



# Stability of metronidazole, tetracycline HCl and famotidine alone and in combination

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## Abstract

Metronidazole, tetracycline HCl and famotidine are commonly used for the treatment of *Helicobacter pylori*-associated peptic ulcer. In this paper, stabilities of these drugs and their combinations in solid and liquid states were studied as part of preformulation in the development of a combination drug delivery system. Solubility studies of metronidazole and tetracycline HCl were investigated, which indicated that both metronidazole and tetracycline HCl have high solubilities at and around pH 2.0. Metronidazole is relatively stable with little degradation in liquid phase. Tetracycline HCl in the dry state is stable when stored at room temperature regardless of exposure to light or humidity in the range of 20–65%. Enhanced temperature associated humidity effect was responsible for the instabilities of tetracycline HCl and famotidine to different extents. Elevated temperature accelerated the degradation of all the drugs in liquid phase but light exposure was not a factor for the degradation. The degradation processes of tetracycline HCl and famotidine were highly dependent on the pH of the solution, and relatively stable profiles were achieved at pH 4.0. No potential incompatibility between the drugs under storage conditions was observed in the development of a new multi-drug delivery tablet.

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## 1. Introduction

*Helicobacter pylori* is identified as the dominant factor in the causes of peptic ulcer (Malfertheiner and

Freston, 1997). Approximately 90–100% of patients with duodenal ulcers and 70–90% of patients with gastric ulcers have been infected by *H. pylori* (Balaban and Peura, 1997; Faigel and Melnyk, 1999). Antibiotics have been strongly recommended by the National Institute of Health (NIH) (Malfertheiner and Freston, 1997) and several antibiotics-based combination regimens for the treatment of *H. pylori*-related peptic ulcers

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have been approved by the Food and Drug Administration (FDA) because of their effectiveness (Balaban and Peura, 1997).

Metronidazole and tetracycline HCl are two antibiotics used along with bismuth in a triple therapy that is believed to be one of the most effective regimens in the eradication of *H. pylori* and is used as a “gold standard therapy” (Balaban and Peura, 1997). However, poor patient compliance significantly reduces the healing rate, and the frequency of dosing, side effects and large number of tablets to be taken daily (e.g. 16 tablets/day) are some of the main concerns (Malferteiner, 1996; Unge, 1996; Howden, 1997).

To overcome these deficiencies, a three-layered matrix tablet based on the gold standard triple therapy with a supplement of an H<sub>2</sub> receptor antagonist has been developed in our laboratory utilizing the principle of geometrical modification of monolithic matrix along with gastroretentive delivery strategies. This novel formulation approach provides for simultaneous delivery of the four actives with different release rates to potentially improve the therapeutic outcome and enhance patient compliance by overcoming the aforementioned limitations. However, one major concern in design of such delivery system from preformulation point of view is the possible incompatibilities between metronidazole and tetracycline HCl when combined in a single layer of three-layered matrix tablet.

The stability and compatibility of the active ingredients are an important concern in the preformulation studies that are conducted during the early stages of dosage form development. Drug substances can undergo the decomposition processes via hydrolysis, oxidation, photolysis, etc. Furthermore, isomerization, including epimerization, is also grouped into this category of decomposition in terms of pharmaceutical instability. For instance, the formation of epitetracycline HCl is the indication of decomposition of its parent drug tetracycline HCl.

The physicochemical characteristics, as well as the pharmacological and pharmacokinetic parameters of the drugs of interest, i.e. metronidazole (Wearley and Anthony, 1984; Moreau, 1995; Erah et al., 1997; Jones et al., 1997; Scheibel and Dyke, 1997; Yao and Moellering, 1999; The Merck Index, 2001; Van der Wouden et al., 2001; Bakshi and Singh, 2003), tetracycline HCl (Ali, 1976; Moreau, 1995; Corral, 1997; Jones

et al., 1997; Murray et al., 1998; Yao and Moellering, 1999), and famotidine (Cooper et al., 1990; Piper et al., 1997; Karalliedde and Henry, 1998; Lacy et al., 2001; Schrefer and Nissen, 2001; The Merck Index, 2001), have been well investigated. In addition, owing to the commercial availability of these dosage forms as single tablets or capsules they appear to be relatively safe and can be given as combination product.

However, when combinations of drugs are used in one formulation, chemical incompatibility issues become one of the most important factors influencing drug stability. Interaction may take place either among different active ingredients or active ingredient and excipients within the formulation, and can be classified as physical or chemical incompatibility according to the mechanism of interaction. The former is always shown as precipitation, complexation, color change, etc., while the latter is chemical reactions, including decomposition.

In view of the stability of the pharmaceutical substances, an adequate stability study is necessary and a requirement prior to submission to the Food and Drug Administration (FDA). In addition, the United State Pharmacopoeia (USP) requirements and the International Conference on Harmonization (ICH) guidelines all provide various techniques that are widely used to monitor the possible decomposition pathways and degradation products. Understanding of the nature of active compounds in a combination formulation is the essential component of a successful product development.

More importantly the stability, physical and chemical compatibility is the primary concern when facing complicated combinations of drugs in the design of the three-layered matrix tablet. Therefore, the stabilities of different drugs and their combinations in solid and liquid states, normal and accelerated conditions were studied in this work. The solubility studies of metronidazole and tetracycline HCl were initially investigated, as this drives the formulation and dissolution studies of the proposed three-layered matrix tablet.

Furthermore, evaluation of the stabilities of combined granulation of metronidazole and tetracycline HCl, and the granulation of colloidal bismuth subcitrate (CBS) and famotidine was performed by the developed and validated HPLC method.

