{(Equation 8.9)}$$

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 64.03 and 292.73, 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 pH 6.8 in 5 min was desired. The values of Ymin and Ymax of percent drug release at pH 6.8 in 5 min (t5) were 4.18 and 8.35, 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.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 8.10)}$$

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 7.11, 7.12 and 7.13.

$di = 1$ if Yi < Ymin (Equation 8.11)

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{if Ymin} \leq \text{Yi} \leq \text{Ymax} \qquad \text{(Equation 8.12)}$$

$di = 0$ if Yi > Ymax (Equation 8.13)

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

$$D = (dI \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \qquad \text{(Equation 8.14)}$$

Where D is overall desirability and d1, d2, d3, d4 are individual desirability

values of measured responses.

8.3.1.8. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

8.3.1.9. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

8.3.1.10. 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.

8.3.1.11. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9.

8.3.1.12. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

8.3.1.13. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

8.3.1.14. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

8.3.2. Results and discussion

8.3.2.1. Experimental design

Preliminary investigations of the process parameters revealed that factors

amount of drug (A), polymer (B) and crosslinking agent (C), highly influenced the bitterness in human volunteers, incorporation efficiency, particle size, drug release at pH, 1.2 and 6.8. Hence A, B and C 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 8.11. 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).

Multiple linear regression analysis (Table 8.11) revealed that C^2, AC and BC terms were insignificant for incorporation efficiency, A^2 and AB terms were insignificant for particle size, BC term was insignificant for drug release at pH 1.2 and bitterness sore, AC and BC terms were insignificant for drug release at pH 6.8. The surface plots are shown in Figure 8.7.

Table 8.12 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 batches of microparticles 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.

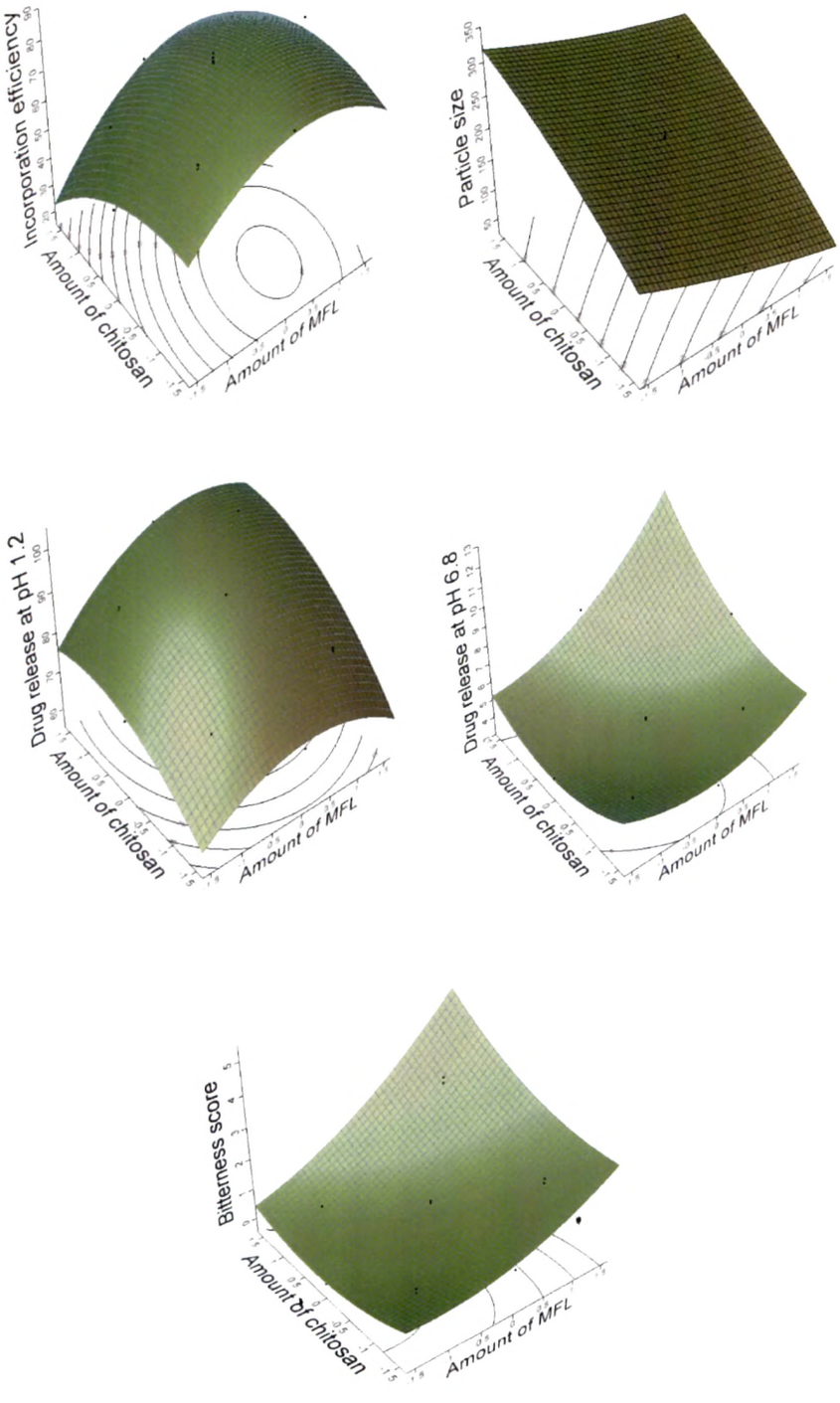

Figure 8.7. Surface plots showing the effect of amount of drug and polymer on particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 8.11. Results of regression analysis

Terms	Incorporation efficiency (%)				Particle size (μm)				Drug release at pH 1.2 (t15 in %)			
	FM		RM		FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	77.19	NA	77.36	NA	206.47	NA	208.71	NA	92.19	NA	92.19	NA
A	7.11	< 0.0001*	7.11	< 0.0001*	-30.06	< 0.0001*	-30.06	< 0.0001*	3.72	< 0.0001*	3.72	< 0.0001*
B	-6.68	< 0.0001*	-6.68	< 0.0001*	49.38	< 0.0001*	49.38	< 0.0001*	6.82	< 0.0001*	6.82	< 0.0001*
C	7.88	< 0.0001*	7.88	< 0.0001*	-32.45	< 0.0001*	-32.45	< 0.0001*	1.03	0.0037	1.03	0.0065
A^2	-6.04	< 0.0001*	-6.06	< 0.0001*	2.94	0.2634	–	–	-3.86	< 0.0001*	-3.86	< 0.0001*
B^2	-3.70	0.0005	-3.72	0.0004	-5.87	0.0398	-6.08	0.0357	-2.74	< 0.0001*	-2.74	< 0.0001*
C^2	0.22	0.7696	–	–	6.25	0.0306	6.04	0.0367	-3.7	< 0.0001*	-3.7	< 0.0001*
AB	2.34	0.0338	2.34	0.0395	3.90	0.2498	–	–	1.55	0.0013	1.55	0.002
AC	1.68	0.1080	–	–	-9.41	0.0146	-9.41	0.0148	1.31	0.0040	1.31	0.0070
BC	1.28	0.2098	–	–	26.17	< 0.0001*	26.17	< 0.0001*	0.69	0.0774	–	–

