Formulation and evaluation of mastic gum as a compression coat for colonic delivery of 5-fluorouracil
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Abstract
Mastic gum has been reported to possess considerable anti-tumor activity against human colorectal cancer. The purpose of this work was to evaluate mastic gum in formulation of colon-specific 5-fluorouracil delivery system for effective treatment of colorectal cancer. Compression coated tablets, containing 5-fluorouracil in the core tablet coated with 200 mg of different coating materials containing various proportions of mastic gum were evaluated for their 5-fluorouracil in vitro release. The results indicated that the concentrations of mastic gum, sodium chloride as well as hydroxypropyl methyl cellulose (HPMC) in the coating materials significantly modify the drug release. The coating material (F6) consisted of 60% mastic gum, 15% sodium chloride and 25% HPMC is considered as a promising formula for achieving colon targeting of 5-fluorouracil. Further, gamma-scintigraphic studies were carried out in healthy male volunteers to evaluate in vivo release of F6. The results showed that tablets remained intact in stomach and small intestine, however partial and complete release of the tracer occurred in the colon. The in-vitro antitumor activity of 5-fluorouracil-mastic gum combination mixed in a ratio representing their concentrations in tablets coated with F6 was carried out against colon cancer cell line using MTT assay. The results revealed that 5-fluorouracil-mastic gum mixture was more effective in arresting cell growth in comparison to that shown by 5-fluorouracil or mastic alone. In conclusion, this new colonic drug delivery system is potentially useful for 5-fluorouracil colon targeting. However, clinical benefits of using mastic in formulation of 5-fluorouracil colonic tablets need further evaluation.

Keywords: Mastic gum, 5-fluorouracil, Compression coating, Colonic delivery.

Introduction
Colorectal cancer is the third most common cancer in the world and the second most common cause of cancer related deaths [1]. A major drawbacks of conventional chemotherapy used in treatment of colorectal cancer is that the drug does not reach the target site in effective concentration [2, 3]. Drug targeting to colon is highly desirable in treatment of colon cancer. The major advantages drug targeting to colon include reduced systemic side effects and higher doses of the applicable medication at the site of drug action.
The different approaches for targeting orally administered drugs to the colon include covalent linkage of a drug with a carrier, coating with pH-sensitive polymers, time-dependent release systems, and enzymatically controlled delivery systems [4-6]. Different natural materials have been used in development of colonic drug delivery systems.

Mastic is a natural oleoresin exudate obtained from the stem and main leaves of a cultivated variety of Pistacia lentiscus var [7]. It has been used extensively for centuries in Mediterranean and Middle Eastern countries both as a dietary supplement and as an herbal remedy. Medical trials have shown that gum mastic may have cytoprotective or antacid effects for the gastrointestinal system, such as relief of ulcers and reducing the intensity of gastric mucosal damage caused by anti-ulcer drugs and aspirin, with little or no side effects [8-10]. Recently, mastic gum was found to possess anti-tumor activity against human colorectal cancer [11, 12]. A major constituent of mastic gum, namely oleanolic acid, is among the best-known triterpenes with biological properties against chemically induced liver injury in laboratory animals, exerting anti-inflammatory and antitumor-promotion effects [13].

Mastic gum has been used as binder in tablets formulation [14, 15]. Also, many authors combined mastic with microcapsule cores in microencapsulation of many substances [16-18]. The previous studies have proved that mastic results in larger/compact particles with no pores and a much slower release; and consequently controlled drug release. However, there is no report regarding the use of mastic gum in design of colonic drug delivery system using compression coating process.

5-fluorouracil is an anticancer agent which is widely used for treatment of colorectal cancer [19]. Many colon specific delivery systems and their expected influences on 5-fluorouracil anticancer activity in treatment of colorectal cancer have been reported by many authors [20-25]. In the present study, mastic gum, in the form of compression coat applied over 5-fluorouracil core tablets, was in vitro and in vivo evaluated as a colonic drug delivery system to maximize the therapeutic outcomes of 5-fluorouracil in treatment of colorectal cancer.