## 2. Materials and methods

### 2.1. Materials and instrument

Metronidazole (Farchemia, Italy), tetracycline HCl (Sichuan Pharmaceutical Co., Ltd., China), famotidine (Spectrum, NJ) and CBS (MCP, CT) were the active agents. The standard samples of metronidazole and tetracycline HCl (USP) were purchased from Spectrum, NJ. Polyvinylpyrrolidone K-25 (PVP, Plasdone<sup>®</sup>, ISP, NJ) and sodium carboxy methylcellulose (NaCMC, Amend, NJ) in low viscosity (31 cps of 2% water solution) were used as binder in wet granulation.

Methanol (MeOH) and monobasic potassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) were purchased from Spectrum, NJ, phosphorous acid (H<sub>3</sub>PO<sub>4</sub>), hydrochloric acid (HCl) and nitric acid (70%) from J.K. Backer, NJ, sodium hydroxide (NaOH) from Amend, NJ. All the solvent and chemicals applied for HPLC were HPLC grade and the remaining were analytical grade agents. Deionized water was used in the study.

An HP-1050 series HPLC (Hewlett-Packard, DE) with a UV detector and a Supelcosil<sup>TM</sup> LC-18 column (4.0 mm × 150 mm, 5 μm, Supelco, USA) were used to perform analytical work. An Accumet 25 pH meter (Fisher Scientific, USA) was used to test pH of solutions. An oscillating sieve machine (Erweka, AR400, Germany), and an air oven (Thelco, GCA, USA) were used for the granulation. Sterifil filter system and Millex AH filter membrane were also used in the study (Millipore, Bedford, MA).

### 2.2. Solubility study of metronidazole and tetracycline HCl

At room temperature (22 °C), saturated solutions were made by dissolving excess amounts of metronidazole or tetracycline HCl into buffer solutions with pH ranging from 1.2 to 8.0, which covers the normal pH range of the human gastrointestinal tract. The pH of the solution was adjusted by hydrochloric acid and sodium hydroxide. Small volumes of these solutions were filtered and diluted as necessary.

These were injected onto a C<sub>18</sub> column and eluted with the mobile phase comprised of methanol–50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 2.5 (40:60, v/v) at a flow rate of 1.0 ml/min. Metronidazole and tetracycline HCl were determined simultaneously at 280 nm. The solubility

profiles for both metronidazole and tetracycline HCl at different pHs were calculated and constructed according to the data ( $n = 2$ ) from HPLC analysis.

### 2.3. Stability and compatibility of drugs in solid state

Granules of metronidazole and tetracycline HCl were made using a wet granulation method by adding 5% polyvinylpyrrolidone (PVP) into the blend of powders of metronidazole and tetracycline HCl (1:1, w/w), and sieving with an oscillating granulator. Granules were dried at a temperature of 60 °C. Dried granules were re-sieved, divided into several parts, precisely weighed, distributed into small storage bottles (open, closed, brown and transparent glass containers) and stored under different conditions (room temperature and refrigerated) respectively as described in Table 1.

Triplicate samples were withdrawn at predetermined time intervals, accurately weighed and dissolved in water to a known volume. Sample solutions were filtered through a 0.45 μm filter, and analyzed by HPLC at 280 nm using a mobile phase consisting of methanol–50 mM KH<sub>2</sub>PO<sub>4</sub>, pH 2.5 (40:60, v/v) at a flow rate of 1.0 ml/min.

Additional batches of granules of metronidazole and tetracycline HCl were made for the stability tests under accelerated conditions, with a 3% (w/v) of sodium carboxy methylcellulose was used as binder. The same procedures were applied to the granulation of famotidine and CBS (1:20, w/w), with the binder concentration of sodium carboxy methylcellulose at 1% (w/v). Dried granules were sieved, divided into small quantities and accurately weighed. Samples were placed in a capped glass container which contained saturated sodium chloride solution to create a 75% relative humidity environment (Nyqvist, 1983), and then stored in a constant temperature water bath at 40 °C.

Samples (triplicate) were removed at predetermined time intervals. Metronidazole and tetracycline HCl granules were dissolved in 10 ml of 0.1 N HCl and instantly diluted to 500 ml of water. Sample solutions were filtered through a 0.45 μm filter. Granules of famotidine and CBS were dissolved in 50 ml of HCl buffer (USP, pH 2.0) and filtered with Sterifil filter system to remove the precipitated bismuth salt. The filtrate was diluted when required and analyzed by HPLC at 280 nm. Mobile phase comprised

Table 1  
The storage conditions of metronidazole and tetracycline HCl granules

Sample no.	Transparent	Brown	Open	Closed	Room temperature	Refrigerated
A	+		+		+	
B	+			+	+	
C		+		+	+	
D	+		+			+
E	+			+		+

of methanol–acetonitrile–50 mM  $\text{KH}_2\text{PO}_4$ , pH 2.5 (10:15:75, v/v/v) at flow rate of 1.0 ml/min was selected in order to get best resolution of all analytes and potential degradation compound(s).

#### 2.4. Stability and compatibility studies of drugs in liquid state

Drug solutions were prepared at the concentrations similar to the amount in the proposed formulations assuming complete dissolution. Approximately 250 mg of metronidazole, 250 mg of tetracycline HCl, and 10 mg of famotidine were dissolved into 1000 ml of USP buffer solution, respectively. The mixture of drugs with identical concentrations as well as the mixture with a supplement of 200 mg CBS were also prepared to investigate the effect of combination of drugs on the aqueous stability by comparing with the treatments containing single drug.

Prepared solutions were stored at different conditions including different pH values, temperature, light and dark (see Table 2). Buffer solutions were prepared at pH 2.0, 4.0, and 6.0 (USP), and water baths were set at 37 and 25 °C, respectively. A florescence lamp was utilized to provide light condition, and aluminum foil, brown paper and chamber cover were used to provide a dark environment.