*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 8.11. ***(Continued)***

Terms	Drug release at pH 6.8 (t5 in %)				Bitterness score			
	FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	4.67	NA	4.67	NA	0.99	NA	0.99	NA
A	1.18	< 0.0001*	1.18	< 0.0001*	0.97	< 0.0001*	0.97	< 0.0001*
B	0.67	< 0.0001*	0.67	< 0.0001*	0.27	< 0.0001*	0.27	< 0.0001*
C	-0.38	< 0.0001*	-0.38	< 0.0001*	-0.42	< 0.0001*	-0.42	< 0.0001*
A^2	0.53	< 0.0001*	0.53	< 0.0001*	0.23	0.0004	0.23	0.0002
B^2	0.53	< 0.0001*	0.53	< 0.0001*	0.23	0.0004	0.23	0.0002
C^2	0.16	0.0172	0.16	0.0108	0.23	0.0004	0.23	0.0002
AB	0.54	< 0.0001*	0.54	< 0.0001*	0.25	0.0013	0.25	0.0007
AC	0.059	0.4460	–	–	0.25	0.0013	0.25	0.0007
BC	0.026	0.7303	–	–	0	1.0000	–	–

*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 8.12 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) 100	PRESS	R^2	Adj-R^2	Pred-R^2	Adeq Precision
Incorporation efficiency (%)	FM	2811.59	9	312.40	42.99	0.01*	474.77	0.97	0.95	0.83	24.44
	RM	2775.23	6	462.54	55.15	0.01*	334.88	0.96	0.94	0.88	27.23
Particle Size (μm)	FM	66067.35	9	7340.82	90.06	0.01*	5551.29	0.98	0.97	0.91	36.04
	RM	65831.19	7	9404.46	107.35	0.01*	4052.73	0.98	0.97	0.93	38.86
Drug release at pH 1.2 (t15 in %)	FM	1277.88	9	11.99	143.99	0.01*	62.78	0.99	0.98	0.95	35.65
	RM	1274.05	8	159.26	127.50	0.01*	67.08	0.98	0.98	0.94	31.61
Drug release at pH 6.8 (t5 in %)	FM	35.92	9	3.99	90.98	0.01*	3.27	0.98	0.97	0.91	30.16
	RM	35.89	7	5.13	130.40	0.01*	2.20	0.98	0.97	0.93	36.07
Bitterness Score	FM	18.69	9	2.08	81.34	0.01*	1.91	0.98	0.97	0.89	29.42
	RM	18.69	8	2.34	100.06	0.01*	1.45	0.98	0.97	0.92	32.52

**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.*

8.3.2.2. Incorporation efficiency

As shown in Table 8.11, the amount of chitosan (B) had a negative coefficient, while the amount of MFL (A) and sodium hydroxide (C) had positive coefficients. This indicates that on increasing the amount of chitosan, incorporation efficiency decreases. The percent incorporation efficiency was found to be in the range between 43 and 89. The percent incorporation efficiency showed a dependence on the extent of crosslinking and amount of drug added. The microparticles with higher amount of drug added exhibited higher incorporation efficiencies. This is due to the accumulation of more drug molecules. The extent of crosslinking has a significant effect on percent incorporation efficiency. As the amount of crosslinking agent increased, an increase in percent incorporation efficiency was observed. This is because at higher extent of crosslinking, there will be a formation of more rigid network structure, which would cause the retention of more of drug molecules during the microsphere preparation (Rokhade et al., 2007).

8.3.2.3. Particle size

As shown in Table 8.11, the amount of chitosan (B) had a positive coefficient, while the amount of MFL (A) and sodium hydroxide (C) had negative coefficients. This indicates that on increasing the amount of chitosan, particle size increases. The particle size is influenced by the orifice of the needle and the viscosity of the chitosan solution. The increased viscosity at higher amount of chitosan resulted in larger particles. A high amount of sodium hydroxide resulted in smaller particle size due to high degree of crosslinking. Similar results were observed for glutaraldehyde (Kumbar et al., 2002; Rokhade et al., 2007).

8.3.2.4. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of MFL (A), chitosan (B) and sodium hydroxide (C) had positive coefficient. This indicates additive effect of amount of MFL, chitosan and sodium hydroxide. 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 improved wettability, provided by the dissolved chitosan (Rane et al., 2007).

For *in vitro* drug release at pH 6.8, the amount of MFL (A) and chitosan (B) had positive coefficient while the amount of sodium hydroxide (C) had negative coefficient. This indicates that on increasing the amount of sodium hydroxide, drug release of microparticles at pH 6.8 decreases. The effect of amount of chitosan on the release of drug was found to be meager. The drug release after 5 min was decreased when the amount of sodium hydroxide was increased. This may be due to the fact that as the amount of sodium hydroxide was increased, it produced microspheres with pronounced cross-linking between polymer chains that retarded the release of drug (Ko et al., 2002; Remunan-Lopez and Bodmeier, 1997). Similar results were observed with glutaraldehyde (Chourasia and Jain, 2004). In addition, the drug release from chitosan microparticles increases with increase in drug content (Bayomi, 2004). Figure 8.8 shows dissolution profile of MFL and optimized microparticles at pH, 1.2 and 6.8.

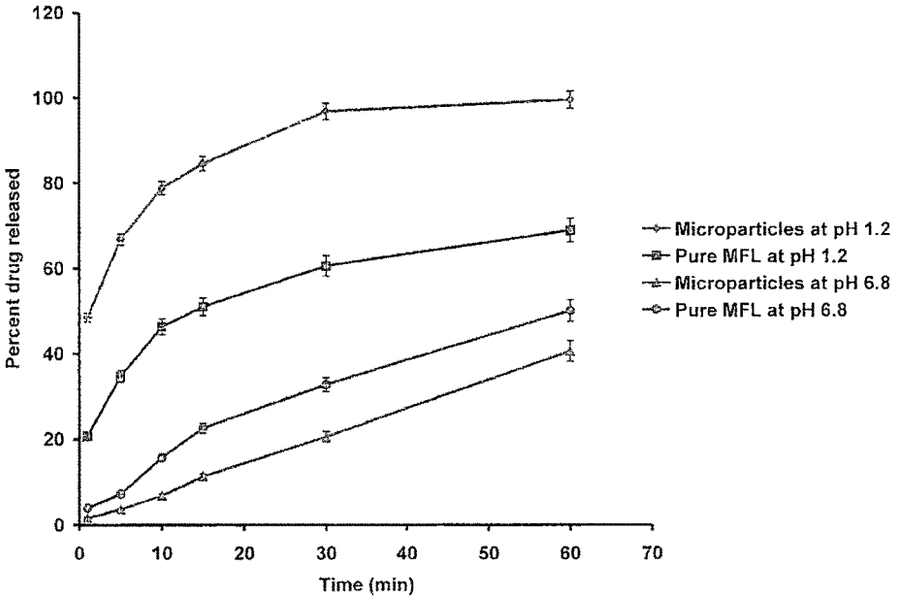

Figure 8.8. Dissolution profile of MFL and optimized microparticles at pH, 1.2 and 6.8

8.3.2.5. Gustatory sensation test

For bitterness score, the amount of MFL (A) and chitosan (B) had positive coefficient, while the amount of sodium hydroxide (C) had negative coefficient. This indicates that on increasing the amount of sodium hydroxide, bitterness score of microparticles decreases. This finding is in agreement with drug release studies carried out at pH 6.8 because the pH of the saliva is 6.8 (Hashimoto et al., 2002). It has been reported that MFL produces bitterness by depolarizing taste cells through K^+ channels (Yamamoto et al., 1998). The microparticles control the drug release at salivary pH and restrict direct interaction of MFL with ionic channels resulting in decreased bitterness of MFL in microparticles. Bitterness score of optimized microparticles is shown in Table 8.13.

