Inclusion complexation with Beta-Cyclodextrin

5.1. Introduction

Reduction of bad tastes by beta-cyclodextrin (CD) is a long known method (Freudenberg et al., 1953; Seiyaku, 1981). The first such observation was already described in 1953 in the very first drug/CD patent by Freudenberg et al. The bad taste of bromoisovaleryl urea was masked by CD complexation (Freudenberg et al., 1953). However the exact mechanism is not yet been explored. The CD itself can not be considered as a tasteless or only slightly sweet substance, although its taste threshold value is lower than that of sucrose (detection: 0.03 and 0.27%, recognition: 0.11 and 0.52%, respectively). A 0.5% CD solution was as sweet as sucrose, and a 2.5% solution as sweet as a 1.71% solution of sucrose (Toda et al., 1985). Sucrose and beta-CD showed an additive effect on sweetness.

The cavity of CD is occupied by water molecules (about 13-14%w/w) both in crystalline state as well as in aqueous solution. Roughly half of this water is so-called 'crystal water' and the other half is 'inclusion water'. The 'crystal water' is located and bound between the adjacent CD molecules, while 'inclusion water' is included into the hydrophobic cavity of CD. Hydrophobic drugs form complex by replacing 'inclusion water' while easily migrating (hydrophilic, well soluble) drugs form complex, assuming replacement of 'crystal water' (Szejtli and Szente, 2005).

CD is the 'host' molecule and an important component of the 'driving force' for the inclusion complex formation is the substitution of high enthalpy water molecules by the 'guest' molecules. As the guest molecule is included into the CD molecule, which is enwrapped into a hydrate shell, the interaction of the guest molecule with cell membranes and receptors is considerably inhibited, resulting in reduced cytotoxicity or reduced taste (Szejtli and Szente, 2005).

There are 2 theoretical possibilities (a) the CD enwraps the bad tasting molecule (=inclusion complexation), impeding its interaction with the taste buds or (b) the CD interacts with the gate-keeper proteins of the taste buds, paralyzing them (Szejtli and Szente, 2005).

In this case, however, all taste sensation (sweet, salt, sour, bitter) would be

extinguished, as long as the adhered CDs are not removed from the taste buds.

The bitter taste of a 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.

The objective of the present work is to study the effect of cyclodextrin for its bitterness masking ability. All three drugs were studied for the cyclodextrin complexation and evaluated for bitterness score.

The complexed drugs increase the bulk for preparing rapid disintegrating tablets (RDTs). To avoid this problem single dose of suspension powder (cachets) were prepared.

5.2. Artemether (ARM)

5.2.1. Experimental

5.2.1.1. Materials

Beta-cyclodextrin (CD) was kindly gifted by Ajanta Pharma Ltd., Mumbai Methanol, hydrochloric acid, potassium chloride, potassium dihydrogen phosphate, sodium hydroxide of analytical grade were purchased from S.D. Fine Chem Ltd, Boisar.

5.2.1.2. Preparation of inclusion complexes

The following binary systems of ARM and β-CD (CD) were prepared in 1:1, 1:5, 1:10, 1:15 and 1:20 molar ratios.

Physical mixtures (PM)

The physical mixture of ARM and CD was obtained by mixing individual components geometrically, that had previously been sieved through sieve no. 44, together with a spatula.

Kneaded system (KS)

The physical mixture of ARM and CD was triturated in a mortar with a small volume of water-methanol (1:1% v/v) solution. The thick slurry was kneaded for 15 min and then dried until dryness. The dried mass was pulverized and sieved through sieve no. 44. Wetting agent (water:methanol, 1:1% v/v) was used mainly to achieve better interaction of ARM with CD during kneading process.

5.2.1.3. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

5.2.1.4. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

5.2.1.5. X-ray powder diffractometry (XRPD)

XRPD study was carried out as mentioned in 3.2.1.5.

5.2.1.6. *In vitro* drug release

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

5.2.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

5.2.1.8. Preparation and evaluation of the dry suspension

The inclusion complex equivalent to 50 mg of ARM was very high to formulate a rapid disintegrating tablet (RDT). Hence suspension powder containing

equivalent of 50 mg of ARM was prepared from inclusion complex and ARM. Xanthan gum was used as suspending agents. Citric acid monohydrate was used as pH modifier.

The following procedure was applied to prepare a suspension powder. The smallest amount of inclusion complex was mixed with the same amount of another excipient, following the principle of the geometric dilution.

To prepare the reconstituted suspension, an appropriate 10 mL of water was added to the suspension powder (cachet) and stirred with spoon until a homogeneous product was obtained.

5.2.1.9. Angle of repose

For measurement of angle of repose of suspension powder, they were passed through a funnel on the horizontal surface. The height (*h*) of the heap formed was measured with a cathetometer and the radius (*r*) of the cone base was also determined. The angle of repose (Φ) was calculated from following equation:

$$\phi = \tan^{-1}\left(\frac{h}{r}\right) \qquad \text{(Equation 5.1)}$$

5.2.1.10. Sedimentation characteristics

To study the sedimentation in suspension, the sedimentation volume was determined as a function of time.

The sedimentation volume, F is defined as the ratio of the final, equilibrium volume of the sediment, Vu to the total volume Vo before settling, as expressed in the following equation:

$$F = \left(\frac{Vu}{Vo}\right) \qquad \text{(Equation 5.2)}$$

In this study, the sedimentation volume was determined as a function of time. 10 mL suspension (height = 12 cm) was decanted in a cylinder of 10 mL with a diameter of 1.5 cm. After 1 hr, the sedimentation volume F was

determined.

5.2.1.11. Gustatory sensation test for suspension powder (cachet)

Suspension powder was reconstituted as mentioned in 5.2.1.8. This reconstituted suspension was evaluated for taste masking by keeping it in mouth for 30 sec and evaluated for bitterness score using a panel of volunteers. For comparison, reconstituted suspension of pure ARM was also subjected to taste evaluation by the panel and the results were compared.

5.2.1.12. Investigation of chemical stability of ARM

Samples were taken everyday and evaluated for their chemical stability. Practically, the suspension powder, containing the suspending agent and other excipients was prepared and divided in 8 bottles. All bottles were reconstituted using distilled water and stored at room temperature. All the samples were analyzed using UV spectrophotometer (Shimadzu, UV visible spectrophotometer 1700).

5.2.1.13. Stability studies

The suspension powder was packed in aluminum foil coated with polyethylene pouches. The cachets of suspension powder were subjected to stability testing according to the International Conference on Harmonization (ICH) guidelines for zone III and IV. The packed cachets were kept for accelerated stability (40^0C/75%RH) in stability chamber (Remi Instruments Ltd, Mumbai) for upto 6 months. A visual inspection (for physical appearance) and drug content estimation were carried out each month for the entire period of the stability testing.

5.2.2. Results and discussion

5.2.2.1. Fourier Transform Infrared Spectroscopy

The FTIR spectrum of ARM, CD, physical mixture and kneaded system in 1:20M are shown in Figure 5.1. The characteristic peak of ARM at 2873 cm^{-1} can be 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} can be assigned to C-H stretching in -CH_3. The FTIR spectra of physical mixture and kneaded system corresponds to the CD with no major peaks corresponding to ARM. This suggests formation of inclusion complexation between the CD and ARM.