Materials and Methods

Materials

Mastic gum (commercial grade) was from Chios' Gum Mastic Growers Association, 5-fluorouracil and magnesium stearate were purchased from Sigma-Aldrich Chemical Company (Milwaukee, WI, USA). Sodium chloride (B.P 98) was purchased from El Nasr Pharmaceutical Chemicals Co., Egypt. Hydroxypropyl methyl cellulose 4000 cp (HPMC) and Avicel PH101 were obtained from Fluka Biochemika Company, Sigma Germany. Diethyleneetriaminepenta acetic acid (DTPA) was obtained from Institute of Isotopes Co. Ltd. Budapest, Hungary. Technetium-99m (99mTc)- DTPA was prepared by radiolabeling DTPA with sodium pertechnetate solution obtained by Elumatic III, Technetium (99mTc) Generator, CIS Bio international, France.

Methods

Physicochemical characterization of mastic gum

Relative solubility

The relative solubility of mastic gum was determined in water, 0.1 N HCl, phosphate buffer (pH 7.4). Two grams of fine powdered mastic gum with 5 ml of each solvent was placed in airtight screwcapped test tubes. The tubes were mounted on a water-bath shaker maintained at 25°C for 24 h. Two ml of supernatant was transferred to a dry porcelain dish and the solvent was evaporated by a mild heat. Increase in the weight of porcelain dish relative to solvent blank gives the amount of material dissolved in the respective solvent. The experiment was repeated three times for each solvent [26].

Swelling index

The swelling index is the volume in ml taken up by the swelling of 1 g of plant material under
specified conditions. The swelling index was determined in water, 0.1 N HCl and phosphate buffer (pH 7.4). An accurately weighed 1 gm of mastic gum powder was introduced into a 25-ml glass-stoppered measuring cylinder of internal diameter 16 mm, and the length of the graduated portion about 125 mm, marked in 0.2-ml divisions from 0 to 25 ml in an upwards direction. Water (25 ml) was added and the mixture was vigorously agitated every 10 min for 1 h and allowed to stand for 3 h at room temperature. The volume in ml occupied by mastic gum was measured and used for calculating swelling index [27].

**Preparation of coating materials**
Mastic particles were pulverized to fine powder by a kitchen mixer and the fine powder were passed through a 450 μm screen. Mastic powder was thoroughly mixed with other coat components in a mortar to prepare seven coating materials. The composition of various coating materials is listed in Table 1. All coating materials were mixed with 1% (w/w) magnesium stearate before compression.

**Formulation of the core tablets**
The core tablets of 5-fluouracil were prepared by direct compression technique. Each core tablet (60 mg) consisted of 25 mg 5-fluouracil, 34.5 mg Avicel PH 101 and 0.5 mg magnesium stearate. The tablets were prepared by using hydraulic press using round flat faced 6 mm punches at constant compression force. The prepared tablets were evaluated for the uniformity of weight and drug content.

**Compression coating of tablets**
The core tablets were compression-coated with 200 mg of each coating materials. About 50% of the coat formulation was placed in the die cavity (diameter 10 mm). The core tablet was then placed in the center of the die cavity, which was filled with the remainder of the coat formulation. Then, it was compressed around the core tablets at constant compression force of 912 kg/cm² using 10 mm flat faced punches. The prepared compression-coated tablets were tested for weight variation, hardness and friability.

**In vitro drug release studies**
5-fluouracil release from the compression coated tablets was assessed by dissolution testing using dissolution apparatus I (Hanson Research, California, USA) at a rotation speed of 50 rpm maintained at 37.0 ± 0.5°C. The dissolution media were 750 ml of 0.1 N HCl (pH 1.2) for the first 2 h and then phosphate buffer pH 7.4 for the next 22 excessive hours. Three milliliter of dissolution medium was withdrawn at 1, 2, 3, 4, 5, 6, 8, 10, 12 and 24 h time intervals and replaced with an equal volume of dissolution media. The collected media was filtered through 0.45 μm membrane, and analyzed for 5-fluouracil contents spectrophotometrically at 265.5 nm.

