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Evaluation of carboxymethyl-β-cyclodextrin with acid function: Improvement of chemical stability, oral bioavailability and bitter taste of famotidine

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ABSTRACT

The objective of the present study was to evaluate the potential influence of carboxymethyl-β-cyclodextrin (CM-β-CyD) on the aqueous solubility, chemical stability and oral bioavailability of famotidine (FMT) as well as on its bitter taste. We examined the effect of the CM-β-CyD on the acidic degradation of FMT compared with that for sulfobutyl-ether-β-cyclodextrin (SBE-β-CyD). The potential use of CM-β-CyD for orally disintegrating tablets (ODTs) was evaluated in vitro and in vivo. A taste perception study was also carried out. A strong stabilizing influence of CM-β-CyD was observed against the acidic degradation, in sharp contrast to SBE-β-CyD which induced a weird destabilizing effect on FMT. ^{13}C NMR was used to investigate the interaction mode between FMT and the 2 CyDs. In vivo study of ODTs indicated a significant increase in C_{max}, AUC and oral bioavailability in the case of FMT-CM-β-CyD tablets, compared with plain drug tablets. However, no significant difference in T_{max} and $t_{1/2}$ was observed. CM-β-CyD complexation appears to be an acceptable strategy for enhancing the oral bioavailability of FMT owing to its dramatic effect on the aqueous solubility and chemical stability of the drug. In addition, it has a pronounced effect on masking the bitter taste of FMT.

1. Introduction

Cyclodextrins (CyDs) possess a unique ability to complex with drugs enabling them to increase solubility, reduce bitterness, enhance stability, and decrease tissue irritation upon dosing (Mosher and Thompson, 2002). One of the most common applications of CyDs cited in the pharmaceutical literature is its ability to enhance drug bioavailability (Carrier et al., 2007). The ability of water-soluble polymers to enhance the solubilizing effect of CyDs and thus possibly reduce the amount of CyD necessary in a given dosage form has been demonstrated (Loftsson et al., 1994; Loftsson et al., 1996; Savolainen et al., 1998; Valero et al., 2003). Formulations containing a water-soluble polymer (e.g., hydroxypropylmethylcellulose, HPMC or polyvinylpyrrolidone, povidone) have been able to achieve bioavailability enhancement equivalent to formulations containing up to 80% less CyD (Savolainen et al., 1998). Such results are generally attributed to a synergistic solubilizing effect of polymer and CyD, which is believed to be due to formation of ternary complexes or co-complexes between the drug, CyD, and polymer (Valero et al., 2003). Solubility can be a key factor in the kinetics of dissolution and can also influence permeation through the intestinal membrane by influencing the concentration of a drug in solution in the intestinal lumen. As many of these studies involve low-solubility compounds, it is difficult to determine if the enhancement of bioavailability is due to the effect of CyDs on dissolution, degradation, or both.

CyDs and their derivatives thereof may enhance drug degradation, depending on the reaction mechanisms and the steric arrangement of the drug in the complex. Drug–CyD complexation can reduce the rate of decomposition of a drug by protecting labile regions from potential reactants in an aqueous environment (Loftsson and Brewster, 1996). Under specific conditions, CyD complexation may accelerate drug degradation depending on the type of the used CyD. For exam-

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ple, CyD has been shown to catalyze the deacetylation and degradation of spiranolactone, the effect was qualitatively correlated with the ionization state of hydroxyl groups on CyDs, which were lower in sulfobutyl-ether-β-cyclodextrin (SBE-β-CyD) (Jarho et al., 2000). In addition, prostaglandin E_1 in neutral and alkaline solution is destabilized by β-CyD, but stabilized by carboxymethylethyl-β-CyD (CME-β-CyD) (Adachi et al., 1992).

Farmotidine (FMT) is a histamine H_2-receptor antagonist used to treat peptic ulcers; gastroesophageal reflux; and conditions where the stomach produces an excess of acid, such as Zollinger–Ellison syndrome (Dollery, 1999). It has been reported that FMT is relatively susceptible to acid-catalyzed hydrolysis. The degradation of FMT in HCl solutions followed pseudo-first-order kinetics (Islam and Narurkar, 1991). The drug has been shown to undergo hydrolysis to a sulfamoyl amide in the presence of excess hydrochloric acid and to a carboxylic acid at elevated temperatures. The degradation products were found to have only weak antagonistic potency (Yanagisawa et al., 1987). A number of possible degradation mechanisms of FMT have been previously proposed (Junnarkar and Stavchansky, 1995). Moreover, Guvener and Ates (1988) investigated the stability of FMT in simulated gastric medium. The drug was found to lose about 34% of the original concentration in 1 h and about 88% in 3 h. To assess the significance of the acid-catalyzed hydrolysis of FMT on the stability of the drug in the stomach after oral administration, important factors such as gastric acidity and gastric emptying time must be considered. The impact of the acid-catalyzed degradation of FMT in the stomach would be expected to be more significant in elderly patients who have a prolonged gastric emptying half-time (123 min). In these patients about 12.0–57.8% of the drug would undergo decomposition in the stomach, thus lowering the amount of intact drug available for absorption (Suleiman et al., 1989). It was reported that poor lipophilicity, poor aqueous solubility and susceptibility to gastric degradation may contribute to the low and variable oral bioavailability of FMT (Islam and Narurkar, 1991). Clinically, the use of FMT is beneficial for elderly patients. Therefore a new FMT preparation that is useful for swallow function deficient patients would be advantageous. The complete bitter-taste-masking of drugs is an extremely important factor in the formulation of oral disintegrating tablets (ODTs) and the palatability of ODTs is a critical factor in ensuring patient compliance (Fu et al., 2004; Khan et al., 2007). In addition, developing a formulation for ODTs in which taste is masked, and drug release is improved, is a major challenge.