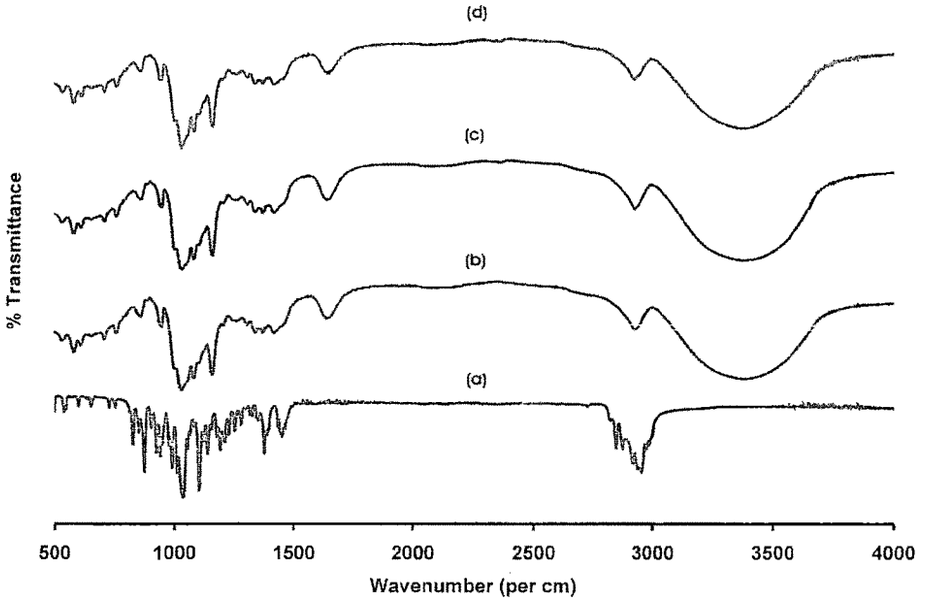

Figure 5.1. FT-IR spectra of (a) ARM, (b) CD, (c) physical mixture and (d) kneaded system

5.2.2.2. Differential Scanning Calorimetry (DSC)

Figure 5.2 shows the DSC curves of ARM, CD, physical mixture and kneaded system in 1:20M. The pure ARM showed 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 characteristic endothermic peak corresponding to melting peak of ARM was abolished in both physical mixture as well as kneaded system. This could

be attributed to higher amount of CD and formation of inclusion complex between the CD and ARM in physical mixture and kneaded system.

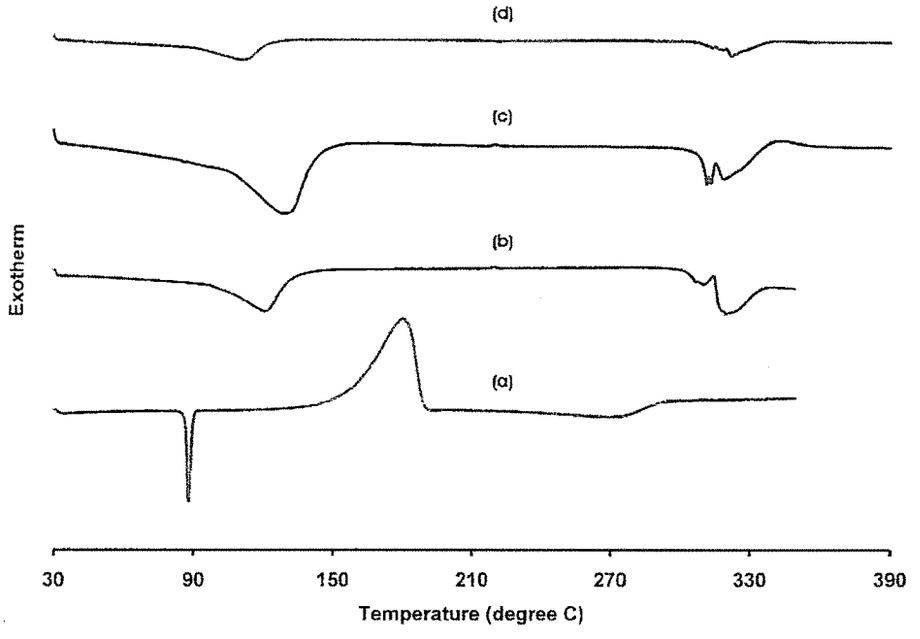

Figure 5.2. DSC curve of (a) ARM, (b) CD, (c) physical mixture and (d) kneaded system

5.2.2.3. X-ray Powder Diffractometry (XRPD)

XRPD analysis was performed to confirm the results of FTIR and DSC studies. XRPD patterns of ARM, CD, physical mixture and kneaded system in 1:20M are shown in Figure 5.3. In x-ray diffractogram of ARM, sharp peaks at a diffraction angle (2θ) of 9.39^0, 10.68^0, 11.16^0, 13.61^0, 17.54^0, 19.11^0, 20.10^0, 21.86^0 and 22.69^0 indicates the presence of crystalline drug. However, the patterns of CD were all amorphous.

The diffractograms of physical mixture and kneaded system, differed from those of ARM, where the characteristic peaks of ARM disappeared, indicating the formation of inclusion complex in physical mixture and kneaded system. This suggests the presence of a new solid phase with lower crystallinity than

the drug. XRPD studies, thus, confirm the findings of DSC studies, indicating formation of a solid form with different properties or ARM-CD inclusion complex in physical mixture and kneaded system.

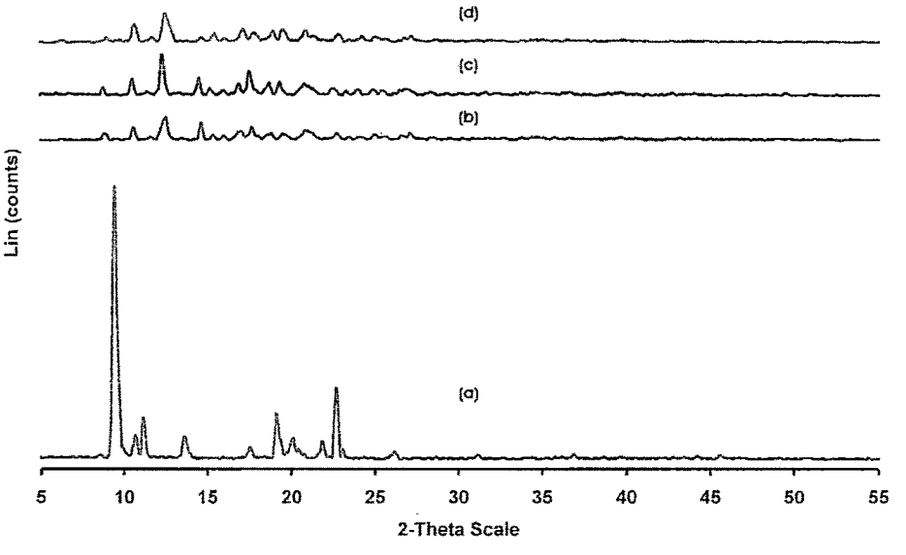

Figure 5.3. XRPD pattern of (a) ARM, (b) CD, (c) physical mixture and (d) kneaded system

5.2.2.4. *In vitro* drug release

When physical mixture or kneaded system was dispersed in a dissolution medium, a very rapid dissolution was observed. Dissolution studies were based on the observation in order to characterize the inclusion complexation between the CD and drug. Figure 5.4 shows the dissolution profiles of pure ARM, CD, physical mixture and kneaded system at pH, 1.2 and 6.8.

The results in terms of dissolution efficiency at 15 min and 60 min along with percent of ARM dissolved at 5 min are reported in Table 5.1. Dissolution studies showed that the increase in the drug release is significant with kneaded system (about 23.33% of drug dissolved in 5 min) and their respective physical mixtures compared (about 16.78% of drug dissolved in 5 min) to pure ARM (about 9.56% of drug dissolved in 5 min) at pH 6.8. Similarly the drug release is significantly improved in kneaded system (about

71.36% of drug dissolved in 5 min) and their respective physical mixtures compared (about 59.50% of drug dissolved in 5 min) to pure ARM (about 7.64% of drug dissolved in 5 min) at pH 1.2. This indicates increased availability of ARM in g.i.t.

