Microencapsulation using Eudragit E-Beta-CD

7.1. Introduction

Eudragit E 100 (EE), is a cationic copolymer based on dimethyl aminoethyl methacrylate and neutral methacrylic esters soluble up to pH 5 (Elisabetta 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.

On the other hand, the bitter taste elimination capacity of beta-cyclodextrin (CD) towards a number of drugs is well known. The bitter taste of a drug substance disappears in the presence of CD, only when the drug molecule which causes the bitter taste is complexed by an appropriate CD molecule. These complex molecules are strongly hydrated on their outer surface, therefore, they don't get attached to the taste-bud receptors on the tongue in oral cavity (Szejtli and Szente, 2005).

Furthermore, the possible use of CD as candidate for co enhancer effect could be considered. Therefore, the objective of present investigation was to graft EE with CD and evaluate its effect on bitter taste masking. Further a palatable formulation has been developed. 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 (Elisabetta et al., 2000; Sinha and Rachna, 2002), so might be EE-CD. 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.

7.2. Artemether (ARM)

7.2.1. Experimental

7.2.1.1. Materials

Eudragit E 100 (EE) (Batch no. G041131159) was a gift from Degussa India Pvt. Ltd., (Mumbai, India). Mono amino beta-cyclodextrin (CD) was a gift from Prof. Giancarlo (University of Turin, Italy). 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. Chloroform, propargyl alcohol, p-toluene sulfonic acid, t-butanol, copper sulphate, ascorbic acid were purchased from Sigma Aldrich (Germany).

7.2.1.2. Grafting of EE-CD

Microwave Assisted Propargylation of EE

2 g EE, 10 mL propargyl alcohol and 1.52 g p-toluene sulfonic acid (2 equivalent) were refluxed at 120^{0}C for 2 hrs in microwave with constant power (200w). Chloroform was added to the concentrate and washed with sodium hydroxide solution 3 times. The chloroform phase was collected and evaporated in rotavapor. The solid was further washed with petroleum ether to remove any impurities, formed during grafting and dried under vacuum.

Microwave and Ultrasound assisted grafting of EE-CD

Microwave in combination with ultrasound was used for the grafting of EE-CD. 950 mg propargylated EE, 25 mL of t-butanol, 342 mcL copper sulphate (0.2 equivalent), 118.7 mg ascorbic acid (0.4 equivalent) and 1000mg mono amino CD (0.5 equivalent) were mixed in 35 mL of water and kept for 2 hrs at 70-75^{0}C in microwave at constant power (95w) with 20.2 frequency of ultrasound (power=30-40w). The solution was washed with diethyl ether. Polymer was present in water phase. Few drops of sodium hydroxide were added and then precipitate was washed with distilled water and centrifuged. The solid collected was further washed with acetone and evaporated to drying using rotavapor.