Okimoto et al. (1996) reported that the binding constants of drugs (either in neutral or cationic form) were consistently greater with the anionic SBE-β-CyD than with the neutral HP-β-CyD. As we completed this study, our goal was to determine the solubility and stability of FMT as a function of two anionic CyDs (SBE-β-CyD and CM-β-CyD) and to elucidate the potential causes of any differences observed between the two compounds. We carried out the kinetic studies of the degradation of FMT in acidic solution (0.1 N HCl) and the effect of pH and temperature on the rate of degradation FMT was examined using the two CyD derivatives, SBE-β-CyD and CM-β-CyD. In this work, the formation of inclusion complexes between FMT and the CyD derivatives was assessed using phase-solubility techniques. The influence of povidone K30 (a water-soluble polymer) on the solubility of CyD complexes also investigated. The complexation of FMT with the CyD derivatives and the mechanism of the impact of CyD on the degradation of FMT were examined using ^{13}C nuclear magnetic resonance spectroscopy (^{13}C NMR). In vitro and in vivo evaluations of ODTs of CM-β-CyD complexes were also performed. Moreover, the masking effects of CM-β-CyD on the bitter taste of FMT was examined.

2. Materials and methods

2.1. Materials

Famotidine (FMT) was supplied by El Mehan pharmaceutical company (MUP), Al-Ismailia, Egypt; Polyvinyl pyrrolidone (povidone K30) was obtained from Tokyo Chemical Industry Co. LTD., Japan; SBE β-CyD (Captisol®) was purchased from Cydex Co., USA; CM-β–CyD was purchased from Sigma Co., USA; Sodium heptane sulfonic acid (SHS) was purchased from Sigma–Aldrich, UK; Acetonitrile for HPLC was obtained from Nacalai Tesque Co., Japan. All other chemicals and solvents used were of pharmaceutical and analytical grade. Double distilled water was used throughout the study.

2.2. Phase-solubility studies

Phase-solubility equilibrium diagrams (in water at 25 °C) were obtained for both binary and ternary systems as per Higuchi and Connors (1965) method. Studies for binary systems were carried out by adding an excess amount of drug to 25 ml aliquots of aqueous solutions containing increasing concentrations of CM-β-CyD and SBE-β-CyD (from 0 to 15 mM) or povidone K30 (from 0 to 3% w/v).

Experiments regarding ternary systems were performed analogously to those for the binary systems, but in the presence of 1% w/v povidone K30 (value obtained from phase-solubility data for the drug: polymer binary system), reported in our previous study (Mady et al., 2010). These series of suspensions were equilibrated for 48 h on a mechanical shaker followed by filtration and analysis. The samples were filtered through a 0.45 μm membrane filter (Millex-HV filter units, Millipore, Bedford, USA) and suitably diluted for analysis. The drug content was determined by UV spectrophotometry (Hitachi, U-2900 Spectrophotometer, Tokyo, Japan) at 265 nm. The presence of CyDs and povidone did not interfere with the spectrophotometric assay of the drug. Each experiment was performed in triplicate.

2.3. Preparation of FMT–CyDs ternary systems

Ternary systems comprised of 1% w/v polymer (povidone K30), drug and CyD derivative were prepared by the solution method. The required amounts of CyD and povidone K30 were first dissolved in double distilled water to give a clear solution. The drug was then dispersed in this aqueous solution of CyD in a molar ratio of (1:1) followed by continuous stirring for 24 h at room temperature. The resulting solution was then subjected to freeze drying using Freeze Dryer FD-1, EYELA, Tokyo Rikakikai, Co., LTD, Japan.

2.4. ^{13}C NMR spectroscopy

^{13}C NMR measurements were conducted on a JEOL JNM-ECA 500 model spectrometer (Tokyo, Japan), with a 5 mm inverse broadband probe, operating at 500 MHz. FMT and its CyDs complexes were dissolved in D_2O with the addition of 5 μL of CD_3COOD (The concentration of the drug was about 5 mg/ml and the pH was 4.2.). The samples were transferred to a capillary and spectra obtained. Chemical shifts are cited as parts per million (ppm) and were calibrated indirectly using tetramethylsilane as an external standard. The chemical shifts of FMT were assigned according to the report of (Barańska et al., 2001).

2.5. Kinetic studies

Studies were initiated by adding 0.1 ml of a 0.01 M stock solution of FMT in *N, N*-dimethylformamide to 9.9 ml of a 0.1 N HCl solution (pH 1.2) in screw-capped vials. The vials were kept in a

thermostated water-bath at 37 ± 0.5 °C. 20 μl samples were withdrawn at appropriate intervals and analyzed for undecomposed FMT by HPLC. To study the effect of SBE-β-CyD and CM-β-CyD on the specific acid catalysis of FMT, accurately weighed amounts of CyDs (0–5% w/v), were added to 10 ml volumetric flasks along with 0.1 ml of a 0.01 M FMT stock solution. The final volume of the flasks was adjusted with 0.1 N HCl. The contents were then transferred to screw-capped vials placed in a thermostated water-bath at 37 ± 0.5 °C. At various intervals, 20 μl aliquots were withdrawn and immediately frozen to stop further reaction and analyzed for undegraded FMT by HPLC. The pseudo-first-order rate constants, k_{obs}, for the degradation were determined from linear plots of the natural logarithm of the remaining FMT vs. time.