### 3. Results and discussion

#### 3.1. Solubility study of metronidazole and tetracycline HCl

The pH solubility profiles for metronidazole and tetracycline HCl are shown in Fig. 1. Overall, the solubility of metronidazole is significantly higher than tetracycline HCl at all the determined pH values. Both metronidazole and tetracycline HCl exhibited high sol-

ubility at a pH < 2.0. For example, at room temperature, pH 1.2, the solubility of metronidazole and tetracycline HCl were 64.80 and 57.96 mg/ml, respectively. The high solubility values at pH 1.2 masked the influence of temperatures on solubility. However, the solubility of tetracycline HCl, being an amphoteric drug, demonstrated a parabolic relationship showing minimum solubility around pH 3.0–6.0 and high solubilities in both the low acid and alkaline range. This may be the result of multiple  $pK_a$  values that tetracycline HCl possesses

Table 2  
Stability studies in liquid state under various conditions

Sample	Storage conditions					
	pH 2.0	pH 4.0	pH 6.0	Light	Dark	37 °C 25 °C
Tetracycline HCl						
T1	+			+		+
T2	+			+		+
T3	+				+	+
T4	+				+	+
T5		+			+	+
T6			+		+	+
Metronidazole						
M1	+			+		+
M2	+			+		+
M3	+				+	+
M4	+				+	+
M5		+			+	+
M6			+		+	+
Famotidine						
F1	+			+		+
F2	+			+		+
F3	+				+	+
F4	+				+	+
F5		+			+	+
F6			+		+	+
Mixture						
TMF	+			+		+
TMFB	+			+		+

Number represent different treatment under defined storage condition. Letters reflected drug(s) involved in the treatment, i.e., T: tetracycline HCl; M: metronidazole; F: famotidine; B: CBS.

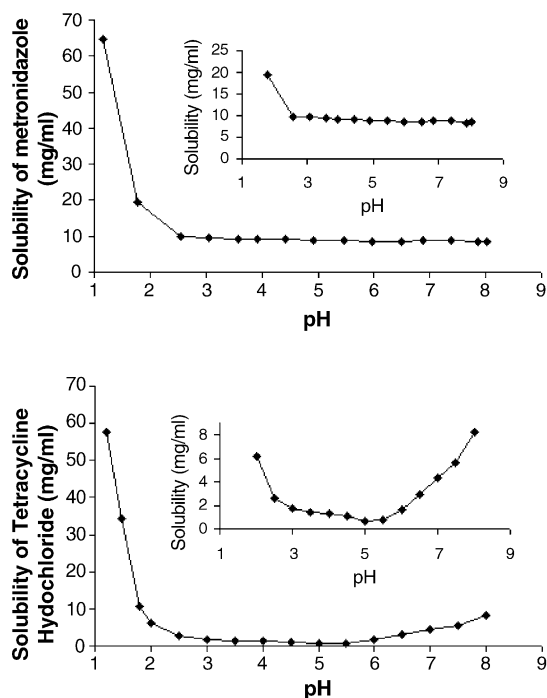


Fig. 1. The effect of various pH values on solubility of metronidazole (upper) and tetracycline HCl (lower) ( $n=2$ ).

(Ali, 1976). Metronidazole, being a weak base, appears to dissolve maximally around  $\text{pH} \leq 2.0$ .

### 3.2. Stability and compatibility of drugs in solid state

#### 3.2.1. Stability and compatibility of metronidazole and tetracycline HCl in solid state under mild conditions

After 8 weeks evaluation, the experimental results showed that in the solid state, metronidazole and tetracycline are compatible in the granular form under all storage conditions selected. When compared to the initial amount of analytes, there were no apparent decreases over a period of storage time and no significant differences in drug stability when granules stored in brown or clear container at refrigerated temperature ( $4^\circ\text{C}$ ) or room temperature ( $25^\circ\text{C}$ ), and open or closed condition (Fig. 2).

Chromatograms also provide the evidence that there was no degradation of analyte for either initial or final samples over a long storage time since no additional

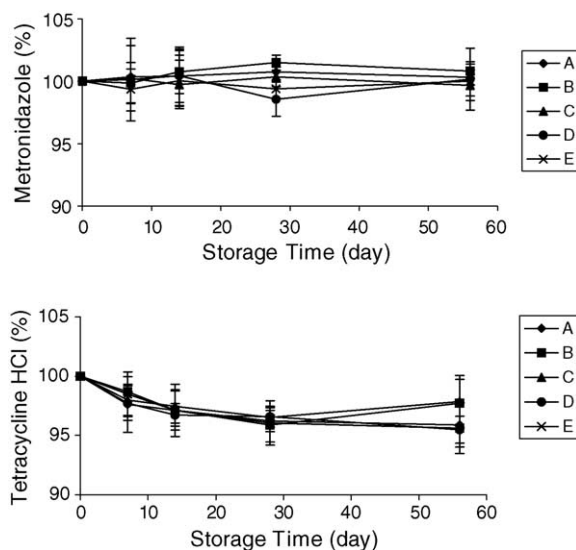


Fig. 2. Relative amount of metronidazole (upper) and tetracycline HCl (lower) in granules of metronidazole and tetracycline HCl under different storage conditions over 8 weeks ( $n=3$ ). Legends A–E are different sample numbers referred to Table 1.

chromatographic peaks were detected and no significant variation in peak area was observed.

The slight decrease of the relative amount of tetracycline HCl may be due to the incomplete extraction of drug from granules possibly due to the binder effect. Lowering the pH of the solution always increased the relative amount of tetracycline HCl due to greater solubility of the drug at low pH.