**In vitro release kinetics mechanisms**
To know the mechanism of drug release from the coated tablets, the obtained in vitro release data were treated according to zero order, first order and Higuchi square root equations [28, 29]. Also, the release data were further analyzed according to Korsmeyer-Pappas model [30] given by the equation

\[ \frac{M_t}{M_\infty} = Kt^n \]

Where \( M_t \) is the amount of drug released at time \( t \), \( M_\infty \) is the amount of drug released at infinite time, \( K \) is the kinetic constant related to the structural and geometric characteristics of the drug delivery system (tablet) and \( n \) is the release exponent indicative of the release mechanism. For cylindrical dosage forms, \( n \leq 0.45 \) corresponds to a Fickian diffusion release (case I, diffusional), \( 0.45 < n \leq 0.89 \) to an anomalous (non-Fickian) transport, \( n = 0.89 \) to a zero-order (case II) release kinetics, and \( n > 0.89 \) to a super Case II transport [31]. To determine the exponent \( n \), only the portion of the release curve where the fractional release of drug is lower than 60% should be taken [32].
Scanning electron microscope
Changes in the surface morphology of compressed tablets before, during and after in vitro release were evaluated by scanning electron microscopy (JEOL JSM – 5500 LV) JEOL Ltd, Japan) by using high vacuum mode. The samples were coated by gold sputter coater (SPI–Module). The pore size distributions and the samples surfaces were investigated by SEM at × 75 magnification.

In vivo release evaluation of the compressed tablets
Gamma scintigraphic imaging is extensively used to evaluate the performance of compression coated tablets for colonic drug delivery systems throughout the gastrointestinal tract of human volunteers [33-35].

Preparation of labeled tablets for in vivo scintigraphic studies:
Each core tablet (average weight 60 mg) for in vivo scintigraphic study consists of sodium chloride (30 mg), Avicel (29.50 mg) and magnesium stearate (0.5 mg). Sodium chloride was dissolved in a solution of 5 millicuri of 99mTc-DTPA, and the obtained solution was evaporated to dryness. The resultant dry powder was then mixed with the remaining excipients and compressed into tablets using 6 mm round, flat punches. The core tablets were then compressed coated with 200 mg of the coating material F6.

In vivo scintigraphic studies
The study was approved by the University protection of human subjects committee, and the protocol complies with the declarations of Helsinki and Tokyo for humans. Four healthy male volunteers participated in this study. After overnight fasting, each volunteer orally swallowed the prepared radiolabeled tablets. The tablets were scanned using a PHILIPS AXIS dual head gamma camera (Phillips Medical System, Cleveland, OH, USA). Anterior and posterior images were taken at regular time intervals (1, 2, 3, 4, 6, 12, and 24 h) after tablet administration.

In vitro evaluation of antitumor activity
Samples were supplied to the Bioassay-Cell Culture Laboratory, National Research Centre, Cairo, Egypt to determine the in vitro antitumor activity of 5-fluorouracil-mastic gum combination comparison with each of 5-fluorouracil and mastic gum. MTT Cell viability assay was carried out with human colon cancer (HCT-116) cell line. This assay measures mitochondrial activity. MTT is a yellow-colored tetrazolium salt that is taken up and cleaved only by metabolically active cells, reducing it to a colored, water-insoluble formazan salt. The solubilized formazan product can be quantified using a 96-well-format spectrophotometer, and the absorbance correlates directly with cell number [42].