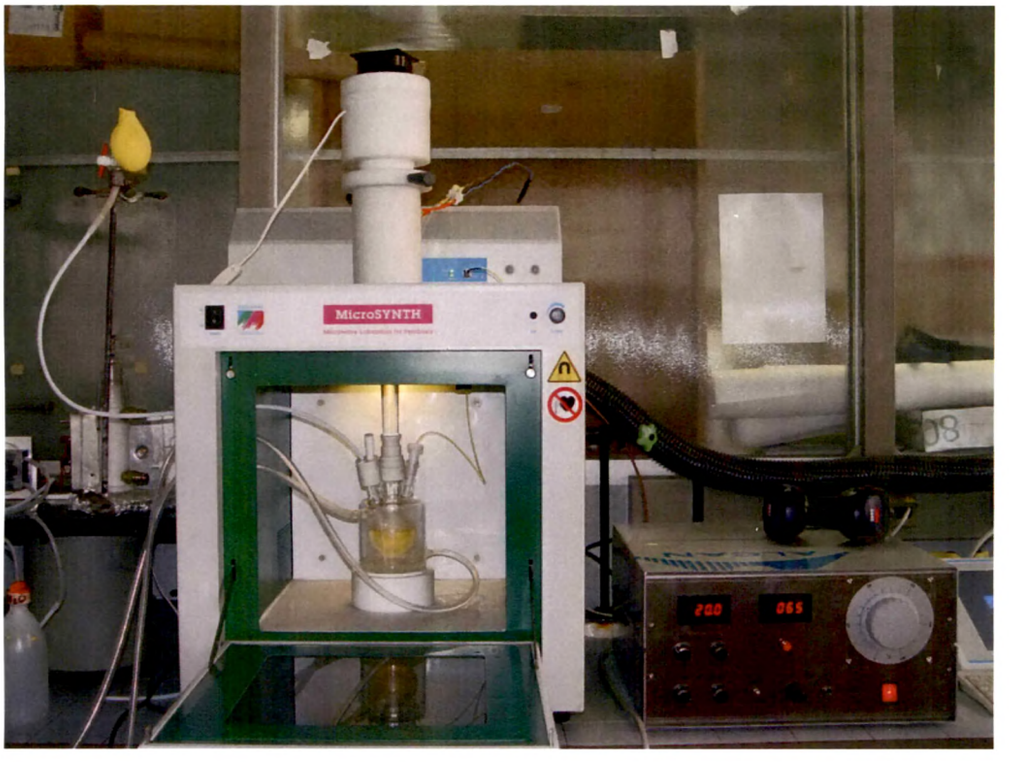


Figure 7.1. Microwave and Ultrasound in combination used for grafting of EE-CD

7.2.1.3. Preparation of Microparticles

Microparticles were prepared by coacervation phase separation method. A concentrated solution of EE-CD (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-CD 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-CD solution. Different concentrations of ARM and EE-CD were used as mentioned in Table 7.1. 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 7.1. 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-CD solution*

7.2.1.4. 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 7.2.

7.2.1.5. Incorporation efficiency

Incorporation efficiency was carried out as mentioned in 6.2.1.4.

7.2.1.6. Particle size analysis

Particle size analysis was carried out as mentioned in 6.2.1.5

7.2.1.7. *In vitro* drug release

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

7.2.1.8. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

Table 7.2. 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					
1	-1	1	54.08 ± 2.74	199.33 ± 5.32	93.22 ± 1.53	4.76 ± 0.68	0
2	0	0	73.53 ± 2.16	112.26 ± 4.51	89.19 ± 1.32	6.64 ± 0.94	1
3	0	1	73.33 ± 1.87	112.89 ± 4.38	97.88 ± 1.14	4.43 ± 0.78	0
4	-1	-1	67.92 ± 1.93	56.47 ± 5.63	85.87 ± 1.68	5.78 ± 0.89	1
5	1	-1	70.24 ± 2.42	59.97 ± 4.49	90.56 ± 1.62	6.25 ± 0.91	3
6	-1	0	61.62 ± 1.69	143.42 ± 5.12	85.51 ± 1.48	7.13 ± 0.72	0
7	1	1	87.58 ± 1.32	59.81 ± 4.18	99.89 ± 1.59	5.86 ± 0.97	0
8	1	0	79.54 ± 2.36	74.22 ± 3.79	91.72 ± 1.27	8.17 ± 0.79	1
9	0	-1	72.67 ± 2.29	48.56 ± 3.42	88.39 ± 1.37	5.28 ± 0.84	2

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

7.2.1.9. 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 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 85.51 and 99.89, respectively. The desirability function of this parameter has been calculated by following equation.

$$di = \left(\frac{Yi - Y\min}{Y\max - Y\min} \right)^{s}$$ (Equation 7.1)

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 48.56 and 199.93, 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 4.43 and 8.17, 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.

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s}$$ (Equation 7.2)

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 8.3, 8.4 and 8.5.

$di = 1$ if Yi < Ymin (Equation 7.3)

$$di = \left(\frac{Y\max - Yi}{Y\max - Y\min} \right)^{s}$$ if Ymin ≤ Yi ≤ Ymax (Equation 7.4)

$di = 0$ if Yi > Ymax (Equation 7.5)

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

$$D = (d1 \times d2 \times d3 \times d4)^{1/4} = \left(\prod_{i=1}^{4} d_i \right)^{1/4} \qquad \text{(Equation 7.6)}$$

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

7.2.1.10. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

7.2.1.11. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

7.2.1.12. 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 50 mg of ARM were prepared from ARM and optimized microparticles. Xanthan gum was 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.

7.2.1.13. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9.

7.2.1.14. Sedimentation characteristics

Sedimentation characteristics were studied as mentioned in 5.2.1.10.

7.2.1.15. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test was carried out as mentioned in 5.2.1.11.

7.2.1.16. Investigation of chemical stability of ARM

Chemical stability of ARM was studied as mentioned in 5.2.1.12.