8.3.2.6. 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 incorporation efficiency and drug release at pH 1.2 have to be maximized, in order to pour desired characteristics in the microparticles. 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 0.66. Table 8.13 enlists the optimized values for independent variables and their responses.

Table 8.13. 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 g	C in mL#						
0.036	0.046	15	79.63 ± 2.73	193.74 ± 2.58	84.17 ± 1.34	3.67 ± 0.84	0	0.66

*A – amount of MFL, B – amount of chitosan, C – amount of NaOH, #mL of 10%w/v sodium hydroxide solution, *Values represent the mean ± SD of 3 experiments, t5 and t15 – percent drug dissolved in 5 and 15 min, respectively.*

8.3.2.7. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of MFL, chitosan, blank microparticles and optimized microparticles are shown in Figure 8.9.

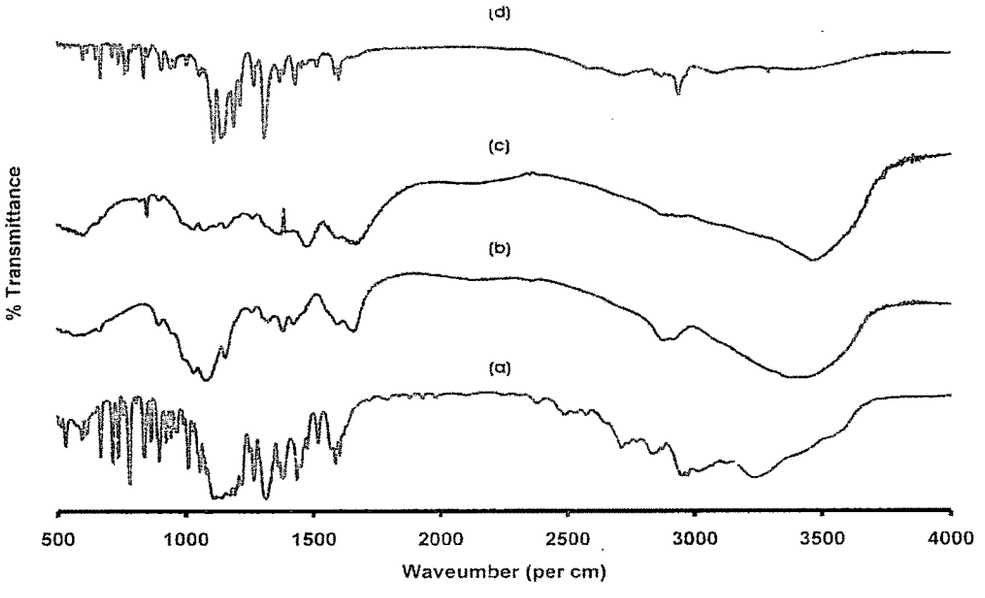

Figure 8.9. FT-IR spectra of (a) MFL, (b) chitosan, (c) blank microparticles and (d) optimized microparticles

Blank microparticles showed shifting of absorption peak of –OH from 3430 to 3461 cm^{-1}, compared to chitosan. In addition the absorption peak of amide band, a C-O stretching mode together with an N-H deformation mode, located at 1658 cm^{-1} shifted to 1666 cm^{-1}, compared to chitosan. The absorption bands of placebo microparticles were again shifted to 3448 cm^{-1} and 1654 cm^{-1}, for –OH stretching and amide band, respectively in optimized microparticles. This finding confirms the crosslinking of chitosan in presence of sodium hydroxide.

The FTIR spectrum of microparticles was found to exhibit some significant difference in the characteristic peaks of MFL, revealing modification of the drug environment. A broad band of bonded N-H stretching vibration of MFL was observed at 3226.46 cm^{-1}. Microparticles showed shifting of this N-H peak to 3290.44 cm^{-1} with reduced intensity. This suggests electrostatic interaction between chitosan and MFL.

8.3.2.8. Differential scanning calorimeter (DSC)

Figure 8.10 shows the DSC curve of MFL, chitosan, blank microparticles and optimized microparticles.

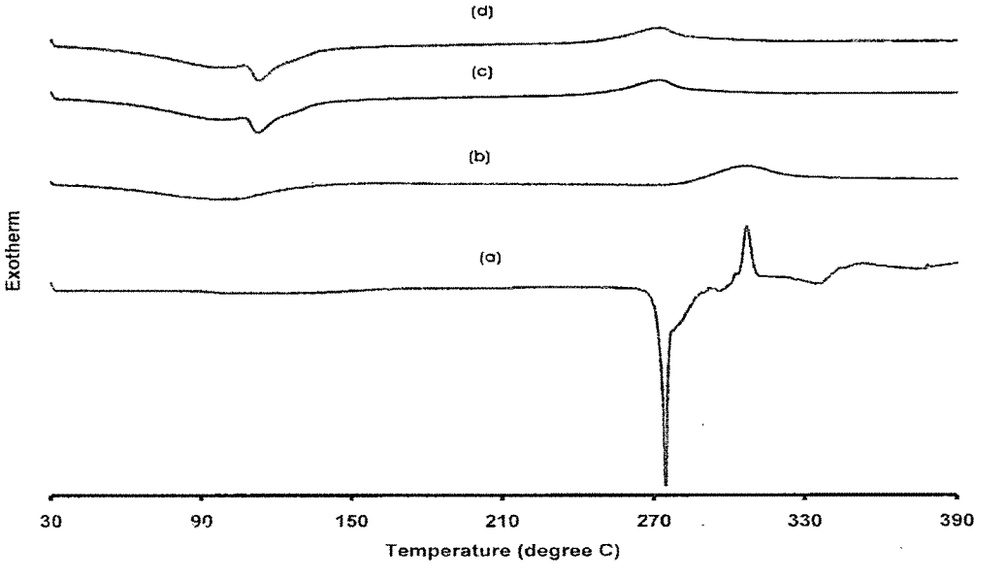

Figure 8.10. DSC curve of (a) MFL, (b) chitosan, (c) blank microparticles and (d) optimized microparticles

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 absent 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.