Table 5.1. Percent dissolution and dissolution efficiency of ARM from binary systems in comparison with pure drug

Formulations	DP5 (%)		DE15 (%)		DE60 (%)	
	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8
ARM	7.64	9.56	9.56	11.20	20.74	23.11
Physical mixture	59.50	16.78	62.53	22.24	82.80	41.41
Kneaded system	71.36	23.33	72.74	28.61	89.08	47.88

DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min

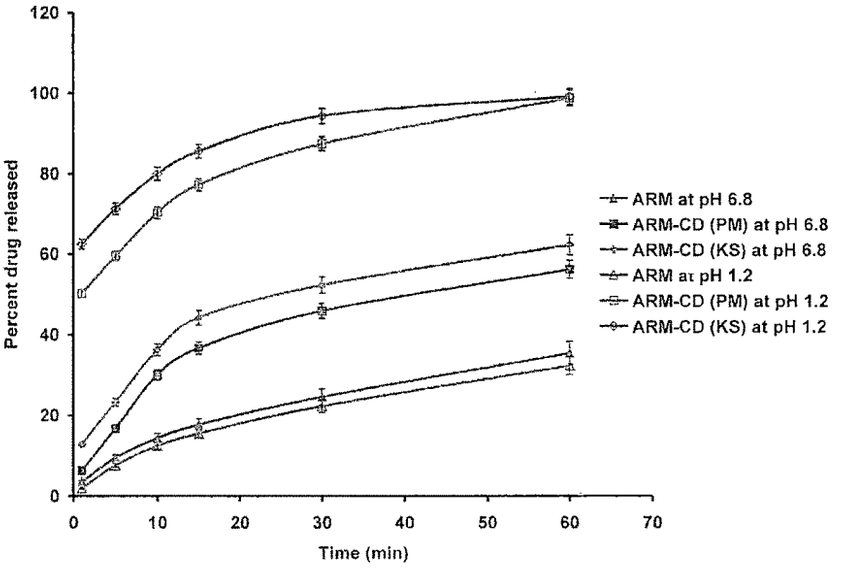

Figure 5.4. Dissolution profile of ARM, physical mixture and kneaded system

The significant improvement in dissolution characteristics of the complexes is justified through the concurrence of several factors: increased particle

selected, based on its bitterness score.

The formula of different suspension powders prepared is summarized in Table 5.3. The formula of optimized suspension powder (DS4) was further used to prepare suspension powder of pure ARM (DS5). The characteristics of suspension powder are summarized in Table 5.4.

Table 5.3. Formulation of suspension powder

Drug/Excipients	Per cachet				
	DS1	DS2	DS3	DS4	DS5
ARM (g)	-	-	-	-	0.050
Physical mixture eq. to 0.050 g ARM (g)	38.05	38.05	38.05	38.05	-
Xanthan gum (g)	0.078	0.156	0.195	0.234	0.234
Lactose (Lactopress) (g)	0.744	0.666	0.627	0.588	38.588
Citric acid (g)	0.039	0.039	0.039	0.039	0.039
Methyl paraben (g)	0.078	0.078	0.078	0.078	0.078
Propyl paraben (g)	0.007	0.007	0.007	0.007	0.007
Sunset yellow FCF (g)	0.004	0.004	0.004	0.004	0.004
Total filled weight per cachet (g)	39.000	39.000	39.000	39.000	39.000

Table 5.4. Physical properties of suspension powder

Parameters	DS1	DS2	DS3	DS4	DS5
Angle of repose (^{0}) ± SD*	38.31 ± 0.48	38.68 ± 0.39	37.87 ± 0.41	38.18 ± 0.46	37.69 ± 0.32
F value (after reconstitution) ± SD*	0.25 ± 0.08	0.56 ± 0.07	0.74 ± 0.08	0.95 ± 0.03	0.96 ± 0.03
pH (after reconstitution)	4.5-4.6	4.6-4.7	4.5-4.6	4.6-4.7	4.5-4.6

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

5.2.2.7. 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 5.5 represents the chemical stability results of ARM in reconstituted suspension. The degradation curves of ARM in reconstituted suspension at room temperature, suggest a 'lag'-time of 5 days. After that period, ARM is degrading.

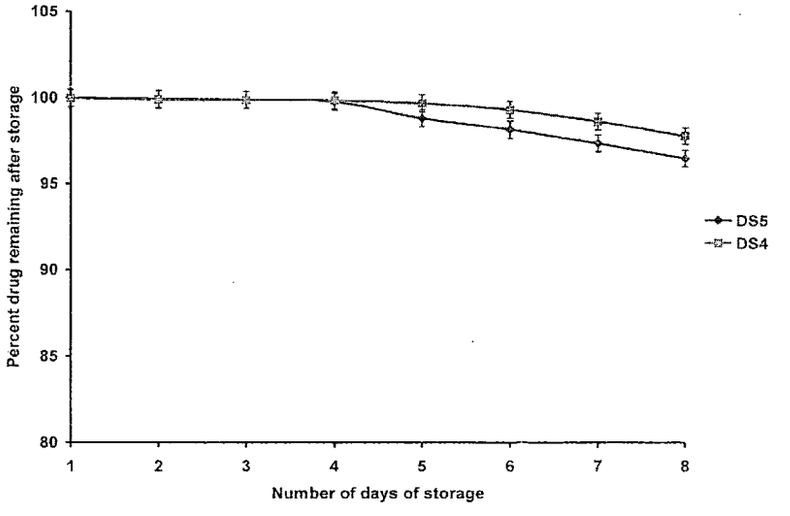

Figure 5.5. Degradation of ARM in DS4 and DS5 after reconstitution as a function of time

5.2.2.8. Gustatory sensation test for suspension powder

The cachets prepared using ARM and the physical mixture of the CD and ARM were subjected to taste evaluation by the same panel of twenty selected volunteers, used for bitterness evaluation of drug and complexes. For DS5, the 5% of panel rated it as very strongly bitter and 95% strongly bitter while DS4 was rated as tasteless by 100% of volunteers of panel (Table 5.5). Though lactose is sweeter than CD, suspension containing pure ARM (DS5) showed bitterness while the suspension containing physical mixture of ARM

and CD (DS4) showed no bitterness. This confirms that the inclusion complex is mainly responsible for bitter taste masking of ARM.

Table 5.5. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
DS5							19	1
DS4	20							

5.2.2.9. 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 DS4 showed little change over time, and so a shelf life up to 1540.18 days (51.33 months) can be proposed.

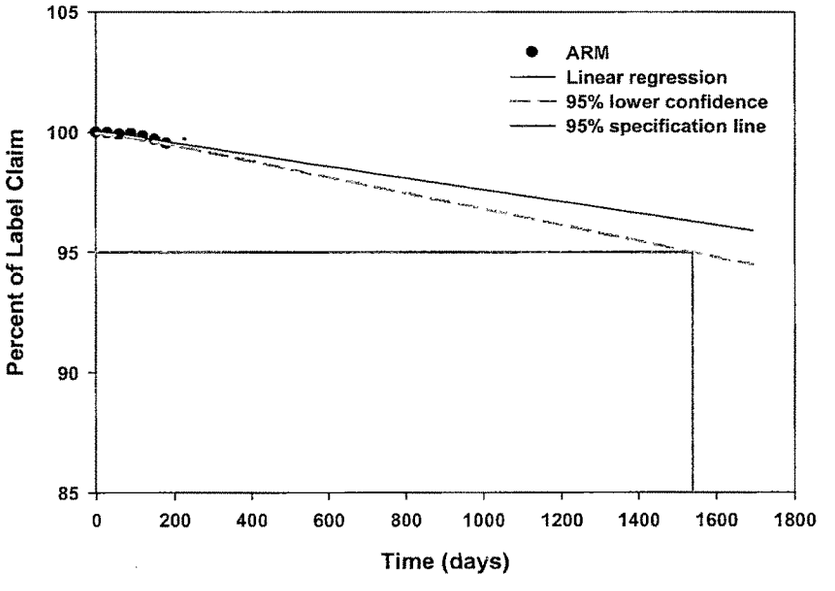

Figure 5.6. Extrapolation of accelerated stability data for shelf life calculation

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).

The study conclusively demonstrated the complete bitter taste masking of ARM with improved dissolution in physical mixture. The FTIR, DSC and XRPD studies indicated inclusion complexation in physical mixture and kneaded system. The physical mixture of the CD and ARM was further incorporated into cachets, based on bitterness score.

5.3. Mefloquine hydrochloride (MFL)

5.3.1.1. Materials

Materials used were as mentioned in 5.2.1.1.