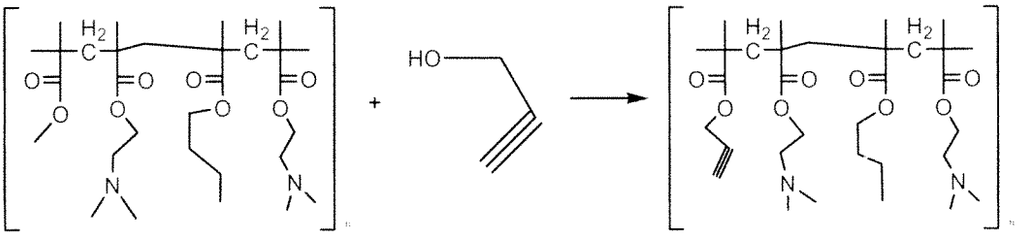
7.2.1.17. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

7.2.2. Results and discussion

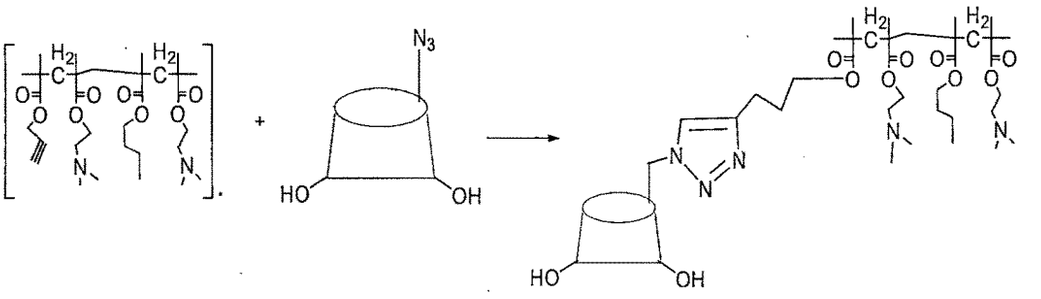
7.2.2.1. Grafting of EE-CD

In first step of grafting, propargylation of EE was carried out in acidic medium as EE get protonated and solublised in acidic medium. The propargylation was carried out at ester group of EE as it was difficult to carry out at dimethyl amino group. In addition, the dimethyl amino group is responsible for the solubility in acidic medium. Thus the newly prepared polymer, EE-CD, retained similar solubility pattern as that of EE. Such reactions takes several days to complete. Microwave offers advantage for such time consuming reactions.



Eudragit E **Propargyl Alcohol** **Propargylated Eudragit E**

Second step of grafting was carried out by 'Click chemistry'. This takes few hours when carried out in microwave and ultrasound. Mono amino CD was reacted with propargylated EE using microwave and ultrasound.



Propargylated EE **Mono amino CD** **EE-CD**

7.2.2.2. 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 7.3. 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 7.3) revealed that A^2 term was insignificant for particle size while A^2 and AB terms were insignificant for drug release at pH 1.2. The term AB was insignificant for drug release at pH 6.8 while A^2 and B^2 terms were insignificant for bitterness score. The surface plots are shown in Figure 7.2.

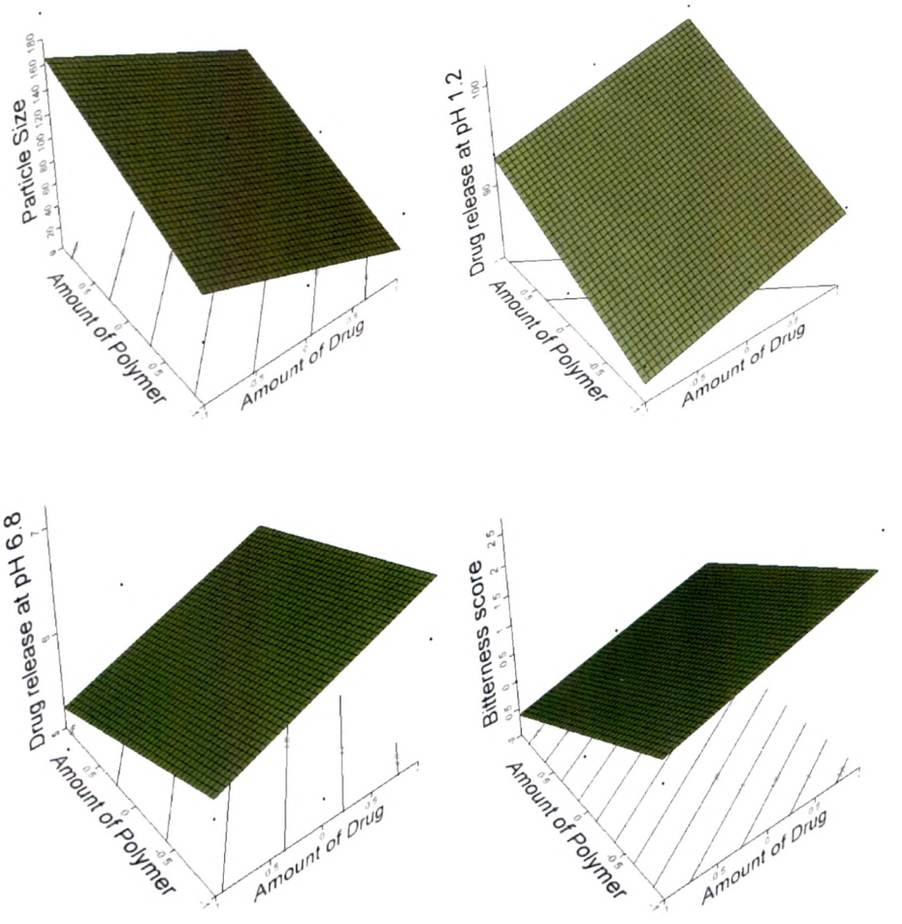


Figure 7.2. Surface plots showing the effect of amount of drug and polymer on particle size, drug release at pH, 1.2 and 6.8 along with bitterness score