8.3.2.9. 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 8.14. The formula of optimized suspension powder (DS84) was further used to prepare suspension powder of plain MFL (DS85). The characteristics of suspension powder are summarized in Table 8.15.

Table 8.14. Formulation of suspension powder

Drug/Excipients	For 6 cachets				
	DS81	**DS82**	**DS83**	**DS84**	**DS85**
MFL (g)	-	-	-	-	0.600
Microparticles eq. to 100 mg MFL (g)	1.716	1.716	1.716	1.716	-
Sodium carboxy methyl cellulose (g)	0.002	0.004	0.006	0.008	0.008
Polyethylene glycol 6000 (g)	0.021	0.021	0.021	0.021	0.021
Microcrystalline cellulose (Avicel PH 302) (g)	0.342	0.340	0.338	0.336	1.452
Citric acid (g)	0.014	0.014	0.014	0.014	0.014
Methyl paraben (g)	0.004	0.004	0.00	0.004	0.004
Propyl paraben (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 8.15. Physical properties of suspension powder

Parameters	DS81	DS82	DS83	DS84	DS85
Angle of repose (^{0}) ± SD*	38.18 ± 0.46	38.37 ± 0.52	38.26 ± 0.48	37.78 ± 0.58	37.93 ± 0.62
F value (after reconstitution) ± SD*	0.17 ± 0.07	0.48 ± 0.09	0.72 ± 0.08	0.96 ± 0.03	0.97 ± 0.02
pH (after reconstitution)	4.5-4.6	4.5-4.6	4.4-4.5	4.4-4.5	4.4-4.5

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

8.3.2.10. 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 DS85, the 5% of panel rated it as very strongly bitter, 85% strongly bitter and 10% moderately bitter. DS84 was rated as tasteless by 100% of volunteers of panel (Table 8.16).

Table 8.16. 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	20							
DS85						2	17	1
DS84	20							

8.3.2.11. 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 DS84 showed little change over time, and so a shelf life up to 1453.29 days (48.44 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).

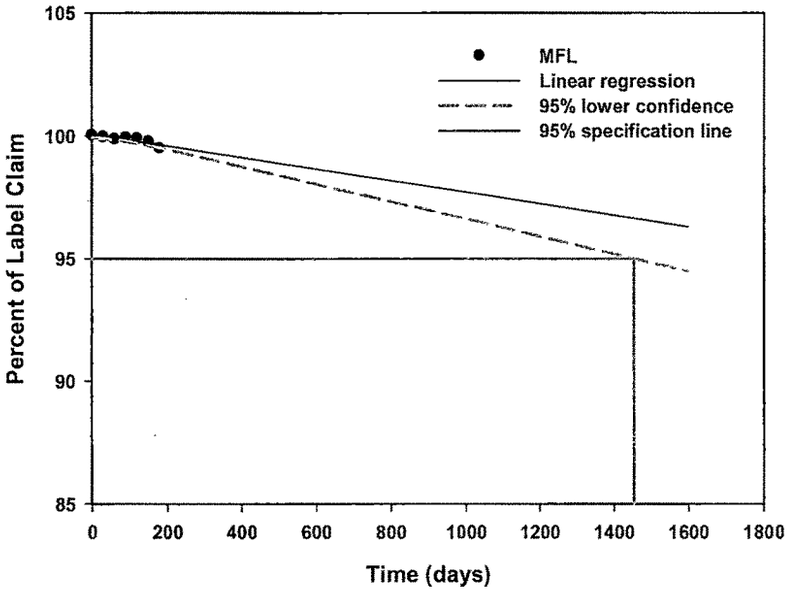

Figure 8.11. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of MFL in microparticles using chitosan as polymer. Present work suggests that all three 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 chitosan, which have significant effect on microparticle's desired properties. The FTIR and DSC studies indicated uniform dispersion of MFL, at the molecular level, in chitosan 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.

8.4. Primaquine Phosphate (PRM)

8.4.1. Experimental

8.4.1.1. Preparation of Microparticles

A concentrated solution of chitosan (1%w/v) was prepared in 1%v/v acetic acid. The required quantity of PRM (46 mg of PRM in 3.2 mL of chitosan solution) was dispersed and mixed with concentrated chitosan solution. The microparticles were prepared by dropping chitosan containing PRM from the dropping device, glass syringe with a 18G×½" flat-cut hypodermic needle to a magnetically stirred (15 mL of 10%w/v) sodium hydroxide solution. The droplets were amputated at a flow rate of 2.5 ml/min into sodium hydroxide solution. Different concentrations of PRM and chitosan were used as mentioned in Table 8.17. 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.

8.4.1.2. Response surface methodology (RSM)

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 8.18.

Table 8.17. Process variables and their levels for Central Composite Design (CCD)

Coded values	Actual Values		
	Amount of PRM (A in g)	Amount of Chitosan (B in g)	Amount of Sod. hydroxide (C in mL*)
-1.63	0.011	0.011	3.15
-1	0.03	0.03	5
0	0.05	0.05	10
1	0.07	0.07	15
1.63	0.114	0.114	24.45

**mL of 10%w/v sodium hydroxide solution*

Table 8.18. Central Composite Design (CCD) with the measured responses

ES	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	C					
1	1	1	-1	31.02 ± 1.19	223.11 ± 3.34	95.43 ± 1.47	9.56 ± 0.94	3
2	-1	1	1	30.67 ± 1.32	252.30 ± 4.62	91.95 ± 1.73	4.32 ± 1.24	0
3	0	0	0	39.96 ± 1.26	183.86 ± 3.67	96.54 ± 1.26	5.14 ± 1.26	1
4	0	0	0	37.19 ± 1.16	184.62 ± 4.87	97.56 ± 1.34	5.73 ± 1.56	1
5	-1	-1	-1	29.41 ± 1.29	236.53 ± 4.56	83.05 ± 1.27	5.30 ± 1.48	2
6	1	-1	1	41.97 ± 1.44	43.68 ± 4.76	87.49 ± 1.19	5.89 ± 1.39	2.5
7	-1	1	-1	23.42 ± 1.15	286.65 ± 4.73	92.72 ± 1.28	5.41 ± 1.10	1
8	0	0	0	38.41 ± 1.27	164.86 ± 3.27	97.12 ± 1.37	5.69 ± 0.91	1
9	1	1	1	42.91 ± 1.37	195.96 ± 3.71	97.41 ± 1.13	7.21 ± 1.82	2.5
10	-1	-1	1	37.43 ± 1.10	84.57 ± 4.91	82.59 ± 1.25	4.88 ± 1.29	0
11	0	0	0	36.74 ± 1.42	187.62 ± 3.38	97.37 ± 1.33	5.92 ± 1.74	1
12	1	-1	-1	34.31 ± 1.06	154.33 ± 3.83	86.51 ± 1.14	7.29 ± 1.37	3+
13	-1.63	0	0	24.16 ± 0.97	278.61 ± 3.48	85.44 ± 1.74	4.34 ± 1.21	0.5
14	0	0	0	37.26 ± 1.52	182.18 ± 4.66	96.72 ± 1.83	5.91 ± 1.82	1
15	0	0	-1.63	33.65 ± 1.49	251.56 ± 4.97	90.91 ± 1.72	6.96 ± 1.18	3
16	1.63	0	0	36.89 ± 1.31	164.19 ± 3.73	91.87 ± 1.53	8.38 ± 1.48	3+
17	0	-1.63	0	37.28 ± 1.12	84.05 ± 3.38	82.35 ± 1.74	5.28 ± 1.19	2
18	0	1.63	0	27.26 ± 0.94	237.92 ± 3.59	98.88 ± 1.96	6.86 ± 1.47	1
19	0	0	1.63	46.82 ± 1.54	87.69 ± 3.92	92.34 ± 1.31	4.61 ± 1.19	0.5
20	0	0	0	36.51 ± 1.17	187.14 ± 3.73	96.89 ± 1.26	5.69 ± 0.96	1

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

8.4.1.3. Incorporation efficiency

Incorporation efficiency was carried out as mentioned in 6.2.1.4.