5.3.1.2. Preparation of inclusion complexes

The following binary systems of MFL and CD were prepared in 1:1, 1:5 and 1:10 molar ratios.

Physical mixture and kneaded system was prepared as mentioned in 5.2.1.2.

5.3.1.3. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

5.3.1.4. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

5.3.1.5. X-ray powder diffractometry (XRPD)

XRPD study was carried out as mentioned in 3.2.1.5.

5.3.1.6. *In vitro* drug release

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

5.3.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

5.3.1.8. Preparation and evaluation of the dry suspension

The inclusion complex equivalent to 100 mg of MFL was very high to formulate a rapid disintegrating tablet (RDT). Hence suspension powder containing equivalent of 100mg of MFL was prepared from inclusion comlex and MFL. Sodium carboxy methyl cellulose (HVP) 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.

5.3.1.9. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9

5.3.1.10. Sedimentation characteristics

Sedimentation characteristics were carried out as mentioned in 5.2.1.10.

5.3.1.11. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test for suspension powder was carried out as mentioned in 5.2.1.11.

5.3.1.12. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

5.3.2. Results and discussion

5.3.2.1. Fourier Transform Infrared Spectroscopy

The FTIR spectrum of MFL, CD, physical mixture and kneaded system in 1:10M are shown in Figure 5.7. The spectrum of MFL is dominated by N-H stretching vibration at 3226 cm^{-1}, quinine ring stretching vibration at 1603,

1363, 1111, and 1069 cm^{-1}, CF_3 stretching vibration at 1316 cm^{-1}. The peaks at 2875 and 2918 cm^{-1} can be assigned to C-H bridge and CH_2 respectively. The peak at 1555 cm^{-1} can be assigned to C=N/C=C. The peaks at 1288 and 1055 cm^{-1} can be assigned to C-N and piperidine ring respectively. The peak at 1174 cm^{-1} is due to the C-C/N-H stretching vibration. The FTIR spectra of physical mixture and kneaded system corresponds to the CD, with no major peaks corresponding to MFL. This suggests formation of inclusion complex between the CD and MFL in physical mixture and kneaded system.

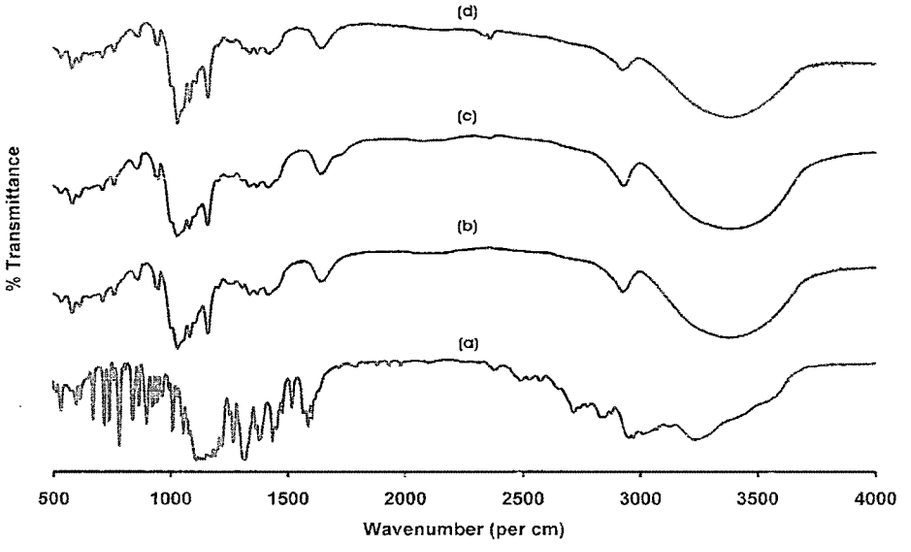

Figure 5.7. FTIR spectra of (a) MFL, (b) CD, (c) physical mixture and (d) kneaded system

5.3.2.2. Differential Scanning Calorimetry (DSC)

Figure 5.8 shows the DSC curves of MFL, CD, physical mixture and kneaded system in 1:10M. The pure MFL showed an endothermic peak at 271.38^{0}C, followed by exothermic peak at 308.36^{0}C.

The 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 characteristic endothermic peak corresponding to melting peak of MFL in was broaden and shifted towards lower temperature, with reduced intensity in physical mixture (254.64^{0}C) and kneaded system (250.81^{0}C). It has been reported that the formation of inclusion complexes is indicated by the disappearance or shift of the endothermic peaks corresponding to the drug melting process (Mielcarek et al., 2006). Hence this shifting of endothermic peak confirms formation of inclusion complex between the CD and MFL in physical mixture and kneaded system.

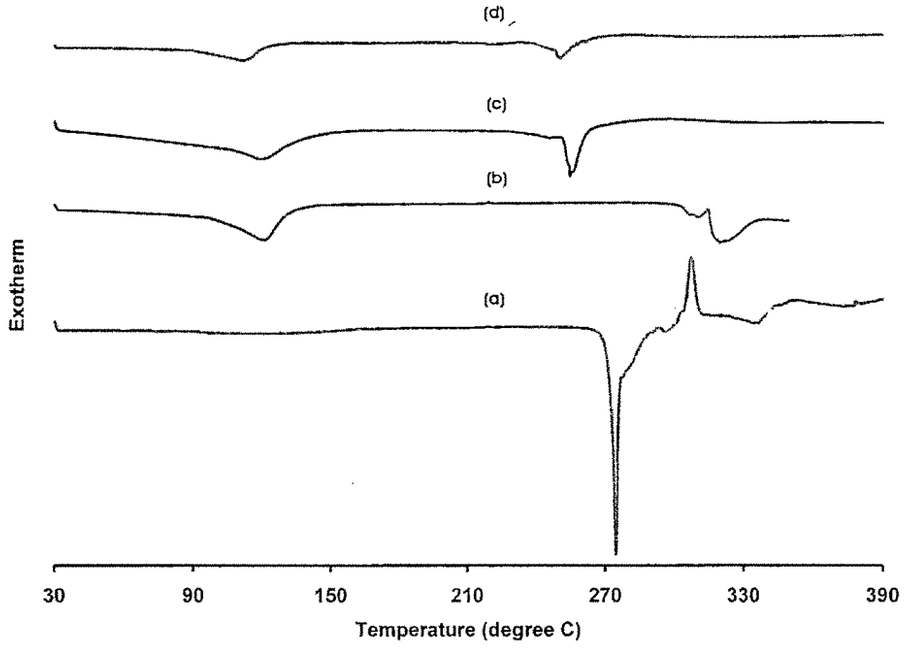

Figure 5.8. DSC curve of (a) MFL, (b) CD, (c) physical mixture and (d) kneaded system

5.3.2.3. X-ray Powder Diffractometry (XRPD)

XRPD analysis was performed to confirm the results of FTIR and DSC studies. XRPD patterns of MFL, CD, physical mixture and kneaded system in 1:10M are shown in Figure 5.9. In x-ray diffractogram of MFL, sharp peaks at a diffraction angle (2θ) of 11.52^0, 14.31^0, 16.37^0, 18.03^0, 20.11^0, 21.26^0, 23.37^0, 25.50^0 and 32.57^0 indicates the presence of crystalline drug. However, the pattern of CD are all amorphous.

The diffractograms of physical mixture and kneaded system, differed from those of MFL, where the characteristic peaks of MFL disappeared, indicating the formation of inclusion complex in physical mixture and kneaded system. This suggests the presence of a new solid phase with lower crystallinity than the drug. XRPD studies, thus confirm the findings of DSC studies, indicating formation of a solid form with different properties or MFL-CD inclusion complex in physical mixture and kneaded system.