#### 3.2.2. Stability studies of metronidazole, tetracycline HCl, and famotidine in solid state under accelerated conditions

In this study, the stability studies are based on the drug combinations where drug–drug compatibility instead of single drug is performed using indicating HPLC methods.

Drugs were separated into two sets as their appearance in the different release layers of the proposed three-layered tablet. This includes the mixture of metronidazole and tetracycline HCl in one layer in the form of a monolithic tablet, and famotidine and CBS in another layer. The solid-state stability study of the four actives was conducted on the granules rather than the tablets. Test was performed over 3 months under the accelerated condition ( $40^\circ\text{C}$ , 75%RH). The stability

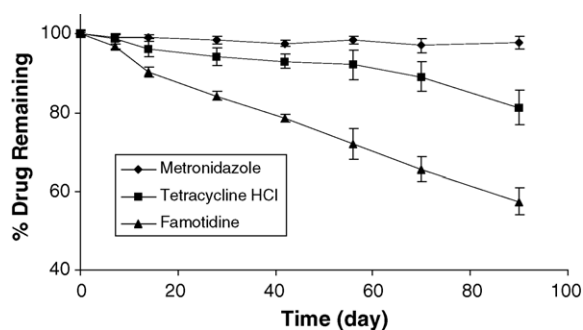


Fig. 3. Stability studies of metronidazole, tetracycline HCl, and famotidine in solid state under accelerated conditions (40 °C, 75%RH) over 90 days ( $n=3$ ).

profiles of metronidazole, tetracycline HCl, and famotidine are presented in Fig. 3.

From the results it appears that metronidazole displayed good stabilities in solid state under the accelerated conditions (40 °C, 75%RH) over 3 months.

Tetracycline HCl was sufficiently stable under normal storage conditions but under accelerated study underwent slow degradation over the 3 months period of studies. Results further indicated that high temperature and humidity both facilitate tetracycline HCl degradation.

The granules containing tetracycline HCl gradually changed color from light yellow to brown and dark brown after exposure to the humidity and heat over 20–30 days. Results also indicated that light, which is the well-known factor responsible for color transformation of tetracycline HCl, is not the only factor to cause color changes.

Under the given accelerated conditions, a relatively rapid degradation was also observed in the samples containing famotidine. The rate of degradation profile for famotidine demonstrated that famotidine was the least stable drug among the tested active ingredients when subjected to accelerated storage conditions.

It is widely accepted that most drug degradations of drug substances follow the first-order kinetics. However, determination of the exact degradation kinetics in a complex nature of this formulation becomes difficult to estimate although not impossible (Martin, 1993).

By analyzing the kinetics of degradation, tetracycline HCl and metronidazole appear to follow the pseudo-first-order degradation. Metronidazole, as shown in Fig. 3, demonstrated negligible degradation

Table 3

Degradation rate constant, % remaining after 7 days and half-life for drugs in solid state under stress condition

Sample	$k$ (per day)	% Remaining after 7 days	$t_{1/2}$ (day)
Metronidazole	0.0003	98.98 ± 0.76	2310
Tetracycline HCl	0.0020	98.85 ± 1.33	346
Famotidine	0.0061	96.92 ± 0.61	114

and in accordance with zero-order or pseudo-first-order kinetics with very slow degradation rate constant (see Table 3). Accordingly, the degradation rate constant for each drug was calculated and the degradation half-life was calculated according to the pseudo-first-order kinetics.

### 3.3. Liquid state stability and compatibility studies of metronidazole, tetracycline HCl, and famotidine

#### 3.3.1. Stability of metronidazole, tetracycline HCl, and famotidine in aqueous solutions

In liquid phase and acidic condition, metronidazole demonstrated to be relatively stable, although a very slight decrease in metronidazole was observed. However, no new peaks were presented in the chromatograms to indicate the possible degradation by products. This finding was consistent with other published HPLC and MS studies which demonstrated slight decomposition of low molecular weight compounds in the presence of acid (Bakshi and Singh, 2003).

The stability profiles as a function of time are shown in Fig. 4. Overall, the degradation of metronidazole is slow and negligible and there is no significant difference under different conditions over 15 days. Results, indicate that metronidazole demonstrated excellent stability in aqueous solutions.

The process of degradation of tetracycline HCl at pH 2.0, 25 °C, and light conditions in the first 7 days however were apparent. The degradation profiles were significantly different in the samples under different storage conditions as shown in Fig. 5 and formation of epitetracycline HCl from tetracycline HCl is shown in Fig. 6.

It has been reported that small amounts of epitetracycline and anhydrotetracycline could be found in new tetracycline preparations and that the formation of these degradants can be retarded but cannot be eliminated even when stored under ideal conditions (Ali,

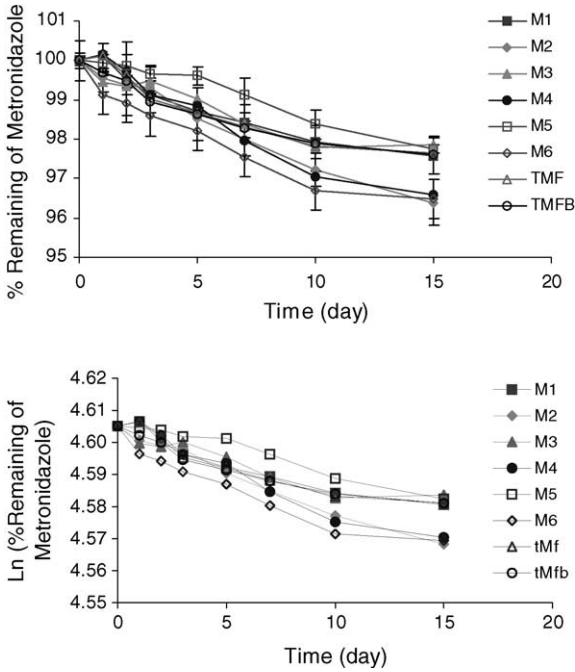


Fig. 4. Stability of metronidazole in liquid state under various storage conditions over time. Upper and lower plots represent the data in normal and natural logarithm scales, respectively. Refer to Table 2 for specific storage conditions of different samples.