8.4.1.4. Particle size analysis

Particle size analysis was carried out as mentioned in 7.2.1.5

8.4.1.5. *In vitro* drug release

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

8.4.1.6. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

8.4.1.7. Optimization of responses using desirability

The percent incorporation efficiency value was targeted to maximize in the procedure, as higher values of this was desired to reduce total weight of microparticles, equivalent to 13.16 mg of PRM (7.5 mg of primaquine base). The values of Ymin and Ymax of percent incorporation efficiency were 23.42 and 46.82, respectively. 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 pH 1.2 in 15 min (t15) was selected. The values of Ymin and Ymax of percent drug release at pH 1.2 in 15 min (t15) were 82.35 and 98.88, respectively. The desirability function of incorporation efficiency and percent drug release at pH 1.2 has been calculated by following equation.

$$di = \left(\frac{Yi - Ymin}{Ymax - Ymin} \right)^{s} \qquad \text{(Equation 8.15)}$$

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 43.68 and 286.65, 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 pH 6.8 in 5 min was desired. The values of Ymin and Ymax of percent drug release at pH 6.8 in 5 min (t5) were 4.32 and 9.56, 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.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{(Equation 8.16)}$$

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 8.17, 8.18 and 8.19.

$$di = 1 \qquad \text{if Yi < Ymin} \qquad \text{(Equation 8.17)}$$

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s} \qquad \text{if Ymin} \leq \text{Yi} \leq \text{Ymax} \qquad \text{(Equation 8.18)}$$

$$di = 0 \qquad \text{if Yi > Ymax} \qquad \text{(Equation 8.19)}$$

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

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \qquad \text{(Equation 8.20)}$$

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

8.4.1.8. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

8.4.1.9. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

8.4.1.10. Preparation of single dose suspension powder (cachets)

Suspension powder containing equivalent of 13.16 mg of PRM (equivalent to 7.5 mg of 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.

8.4.1.11. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9.

8.4.1.12. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

8.4.1.13. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

8.4.1.14. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

8.4.2. Results and discussion

8.4.2.1. 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, incorporation efficiency, particle size, drug release at pH, 1.2 and 6.8. Hence A, B and C 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 8.19. 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 8.19) revealed that AC and BC terms were insignificant for incorporation efficiency, C^2, AB and AC terms are insignificant for particle size. AB and BC terms were insignificant for drug release at pH 1.2 while C^2 term was insignificant for drug release at pH 6.8. AB term was insignificant for bitterness score. The surface plots are shown in Figure 8.12.

Table 8.20 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 batches of microparticles 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.

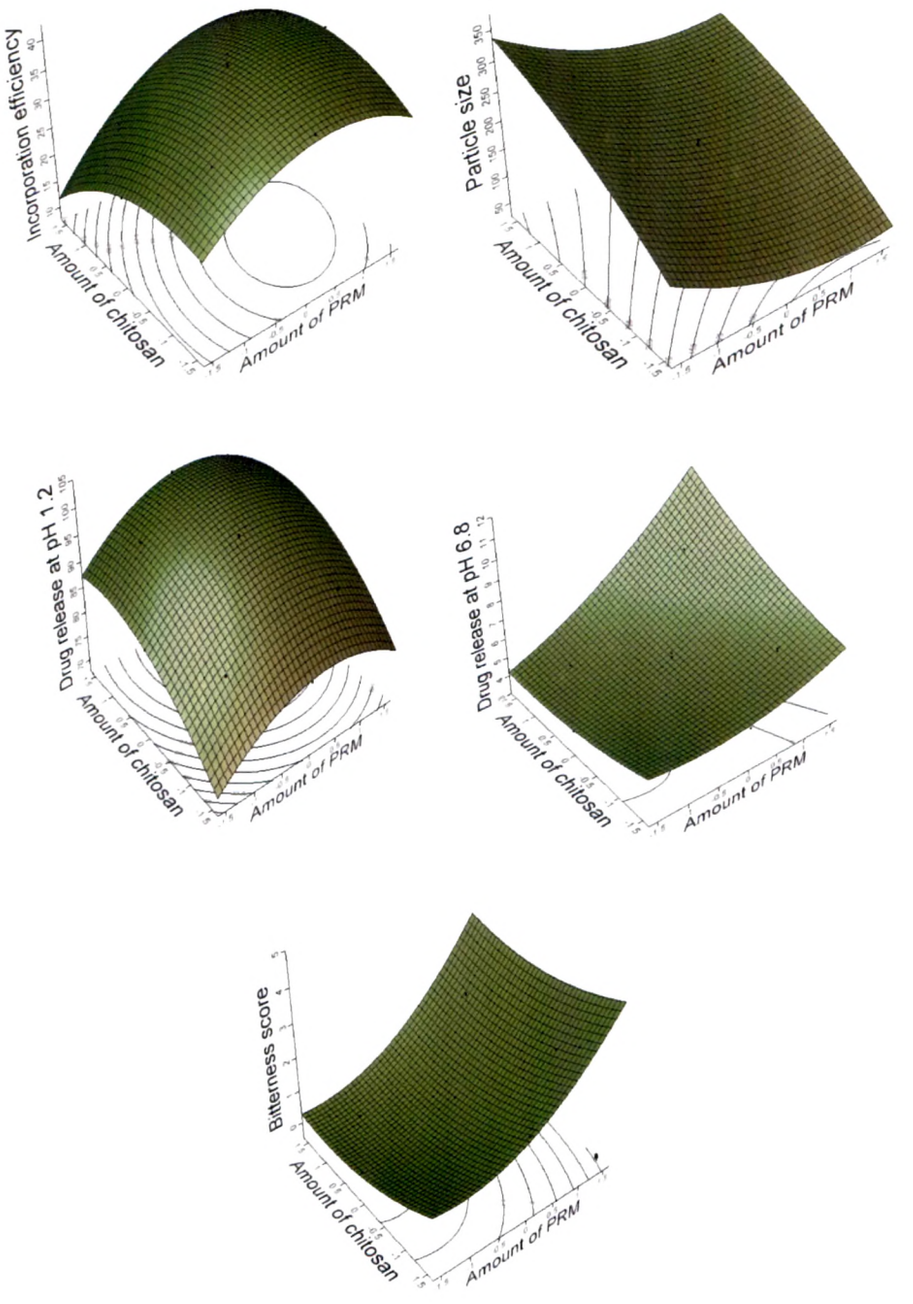

Figure 8.12. Surface plots showing the effect of amount of drug and polymer on particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 8.19. Results of regression analysis