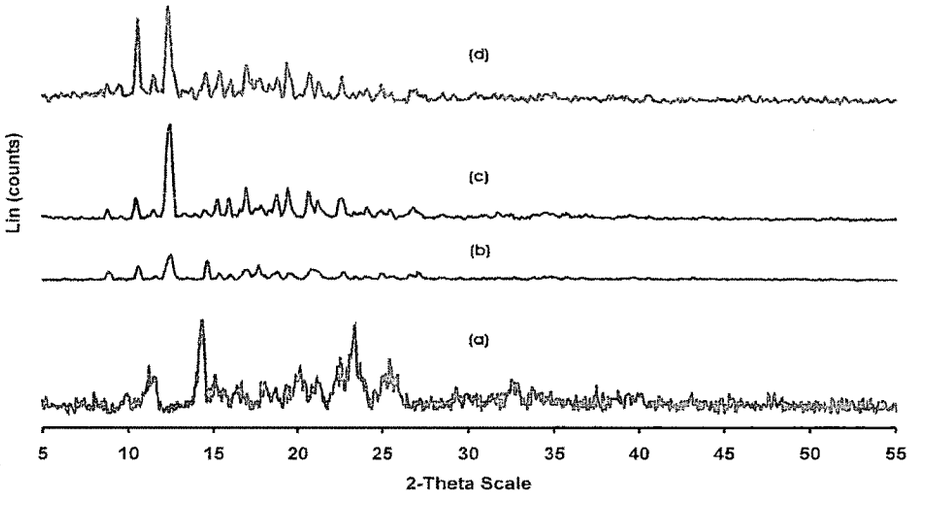

Figure 5.9. XRPD pattern of (a) MFL, (b) CD, (c) physical mixture and (d) kneaded system

5.3.2.4. *In vitro* drug release

When physical mixture or kneaded system was dispersed in a dissolution medium, a very rapid dissolution was observed. Dissolution studies were based on the observation in order to characterize the inclusion complexation between the CD and drug. Figure 5.10 shows the dissolution profiles of pure MFL, CD, physical mixture and kneaded system at both pH, 1.2 and 6.8.

The results in terms of dissolution efficiency and percent of MFL dissolved at 5 min are reported in Table 5.6. Drug release studies showed that the increase in the drug release is significant with kneaded system (about 52.28% of drug dissolved in 5 min) and their respective physical mixtures compared (about

43.43% of drug dissolved in 5 min) to pure MFL (about 8.24% of drug dissolved in 5 min) at pH 6.8. Similarly the drug release is significantly improved in kneaded system (about 76.34% of drug dissolved in 5 min) and their respective physical mixtures compared (about 64.37% of drug dissolved in 5 min) to pure MFL (about 34.78% of drug dissolved in 5 min) at pH 1.2. This indicates increased availability of MFL in g.i.t.

Table 5.6. Percent dissolution and dissolution efficiency of ARM from binary systems in comparison with pure drug

Formulations	DP5 (%)		DE15 (%)		DE60 (%)	
	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8
MFL	34.78	8.24	37.80	12.14	55.82	30.71
Physical mixture	64.37	43.43	66.04	46.19	83.92	64.58
Kneaded system	76.34	52.28	77.16	54.08	91.04	72.22

DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min

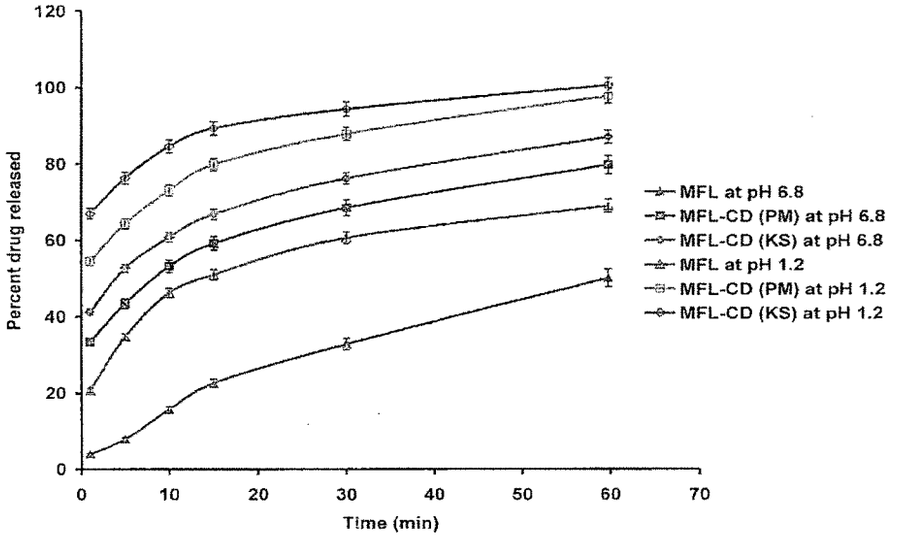

Figure 5.10. Dissolution profile of MFL, physical mixture and kneaded system

The significant improvement in dissolution characteristics of the complexes is justified through the concurrence of several factors: increased particle wettability, and reduction of crystallinity of the product (Mura et al., 2002; Naidu et al., 2004; Otero-Espinar et al., 1991). Improved dissolution may be attributed to the high energetic amorphous state and reduction in crystallinity of the MFL following complexation in physical mixture and kneaded system, which was confirmed by XRPD and DSC studies.

5.3.2.5. Gustatory sensation test

Bitterness evaluation results made by the consents of trained persons, are listed in Table 5.7. No bitterness was imparted in physical mixture with reference to pure drug and kneaded system. It has been reported that MFL depolarize taste cells by closing K^+ channel and produce bitterness (Yamamoto et al., 1998). In addition, it has been reported that the CD enwraps bitter tasting drug, impeding its interaction with the taste buds (Szejtli and Szente, 2005). This complexed MFL is strongly hydrated on the outer surface, therefore it didn't interact with K^+ channel and thus reduces bitterness. Further the sweet taste of CD imparted additive effect. Surprisingly, kneaded system showed high bitterness score. This might be because of the reduced particle size of MFL, due to kneading, confirmed by XRPD studies. These small complexed drug particles might be retained on the tongue for longer period and attached to K^+ channel, which results in bitterness.

Table 5.7. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
Pure MFL							18	2
Physical Mixture	20							
Kneaded systems						2	17	1

5.3.2.6. Preparation and evaluation of dry suspension

To formulate a dry suspension of MFL, the 1:10M physical mixture was

selected, based on its bitterness score.

The formula of different suspension powders prepared is summarized in Table 5.8. The formula of optimized suspension powder (DS14) was further used to prepare suspension powder of pure MFL (DS15). The characteristics of suspension powder are summarized in Table 5.9.

Table 5.8. Formulation of suspension powder

Drug/Excipients	Per cachet				
	DS11	DS12	DS13	DS14	DS15
MFL (g)	-	-	-	-	0.100
Physical mixture eq. to 100 mg MFL	2.836	2.836	2.836	2.836	-
Sodium carboxy methyl cellulose (g)	0.015	0.021	0.024	0.027	0.027
Lactose (Lactopress) (g)	0.099	0.093	0.090	0.087	2.823
Polyethylene glycol 6000 (g)	0.015	0.015	0.015	0.015	0.015
Citric acid (g)	0.027	0.027	0.027	0.027	0.027
Methyl paraben (g)	0.006	0.006	0.006	0.006	0.006
Propyl paraben (g)	0.001	0.001	0.001	0.001	0.001
Sunset yellow FCF (g)	0.001	0.001	0.001	0.001	0.001
Total filled weight per cachet (g)	3.000	3.000	3.000	3.000	3.000

Table 5.9. Physical properties of suspension powder

Parameters	DS11	DS12	DS13	DS14	DS15
Angle of repose (^{0}) ± SD*	37.42 ± 0.52	37.59 ± 0.46	37.29 ± 0.39	37.73 ± 0.44	38.17 ± 0.38
F value (after reconstitution) ± SD*	0.21 ± 0.09	0.48 ± 0.08	0.78 ± 0.09	0.96 ± 0.02	0.97 ± 0.02
pH (after reconstitution)	4.5-4.6	4.5-4.6	4.5-4.6	4.6-4.7	4.6-4.7

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

5.3.2.7. Gustatory sensation test for suspension powder

The cachets prepared using MFL and the physical mixture of the CD and MFL were subjected to taste evaluation by the same panel of twenty selected volunteers. For DS15, the 5% of panel rated it as very strongly bitter, 85% strongly bitter and 10% moderate to strong bitter while DS14 was rated as tasteless by 100% of volunteers of panel (Table 5.10). Though lactose is sweeter than CD, suspension containing pure MFL (DS15) showed bitterness while the suspension containing physical mixture of the CD and MFL (DS14) showed no bitterness. This confirms that the inclusion complex is mainly responsible for masking the bitter taste of MFL.