Human colon cancer (HCT-116) cells were suspended in RPMI 1640 medium containing 1% antibiotic-antimycotic mixture (10,000U/ml Potassium Penicillin, 10,000μg/ml Streptomycin Sulfate and 25μg/ml Amphotericin B) and 1% L-glutamine in 96-well flat bottom microplate at 37°C under 5% CO2. The medium was aspirated, fresh medium (without serum) was added and cells were incubated either alone or with DMSO alone (control) or with different concentrations of samples to give a final concentration of (12.5, 25, 50 and 100μg/ml) of 5-fluorouracil, mastic gum and a mixture of 5-fluorouracil and mastic gum mixed in a ratio representing their concentrations in the compression coated tablets. After 48 h of incubation, medium was aspirated, 40 μL MTT salt (2.5μg/ml) were added to each well and incubated for further four hours at 37°C under 5% CO2. To stop the reaction and dissolve the formed crystals, 200 μL of 10% Sodium lauryl sulphate in deionized water were added to each well and incubated overnight at 37°C. The absorbance was measured using a microplate reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595nm. DMSO was used as a solvent for dissolution of samples and its final concentration was less than 0.2%. Cell viability was calculated as the percentage of absorbance in wells with the treated cells to that of control cells (cells treated with DMSO only).
A probit analysis was carried for IC$_{50}$ (the concentration that inhibited cell growth by 50%) determination using SPSS 11 program.

**Results and Discussion**

**Solubility and swelling index studies**
Relative solubility study of mastic gum in different solvents indicates that mastic gum is insoluble in water, 0.1 N HCl and phosphate buffer (pH 7.4). The results of swelling index revealed that mastic gum showed no swelling ability up on contact with water, 0.1 N HCl or phosphate buffer (pH 7.4). This may be attributed to the hydrophobic nature of mastic gum which prevents its possible hydration by the swelling medium.

**Physical properties of tablets**
The prepared core tablets complied with the requirements of The United States Pharmacopeia [36] with regard to the uniformity of weight and drug content. The mechanical properties of the compressed coated tablets are list in Table 1. All compression coated tablets showed excellent mechanical properties expressed by high hardness values and minimal % friability.

**In vitro release studies**
Figure 1 shows the release profiles of 5-fluouracil from tablets coated with coating materials F1, F2, F3 and F4. The results indicated that tablets coated with F1 (100% mastic) showed no release of drug and remained intact over the release time. This may be attributed to the hydrophobic nature and the strong binding property of mastic gum that result in formation of highly impermeable coats [18, 37]. To modify the drug release, water soluble pore inducer, sodium chloride [38] was added to the coating materials in 3 different concentrations of 15%, 30% and 45% of the coat weight to form 3 coating materials F2, F3 and F4, respectively.

<table>
<thead>
<tr>
<th>Table 1. Composition of coating materials and mechanical properties of the 5-fluouracil compressed coated tablets.</th>
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<tr>
<td><strong>Composition of coating materials</strong>* (%)</td>
</tr>
<tr>
<td><strong>Code</strong></td>
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<tr>
<td>F1</td>
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<td>F2</td>
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<td>F5</td>
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<td>F6</td>
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<td>F7</td>
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</table>

*All coating materials were mixed with 1 % (w/w) magnesium stearate before compression.*

![Figure 1](https://example.com/figure1.png)

Figure 1. Release profiles of 5-fluouracil from compression-coated tablets with F1, F2, F3 and F4 in 0.1 N HCl for 2 h and phosphate buffer (pH 7.4) till the end of 24 h.
The cumulative percentage of the drug released from F2 was 8.44% at the end of the release period. The lower release from F2 can be explained due to the higher mastic content that may prevent complete dissolution of sodium chloride particles which is surrounded by the hydrophobic mastic particles leading to suppression of drug release. Further increase of sodium chloride content in the coats to 30% and 45% (F3 and F4, respectively) was accompanied by a significantly higher rate and extent of drug release. The results revealed that as the amount of sodium chloride increased in the coat formulation, the amount of drug release increased. This could be explained because of increased porosity in the tablet coat as a result of sodium chloride dissolution. Moreover, the higher release rate of 5-fluorouracil from tablets coated with F3 and F4 may be attributed to the higher sodium chloride content may pump more dissolution medium inside the tablet coat, thereby increasing the internal osmotic pressure, and making the coat present fine cracks that allow the rapid drug release.