Terms	Incorporation efficiency (%)				Particle size (μm)				Drug release at pH 1.2 (t15 in %)			
	FM		RM		FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	37.68	NA	37.68	NA	181.69	NA	178.69	NA	97.02	NA	97.02	NA
A	3.76	< 0.0001*	3.76	< 0.0001*	-32.24	< 0.0001*	-32.24	< 0.0001*	2.03	< 0.0001*	2.03	< 0.0001*
B	-2.36	< 0.0001*	-2.36	< 0.0001*	51.76	< 0.0001*	51.76	< 0.0001*	4.86	< 0.0001*	4.86	< 0.0001*
C	4.22	< 0.0001*	4.22	< 0.0001*	-44.38	< 0.0001*	-44.38	< 0.0001*	0.30	0.0196	0.30	0.0115
A^2	-2.69	< 0.0001*	-2.69	< 0.0001*	14.96	0.0004	15.28	0.0003	-3.10	< 0.0001*	-3.10	< 0.0001*
B^2	-2.04	0.0002	-2.04	0.0001	-7.69	0.0221	-7.37	0.0319	-2.36	< 0.0001*	-2.36	< 0.0001*
C^2	0.95	0.0227	0.95	0.0209	-4.45	0.1485	–	–	-1.98	< 0.0001*	-1.98	< 0.0001*
AB	1.30	0.0169	1.30	0.0152	0.40	0.9147	–	–	-0.024	0.8703	–	–
AC	0.53	0.2660	–	–	6.06	0.1280	–	–	0.52	0.0042	0.52	0.0019
BC	0.43	0.3633	–	–	25.14	< 0.0001*	25.14	< 0.0001*	0.086	0.5567	–	–

*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 8.19. ***(Continued)***

Terms	Drug release at pH 6.8 (t5 in %)				Bitterness score			
	FM		RM		FM		RM	
	EC	Prob > F	EC	Prob > F	EC	Prob > F	EC	Prob > F
Intercept	5.72	NA	5.72	NA	1.00	NA	1.00	NA
A	1.25	< 0.0001*	1.25	< 0.0001*	1.00	< 0.0001*	1.00	< 0.0001*
B	0.43	< 0.0001*	0.43	< 0.0001*	-0.23	0.0004	-0.23	0.0003
C	-0.68	< 0.0001*	-0.68	< 0.0001*	-0.64	< 0.0001*	-0.64	< 0.0001*
A^2	0.28	0.0010	0.28	0.0009	0.37	< 0.0001*	0.37	< 0.0001*
B^2	0.17	0.0182	0.17	0.0195	0.18	0.0028	0.18	0.0024
C^2	0.065	0.3104	–	–	0.27	0.0001	0.27	< 0.0001*
AB	0.50	< 0.0001*	0.50	< 0.0001*	0.063	0.3134	–	–
AC	-0.28	0.0051	-0.28	0.0046	0.19	0.0097	0.19	0.0090
BC	-0.20	0.0273	-0.20	0.0262	0.19	0.0097	0.19	0.0090

*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 8.20 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) 100	PRESS	R^2	Adj-R^2	Pred-R^2	Adeq Precision
Incorporation efficiency (%)	FM	679.59	9	75.51	45.77	0.01*	79.93	0.97	0.95	0.88	27.24
	RM	675.81	7	96.54	57.12	0.01*	57.63	0.97	0.95	0.91	30.22
Particle Size (μm)	FM	85492.53	9	9499.17	88.95	0.01*	6167.61	0.98	0.97	0.92	35.13
	RM	84935.34	6	14155.89	113.24	0.01*	4270.96	0.98	0.97	0.95	38.81
Drug release at pH 1.2 (t15 in %)	FM	596.59	9	66.29	411.92	0.01*	7.95	0.99	0.99	0.98	57.49
	RM	596.52	7	85.22	611.15	0.01*	5.06	0.99	0.99	0.99	68.59
Drug release at pH 6.8 (t5 in %)	FM	33.78	9	3.75	76.35	0.01*	1.25	0.98	0.97	0.96	33.64
	RM	33.75	8	4.22	84.67	0.01*	1.43	0.98	0.97	0.95	35.24
Bitterness Score	FM	23.17	9	2.57	92.84	0.01*	2.16	0.98	0.97	0.90	31.99
	RM	23.14	8	2.89	103.11	0.01*	1.71	0.98	0.97	0.92	33.52

**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.*

8.4.2.2. Incorporation efficiency

As shown in Table 8.19, the amount of chitosan (B) had a negative coefficient, while the amount of PRM (A) and sodium hydroxide (C) had positive coefficients. This indicates that on increasing the amount of chitosan, incorporation efficiency decreases. The percent incorporation efficiency was found to be in the range between 23.42 and 46.82. The percent incorporation efficiency showed a dependence on the extent of crosslinking and amount of drug added. The microparticles with higher amount of drug added exhibited higher incorporation efficiencies. This is due to the accumulation of more drug molecules. The extent of crosslinking has a significant effect on percent incorporation efficiency. As the amount of crosslinking agent increased, an increase in percent incorporation efficiency was observed. This is because at higher extent of crosslinking, there will be a formation of more rigid network structure, which would cause the retention of more of drug molecules during the microsphere preparation (Rokhade et al., 2007).

8.4.2.3. Particle size

As shown in Table 8.19, the amount of chitosan (B) had a positive coefficient, while the amount of PRM (A) and sodium hydroxide (C) had negative coefficients. This indicates that on increasing the amount of chitosan, particle size increases. The particle size is influenced by the orifice of the needle and the viscosity of the chitosan solution. The increased viscosity at higher amount of chitosan resulted in larger particles. A high amount of sodium hydroxide resulted in smaller particle size due to high degree of crosslinking. Similar results were observed for glutaraldehyde (Kumbar et al., 2002; Rokhade et al., 2007).

8.4.2.4. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of PRM (A), chitosan (B) and sodium hydroxide (C) had positive coefficient. This indicates additive effect of amount of PRM, chitosan and sodium hydroxide. 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 improved

wettability, provided by the dissolved chitosan (Rane et al., 2007). Figure 8.13 shows dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8.