Table 5.10. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
DS15						2	17	1
DS14	20							

5.3.2.8. 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 DS14 showed little change over time, and so a shelf life up to 1582.32 days (52.74 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).

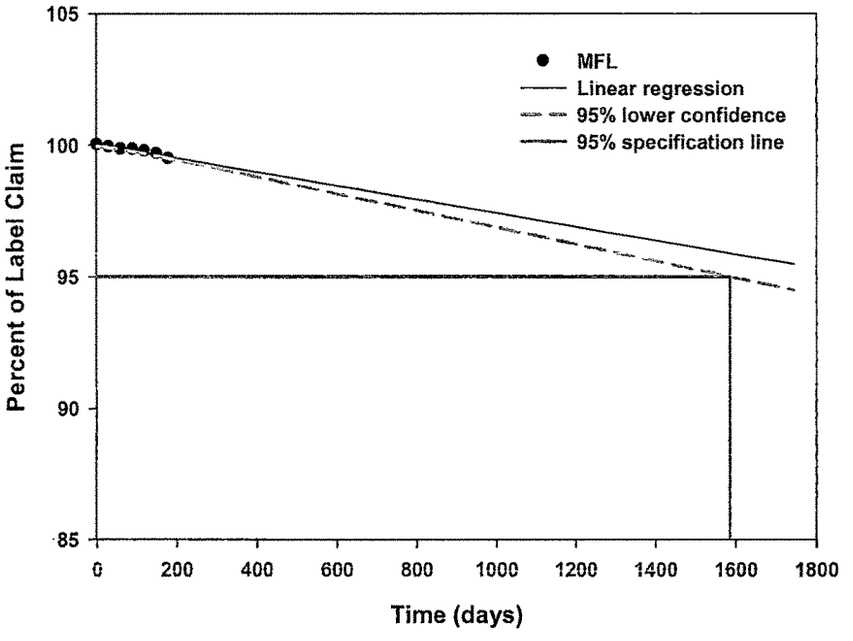

Figure 5.11. Extrapolation of accelerated stability data for shelf life calculation

The study conclusively demonstrated the complete taste masking of MFL with improved dissolution in physical mixture. The FTIR, DSC and XRPD studies indicated inclusion complexation in physical mixture and kneaded system. The physical mixture of the CD and MFL was further incorporated into cachets, based on bitterness score.

5.4. Primaquine Phosphate (PRM)

5.4.1.1. Materials

Materials used were as mentioned in 5.2.1.1.

5.4.1.2. Preparation of inclusion complexes

The following binary systems of PRM and CD were prepared in 1:1, 1:5, 1:10, 1:15, 1:20 and 1:25 molar ratios.

Physical mixture and kneaded system was prepared as mentioned in 5.2.1.2.

5.4.1.3. Fourier transform infra-red spectroscopy (FTIR)

FTIR study was carried out as mentioned in 3.2.1.3.

5.4.1.4. Differential scanning calorimeter (DSC)

DSC study was carried out as mentioned in 3.2.1.4.

5.4.1.5. X-ray powder diffractometry (XRPD)

XRPD study was carried out as mentioned in 3.2.1.5.

5.4.1.6. *In vitro* drug release

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

5.4.1.7. Gustatory sensation test

Gustatory sensation test was carried out as mentioned in 3.2.1.8.

5.4.1.8. Preparation and evaluation of the dry suspension

The inclusion complexes equivalent to 13.12 mg of PRM was very high to formulate a rapid disintegrating tablet (RDT). Hence suspension powder containing equivalent of 13.12 mg of PRM (equivalent to 7.5 mg primaquine base) was prepared from the inclusion complex and PRM. Sodium carboxy methyl cellulose (HVP) 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.

5.4.1.9. Angle of repose

Angle of repose was studied as mentioned in 5.2.1.9

5.4.1.10. Sedimentation characteristics

Sedimentation characteristics were carried out as mentioned in 5.2.1.10.

5.4.1.11. Gustatory sensation test for suspension powder (cachet)

Gustatory sensation test for suspension powder was carried out as mentioned in 5.2.1.11.

5.4.1.12. Stability studies

Stability studies were carried out as mentioned in 5.2.1.13.

5.4.2. Results and discussion

5.4.2.1. Fourier Transform Infrared Spectroscopy (FTIR)

The FTIR spectrum of PRM, CD, physical mixture and kneaded system in 1:25M are shown in Figure 5.12.

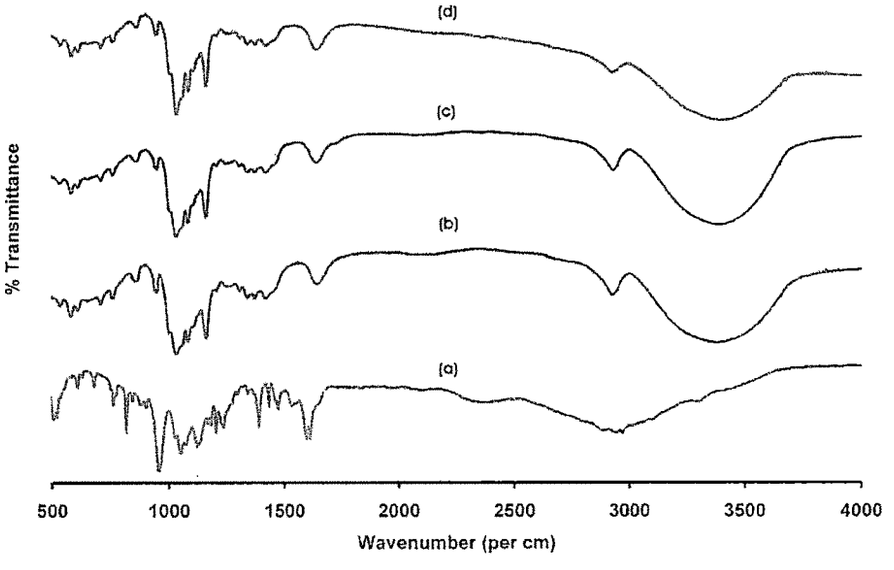

Figure 5.12. FTIR spectra of (a) PRM, (b) CD, (c) physical mixture and (d) kneaded system

The characteristic peaks of PRM at 2968 and 2878 cm^{-1} were 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 spectra of physical mixture and kneaded system corresponds to the CD, with no major peaks corresponding to PRM. This suggests formation of inclusion complexation between the CD and PRM in physical mixture and kneaded system.

5.4.2.2. Differential Scanning Calorimetry (DSC)

Figure 5.13 shows the DSC curves of PRM, CD, physical mixture and kneaded system. The pure PRM showed a sharp endothermic peak at 202.68^{0}C.