The apparent first-order rate constants were determined at various pH values ranging from 1.2 to 2.5, using HCl and glycine-HCl buffer for pH values 1.2–2.0 and 2.5, respectively. All pH measurements were made using a digital pH meter (Horiba pH-meter F-21, Kyoto, Japan). To assess the kinetics of degradation of FMT in 0.1 N HCl as a function of temperature, flasks, containing FMT with or without CyD derivatives, were placed in a water-bath that had been previously adjusted to 298, 310 or 323 K. Thermodynamic activation parameters were calculated from the plot of the logarithm of the degradation rate constant (lnk) vs. the reciprocal values of temperature (1/*T*).

2.6. HPLC

Quantitative determinations of FMT were carried out by HPLC using a Hitachi L-6000 pump, a Hitachi L-4000 UV detector operated at 269 nm, Hitachi D-2500 Chromato integrator, Japan. A 50-μl injection loop, an YMC-Pack ODS-AM 303 250 mm × 4.6 mm (5 μm, 120 Å) column and LiChrospher 100 RP-18(e) (Merck) guard column were used in the HPLC analysis. The mobile phase was developed and validated. It consisted of 70: 30 0.1 mol/l aqueous acetic acid solution (containing 6.07 g/l sodium heptane sulfonic acid) (pH 4.6): acetonitrile. The retention time for the FMT peak was 5.9 min at a flow rate of 0.9 ml/min.

2.7. Tablet formulation and characterization

Oral disintegrating tablets (ODTs) containing FMT (20 mg) or the CM-β-CyD complex equivalent to (20 mg) were compressed on a single rotary tabletting press using an 8-mm round shaped flat punch by the direct compression technique (Fu et al., 2004). ODTs were prepared using crospovidone as a superdisintegrant and anhydrous lactose as a diluent. The prepared ODTs were evaluated for hardness (Hardness tester Hardness tester, Fujiwara Seisakusho, LTD, Tokyo, Japan), disintegration time (Riken's disintegration tester, Miyamoto Riken Ind. Co., Ltd, Osaka, Japan) and weight variation.

2.8. In vivo study of ODT

CyDs have been utilized extensively in pharmaceutical formulations to enhance oral bioavailability. Typically, a complex of the drug and CyD is determined, and the resulting pharmacokinetic parameters are compared to those for the case where the drug alone is administered. To evaluate the effects of CM-β-CyD on the release profile in vivo, the bioequivalence of formulation 1 (FMT-ODTs) and formulation 2 (CM-β-CyD-ODTs) were determined in rats. A single-dose, randomized study was conducted using 16 rats (Wistar rats, male, weight 250–350 g). The rats were divided into two groups and fasted overnight. The tablets were dispersed in water and immediately administered at a dose of 15 mg/kg per animal through oral gavage. Heparinized samples of venous blood (0.3 ml) were then collected from tail vein at 15, 30, 60, 120, 180, 240, 360 and 480 min. Plasma samples were obtained by centrifugation and stored frozen at −20 °C until HPLC analysis. The plasma samples were prepared for HPLC analysis as previously reported, with minor modifications (Zarghi et al., 2005). In brief: a mixture of plasma (50 μl), 46% perchloric acid solution (30 μl) and ranitidine (as internal standard) at a concentration of 6 μg/ml (20 μl) was prepared using a vortex mixer for 2 min. The mixture was then centrifuged at 10,000 × *g* for 5 min. Linearity was performed with a five-point calibration curve. The method was found to be linear over the examined concentration range 50–800 ng/ml. The average calibration equation can be described by: $y = 0.0958x + 0.0004$, with a correlation coefficient of 0.9998. Where the *y* is the ratio of the peak area of FMT and internal standard and *x* is the concentration (ng/ml). The limit of detection (LOD) was 10 ng/ml. The retention time for FMT was 5.9 min. A two-compartment model was used in the pharmacokinetic analyses after administration of the tablets. Pharmacokinetic parameters were estimated by curve-fitting. The two-compartment model can be described by the following equation:

$$C = \frac{FDka}{V(ka - ke)}(\exp(-ket) - \exp(-kat)) \quad (1)$$

where *C* is the concentration, *t* is the time after administration of drug, *F* is the bioavailability, *V* is the distribution volume, and *ka* and *ke* are rate constants for the absorption and elimination phase, respectively. The pharmacokinetics and statistical analyses were computed by fitting using the Practical Pharmacokinetic Program (MULTI, a normal least square program (Yamaoka et al., 1981). C_{max} (the maximum plasma concentration) and T_{max} (time point of maximum plasma concentration) were obtained directly from the measured data; the $AUC_{0-\infty}$ (area under the plasma concentration–time curve from 0 to ∞) was calculated according to the trapezoidal rule and $t_{1/2}$ (half-life of drug elimination during the terminal phase) were calculated.

2.9. Gustatory sensation test and in vivo oral disintegration time

A gustatory sensation test was carried out according to the method described by Mou-ying et al. (1991). Eight healthy human volunteers, of either sex; in the age group of 23–27 years were selected. The protocol for the investigation was read and signed by the volunteers before starting the study. They were asked to rinse their mouth with a cup of water (200 ml). Before testing, the volunteers ($n = 8$) were asked to retain the reference solutions in their mouths for 10 s, and to provide information on their concentrations and their bitterness intensities. All unknown samples were randomly supplied to each volunteer in a blind manner. They were then asked to taste the ODTs and to assign a bitterness score to each tablet. All tablets were kept in the mouth of the volunteers until it disintegrated. After tasting the tablets, they expectorate and the bitterness level was recorded and subjects then gargled well. The wash out period between testing samples was 15 min.

A numerical scale was used with the following values: 0 = tasteless, 1 = slightly bitter, 2 = moderately bitter, 3 = bitter, 4 = strongly bitter. This numerical scale was validated by testing samples randomly. The oral cavity was rinsed with distilled water three times to avoid bias.