Table 7.3. 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	104.88	NA	104.88	NA	89.27	NA	88.81	NA	6.73	NA	6.73	NA	0.78	NA	0.33	NA
A	-34.20	0.0012	-34.20	0.0005	2.93	0.0005	2.93	0.0002	0.44	0.0034	0.44	0.0051	0.50	0.0079	0.50	0.0071
B	34.50	0.0011	34.50	0.0005	4.36	0.0001	4.36	< 0.0001*	-0.38	0.0051	-0.38	0.0085	-1.00	0.0010	-1.00	0.0003
A^2	7.63	0.2116	–	–	-0.69	0.1061	–	–	0.88	0.0022	0.88	0.0029	0.17	0.3081	–	–
B^2	-20.46	0.0240	-20.46	0.0224	3.83	0.0011	3.83	0.0006	-1.92	0.0002	-1.92	0.0001	0.33	0.0917	–	–
AB	-35.75	0.0019	-35.75	0.0009	0.50	0.1034	–	–	0.16	0.0862	–	–	-0.50	0.0138	-0.50	0.0158

*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 7.4 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	20230.35	5	4046.07	87.00	0.0019	1118.15	0.99	0.98	0.94	26.32
	RM	20113.82	4	5028.45	78.55	0.0005	1718.69	0.98	0.97	0.91	23.56
Drug release at pH 1.2 (t15 in %)	FM	196.84	5	39.37	215.49	0.0005	6.62	0.99	0.99	0.96	41.77
	RM	194.91	3	64.97	130.72	< 0.0001	8.29	0.98	0.97	0.95	31.02
Drug release at pH 6.8 (t5 in %)	FM	10.99	5	2.20	140.57	0.0009	0.49	0.99	0.98	0.95	35.32
	RM	10.89	4	2.72	74.53	0.0005	0.74	0.98	0.97	0.93	25.31
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 7.4 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.

7.2.2.3. 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 7.2. The high content of ARM in the microparticles was believed to be due to poor solubility of ARM in EE-CD solution. Incorporation efficacy improves with increase in polymer (Satturwar et al., 2002). However higher quantity of EE-CD solution prepared in 1%v/v acetic acid, showed solubilization of ARM. This resulted in decreased incorporation efficiency (Rama Rao et al., 2005). This finding suggests that the EE-CD is suitable for the preparation of microparticles of a poorly water-soluble drug, such as ARM.

7.2.2.4. Particle size

For particle size, the amount of ARM (A) is negative while amount of EE-CD (B) is positive. This indicates that on increasing the amount of EE-CD, particle size increases. It was observed that the polymer viscosity influenced particle size (Satturwar et al., 2002). Increasing the amount of EE-CD 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.

7.2.2.5. *In vitro* drug release

For *in vitro* drug release at pH 1.2, the amount of ARM (A) and EE-CD (B) are positive. This indicates additive effect of amount of ARM and EE-CD. 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-CD, and improved wettability, provided by the dissolved EE-CD (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-CD (B) is negative. This indicates that on increasing the amount of EE-CD, drug release from microparticles decreases. As the amount of EE-CD 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-CD 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 7.3 shows dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8.