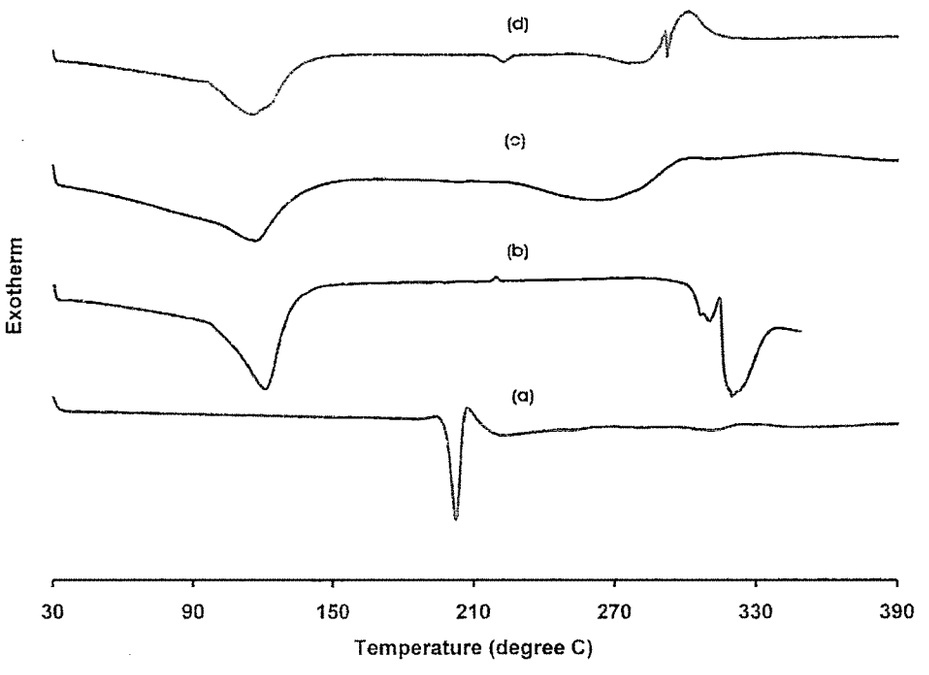

Figure 5.13. DSC curve of (a) PRM, (b) CD, (c) physical mixture and (d) kneaded system

The 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 characteristic endothermic peak corresponding to melting peak of PRM was broaden and shifted towards higher temperature, with reduced intensity

in physical mixture (267.35^{0}C) and kneaded system (276.77^{0}C). It has been reported that the formation of inclusion complexes is indicated by the disappearance or shift of the endothermic peaks corresponding to the drug melting process (Mielcarek et al., 2006). Hence this shifting of endothermic peak confirms formation of inclusion complex between the CD and PRM in physical mixture and kneaded system.

5.4.2.3. X-ray Powder Diffractometry (XRPD)

XRPD analysis was performed to confirm the results of DSC studies. XRPD patterns of PRM, CD, physical mixture and kneaded system in 1:25M are shown in Figure 5.14. In x-ray diffractogram of PRM, sharp peaks at a diffraction angle (2θ) of 10.26^0, 14.35^0, 16.34^0, 18.26^0, 20.54^0, 21.69^0, 22.51^0, 24.87^0, 26.37^0 28.93^0, 30.12^0 and 32.15^0 indicates the presence of crystalline drug. However, the patterns of CD were all amorphous.

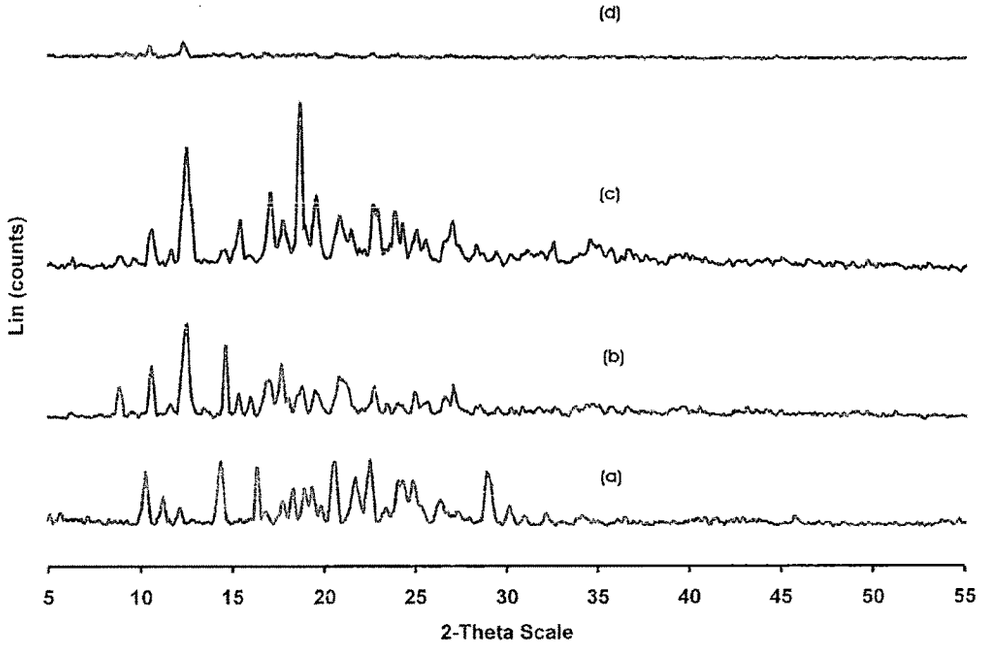

Figure 5.14. XRPD pattern of (a) PRM, (b) CD, (c) physical mixture and (d) kneaded system

The diffractograms of physical mixture and kneaded system, differed from

those of PRM, where the characteristic peaks of PRM disappeared, indicating the formation of inclusion complex in these systems. In addition, new peaks at a diffraction angle (2θ) of 10.58^0, 12.46^0, 15.43^0, 17.05^0, 18.66^0, 19.53, 20.87^0, 22.75^0, 23.88^0 and 27.07^0 were observed. This suggests the presence of a new solid phase with lower crystallinity. XRPD studies, thus, confirm the findings of DSC patterns indicating formation of a solid form with different properties or PRM-CD inclusion complex in physical mixture and kneaded system.

5.4.2.4. *In vitro* drug release

When physical mixture or kneaded system was dispersed in a dissolution medium, a very rapid dissolution was observed. Dissolution studies were based on the observation in order to characterize the inclusion complexation between the CD and drug. Figure 5.15 shows the dissolution profiles of pure PRM, CD, physical mixture and kneaded system at pH, 1.2 and 6.8.

Table 5.11. Percent dissolution and dissolution efficiency of PRM from binary systems in comparison with pure drug

Formulations	DP5 (%)		DE15 (%)		DE60 (%)	
	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8	At pH 1.2	At pH 6.8
PRM	69.15	98.26	67.32	94.51	72.12	98.37
Physical mixture	78.82	96.28	77.39	93.43	86.52	97.35
Kneaded system	85.53	96.92	83.82	94.10	92.23	97.70

DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min

The results in terms of dissolution efficiency and percent of PRM dissolved at 5 min are reported in Table 5.11. Dissolution studies showed that the drug release was slightly decreased in kneaded system (about 96.92% of drug dissolved in 5 min) and their respective physical mixtures compared (about 96.28% of drug dissolved in 5 min) to pure PRM (about 98.26% of drug dissolved in 5 min) at pH 6.8. However the drug release is significantly

improved in kneaded system (about 85.53% of drug dissolved in 5 min) and their respective physical mixtures compared (about 78.82% of drug dissolved in 5 min) to pure PRM (about 69.15% of drug dissolved in 5 min) at pH 1.2. This indicates increased availability of PRM in g.i.t.

The significant improvement in dissolution characteristics of the complexes is justified through the concurrence of several factors: increased particle wettability, and reduction of crystallinity of the product (Mura et al., 2002; Naidu et al., 2004; Otero-Espinar et al., 1991). Improved dissolution may be attributed to the high energetic amorphous state and reduction in crystallinity of the PRM following complexation in physical mixture and kneaded system, which was confirmed by XRPD and DSC studies.