The disintegration time of ODTs is measured utilizing conventional tests for tablets that are described in the Pharmacopoeias (including USP and EP). However, it is difficult to assess the disintegration rate for an ODT with these tests due to its rapid disintegration rate even in a small amount of water. Thus, the disintegration rate obtained from the conventional disintegration tests appears not to be reflective of the disintegration rate in the human mouth.

Nevertheless, this standard compendial test faces many limitations in discriminating between different ODTs as their

Table 1
Values of the apparent stability constant (Kc) and complexation efficiency (CE) of different binary and ternary systems of SBE- and CM-β-CyDs.

Type of CyD	Kc (M^{-1})	CE
SBE- β-CyD binary	370 ± 9	0.79 ± 0.11
CM- β-CyD binary	414 ± 4	1.01 ± 0.06
SBE- β-CyD ternary	641 ± 7	1.91 ± 0.21
CM- β-CyD ternary	657 ± 3	2.24 ± 0.22

Kc: stability constant; CE: complexation efficiency.

disintegration time is very short and also because of the strong agitation and large volume of water used during this test (Watanabe et al., 1995). As a result, measurements of disintegration time in the oral cavity were carried out in human volunteers (Fukami et al., 2005) who were administered the formulae for the evaluation of bitter taste. The ODT was placed on the subject's tongue and a stopwatch was started immediately, as soon as the tablet came into contact with the tongue. The subjects were instructed to gently move the tablet against the upper part of the mouth with their tongue and to cause a gentle tumbling action on the tablet. It was emphasized to the subjects that this is a gentle motion without biting on the tablet or tumbling it from side to side. Immediately after the last noticeable granule had disintegrated, the time was recorded. Swallowing of saliva was prohibited during the test, and saliva was also rinsed from the mouth after each measurement.

3. Results and discussion

3.1. Solubility studies

The interaction of FMT with the two β-CyDs was studied using the phase-solubility method. The solubility of FMT was significantly enhanced by the two types of CyDs in the rank order of CM-β-CyD > SBE-β-CyD, and was linearly dependent on CyD concentration (A_L type). A 1:1 complex was assumed and Eqs. (2) and (3) were used to estimate the apparent binding constant, Kc and the complexation efficiency, CE:

$$\mathrm{Kc} = \frac{\mathrm{Slope}}{S_o(1 - \mathrm{Slope})} \quad (2)$$

$$\mathrm{CE} = \frac{\mathrm{Slope}}{(1 - \mathrm{Slope})} \quad (3)$$

The estimated Kc values for CM-β-CyD systems were slightly greater than the systems of SBE-β-CyD (either in the absence or the presence of povidone K30), as shown in Table 1.

3.2. Stability studies

While most studies in the literature have focused on differences in the binding constants of drugs to various CyDs, several reports discussed the effect of the neutral CyD on the degradation of FMT. Islam and Narurkar (1991) revealed that increasing the HP-β-CyD concentration resulted in a decrease in the rate of degradation of FMT at pH 2.2. However, there are no previous reports dealing with the effect of anionic CyD (SBE-β-CyD or CM-β-CyD) on the acidic degradation of FMT. Fig. 1 shows a comparison between the degradation profile of free FMT, FMT complexed with either SBE-β-CyD or CM-β-CyD. This plot indicates that free FMT degrades more rapidly than complexed FMT in case of CM-β-CyD and that complex formation increases the stability of FMT. However, in case of SBE-β-CyD, the complexed FMT degraded slightly faster than the free FMT, indicating that the complexation of FMT with SBE-β-CyD is not favorable from the point of view of stability.

The degradation of FMT in acidic medium followed pseudo-first-order kinetics in the presence and in the absence of the two