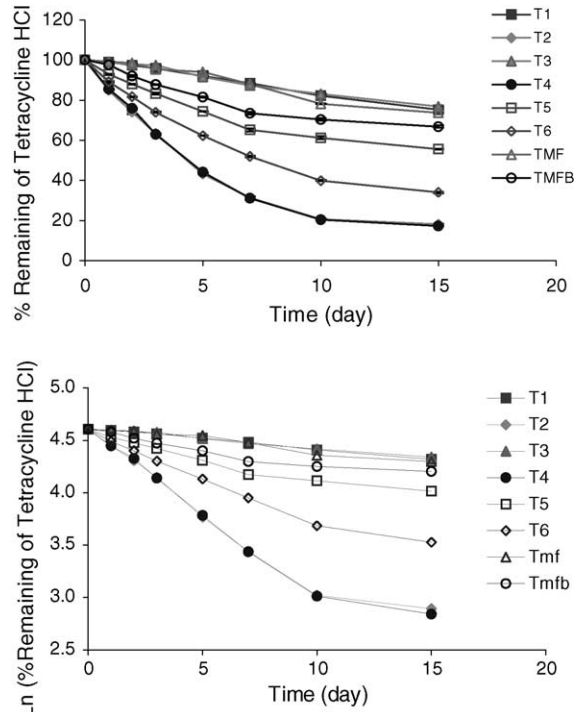


Fig. 5. Stability of tetracycline HCl in liquid state under various storage conditions over time. Upper and lower plots represent the data in normal and natural logarithm scales, respectively. Refer to Table 2 for specific storage conditions of different samples.

1976). Our initial HPLC analysis demonstrated that the standard samples of tetracycline HCl contains more than 98.0% of tetracycline HCl and less than 1.64% of epitetracycline HCl.

It was observed that epitetracycline HCl in T1, T3, TMF, and TMFB was formed and increased with time in a zero-order manner (Fig. 6). The parabolic profiles were also observed for treatments T2, T4, T5, and T6, which exhibited a rapid increase, followed with a flat (T2, T4, T5) or slow descent (T6). It is apparent that the elevated temperature and pH, regardless of light or dark condition, initially increased the formation of epitetracycline HCl. At the same time it is possible that the formed degradation compound might be transformed back to its parent drug or may further degrade into other compounds resulting in the profile descent.

Famotidine was the least stable compound in this drug combination system. Degradation chromatograms of famotidine demonstrated that famotidine stored in the aqueous solution at pH 2.0, 25 °C and under light conditions is extremely unstable.

The stability profiles of famotidine versus time in aqueous solution under various conditions are presented in Fig. 7. Figures indicate that the stability of famotidine is strongly dependent on the changes of temperature and pH in aqueous solutions and appears

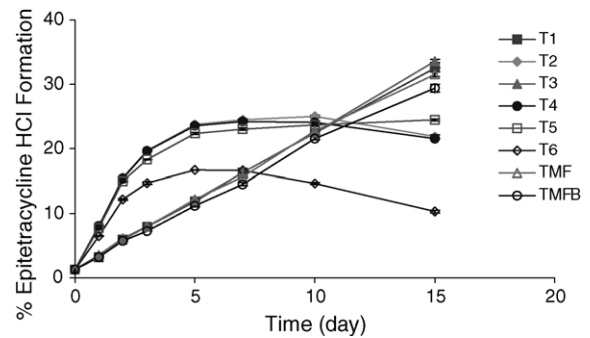


Fig. 6. Epitetracycline HCl formation from tetracycline HCl in liquid state under various storage conditions over a period of time ( $n = 3$ ). Refer to Table 2 for specific storage conditions of different samples.

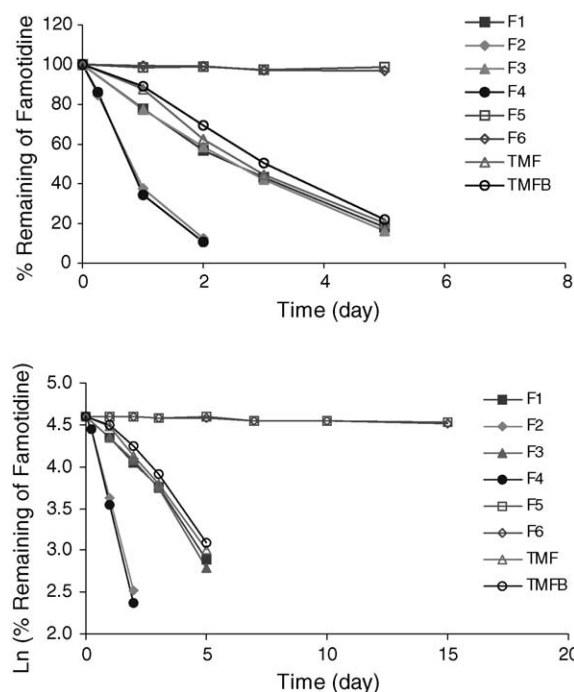


Fig. 7. Stability of famotidine in liquid state under various storage conditions over time. Upper and lower plots represent the data in normal and natural logarithm scales, respectively. Refer to Table 2 for specific storage conditions of different samples.

to be independent of exposure to light. The presence of other drugs in the study did not influence on the stability of famotidine and overall result show good chemical compatibilities.

