A new intragastric delivery system for the treatment of 
*Helicobacter pylori* associated gastric ulcer: *in vitro* evaluation

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Abstract

A new strategy is proposed for the triple drug treatment (tetracycline, metronidazole and bismuth salt) of *Helicobacter pylori* associated peptic ulcers. The design of the delivery system was based on the swellable asymmetric triple layer tablet approach, with floating feature in order to prolong the gastric retention time of the delivery system. Hydroxypropylmethylcellulose and poly(ethylene oxide) were the major rate-controlling polymeric excipients. Tetracycline and metronidazole were incorporated into the core layer of the triple-layer matrix for controlled delivery, while bismuth salt could be included in one of the outer layers for instant release. The concentration of tetracycline and metronidazole released over time was determined simultaneously on a gradient high-performance liquid chromatography system. Results demonstrated that sustained delivery of tetracycline and metronidazole over 6–8 h can be easily achieved while the tablet remained afloat. The floating aspect was envisaged to extend the gastric retention time of the designed system to maintain effective localized concentration of tetracycline and metronidazole. Additionally, the developed HPLC method for the concurrent determination of tetracycline and metronidazole was proved to be rapid and accurate. The developed delivery system has potential to increase the efficacy of the therapy and improve patient compliance. © 1999 Elsevier Science B.V. All rights reserved.

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1. Introduction

It has become apparent that consumption of nonsteroidal anti-inflammatory drugs (NSAIDs) and stomach colonization by *Helicobacter pylori* (*H.* *pylori*) are the two most common causes of peptic ulcer disease [1–5]. In the United States, peptic ulcer disease affects 10% of population at some point in their lives [6]. The prevention and management of NSAID related gastrointestinal (GI) complications are well recognized and in many cases successfully treated [1]. However, the understanding and treatment of *H. pylori*-induced ulcers are still in progress. During the early 1980s, Marshall and Warren [7] for the first time isolated a spiral, urease-producing, flagellate gram-negative bacterium which was later

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identified as *Helicobacter pylori*, a causative factor in the etiology of peptic ulcer disease. *H. pylori* appears to be responsible for 95% of the cases of gastritis and 65% of gastric ulcers [8]. Although most individuals with *H. pylori* are asymptomatic, there is now convincing evidence that this bacterium is the major etiologic factor in chronic dyspepsia, *H. pylori*-positive duodenal and gastric ulcers and gastric malignancy [9,10]. Consequently, *H. pylori* eradication is now recognized to be the correct approach along with conventional therapies in the treatment of the disease. Options that have been considered to treat peptic ulcer disease include taking drugs such as antacids, H2-blockers, antimuscarinics, proton pump inhibitors and combination therapy for gastritis associated with *H. pylori*.

The eradication of *H. pylori* is limited by its unique characteristics. Once acquired, it penetrates the gastric mucus layer and fixes itself to various phospholipids and glycolipids on the epithelial surface, including phosphatidylyethanolamine [11], GM3 ganglioside [12] and Lewisb antigen [13]. Therefore, the organism exclusively resides on the luminal surface of the gastric mucosa under the mucus gel layer in the acidic environment of the stomach (see the schematics, Fig. 1). The organism is catalase positive, oxidative positive and urease positive. As a result, urea is broken down into bicarbonate and ammonia, which protects the bacterium in the acid milieu of stomach [15] and causes gastric epithelial injury [16]. For effective *H. pylori* eradication, therapeutic agents have to penetrate the gastric mucus layer to disrupt and inhibit the mechanism of colonization. This requires targeted drug delivery within the stomach environment. Although most antibiotics have very low in-vitro minimum inhibitory concentrations (MIC) against *H. pylori* (MIC90 ≤ 1 mg/L) [17], no single antibiotics has been able to eradicate this organism effectively. Currently, a drug combination namely “triple therapy” with bismuth salt, metronidazole and either tetracycline or amoxycillin with healing rates of up to 94% has been successfully used [3,18,19]. The principle of triple therapy is to attack *H. pylori* luminally as well as systemically. The current treatment is based on frequent administration (4 times daily) of individual dosage forms of bismuth, tetracycline and metronidazole (Helidac Therapy, consisting of 262.4 mg bismuth subsalicylate, 500 mg tetracycline and 250 mg metronidazole). The associated limitations are the complex dosing regimen/frequency, large amount of dosage forms and reduced patient compliance. Therefore, a successful therapy not only includes the selection of the right drugs but also the timing and frequency as well as the formulation of the delivery system.