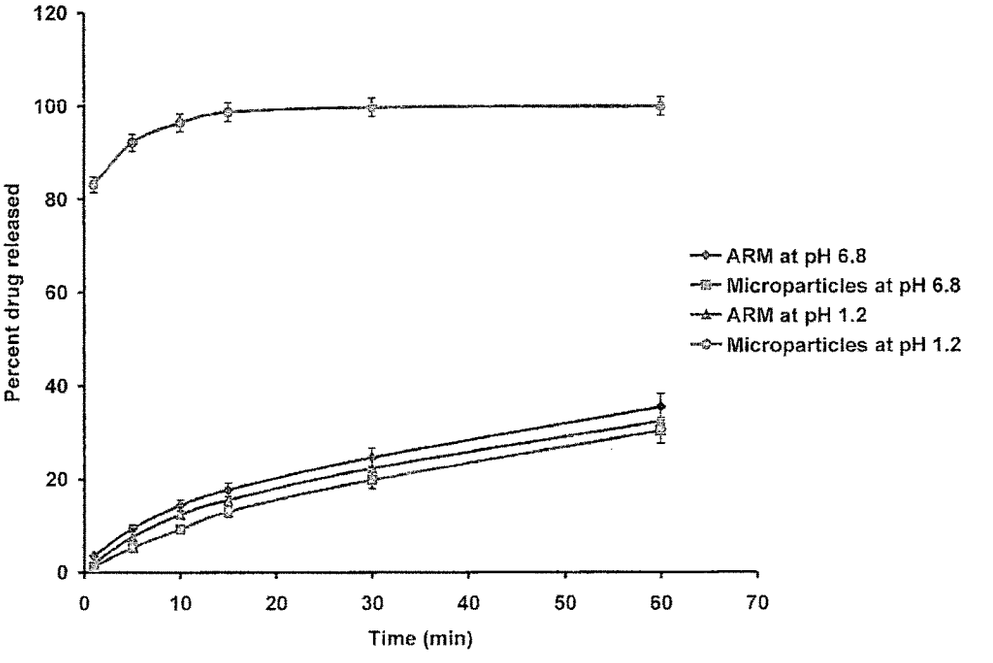


Figure 7.3. Dissolution profile of ARM and optimized microparticles at pH, 1.2 and 6.8

7.2.2.6. Gustatory sensation test

For bitterness score, the amount of ARM (A) is positive while amount of EE-CD (B) is negative. This indicates that on increasing amount of EE-CD, 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-CD is expected to behave as insoluble at pH 6.8 and showed decreased drug release in microparticles. Thus EE-CD 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 7.5.

7.2.2.7. 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. 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 individuai desirability of each of the responses using DOE v6.0.5 (Stat-Ease, Inc.). The optimized batch was identified with a desirability value of 0.89. Table 7.5 enlists the optimized values for independent variables and their responses.

Table 7.5. Optimum levels of independent variables and their responses

Actual values of independent variables for optimized batch		Incorporation efficiency (%) ± SD*	Particl e size (μm) ± SD*	Drug release at pH 1.2 (t15 in %) ± SD*	Drug releas e at pH 6.8 (t5 in %) ± SD*	Bitterness score	Overall Desirability
A in g	B in mL#						
0.04	15	84.17 ± 2.78	64.52 ± 1.63	98.39 ± 1.42	5.26 ± 0.89	0	0.89

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

7.2.2.8. Fourier transform infra-red spectroscopy (FTIR)

The FT-IR spectrum of ARM, EE-CD, physical mixture of ARM and EE-CD along with optimized microparticles are shown in Figure 7.4.

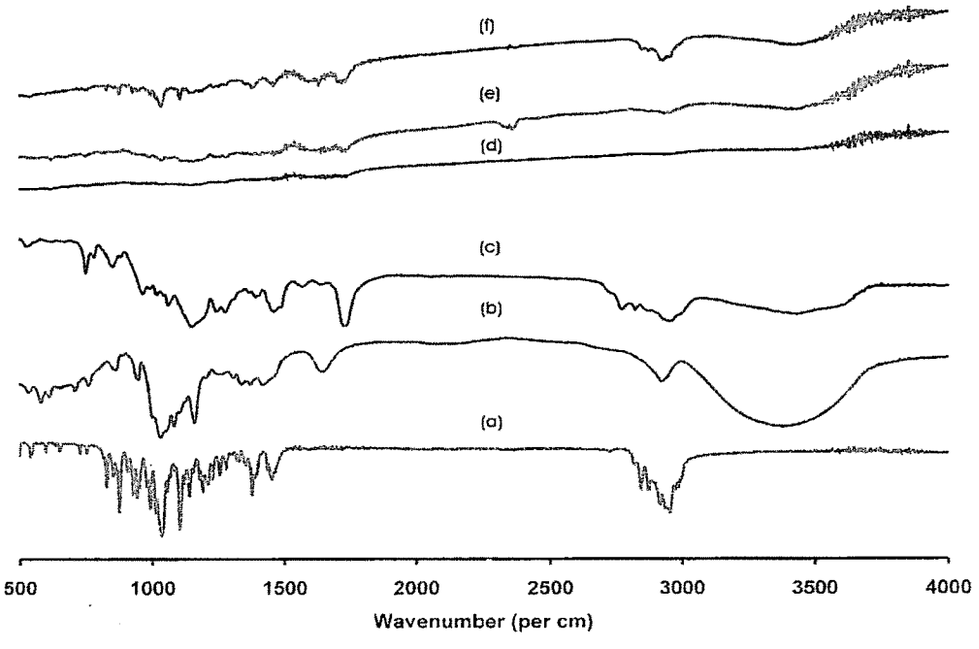


Figure 7.4. FT-IR spectra of (a) ARM, (b) CD (c) EE (d) EE-CD, (e) physical mixture of ARM and EE-CD (f) optimized microparticles

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. As can be seen, the spectrum of EE exhibits the characteristic bands at 2772 and 2822 cm^{-1} corresponding to the absorption by nonionized dimethylamino groups of the copolymer. The spectrum of CD also exhibits the vibration of free OH at 3380 cm^{-1} and those of the bound OH at 2924 cm^{-1}, a shorter band between 1550 and 1700 cm^{-1}, and a large band which displayed distinct peaks in the region of 900–1200 cm^{-1}.