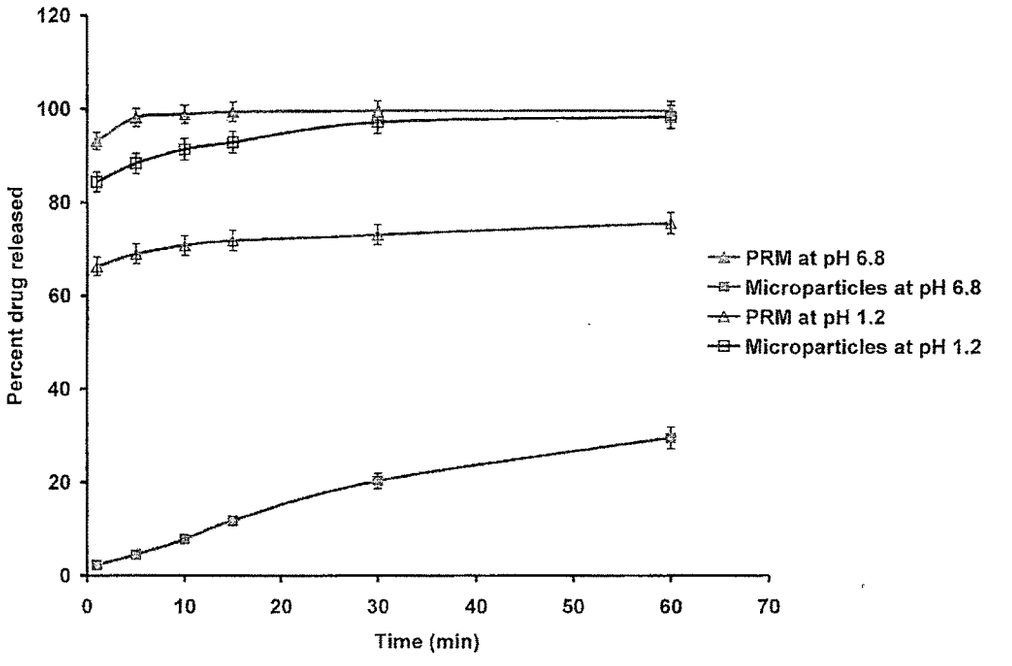

Figure 8.13. Dissolution profile of PRM and optimized microparticles at pH, 1.2 and 6.8

For *in vitro* drug release at pH 6.8, the amount of PRM (A) and chitosan (B) had positive coefficient while the amount of sodium hydroxide (C) had negative coefficient. This indicates that on increasing the amount of sodium hydroxide, drug release of microparticles at pH 6.8 decreases. The effect of amount of chitosan on the release of drug was found to be meager. The drug release after 5 min was decreased when the amount of sodium hydroxide was increased, which is due to the fact that as the amount of sodium hydroxide was increased, it produced microspheres with pronounced cross-linking between polymer chains that retarded the release of drug (Ko et al., 2002; Remunan-Lopez and Bodmeier, 1997). Similar results were observed with glutaraldehyde (Chourasia and Jain, 2004). In addition, the drug release from chitosan microparticles increases with increase in drug content (Bayomi, 2004).

8.4.2.5. Gustatory sensation test

For bitterness score, the amount of PRM (A) had positive coefficient, while the amount of chitosan (B) and sodium hydroxide (C) had negative coefficient. This indicates that on increasing the amount of sodium hydroxide, bitterness score of microparticles decreases. This finding is in agreement with drug release studies carried out at pH 6.8 because the pH of the saliva is 6.8 (Hashimoto et al., 2002). It has been reported that PRM produces bitterness by depolarizing taste cells through K^+ channels (Yamamoto et al., 1998). The microparticles control the drug release at salivary pH and restrict direct interaction of PRM with K^+ channels resulting in decreased bitterness of PRM in microparticles. Bitterness score of optimized microparticles is shown in Table 8.21.

8.4.2.6. 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 incorporation efficiency and drug release at pH 1.2 have to be maximized, in order to pour desired characteristics in the microparticles. 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 0.73. Table 8.21 enlists the optimized values for independent variables and their responses.

Table 8.21. 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 g	C in mL#						
0.046	0.032	15	38.66 ± 1.87	153.19 ± 2.51	92.72 ± 1.83	4.55 ± 0.69	0	0.73

*A – amount of PRM, B – amount of chitosan, C – amount of NaOH, #mL of 10%w/v sodium hydroxide solution, *Values represent the mean ± SD of 3 experiments, t5 and t15 – percent drug dissolved in 5 and 15 minute respectively.*

8.4.2.7. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of PRM, chitosan, blank microparticles and optimized microparticles are shown in Figure 8.14. Blank microparticles showed shifting of absorption peak of –OH from 3430 to 3461 cm^{-1}, compared to chitosan. In addition the absorption peak of amide band, a C-O stretching mode together with an N-H deformation mode, located at 1658 cm^{-1} shifted to 1666 cm^{-1}, compared to chitosan. The absorption bands of placebo microparticles were again shifted to 3448 cm^{-1} and 1654 cm^{-1}, for –OH stretching and amide band, respectively in optimized microparticles. This finding confirms the crosslinking of chitosan in presence of sodium hydroxide.

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 FTIR spectrum of microparticles found to exhibit some significant difference in the characteristic peaks of PRM, revealing modification of the drug environment. A sharp band (doublet) at 1612 and 1595 cm^{-1} was observed in PRM. Microparticles showed conversion of this band in singlet at 1614 cm^{-1}. Further the C-H def in methylene observed at 1455 cm^{-1}, shifted to 1470cm^{-1} in

microparticles with reduced intensity. This suggests electrostatic interaction between chitosan and PRM.

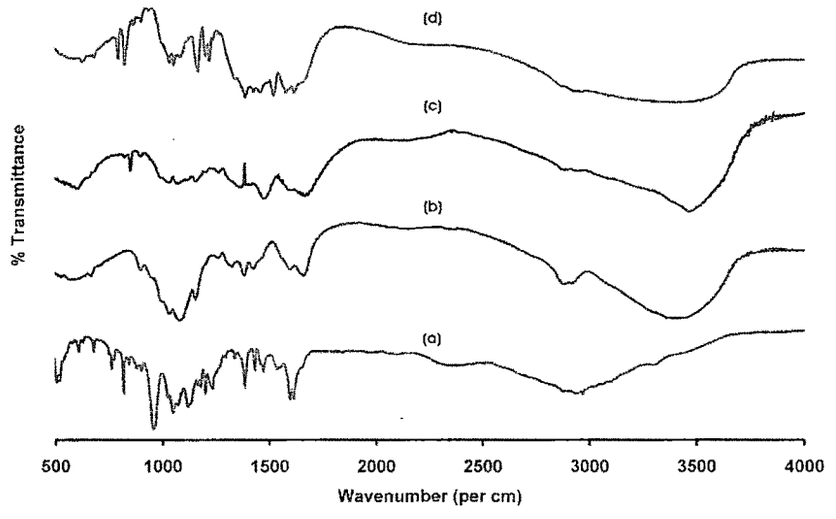

Figure 8.14. FT-IR spectra of (a) PRM, (b) chitosan, (c) blank microparticles and (d) optimized microparticles