The objective of this study is to develop a new floatable drug delivery system for controlled delivery of drugs commonly referred to as “standard triple therapy”. The bismuth salt will rapidly dissolve while sustained delivery of both tetracycline and metronidazole will follow. The floating feature is incorporated for possible prolongation of the gastric retention time of the delivery system [20–23], thus increasing localized concentration and effects of the antibiotics. Schematics of the system design and a brief description of its associated macroscopic changes during drug release are presented in Fig. 2.

2. Materials and methods

2.1. Materials

Poly(ethylene oxides) (PEO, polyox®-WSR) with average molecular weight of 1×10⁶ and 7×10⁵ were
ronidazole, individually and in combination is given in Table 1. All ingredients were passed through a #20 US standard sieve, the particulate mixture for each layer was blended in a cube-mixer for fifteen min. 0.1% magnesium stearate was added and mixed for additional five min. The tablets were produced using a Carver laboratory press (Model C, Fred S Carver Inc., Wabash, IN) with 10 mm diameter flat faced tooling. The powder mix of each layer was transferred into the die manually, the first and second layer were compressed up to 900 lbs and finally after addition of the third layer the total die content was compressed to 5000 lbs. The full compression cycle of one minute was used in each case. In the formulation I (Table 1) in the rapidly dissolving layer bismuth salt was replaced with an equivalent amount of lactose to simplify analytical measurements. The lactose or bismuth salt containing layer disintegrated within 10–15 min.

2.3. In-vitro buoyancy lag time determination and floating capacity optimization

The gas-generating layer consisted of 120 mg poly(ethylene oxide) Polyox® WSR-303 (MW=7×10^5), 20 mg HPMC K4M, and a mixture of sodium bicarbonate: calcium carbonate (1:2 ratio). To determine and optimize the floating lag time and buoyancy duration of the delivery system, 20, 30, 40, 50 and 60 mg gas-generating salt mixtures were

Table 1

<table>
<thead>
<tr>
<th>Layer</th>
<th>Function</th>
<th>Total weight (mg)</th>
<th>Components</th>
<th>Formulation (% w/w)</th>
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| 1     | Swellable gas generating layer | 200    | PEO MW=7×10^5 | 60 | 60 | 60 |
|       |                                   |        | HPMC K4M      | 10 | 10 | 10 |
|       |                                   |        | Calcium carbonate | 20 | 20 | 20 |
|       |                                   |        | Sodium bicarbonate | 10 | 10 | 10 |
| 2     | Swellable/sustainable drug(s) containing layer | 500 | PEO MW=1×10^5 | 15 | 15 | 15 |
|       |                                   |        | Tetracycline hydrochloride | 50 | 50 | 0 |
|       |                                   |        | Metronidazole   | 25 | 0  | 25 |
|       |                                   |        | Lactose         | 10 | 35 | 60 |
| 3     | Rapidly dissolving drug layer | 100    | Lactose         | 38 | 78 | 78 |
|       |                                   |        | Bismuth salts   | 60 | 60 | 0  |
|       |                                   |        | Ac-Di-Sol       | 2  | 2  | 2  |
|       |                                   |        | PEO MW=1×10^5  | 0  | 20 | 20 |

1% magnesium stearate was used as lubricant in each layer.

The solubility of tetracycline and metronidazole in 0.1 M HCl (pH=1.8) solution at 37°C was 99.7 mg/ml and 24.3 mg/ml respectively.