The spectrum of EE-CD exhibits certain specific features. In particular, the EE-CD spectrum contains bands corresponding to dimethyl amino group of EE and OH group of CD with decreased percent transmittance. However this decrease in frequency of a specific peak of EE and CD suggest grafting of EE with CD.

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

7.2.2.9. Differential scanning calorimeter (DSC)

Figure 7.5 shows the DSC curve of ARM, EE-CD, physical mixture of ARM and EE-CD along with optimized microparticles. The pure ARM shows an endothermic peak at 87.94^{0}C, followed by exothermic peak at 180.28^{0}C.

The DSC curve of CD displayed a wide and strong endothermic effect in the 100–130^{0}C interval (peak Tmax = 121.03^{0}C), which may be ascribed to dehydration (Li et al., 2005). Moreover, the melting peak of the CD was Tmax = 319.66^{0}C.

The DSC curve of EE-CD showed shifting of dehydration curve of CD in the 66-117^{0}C. interval (peak Tmax = 94.84^{0}C). Moreover, the melting peak of CD was disappeared. This confirmed the results obtained from FTIR studies.

The endothermic peak corresponding to melting peak of ARM was broadened and shifted towards lower temperature, with reduced intensity in physical mixture while it was absent in 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.

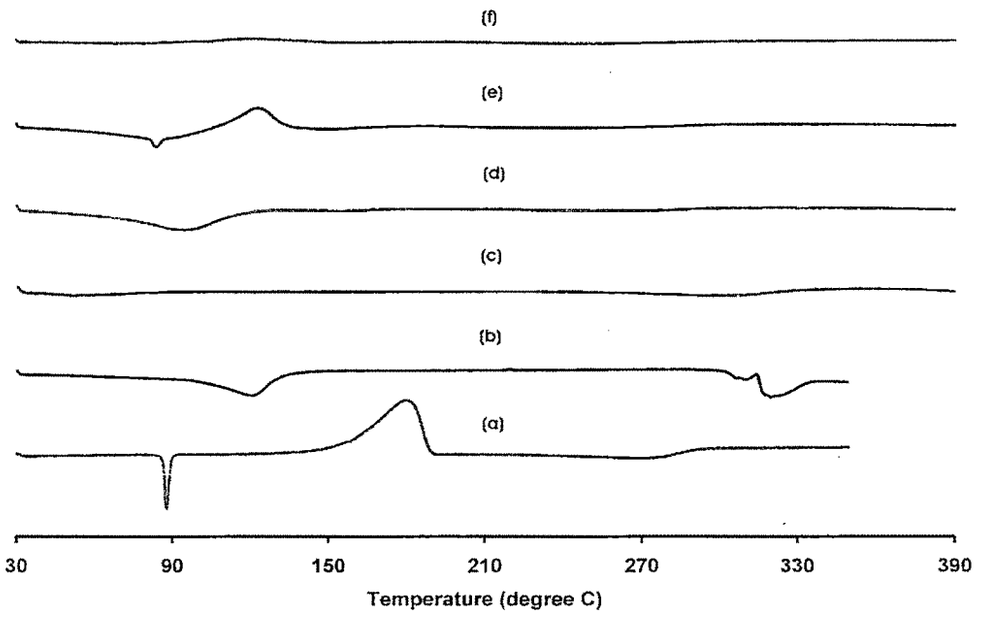


Figure 7.5. DSC curve of (a) ARM, (b) CD (c) EE (d) EE-CD, (e) physical mixture of ARM and EE-CD (f) optimized microparticles

7.2.2.10. 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 7.6. The formula of optimized suspension powder (DS64) was further used to prepare suspension powder of plain ARM (DS65). The characteristics of suspension powder are summarized in Table 7.7.