To avoid development of such coat cracks during dissolution and to modify the drug release, 15% sodium chloride in addition to different concentrations of HPMC have been used in formulation of the coating materials F5, F6 and F7 (Table 1). HPMC, a semi-synthetic cellulose derivative, is widely used in oral controlled release tablet formulations [39, 40]. HPMC upon contact with the dissolution medium form a rate-controlling gel that may fill the pores developed in tablet coat as a result of sodium chloride dissolution. The high viscosity in the pores serves to retard the diffusion of the drug at the early stages of release. At the later stages of release, the polymer gel becomes dissipated and resistance to diffusion is decreased [30].

The release profiles of 5-fluorouracil from tablets coated with F5 and F6 (Figure 2) revealed that the percentage of drug released at the end of 5th h which is the expected time for the arrival of the dosage form in the colon, were found to be 3% and 7%, respectively. This significant decrease in the rate of drug release may be attributed the higher mastic content in F5 and F6 would reduce the free water volume in the coats and increase the gel viscosity inside the pores causing subsequent reduction in drug release.

While decreasing mastic content to 50% in F7 coat together with increasing HPMC content to 35% significantly increase the release rate during the early stages of release. The lower mastic content in addition to sodium chloride content may result in more hydration of HPMC and consequently decrease the viscosity inside the pores leading to rapid drug release. The release profile of 5-fluorouracil from tablets coated with F6 (Figure 2) indicated that, the cumulative percent drug release was found to be 7% in the first 5 h of the study and a total of 98.80% of drug was released in 24 h. This coat formula seems quite promising for achieving colon targeting of 5-fluorouracil. Further, the colonic release of the drug from compressed coated tablets with F6 may be confirmed by continuing the studies for in vivo release.

To determine the mechanism of drug release, the in vitro release data were fitted into various kinetic models. Table 2 shows the result of drug release kinetics of various compressed coated tablets. It can be observed that the drug release data of all coat formulations were best fitted to zero order equation. These results are in good agreement with that reported by Landgraf et al. [41], the authors found that when a cylinder, comprising impenetrable outer walls, contains an opening of fixed area. Ingredients contained within such a cylinder will escape from containment only through this fixed area and release will consequently approach zero-order kinetics. Moreover, the release data were analyzed using the exponential relationship previously presented. The values of release exponent n for all studied coat formulations were > 1 which indicates that the release pattern of 5-fluorouracil was characterized by super case II transport, which means the drug release rate is
characterized by zero order release. In this case, the higher values for $n$ together with the good fitting of the zero-order model indicate significant contribution of erosion as a mechanism for drug release.

Figure 2. Release profiles of 5-fluorouracil from compression-coated tablets with F5, F6 and F7 in 0.1 N HCl for 2 h and phosphate buffer (pH 7.4) till the end of 24 h.

Table 2. Fitting of release kinetic models to 5-fluorouracil release data.

<table>
<thead>
<tr>
<th>Zero order</th>
<th>First order</th>
<th>Higuchi model</th>
<th>Korsemeyer model$^*$</th>
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<tbody>
<tr>
<td>R²</td>
<td>R²</td>
<td>R²</td>
<td>R²</td>
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<tr>
<td>F2</td>
<td>0.996</td>
<td>0.996</td>
<td>0.968</td>
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<tr>
<td>F3</td>
<td>0.986</td>
<td>0.966</td>
<td>0.964</td>
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<tr>
<td>F4</td>
<td>0.990</td>
<td>0.899</td>
<td>0.988</td>
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<td>F5</td>
<td>0.998</td>
<td>0.989</td>
<td>0.982</td>
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<tr>
<td>F6</td>
<td>0.995</td>
<td>0.994</td>
<td>0.995</td>
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<tr>
<td>F7</td>
<td>0.998</td>
<td>0.989</td>
<td>0.984</td>
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$^*$Release exponent evaluated for < 60% released drug