Bismuth salts was replaced with lactose for simplicity of analysis.
incorporated into the gas-generating layer while the weight of other formulation components were kept constant. The total weight of the entire tablet was maintained around 800 mg. The buoyancy lag time and the duration of buoyancy were determined in the USP 23 dissolution apparatus II in an acid environment as is outlined in the drug release section. The time interval between the introduction of the tablet into the dissolution medium and its buoyancy to the top of dissolution medium was taken as buoyancy lag time and the duration of system floatation was observed visually.

2.4. Individual drug release measurements

The in-vitro release studies were conducted in accordance with USP 23 apparatus II procedure (VK 7000, Vankel Industries, Inc., Edison, NJ) at 37°C in 900 ml 0.1 M HCl solution (pH=1.8). The paddle speed was 50 rotation per minute. As part of the formulation development the initial formulation contained either tetracycline or metronidazole (i.e., formulations II and III). The amount of tetracycline or metronidazole released from their corresponding formulations was independently measured on a HP8451A photo-diode array spectrophotometer at 356 and 278 nm respectively.

2.5. Chromatographic analysis of tetracycline and metronidazole mixture

The concomitant release of tetracycline and metronidazole was determined on a high-performance liquid chromatography method developed in this study. Samples (2 ml) were withdrawn at 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5 h during the dissolution process and kept frozen until analysis. 0.1 M HCl solution (2 ml) was added to the dissolution vessel after each sampling period to maintain constant volume.

A Waters gradient HPLC system (model 600E) with a tunable UV detector (model 486) was used. The column was a Nova-Pac C-18 (3.9×150 mm, 4 micron Waters, Milford, MA). The mobile phase consisted of a mixture of 0.2 M ammonium oxalate, diethylformamide, and 0.1 M EDTA. The pH was adjusted to 6.2 with a 0.4 M tetrabutylammonium hydroxide. All experiments were run under isocratic conditions at a flow-rate of 1.0 ml/min. The absorption wavelength of the detector was set at 280 nm. Five standard solutions were prepared from the original stock solution containing 18.1 mg metronidazole and 36.2 mg tetracycline per 100 ml of 0.1 M HCl solution (pH=1.8). A volume of 20 μl standard solution was injected for the HPLC analysis. Each sample was analyzed in four replicates to obtain the statistical data. 20 μl aliquot of sample solution from dissolution studies was directly injected into the HPLC. A typical chromatogram of the sample is shown in Fig. 3.

3. Results and discussion

3.1. In-vitro buoyancy lag time determination and floating capacity optimization

It has been reported that floating delivery systems can prolong the gastric retention time and thus increase the overall drug bioavailability for certain drugs [20–23]. In this work, for greater localized effect of tetracycline and metronidazole, the floating strategy was taken into consideration in the design of delivery system. The floatation was accomplished by incorporating gas-generating salts such as sodium bicarbonate and calcium carbonate into a swellable hydrophilic layer (i.e., gas generating layer, see Fig. 2). The overall makeup of this particular matrix is of swellable hydrophilic polymers. As the dissolution medium was imbibed into the matrix, the interaction
of acidic fluid with sodium bicarbonate/calcium carbonate resulted in the formation and entrapment of carbon dioxide gas within the swollen gel thus causing floatation as the matrix volume expanded and its density decreased. It was observed that all the tablets ascended to the upper one third of the dissolution vessels within a short time, and remained floated until the completion of release studies. The relationships between the amount of gas-generating salt blends and the buoyancy lag time as well as the duration of system buoyancy are shown in Fig. 4, panels a and b. It is observed that the buoyancy lag time for this system is in the range of 17–28 min and no floatation was achieved below the minimum gas-generating quantity of 20 mg within 60 min. Also the system was afloat over the entire dissolution period in each case. The result implied that the thickness of gas-generating layer can vary over a wide range and as a result the desired release duration can be easily achieved. Since larger tablets might have the potential to stay in stomach for a longer time period [23], in this work maximum amount of gas-generating mixture was incorporated into the gas-generating layer in the formulation as shown in Table 1.