Table 7.6. Formulation of suspension powder

Drug/Excipients	For 6 cachets				
	DS61	DS62	DS63	DS64	DS65
ARM (g)	-	-	-	-	0.300
Microparticles eq. to 0.050 g ARM (g)	1.693	1.693	1.693	1.693	-
Xanthan gum (g)	0.004	0.006	0.009	0.012	0.012
Microcrystalline cellulose (Avicel PH 302) (g)	0.381	0.379	0.376	0.373	1.766
Citric acid (g)	0.016	0.016	0.016	0.016	0.016
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 7.7. Physical properties of suspension powder

Parameters	DS61	DS62	DS63	DS64	DS66
Angle of repose (^{0}) ± SD*	32.14 ± 0.32	31.69 ± 0.43	31.69 ± 0.47	32.36 ± 0.38	33.42 ± 0.52
F value (after reconstitution) ± SD*	0.36 ± 0.06	0.58 ± 0.03	0.83 ± 0.07	0.95 ± 0.03	0.95 ± 0.04
pH (after reconstitution)	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*

7.2.2.11. 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 7.6 represents the chemical stability results of ARM in reconstituted

suspension. The degradation curves of ARM in reconstituted suspension (Figure 7.6) at room temperature, suggest a 'lag'-time of 5 days. After that period, ARM is degrading.

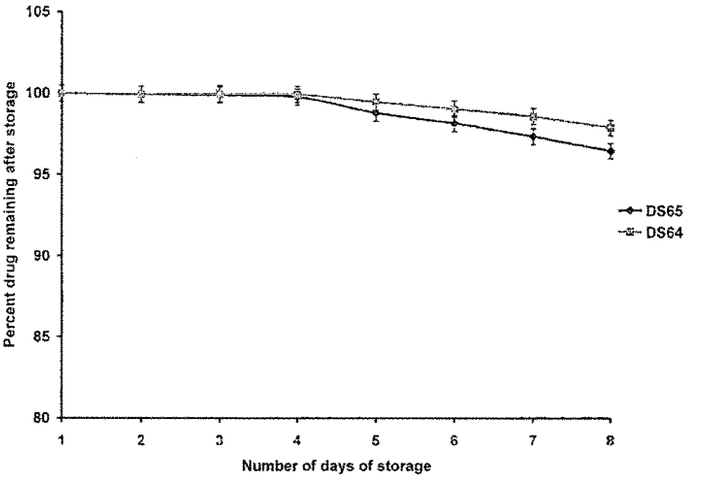


Figure 7.6. Degradation of ARM in DS64 and DS65 after reconstitution as a function of time

7.2.2.12. 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 DS65, the 85% strongly bitter and 15% moderate to strong bitter. DS64 was rated as tasteless by 100% of volunteers of panel (Table 7.8).

Table 7.8. 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							
DS65						3	17	
DS64	20							

7.2.2.13. 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 DS64 showed little change over time, and so a shelf life up to 1580.05 days (52.66 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).

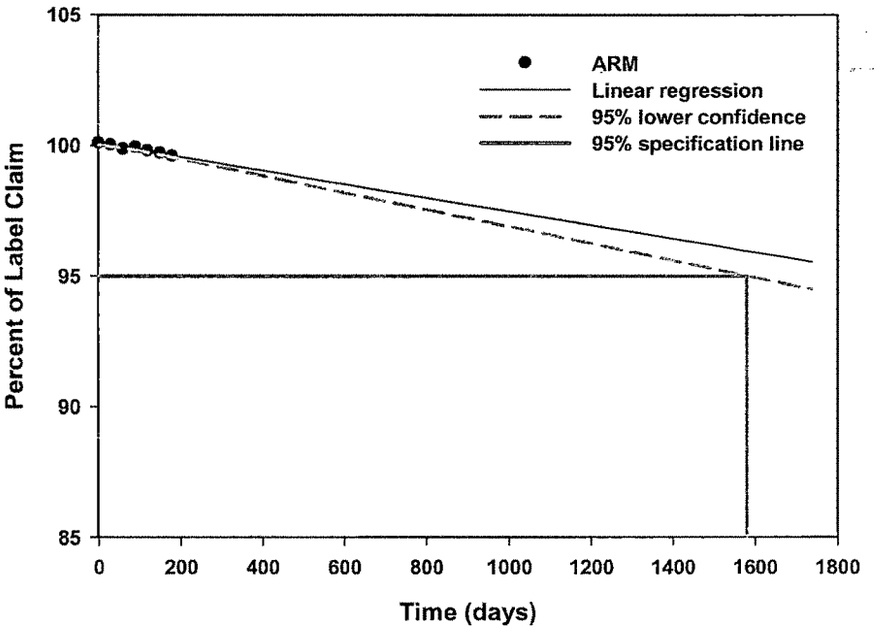


Figure 7.7. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated complete taste masking of ARM in microparticles using EE-CD 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 ARM and EE-CD, which have significant effect on microparticle's desired properties. The FTIR

and DSC studies indicated uniform dispersion of ARM, at the molecular level, in EE-CD 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.