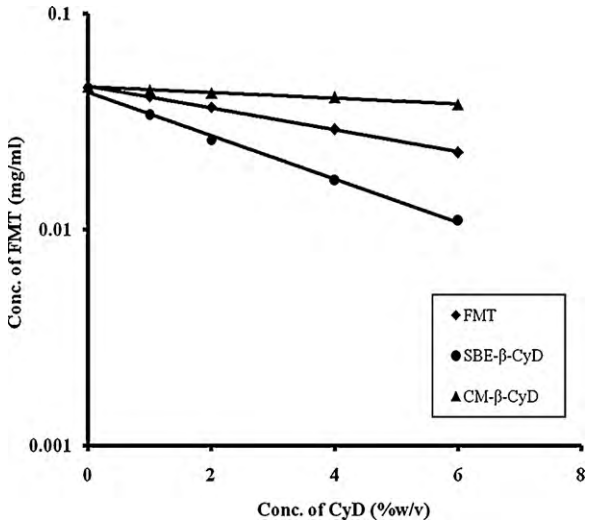


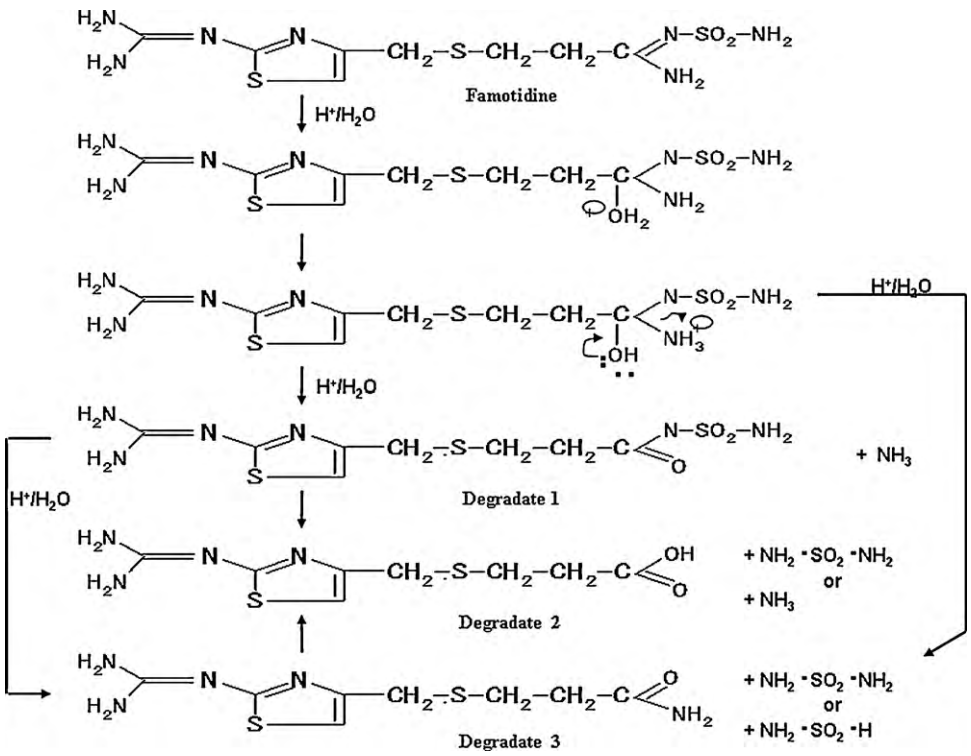
Fig. 1. Plot showing the pseudo-first-order acidic degradation of FMT at 37 ± 0.5 °C in the absence and presence of β-CyDs (2% w/v) at pH 1.2 (logarithmic scale).

CyDs. As shown in Table 2, the degradation rate constant of FMT, k_{obs}, increased with increasing the concentration of SBE-β-CyD. However, k_{obs} decreased with increasing the concentration of CM-β-CyD. When inclusion complexes are formed, the non-polar cavity of CyDs provides an unfavorable microenvironment for the formation of a polar transition state (see Scheme 1); therefore, the degradation of FMT, when in the form of an inclusion complex may be retarded, simply because it does not favor charge separation. This may be the principle behind the CM-β-CyD stabilization effect. Moreover, upon measurement of the pH of solutions of FMT at different acidic pH values (1.2, 1.6, 2 and 2.5), we found that the measured pH values of the previous solutions with the addition of 2% w/v CM-β-CyD increased to 2, 2.3, 2.8 and 3.1, respectively. However, the measured pH values with the addition of 2% w/v SBE-β-CyD were 1.4, 1.6, 2 and 2.5, respectively (Fig. 2). This provides information on the buffer effect induced by CM-β-CyD. Another possible cause for the differences observed between SBE-β-CyD and CM-β-CyD is that the inclusion modes between FMT and the two CyDs are different. This was subsequently clarified in the NMR study and by thermodynamic analysis data. FMT may be positioned in a more hydrophobic environment with CM-β-CyD than SBE-β-CyD. It was reported that the k_{obs} value is directly proportional to the hydrogen ion concentration (Suleiman et al., 1989). At a pH of less than 4, FMT exists entirely in the protonated form with H^+ being the exclusive catalyst (Islam and Narurkar, 1991). As a result, an increase in pH leads to a decrease in the hydrogen ion concentration and, hence, a decrease in k_{obs}. However, the presence of SBE-β-CyD in an acidic solution of FMT induced an increase in the degradation rate. This may be attributed to the increase in H^{+1} ion

Table 2
Observed pseudo-first-order rate constants (k_{obs}) for FMT degradation in 0.1 N HCl (pH 1.2) at 37 ± 0.5 °C in the presence of different concentrations of CyDs.

Type of CyD	Concentration of CyD (% w/v)	k_{obs} (h^{-1})[a]
FMT alone	–	0.116
SBE-β-CyD	0.2	0.172
	0.5	0.203
	2	0.257
	5	0.273
CM-β-CyD	0.2	0.077
	0.5	0.031
	2	0.019
	5	0.007

[a] k_{obs} is calculated from the slope of Log concentration vs. time curve.



Scheme 1. The proposed scheme for acid-catalyzed hydrolysis of FMT (Junnarkar and Stavchansky, 1995).

concentration due to the interaction between SBE-β-CyD and FMT in 0.1 N HCl.

From another viewpoint, in the proposed scheme for the acid-catalyzed hydrolysis of FMT reported previously (Junnarkar and Stavchansky, 1995), the degradation products of FMT (including ammonium ion) together with the acetate group from CM-β-CyD may have resulted in the formation of ammonium acetate buffer which represents a good medium for stabilizating FMT against further degradation and thus would result in a stabilizating effect. On the other hand, the degradation products of FMT, in presence of the sulfite group of SBE-β-CyD, could result in the formation of ammonium bisulfate which has no buffering effect and the acidic medium could continue to promote the degradation of FMT.