3.2. Experimental optimization of matrix formulations for controlled delivery of tetracycline and metronidazole

The principle of the triple-layer configuration delivery system has been discussed previously [24]. It consisted of a drug-containing layer sandwiched with two different barrier layers. The barrier layers were also designed to erode away at variable rates. When the matrix system was exposed to dissolution medium, the rapidly dissolving layer goes into the solution and the remaining layers start swelling as a result of fluid ingress. Due to the gradual and continuous generation of gas in the first layer the matrix gradually floated [25]. At the same time drug release rate was controlled by polymer swelling, matrix erosion, drug diffusion and the gel thickness dynamics.

Individual triple-layer tablets of tetracycline and metronidazole were designed (formulation II and III in Table 1) to float and provide linear and complete drug release within 8 h. The fraction and amount of tetracycline and metronidazole released from corresponding triple-layer tablets are presented in Fig. 5, panels a and b. Both drugs were able to be delivered at constant rates up to 90% of the total loading dose.

3.3. Design and in-vitro evaluation of delivery system for the controlled and concomitant release of tetracycline and metronidazole

Based on the results from individual asymmetric triple-layer tablets for tetracycline and metronidazole, a triple-layer delivery system containing both drugs was developed (formulation I in Table 1). Its schematics as well as the actual matrix photo-
and $K_2$ are constants denoting the significance of diffusion-controlled and Case-II transport contribution respectively. A nonlinear regression analysis of release data fitted to Eq. (2) was performed (Sigma-plo-t for Windows, version 4.0, Jandel Scientific, California), and the calculated $K_1$ and $K_2$ values were $0.2836$ and $0.0398$ ($K_1/K_2 = 7.1$) for tetracycline, and $0.2623$ and $0.0413$ ($K_1/K_2 = 6.4$) for metronidazole. These values indicated that both diffusion and polymer relaxation-erosion were taking place. Overall, the diffusion mechanism was the dominant process of drug release.

Further inspection of Fig. 7 reveals that the concomitant release of tetracycline and metronidazole exhibited biphasic patterns. The initial release of both drugs overlapped (i.e., the first 2 h), followed by a parallel and sustained delivery for the remainder of the dissolution period. The disappearance of the rapidly dissolving layer together with the high solubility of tetracycline (99.7 mg/ml in 0.1 M HCl solution at 37°C) and metronidazole (24.3 mg/ml in 0.1 M HCl solution at 37°C) are the major factors contributing to the initial phase of release. This is due to the matrix swelling and rapid drug diffusion. On the other hand, the second phase of release in addition to drug solubility and formation of new surfaces can be attributed to the polymer swelling/erosion characteristics as the disentanglement threshold is crossed. Dissolution of hydrophilic
polymers generally occurs via two distinctive processes, swelling and subsequent erosion (i.e., chain disentanglement) at the dissolution front. Transition from glassy to rubbery phase occurs as a result of water ingress, leading to the formation of the polymer gel. The polymer dissolution occurs once the swollen polymeric chains reach a disentanglement threshold value [27,28] at the periphery of gel layer. Therefore, drug release from swellable polymeric systems in the late-time period is predominantly governed by polymer relaxation and erosion. This is consistent with the data presented in Fig. 8. Therefore, the biphasic release pattern observed in Fig. 7 may be attributed to this phenomenon. In addition, the incorporation of soluble drugs and excipients as expected will enhance water uptake and the degree of polymer erosion.

4. Conclusion

This study has demonstrated that a triple-layer technology can be rationally designed for instant drug release as well as sustained delivery of tetracycline and metronidazole over 6–8 h. The floating feature could possibly prolong the gastric retention time of this system to maintain high localized concentration of tetracycline and metronidazole. This aspect requires further work. The developed delivery system has a potential to increase the efficacy of the therapy for *H. pylori* associated ulcers and to improve patient compliance.

References