The results obtained from EE-CD did not show any significant difference in terms of incorporation efficiency and drug release at pH, 1.2 and 6.8, compared to EE. Further the grafting process is multi-step process and need sophisticated instruments. Hence further studies for MFL and PRM were not tried.

7.3. Summary

	ARM-EE	ARM-EE-CD
Excipient required per cachet	50 mg ARM + 236.40 mg EE	50 mg ARM + 232.23 mg EE-CD
Incorporation efficiency (%) ± SD*	82.93 ± 1.32	84.17 ± 2.78
Particle size (μm) ± SD*	85.89 ± 1.53	64.52 ± 1.63
Drug release at pH 1.2 (t15 in %) ± SD*	89.42 ± 1.27	98.39 ± 1.42
Drug release at pH 6.8 (t5 in %) ± SD*	4.17 ± 0.72	5.26 ± 0.89
Bitterness score (for microparticles)	0	0
Overall desirability	0.88	0.89
Total filled weight per cachet (g)	0.350	0.350
Angle of repose (^{0}) ± SD*	31.69 ± 0.41	32.36 ± 0.38
F value (after reconstitution) ± SD*	0.94 ± 0.04	0.95 ± 0.03
pH (after reconstitution)	4.6-4.7	4.5-4.6
Bitterness score (for dry suspension)	0	0
Shelf life (months)	51.36	52.66

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

7.4. References

Bolton, S. and Charles, S., 2004. Pharmaceutical statistics. Marcel Dekker Inc, New york, NY: pp. 249.

Elisabetta, E., Roberta, R., Rita, C., Franco, C. and Claudio, N., 2000. Production of Eudragit microparticles by spray-drying technique: Influence of experimental parameters on morphological and dimensional characteristics. *Pharm Dev Tech*, 5(2): 267-278.

Gohel, M.C. and Panchal, M.K., 2002. Novel use of similarity factor f_2 and S_d for the development of diltiazem HCL modified release tablets using a 3^2 factorial design. *Drug Dev Ind Pharm*, 28: 77-87.

Hashimoto, Y., Tanaka, M., Kishimoto, H., Shiozawa, H., Hasegawa, K., Matsuyama, K. and Uchida, T., 2002. Preparation, characterization and taste masking properties of polyvinylacetal diethylamino acetate microsphere containing trimebutine. *J Pharm Pharmacol*, 54: 1323-1328.

Li, N., Zhang, Y.-H., Wu, Y.-N., Xiong, X.-L. and Zhang, Y.-H., 2005. Inclusion complex of trimethoprim with beta-cyclodextrin. *J Pharm Biomed Anal*, 39: 824-829.

Mingshi, Y., Fude, C., Bengang, Y., Jian, Y., Liang, W., Liqiang, Z. and Yoshiaki, K., 2004. A novel pH-dependent gradient-release delivery system for nitrendipine I. Manufacturing, evaluation in vitro and bioavailability in healthy dogs. *J Contr Release*, 98: 219- 229.

Rama Rao, K., Senapati, P. and Das, M.K., 2005. Formulation and in vitro evaluation of ethyl cellulose microspheres containing zidovudine. *J Microencapsul*, 22(8): 863-876.

Rao, V.M., Engha, K. and Qiu, Y., 2003. Design of pH-independent controlled release matrix tablets for acidic drugs. *Int J Pharm*, 252: 81-86.

Satturwar, P.M., Mandaogade, P.M. and Dorle, A.K., 2002. A novel method for preparation of Eudragit RL microcapsules. *J Microencapsul*, 19(4): 407-413.

Shah, T.J., Amin, A.F., Parikh, J.R. and Parikh, R.H., 2007. Process optimisation and characterisation of poloxamer solid dispersion of a poorly water soluble drug. *AAPS PharmSciTech*, 8(2): article 29.

Sinha, V.R. and Rachna, K., 2002. Binders for colon specific drug delivery: an in vitro evaluation. *Int J Pharm*, 249: 23-31.

Szejtli, J. and Szente, L., 2005. Elimination of bitter, disgusting tastes of drugs and foods by cyclodextrins. *Eur J Pharm Biopharm*, 61: 115-125.

Valizadeh, H., Nokhodchi, A., Qarakhani, N., Zakeri-Milani, P., Azarmi, S., Hassanzadeh, D. and Lo¨Benberg, R., 2004. Physicochemical characterization of solid dispersions of indomethacin with PEG 6000, Myrj 52, lactose, sorbitol, dextrin and Eudragit E100. *Drug Dev Ind Pharm*, 30(3): 303-317.

Yamamoto, T., Nagai, T., Shimura, T. and Yasoshima, Y., 1998. Roles of chemical mediators in taste system. *Jpn J Pharmacol*, 76: 325-348.