### 3.3.2. Effects of light and temperature on stability

The combined effects of light and temperature on the drug stability were investigated on samples stored in buffer at pH 2.0. The plots of percentage remaining of metronidazole, tetracycline HCl, and famotidine as a function of storage time are shown in Fig. 8.

It was noticed that the degradation in the samples containing the different drugs was independent of light, but was accelerated by the temperature increase. For example, similar profiles were obtained in T1 (25 °C, light) and T3 (25 °C, dark), and T2 (37 °C, light) and T4 (37 °C, dark), respectively when stored at same temperature regardless of exposure to the light. However, the increased storage temperature in T2 and T4 resulted in a faster degradation compared to the samples T1 and T3 that were stored at lower temperature.

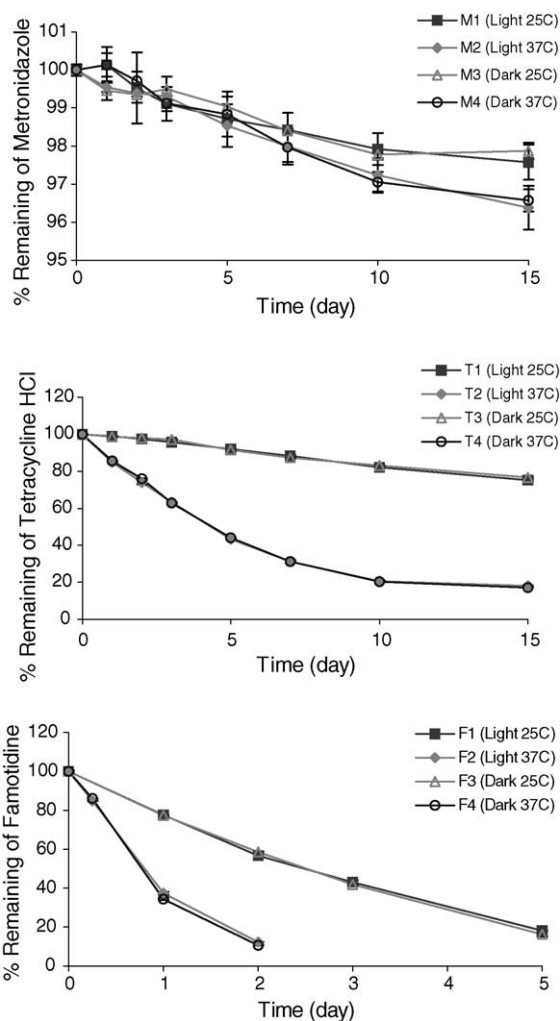


Fig. 8. Effects of light and temperature on stability of metronidazole (top), tetracycline HCl (middle), and famotidine (bottom) ( $n=3$ ). Refer to Table 2 for specific storage conditions of different samples.

All the tested drugs except metronidazole, underwent different degrees of degradation when subjected to thermal stress. In all the samples tested, there was no evidence of light-induced drug decomposition.

The results obtained for metronidazole in this study were in conflict with reported literature (Bakshi and Singh, 2003), where it is stated that metronidazole undergoes photolysis under photolytic conditions.

Based on the results presented here it is evident that none of the drugs tested show sensitivity to light, how-



ever, generally it is prudent to protect drugs against excessive light exposure.

3.3.3. Effect of pH on stability of drugs in the liquid state

The percentages remaining of metronidazole, tetracycline HCl, and famotidine versus the storage time are plotted and shown in Fig. 9.

It is apparent that pH value of the aqueous solution was the critical factor on the stability of all three of the drugs. Overall, metronidazole was relatively stable under all the pH conditions. Only small variations be-

tween the profiles were observed. Specifically, pH 4.0 provided the most stable condition, while slight acceleration of the degradation was noticed in either pH 2.0 or 6.0 conditions, which is in agreement with published report (Erah et al., 1997).

The results provide assurance that metronidazole will remain stable during the dissolution studies under all conditions.

The degradation profiles of tetracycline HCl demonstrated that the stability of tetracycline HCl was strongly dependent on the pH of the solution. Degrada-

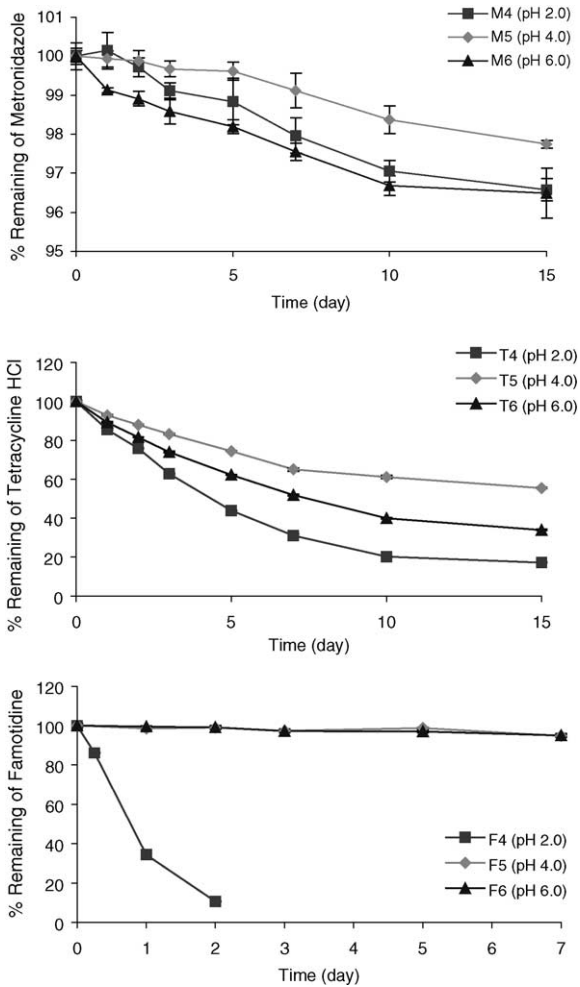


Fig. 9. Effects of pH on stability of tetracycline HCl (top), metronidazole (middle), and famotidine (bottom) ( $n = 3$ ). Refer to Table 2 for specific storage conditions of different samples.