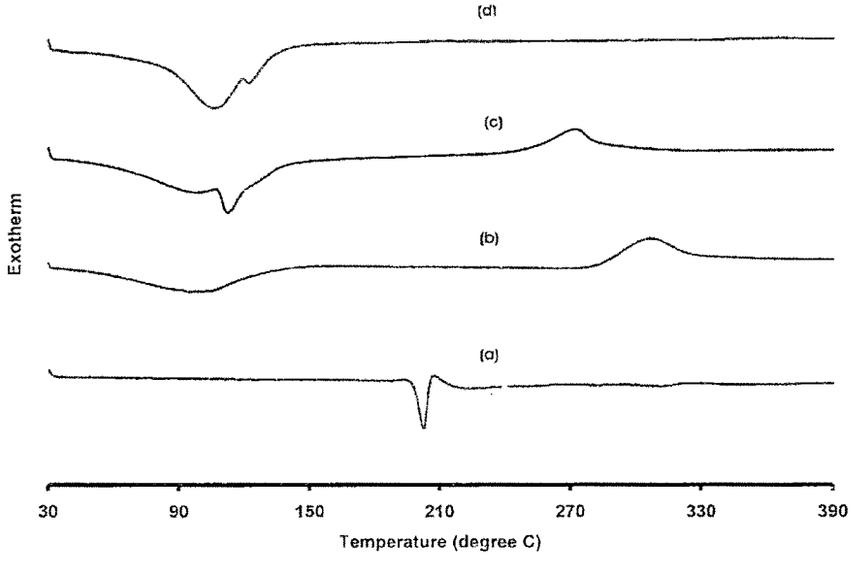

Figure 8.15. DSC curve of (a) PRM, (b) chitosan, (c) blank microparticles and (d) optimized microparticles

8.4.2.8. Differential scanning calorimeter (DSC)

Figure 8.15 shows the DSC curve of PRM, chitosan, 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 absent 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.

8.4.2.9. 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 8.22. The formula of optimized suspension powder (DS94) was further used to prepare suspension powder of plain PRM (DS95). The characteristics of suspension powder are summarized in Table 8.23.

Table 8.22. Formulation of suspension powder

Drug/Excipients	For 6 cachets				
	DS91	**DS92**	**DS93**	**DS94**	**DS95**
PRM (g)	-	-	-	-	0.078
Microparticles eq. to 13.16 mg PRM (g)	0.345	0.345	0.345	0.345	-
Sodium carboxy methyl cellulose (g)	0.001	0.002	0.003	0.004	0.004
Granular Lactose (Lactopress) (g)	0.241	0.240	0.239	0.238	0.512
Citric acid (g)	0.006	0.006	0.006	0.006	0.006
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)	0.600	0.600	0.600	0.600	0.600

Table 8.23. Physical properties of suspension powder

Parameters	DS91	DS92	DS93	DS94	DS95
Angle of repose (^{0}) ± SD*	34.62 ± 0.32	35.15 ± 0.38	34.87 ± 0.27	35.74 ± 0.48	35.89 ± 0.42
F value (after reconstitution) ± SD*	0.18 ± 0.09	0.36 ± 0.06	0.78 ± 0.05	0.95 ± 0.04	0.96 ± 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.*

8.4.2.10. 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 DS95, the 5% of panel rated it as very strongly bitter, 95% strongly bitter. DS94 was rated as tasteless by 100% of volunteers of panel (Table 8.24).

Table 8.24. 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							
DS95							19	1
DS94	20							

8.4.2.11. 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 DS94 showed little change over time, and so a shelf life up to 1448.10 days (48.27 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).

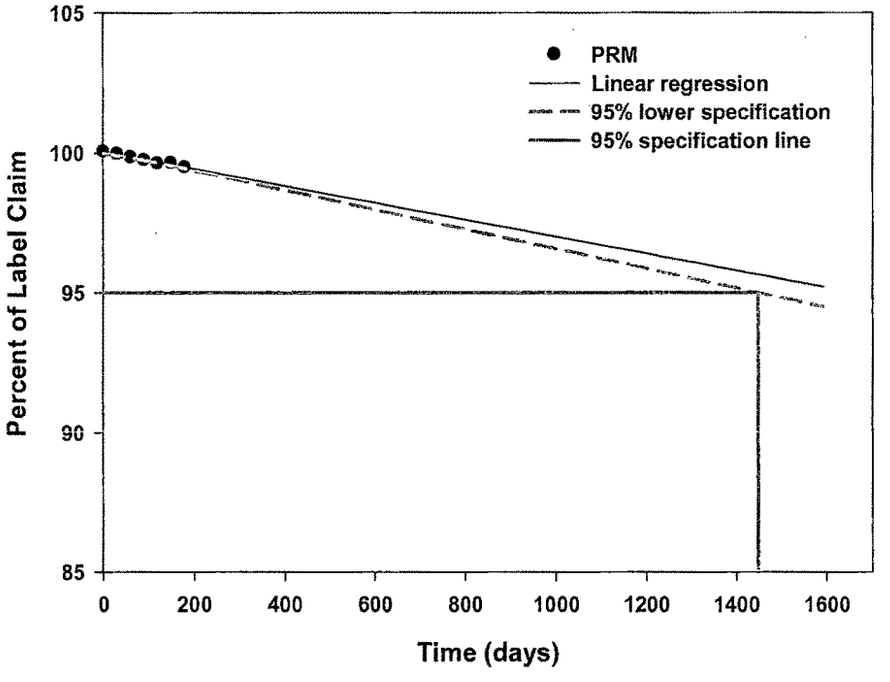

Figure 8.16. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of PRM in microparticles using chitosan as polymer. Present work suggests that all three 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, chitosan and NaOH, which have significant effect on microparticle's desired properties. The FTIR and DSC studies indicated uniform dispersion of PRM, at the molecular level, in chitosan microparticles. These taste masked microparticles further incorporated into cachets to avoid rupturing of microparticles on compression and leaching of drug after reconstitution on storage.

8.5. Summary

	ARM-Chi	MFL-Chi	PRM-Chi
Chitosan required per cachet	50 mg ARM + 88.80 mg chitosan	100 mg MFL + 186 mg Chitosan	13.16 mg PRM + 331.84 mg Chitosan
Incorporation efficiency (%) ± SD*	83.43 ± 2.2	79.63 ± 2.73	38.66 ± 1.87
Particle size (μm) ± SD*	234.87 ± 2.71	193.74 ± 2.58	153.19 ± 2.51
Drug release at pH 1.2 (t15 in %) ± SD*	80.29 ± 1.36	84.17 ± 1.34	92.72 ± 1.83
Drug release at pH 6.8 (t5 in %) ± SD*	3.57 ± 1.18	3.67 ± 0.84	4.55 ± 0.69
Bitterness score (for microparticles microparticles)	0	0	0
Overall desirability	0.70	0.66	0.73
Total filled weight per cachet (g)	0.200	0.350	0.100
Angle of repose (^{0}) ± SD*	34.43 ± 0.59	37.78 ± 0.58	35.74 ± 0.48
F value (after reconstitution) ± SD*	0.96 ± 0.02	0.96 ± 0.03	0.95 ± 0.04
pH (after reconstitution)	4.5-4.6	4.4-4.5	4.6-4.7
Bitterness score (for dry suspension)	0	0	0
Shelf life (months)	46.54	48.44	48.27

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

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