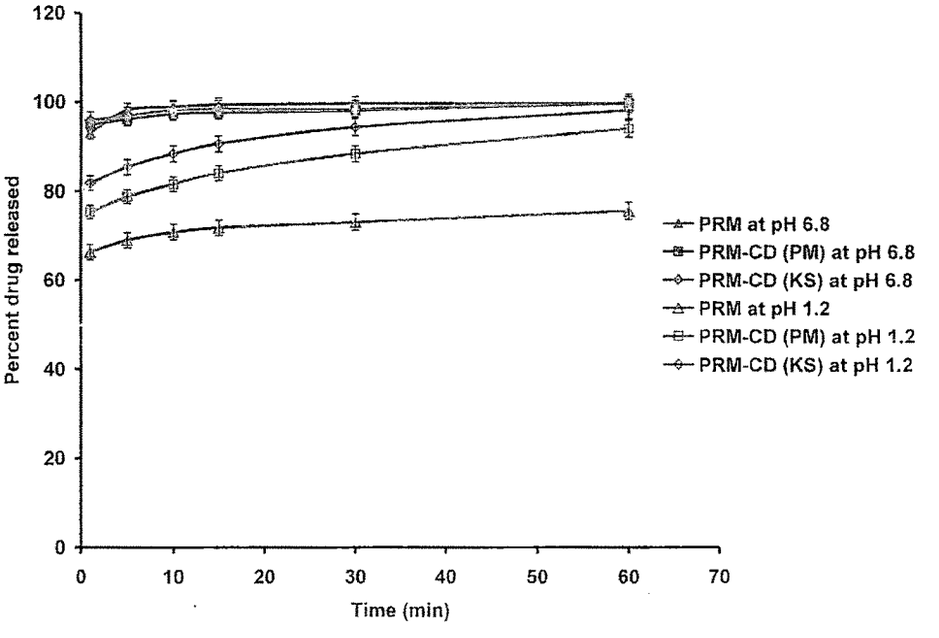

Figure 5.15. Dissolution profile of PRM, physical mixture and kneaded system

5.4.2.5. Gustatory sensation test

Bitterness evaluation results made by the consents of trained persons, are listed in Table 5.12. No bitterness was imparted in physical mixture with reference to pure drug and kneaded system. It has been reported that PRM

depolarize taste cells by closing K^+ channel and produce bitterness (Yamamoto et al., 1998). In addition, it has been reported that the CD enwraps bitter tasting drug, impeding its interaction with the taste buds (Szejtli and Szente, 2005). This complexed PRM is strongly hydrated on the outer surface, therefore it didn't interact with K^+ channel and thus reduces bitterness. Further the sweet taste of CD imparted additive effect. Surprisingly, kneaded system showed high bitterness score. This might be because of the reduced particle size of PRM, due to kneading, confirmed by XRPD studies. These small complexed drug particles might be retained on the tongue for longer period and attached to K^+ channel, which results in bitterness.

Table 5.12. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
Pure PRM							19	1
Physical Mixture	20							
Kneaded systems							18	2

5.4.2.6. Preparation and evaluation of dry suspension

To formulate a dry suspension of PRM, the 1:25M physical mixture was selected, based on its bitterness score.

The formula of different suspension powders prepared is summarized in Table 5.13. The formula of optimized suspension powder (DS24) was further used to prepare suspension powder of pure PRM (DS25). The characteristics of suspension powder are summarized in Table 5.14.

Table 5.13. Formulation of suspension powder

Drug/Excipients	Per cachet				
	DS21	DS22	DS23	DS24	DS25
PRM (g)	-	-	-	-	0.013
Physical mixture eq. to 13.16 mg PRM (g)	0.817	0.817	0.817	0.817	-
Sodium carboxy methyl cellulose (g)	0.002	0.003	0.004	0.005	0.005
Microcrystalline cellulose (Avicel PH 302) (g)	0.071	0.070	0.069	0.068	0.871
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
Sunset yellow FCF (g)	0.001	0.001	0.001	0.001	0.001
Total filled weight per cachet (g)	0.900	0.900	0.900	0.900	0.900

Table 5.14. Physical properties of suspension powder

Parameters	DS21	DS22	DS23	DS24	DS25
Angle of repose (^{0}) ± SD*	37.32 ± 0.53	38.14 ± 0.44	37.78 ± 0.48	37.56 ± 0.32	37.68 ± 0.43
F value (after reconstitution) ± SD*	0.34 ± 0.08	0.68 ± 0.09	0.83 ± 0.07	0.94 ± 0.04	0.96 ± 0.02
pH (after reconstitution)	4.5-4.6	4.5-4.6	4.5-4.6	4.6-4.7	4.6-4.7

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

5.4.2.7. Gustatory sensation test for suspension powder

The cachets prepared using PRM and the physical mixture of the CD and PRM were subjected to taste evaluation by the same panel of twenty selected volunteers. For DS25, the 10% of panel rated it as very strongly bitter, 85% strongly bitter and 5% moderate to strong bitter while DS24 was rated as

tasteless by 100% of volunteers of panel (Table 5.15).

Table 5.15. Bitterness score evaluation by a panel of twenty human volunteers

Formulations	Number of volunteers rating the preparation as							
	0	0.5	1	1.5	2	2.5	3	3+
DS25						1	17	2
DS24	20							

5.4.2.8. 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.

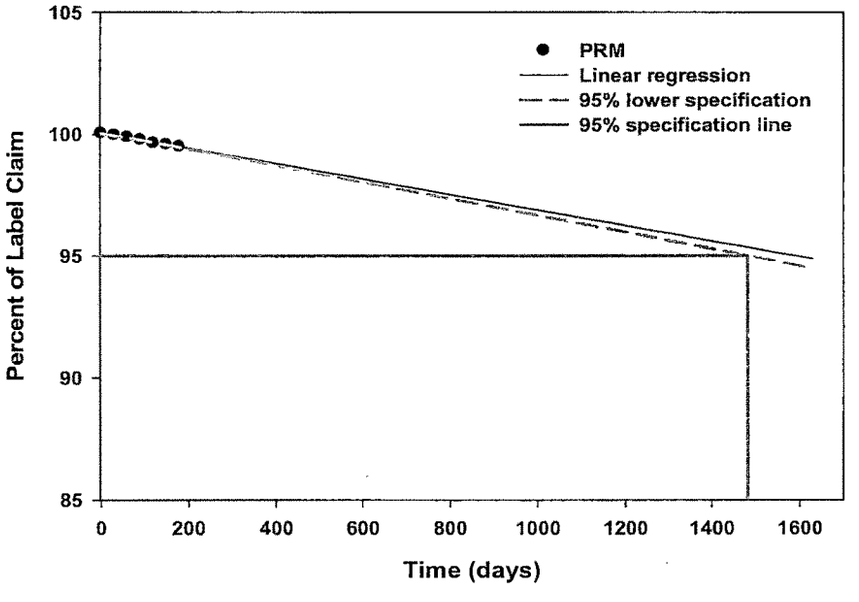

Figure 5.16. Extrapolation of accelerated stability data for shelf life calculation

The accelerated stability data of DS24 showed little change over time, and so a shelf life up to 1480.10 days (49.33 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).

The study conclusively demonstrated the complete taste masking of PRM with improved dissolution in physical mixture. The FTIR, DSC and XRPD studies indicated inclusion complexation in physical mixture and kneaded system. The physical mixture of the CD and PRM was further incorporated into cachets, based on bitterness score.

5.5. Summary

	ARM-CD	MFL-CD	PRM-CD
CD required per cachet	50 mg ARM + 3800 mg CD	100 mg MFL + 2736 mg CD	13.16 mg PRM + 817 mg CD
Mole ratio	1:20	1:10	1:25
DP5 at pH 1.2 (%)	59.50	64.37	78.82
DP5 at pH 6.8 (%)	16.78	43.43	96.20
DE60 at pH 1.2 (%)	82.80	83.92	86.52
DE60 at pH 6.8 (%)	41.41	64.58	97.35
Bitterness score (for physical mixture)	0	0	0
Total filled weight per cachet (g)	39.00	3.00	0.900
Angle of repose (^{0}) ± SD*	38.18 ± 0.46	37.73 ± 0.44	37.56 ± 0.32
F value (after reconstitution) ± SD*	0.95 ± 0.03	0.96 ± 0.02	0.94 ± 0.04
pH (after reconstitution)	4.6-4.7	4.6-4.7	4.6-4.7
Bitterness score (for cachet)	0	0	0
Shelf life (months)	51.33	52.7	49.33

* Values represent mean ± SD of 3 experiments, *DP5 – Percent drug dissolved at 5 min, DE15 and DE60 – dissolution efficiency at 15 and 60 min*

5.6. References

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