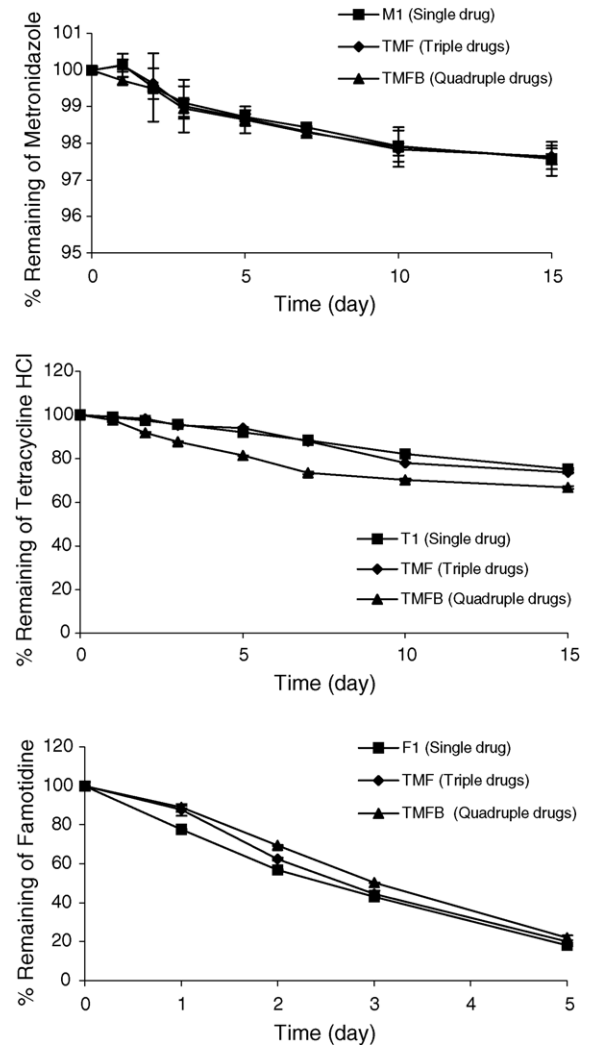


Fig. 10. Effects of drug combination on stability of tetracycline HCl (top), metronidazole (middle), and famotidine (bottom) ( $n = 3$ ). Refer to Table 2 for specific storage conditions of different samples.

dation profiles from different pHs exhibited significant differences in their degradation kinetics. In all the samples, the most rapid degradation occurred at pH 2.0, and followed by the sample stored at pH 6.0. The sample that retarded the process of degradation was achieved at pH 4.0. These observations are consistent with pH solubility profile of tetracycline HCl which behaves similar to amphoteric substances.

Therefore, both metronidazole and tetracycline HCl demonstrated their greatest stability around pH 4.0. It should be noted that in an in vivo situation the reduced acidity of the environment created by famotidine would enhance tetracycline stability and efficacy against *H. pylori* (Malferteiner, 1996; Balaban and Peura, 1997; Howden, 1997; Seppala et al., 2000; Dore et al., 2002). Hence, the absorption of famotidine may increase gastric pH to or greater than 4.0 which could be conducive to greater efficacy of drugs delivered locally.

### 3.3.4. Effect of drug combination on stability in the liquid state

The effect of drug combination on drug stability was investigated on samples stored at 25 °C and under light conditions. The plots of percentage remaining of metronidazole, tetracycline HCl, and famotidine combination during storage time are given in Fig. 10.

The results showed that mixture of metronidazole and tetracycline HCl would not cause incompatibility.

Tetracycline tends to form a complex (chelate) with many metal ions and this formation will interfere with drug absorption (Ali, 1976; Mayersohn, 1996; Corral, 1997; Yao and Moellering, 1999). Accordingly, a major concern is the possible complexation between bismuth salt and tetracycline, which may result in a decrease in availability of free tetracycline for both local effect as well as absorption.

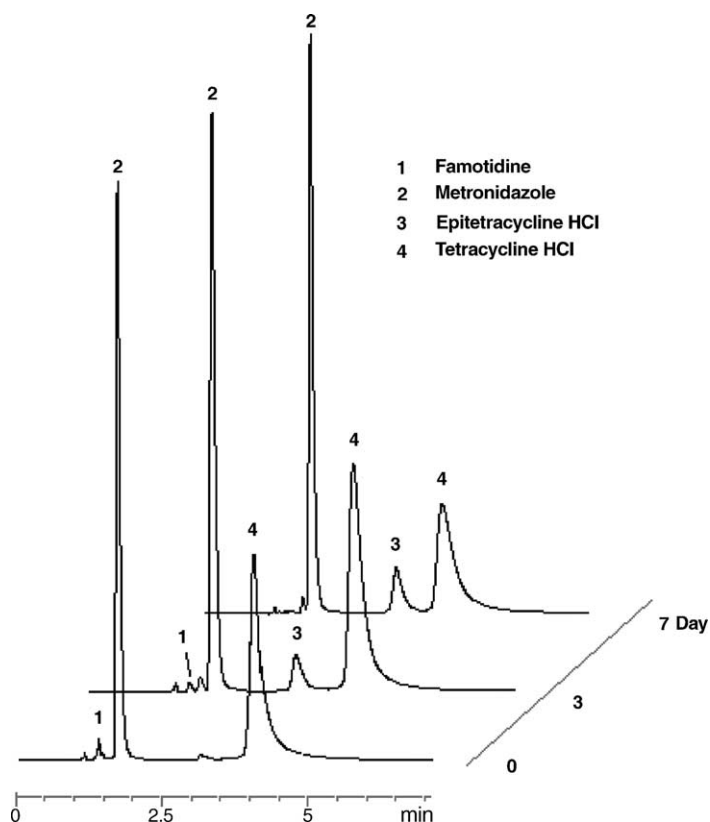


Fig. 11. Chromatograms of sample (TMFB) stored at pH 2.0, 25 °C and light conditions after 0, 3, and 7 days, respectively. Note the disappearance of peak 1 after 7 days and changes in peaks 3 and 4 on days 3 and 7.