The apparent first-order rate constant for the degradation of FMT in aqueous solution was measured as a function of temperature. The rate of degradation of FMT alone was markedly accelerated with increasing temperature (Fig. 3). The effects of CM-β-CyD and SBE-β-CyD complexation on acidic degradation rate were also examined at temperatures of 298, 310 and 323 K. The rate of degradation of FMT alone was significantly increased when compared with the value when it was complexed with CM-β-CyD. In contrast, the rate of degradation of FMT was found to be increased in solutions containing SBE-β-CyD. The rate of degradation of FMT in the presence of either SBE-β-CyD or CM-β-CyD (1:1 molar ratio) also increased with increasing the temperature. Since temperature is one of the primary factors affecting drug stability, Arrhenius developed and described the effect of temperature on

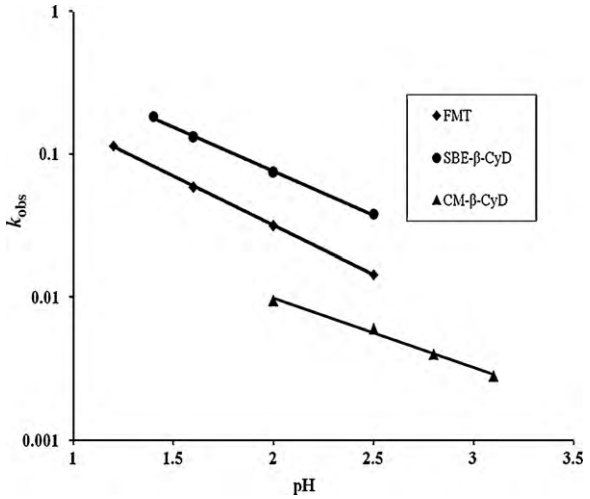


Fig. 2. pH-rate profiles for the degradation of FMT in the absence and presence of β-CyDs (2% w/v) (logarithmic scale).

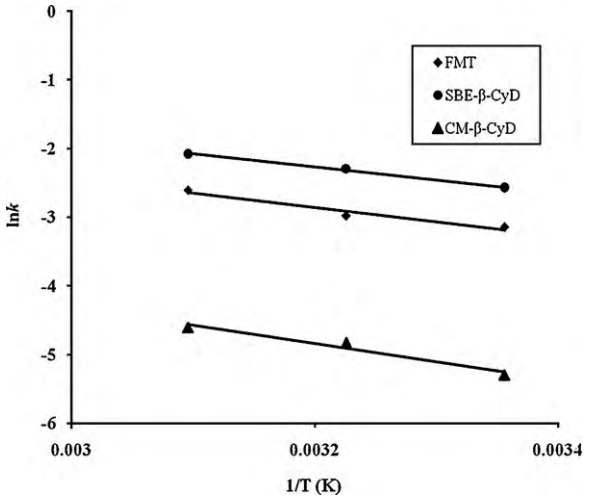


Fig. 3. Arrhenius plots for acidic degradation of FMT in the absence and presence of β-CyDs.

Table 3
Effect of β-CyDs on the thermodynamic parameters for the degradation of FMT in acidic medium.

	ΔH^* (kcal mol^{-1})	ΔS^* (e.u.)	ΔG^* (kcal mol^{-1})
FMT	3.57	7.64	1.29
SBE-β-CyD	3.18	7.57	0.92
CM-β-CyD	4.71	7.34	2.52

ΔH^* = activation enthalpy change, ΔS^* = activation entropy change and ΔG^* = activation free energy change.

rate processes by the following equations:

$$\ln\ k = \ln A - \frac{\mathrm{Ea}}{RT} \quad (4)$$

$$\Delta H* = \mathrm{Ea} - RT \quad (5)$$

$$\ln\ k = \frac{\Delta S*}{R} - \frac{\Delta H*}{RT} \quad (6)$$

$$\Delta G* = \Delta H* - T\Delta S* \quad (7)$$

where, k = the degradation rate constant, A = coefficient of frequency, Ea = activation energy, ΔH^* = activation enthalpy change, ΔS^* = activation entropy change, ΔG^* = activation free energy change, R = universal gas constant, T = absolute temperature in K. The plot of ln k vs. $1/T$ has been traditionally used to describe the temperature dependency for various equilibrium processes (Jarho et al., 1995; Mallick et al., 2007, 2008). The effect of temperature on equilibrium rate constants was obtained using the Arrhenius equation. The rate constants were employed for the assessment of thermodynamic parameters, in the presence and absence of CyDs. Various thermodynamic parameters were calculated (Table 3). It is apparent that the deceleration and acceleration of FMT degradation by the addition of CM-β-CyD and SBE-β-CyD, respectively, are controlled by changes in Ea, ΔH^* and ΔG^*, i.e. an increase in Ea, ΔH^* and ΔG^* in the former CyD and a decrease in the later CyD, with small changes in the ΔS^*. These results support the conclusion that the non-polar cavity of CM-β-CyD provides an unfavorable microenvironment for the formation of a polar transition state of FMT, whereas the large negative charges of SBE-β-CyD stabilize the cationic transition species of FMT.

3.3. ^{13}C NMR studies

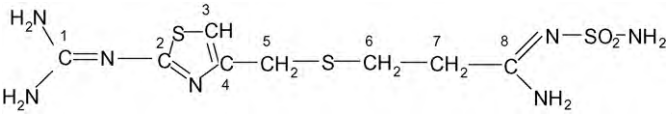
Host/guest interactions of CyDs with drugs have been investigated using a number of chemical and physical techniques such as spectroscopy, potentiometric titration, kinetic studies and solubility methods (Hirayama and Uekama, 1987; Connors, 1997).

Among these techniques, NMR spectroscopy is particularly useful for the structure determination of CyD inclusion complexes in solution (Kimura et al., 1999). Table 4 shows the ^{13}C chemical shift displacement of FMT in the presence of SBE-β-CyD and CM-β-CyD. The shift changes for the SBE-β-CyD system were larger than those for the CM-β-CyD system, probably because of the large negative charges of the former host, compared with that of the later host. When the ^{13}C shift changes were compared in the FMT molecule, large shifts were observed around the guanidinothiazole moiety (C1, C2 and C3) rather than around the reactive site of FMT (propionamide portion, C7 and C8, see Scheme 1) in the case of SBE-β-CyD system. In contrast, relatively large shifts were observed for the C5, C7 and C8 carbons of the methylthiopropioamide moiety, compared with the C1 and C2 carbons of the guanidine moiety in the case of the CM-β-CyD system. These results suggest that the inclusion sites of FMT were slightly different between SBE-β-CyD and CM-β-CyD, i.e. SBE-β-CyD preferably interacts around the gaunidinothiazole moiety, whereas CM-β-CyD around the propionamide moiety of the drug. This different inclusion mode may be at least partly reflected in the different effects of SBE-β-CyD and CM-β-CyD in resisting FMT degradation.