Scanning electron microscope

SEM photomicrographs of the coat surface at different time intervals showing the progress of pore size in the compression coated tablets of F6. Before dissolution, the coat surface was intact without pores (Figure 3, A), while after 6 h the pores had formed throughout the coat with average pore diameter of 66.42 ± 6.24 µm (Figure 3, B). The pores diameters increased with respect to time to be 285.31± 12.43 µm after 24 h of the dissolution as indicated by photographs at

Figure 3. C. The increase in the pores diameter with respect to time increase reflects the prevalence of erosion as a mechanism for drug release.

Figure 3. SEM photographs of compression coated tablets showing surface morphology of F6 coat; (A) before dissolution, (B) after 6 h, and (C) after 24 h dissolution in 0.1 N HCl for 2 h and phosphate buffer (pH 7.4) till the end of 24 h.
**In-vivo scintigraphic studies**
From γ-scintigraphic images taken at regular time intervals in all volunteers, the observed time for initiation of release and distribution of the tracer through gastrointestinal tract (GIT) were closely similar. Figure 4 is a group of representative images for initiation of release and distribution of the traces in GIT in one volunteer. From the images, it was found that the tablets remained intact in stomach (after 1 and 2 h) and small intestine (after 2 and 4 h). On entering the colon, at 6 h the tablets started their tracer release and the release was increased gradually in the colon with increasing the time and finally the uniform distribution of the tracer along the entire colon was achieved after 24 h.

**In vitro evaluation of antitumor activity**
The in-vitro antitumor activity of 5-fluorouracil, mastic gum and equivalent dose of mixture of 5-fluorouracil and mastic gum mixed in a ratio representing their concentrations in the compression coated tablets against MCT-116 cells was qualified using the MTT assay. The results of the MTT cell viability assay are shown in Figure 5. The results demonstrated that treating cells with all tested samples have shown dose dependent growth inhibitory effect. It is obvious that the combination of drug and mastic gum was more effective in arresting cell growth with significant loss of viability at a concentration of 100μg/ml in comparison to that shown by 5-fluorouracil or mastic gum alone. The IC₅₀ of 5-fluorouracil-mastic gum combination was found to be 63.4μg/ml while that of 5-fluorouracil and mastic gum alone were 94 and 107 μg/ml, respectively. This accounts for 32.55 % reduction in the IC₅₀ value of drug-mastic gum combination in comparison with 5-fluorouracil alone. These results show that a combination of 5-fluorouracil and mastic gum is more effective than 5-fluorouracil alone against MCT-116 colorectal cancer cells.

![Figure 4. Representative gamma scintigraphic images taken at regular time intervals for one volunteer.](image)

![Figure 5. Cell viability of human colonic cancer (HCT-116) cell line after 48 h of incubation with different concentrations of 5-fluorouracil, mastic gum and a mixture of 5-fluorouracil and mastic gum relative to control (Cells with DMSO alone).](image)
Conclusions
In this study, mastic gum was used as a carrier for 5-flourouracil colonic delivery. Based on results of in vitro drug release as well as in vivo gamma scintigraphic release study, compression coated tablets, containing 5-flourouracil in the core tablet and coated with 200 mg of coating materials consisted of 60% mastic gum, 15% sodium chloride and 25% HPMC could produce a successful targeting of 5-flourouracil to the colon. The combination of 5-flourouracil and mastic gum significantly increased the in vitro antitumor activity of 5-flourouracil against colon cancer cells. Therefore, use of mastic gum as a carrier for colonic delivery of chemotherapeutic drugs may be beneficial for the treatment of colorectal cancer. However, the clinical benefits of using mastic gum in formulation of 5-flourouracil colonic tablets need further evaluation.

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