Recent report (Healy et al., 1997) investigating the possible interaction between tetracycline and bismuth subsalicylate along with metronidazole used for the treatment of *Helicobacter pylori* infections provides valuable information. Both in vitro and in vivo data indicated that the bismuth salt was not responsible for the observed decrease of tetracycline bioavailability by forming a complex with tetracycline as previously postulated.

In the liquid state stability study, an apparent decrease of tetracycline HCl in combination with other drugs including CBS (TMFB) was observed when compared with drug samples alone (T1) or when combined with drugs without the bismuth salt (TMB). The decrease was insignificant initially, however, it increased significantly with the passage of time. It is assumed that tetracycline HCl might be physically adsorbed to the CBS as its extremely large surface area with a delayed equilibrium, as there was no apparent decrease of tetracycline HCl during the first day, this kind of complex formation will not impact drug release during dissolution studies from the proposed three-layered tablet.

The chromatograms of the samples containing four drugs (TMFB) are shown in Fig. 11. Figure clearly demonstrates that metronidazole is stable, however, famotidine and tetracycline HCl show changes in shape and size of the chromatograms. Both tetracycline and epitetracycline peaks change while famotidine peak disappears after 7 days with formation of a new peak close to that of metronidazole (see Fig. 11, data for day 3 and 7).

The degradation profiles for the sample containing the combination of the drugs did not show a significant difference with the one containing the drug alone. Tetracycline HCl exhibited a small decrease after contact with CBS after 1 day which does not constitute a major problem in this work. Accordingly based on the above drug incompatibility and degradation profiles, it is evident that during dosage form development and release evaluation drugs remain stable and compatible in actual formulation design and delivery system.

### 3.3.5. The kinetic of the drug degradation

By comparing the regression coefficient values ( $R$ ) of degradation profiles plotted in logarithmic scale (pseudo-first-order kinetics) with the ones in normal scale (zero-order kinetics) the degradation

Table 4

Degradation rate constants, % remaining after 1 day, and half-life for drugs in liquid state under various storage conditions

Sample	$k$ (per day)	% Remaining after 1 day	$t_{1/2}$ (day)
T1	0.0197	98.95 ± 0.17	35.18
T2	0.1241	84.94 ± 0.12	5.58
T3	0.0187	98.82 ± 0.29	37.06
T4	0.1275	85.52 ± 0.27	5.44
T5	0.0403	92.82 ± 0.39	17.20
T6	0.0749	89.17 ± 0.44	9.25
Tmf	0.0097	99.19 ± 0.23	30.94
Tmfb	0.0125	97.68 ± 0.31	24.06
M1	0.0018	100.13 ± 0.32	385.00
M2	0.0025	99.55 ± 0.12	277.20
M3	0.0015	99.46 ± 0.25	462.00
M4	0.0026	100.14 ± 0.47	266.54
M5	0.0016	99.93 ± 0.21	433.13
M6	0.0023	99.13 ± 0.06	301.30
tMf	0.0018	100.13 ± 0.17	385.00
tMfb	0.0016	99.71 ± 0.10	433.13
F1	0.3410	77.67 ± 0.32	2.03
F2	1.0642	37.46 ± 0.63	0.65
F3	0.3625	77.37 ± 0.53	1.91
F4	1.1450	34.43 ± 0.85	0.61
F5	0.0045	98.58 ± 0.76	154.00
F6	0.0059	99.50 ± 0.35	117.46
tmF	0.3305	87.72 ± 3.01	2.10
tmFb	0.3092	89.12 ± 0.65	2.24

profiles and comparing the regression coefficient values ( $R$ ) among pseudo-first-order and zero-order kinetics, it is apparent that almost all the degradation profiles followed the pseudo-first-order kinetics. Consequently, the degradation rate constants, percentage of drug remaining after 1 day, and half-lives of the drugs in different samples were calculated as shown in Table 4.

## 4. Conclusions

The results from the solubility study indicate that both metronidazole and tetracycline HCl showed good solubilities at low pH. Therefore it should be fairly easy to combine these drugs together into a core layer of the three-layered tablet that will release these two antibiotics simultaneously in the acidic environment of the stomach.

The results of the stability studies in solid state indicated that metronidazole and tetracycline HCl were

stable in the dry state when stored at room temperature regardless of exposure to light or humidity in the range of 20–65%. Under accelerated conditions, the elevated temperature and humidity were only responsible for the instabilities of tetracycline HCl and famotidine to different extents. No potential incompatibility between the drugs in granular forms was noted under the various storage conditions.

Degradation rate of drug stored in liquid phase was particularly by hydrolysis. Metronidazole is relatively stable material, with insignificant differences observed between the degradation profiles of various samples tested. Elevated temperature accelerated the degradation of all the drugs, however, light exposure was not a factor for the degradation. The degradation processes of tetracycline HCl and famotidine were highly dependent on the pH of the solution, although relatively stable profiles for the drugs were achieved at pH 4.0. In general in solid state and well-protected storage conditions combinations of metronidazole and tetracycline HCl, famotidine along with bismuth subcitrate does not present any major problem in the development of a new multi-drug delivery system.

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