3.4. Physical properties of ODTs

Since the ability to swallow deteriorates with age, many elderly patients find it difficult to swallow solid dosage forms of drugs that are currently available, such as tablets and capsules. To address this problem, a fast-disintegrating, user-friendly dosage form has been developed (Watanabe et al., 1995). This formulation disintegrates immediately in the mouth so that patients can take it without water, similar to a liquid formulation. This will be convenient for the patients and enhance compliance, especially for those who have difficulty in swallowing solid dosage forms, or do not have ready access to water. Many companies have developed various types of oral disintegrating dosage forms. ODTs require not only rapid disintegration in the oral cavity but also a high hardness (more than 2 kg) to resist the strength to handle. To improve the physicochemical properties of the tablets, tablets containing FMT (20 mg), lactose (75% w/w), crospovidone (5% w/w) as a superdisintegrant and magnesium stearate (as a lubricant) (1%w/w) were prepared by direct compression with a compression force of 5 KN. Crospovidone has excellent wicking characteristics, although it swells only to a small extent. The hardness of the resulting tablets was around 3.5 kg and the disintegrating time was about 26 s. Tablets containing an equivalent amount of CM-β-CyD complex, lactose (75% w/w), crospovidone (5%w/w) as a superdisintegrant and magnesium stearate (1% w/w) were prepared by direct compression with compression force of 2 KN. The hardness of the resulting tablets was about 4 kg and its disintegrating time was 43 s. The compression force used in the case of CM-β-CyD tablets was decreased in order to decrease the disintegration time because we observed a prolongation in the disintegration time to more than 1.5 min when 5 KN was used as the compression force. All of the prepared tablet

Table 4
FMT carbon-13 chemical shift changes in the presence of β-CyDs.



Assignment	Chemical shifts of free drug δ (ppm)	Change of chemical shift $\Delta\delta$ (ppm) (δ complexed-δ free)	
		SBE-β-CyD	CM-β-CyD
C1	154.3	3.13	−0.04
C2	177.48	1.15	−0.16
C3	111.11	−5.15	−1.11
C4	148.42	−0.21	0.39
C5	36.58	−0.40	−0.62
C6	30.65	0.73	0.11
C7	27.59	0.06	0.38
C8	167.25	0.52	0.57

Table 5
Physical properties of the oral disintegrating tablets.

Formulation	Weight variation (mg)	Hardness (kg/cm^2)	In vitro disintegration time (s)	In vivo disintegration time (s)
Formulation 1	150 ± 2	3.5	26	21
Formulation 2	150 ± 2	4	43	30

Formulation 1: FMT-ODT and formulation 2: CM-β-CyD ternary system-ODT.

Table 6
Bitterness scores, as evaluated by a panel composed of eight human volunteers.

Formulations	Number of volunteers rating the preparation as				
	0	1	2	3	4
Formulation 1				3	5
Formulation 2	2	6			

Formulation 1: FMT-ODT and Formulation 2: CM-β-CyD ternary system-ODT.

formulations met the USP 27 requirements for weight variation. Content uniformity was found to be good when the percentage of drug content was more than 98%.

In general, the disintegration time of tablets in the mouth is related to the rate of penetration of water into the tablet. According to the literature (Schiermeier and Schmidt, 2002), the oral disintegration time of ODT is 1 min or less; however, the most suitable disintegration time was not confirmed. Upon comparing the disintegration time of the three formulas, measured either in vitro or in vivo, we found that formulation 2 disintegrates more slowly than formulation 1. However, both formulations disintegrate within 1 min. (Table 5)

3.5. Human gustatory sensation test

The results of the taste-masking test are listed in Table 6. The mean score of 1 for the formulation 2 indicated that the formulation containing CM-β-CyD sufficiently alleviated the bitterness of FMT tablets, compared with a mean score 4 for formulation 1 which contained the drug alone. Similar masking effects of the bitter taste of FMT using SBE-β-CyD were found at this laboratory (Mady et al., 2010). The gustatory sensation tests obtained here clearly indicate that the significantly lower bitter taste by CM-β-CyD can be due to masking taste effects of CM-β-CyD, rather than the chemical stabilization effect of CM-β-CyD. Because FMT is stable in these experimental conditions (pH in saliva may be pH 6.0–8.0).

3.6. Pharmacokinetic analysis

The HPLC assay was validated and was found to have a good linearity in the concentration range from 10 to 800 ng/ml with acceptable within- and between-day reproducibility. The lower limit of FMT quantification in plasma was 10 ng/ml. The mean plasma concentration–time profile of FMT following the administration of formulation 1(FMT alone ODT) and formulation 2 (CM-β-CyD complex ODT) is shown in Fig. 4. Moreover, the pharmacokinetic parameters that were determined from the FMT plasma concentration–time data are presented in Table 7. The

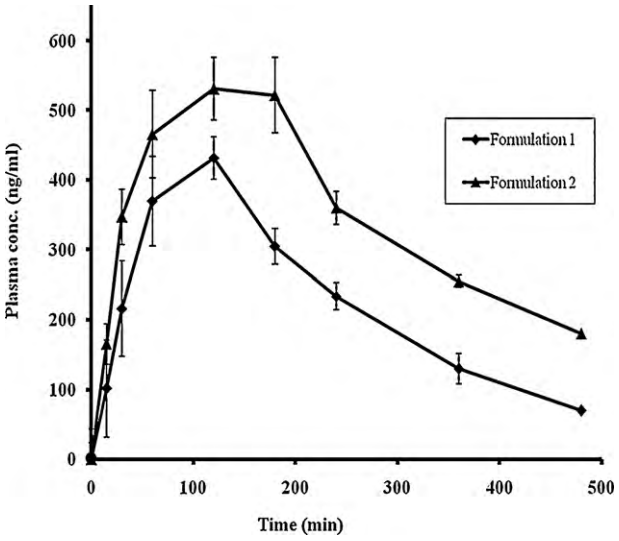


Fig. 4. Mean plasma pharmacokinetic profile of FMT (±S.D.) in rats following an oral administration of ODT of FMT (15 mg/kg). Formulation 1: FMT-ODT and formulation 2: CM-β-CyD-ODT.

reported increase in bioavailability is typically expressed as a change in the area under the plasma concentration vs. time curve (AUC) value, a change in the time to reach maximum plasma levels of the given compound (T_{max}), and/or the maximum plasma level achieved (C_{max}) (Kikuchi et al., 1987; Savolainen et al., 1998; Wong and Yuen, 2001; Elkheshen et al., 2002). The mean peak plasma concentration (C_{max}) for formulation 1 was 325.5 ± 51.5 ng/ml with mean T_{max} of 1.9 ± 0.3 h. The $AUC_{(0-\infty)}$ was 1895.9 ± 165.5 ng h/ml. The mean elimination half-life ($t_{1/2}$) was 0.9 ± 0.2 h. On the other hand, the mean peak plasma concentration (C_{max}) for formulation 2 was 561.5 ± 21.3 ng/ml and the mean time of peak plasma concentration (T_{max}) was 1.9 ± 0.2 h. Also, $AUC_{(0-\infty)}$ was found to be 3128.9 ± 295.3 ng h/ml. In addition, the mean elimination half-life ($t_{1/2}$) was 0.9 ± 0.1 h.

Our study indicates that the ratio of the AUC with CM-β-CyD to the AUC of the drug alone is 1.6 and is accompanied by an increase in the C_{max} value, with no detectable change in T_{max}. In order to compare the mean bioavailability and pharmacokinetic parameters of the tested formulae, the mean C_{max}, T_{max}, $AUC_{(0-\infty)}$ and $t_{1/2}$ were analyzed using the ANOVA test followed by the Bonferroni inequality test, the results revealed a significant difference in C_{max}, $AUC_{(0-\infty)}$ between formulations 1 and 2 ($p < 0.01$) In addition, no significant difference was found in T_{max} and elimination $t_{1/2}$.

Table 7
Pharmacokinetic parameters of FMT (mean ±S.D.) following the oral administration of formulation 1and 2 ($n = 8$).

Pharmacokinetic parameters	Formulation 1 (mean ±S.D.)	Formulation 2 (mean ±S.D.)
$AUC_{(0-\infty)}$ (ng h/ml)	1895.9 ± 165.5	3128.9 ± 295.3
C_{max} (ng/ml)	325.5 ± 51.5	561.5 ± 21.3
T_{max} (h)	2 ± 0.25	1.9 ± 0.3
$t_{1/2\beta}$ (h)	0.94 ± 0.17	0.94 ± 0.12
MRT (h)	4.4 ± 0.5	4.2 ± 0.3
F (%)	47.6 ± 7.1	78.6 ± 4.9

Formulation 1: FMT-ODT and Formulation 2: CM-β-CyD ternary system-ODT. F (%) is the relative bioavailability.

The increase in the oral bioavailability of CM-β-CyD-ODT compared with FMT-ODT might be attributed to the higher dissolution rate and the stabilization effect against acidic degradation, as the result of complexation with CM-β-CyD.

The relative bioavailability (F) of any oral dosage forms is calculated as follows:

$$F(\%) = \frac{[\mathrm{AUC}]\mathrm{oral}}{[\mathrm{AUC}]iv} \times 100 \quad (8)$$

We found that the relative bioavailability of formulation 1 and 2 was 47.6 ± 7.1 and $78.6 \pm 4.9\%$, respectively. F (%) of formulation 2 is higher than that of formulation 1 by about 31% due to the stabilization effect of CM-β-CyD against the acidic degradation of FMT. This result is in good agreement with the in vitro kinetic data.

Since, CyDs were previously reported as potent absorption enhancers (Dotsikas and Loukas, 2002), this may be another reasonable explanation for the improvement effect in the oral bioavailabilty of FMT in CM-β-CyD-ODT.

4. Conclusions

In conclusion, the present study examined the effect of SBE-β-CyD and CM-β-CyD on the degradation of FMT in acidic medium. The results suggest that CM-β-CyD had a pronounced stabilizing influence on the acidic degradation of FMT. The acid-catalyzed degradation of FMT was accelerated slightly in solutions containing SBE-β-CyD. These conflicting results can be explained by the different inclusion modes for the FMT molecule and the two β-CyDs. This favorable stabilizing effect by CM-β-CyD played an important role in enhancing the oral bioavailability of FMT. Furthermore, the present study highlights the usefulness of the direct compression technique for CyD complexes, along with the use of crospovidone as a superdisintegrant and lactose as a diluent as a simple and easy method for formulating the ODT of FMT in preparing tablets with an acceptable physical properties and an acceptable masking of the bitter taste. The most important effect for the formulation of CM-β-CyD-ODT is the improvement in the oral bioavailability of FMT. This can be attributed to the stabilization effect of CM-β-CyD on the acidic degradation of FMT.

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