**In vitro, in vivo** evaluation of inter polymer complexes between carboxymethyl fenugreek gum and chitosan or carboxymethyl guar gum and chitosan for colon delivery of tamoxifen

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**ABSTRACT**

**Objective:** To develop colon targeted tamoxifen tablets employing interpolymer complexes (IPC) between carboxymethyl fenugreek gum (CMF) and chitosan (CH) or carboxymethyl guar gum (CMG) and CH. **Methods:** IPC between CMF and CH or CMG or CH was obtained as a result of reaction between negatively charged –COO⁻ group of CMF or CMG and positively charged –NH₃⁺ group of CH. These interactions were characterized by FTIR spectroscopy. The core tablets were coated with various ratios of CMF:CH and CMG:CH IPC solutions. The coated tablets were subjected to **in vitro** dissolution studies in the presence or absence rat cecal contents. The selected coated tablets were evaluated for their pharmacokinetic behavior by oral administration to rats. **Results:** The tablets coated with CMF:CH 40:60 and CMG:CH 50:50 were capable of protecting drug release in pH 1.2 (stomach) and pH 7.4 (small intestine) and releasing, respectively, 91% and 94% tamoxifen in pH 6.8 in the presence of rat cecal contents. The oral administration of these tablets to rats showed a tmax of 10 hrs as compared to 2 hrs in case of uncoated tablets. **Conclusions:** The tablets coated with CMF–CH ratio of 40:60 or CMG–CH ratio of 50:50 were able to protect drug release in stomach and small intestine and deliver tamoxifen after the tablets reached colon.

**1. Introduction**

The colon as a site of drug delivery offers numerous therapeutic advantages on account of a near neutral pH and much longer transit time. The successful targeted delivery of drugs to the colon via the gastrointestinal tract (GIT) requires the protection of a drug from release and degradation in the stomach and small intestine and release in the proximal colon. This might be achieved by the use of suitably designed colonic drug delivery systems (CDDS) that can protect the drug during its transfer to the colon [1]. Among the different approaches to achieve colon specific drug delivery, the use of polysaccharides, which are specifically biodegraded by colonic bacterial enzymes, holds promise [2-3]. The important bacteria present in the colon such as Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Lactobacillus, Clostridium secrete a wide range of reductive and hydrolytic enzymes such as β-glucuronidase, β-xylosidase, β-galactosidase, α-arabinosidase, nitroreductase, azoreductase, deaminase and urea hydroxylase [4].

Chitosan (CH) is a linear polysaccharide obtained from deacetylation of chitin. Due to amino groups, it carries a net positive charge and can be easily cross–linked with other anions, oppositely charged drugs and polymers [5,6]. The native form of chitosan is soluble in acid so can’t be used alone to formulate colon specific drug delivery systems. Complexation of chitosan with anionic polymers has been reported to be used for colon targeting. Chitosan–chondroitin sulphate cross linked films have been successfully reported to coat tablets for colon delivery of busulonide [7].

Guar gum (GG) is a naturally occurring galactomannan...
polysaccharide. It consists of high molecular weight hydrocolloidal polysaccharide, composed of galactose and mannose units combined through glycosidic linkages and shows degradation in the large intestine. Fenugreek gum (FG) is a galactomannan polysaccharide that is extracted from fenugreek seeds. The ratio of mannose to galactose in galactomannans is different in these gums – fenugreek gum has a mannose:galactose ratio of 1:1 and guar gum has a 2:1 ratio [8]. Guar gum has been used to formulate colonic specific drug delivery however, when used alone it is required in very high concentrations (60% w/w) [9]. Cross linking of guar gum has been reported to reduce the swelling of guar gum and the in vitro release in different pH media has been reported to be affected by changes in guar gum concentration and cross linking agent concentration [10]. Carboxymethyl guar gum (CMG) and carboxymethyl fenugreek gum (CMF) are the anionic derivatives of GG and FG, respectively. The incorporation of the carboxylic group incorporates anionic character to the gums and allows the formation of interpolymer complexation with cationic polymers.

Cancer is a major human health problem worldwide and it is the second leading cause of death in USA. The lifetime risk for a woman of developing breast cancer is 12.6% for whites and 10.2% for blacks in the United States. The international incidence of female breast cancer will reach approximately 3.2 million new cases per year by 2050 [11,12]. Tamoxifen, a non-steroidal antiestrogen and a selective estrogen receptor modulator has been the clinical choice for the antiestrogenic treatment of metastasis for advanced breast cancer for more than 20 years [13,14]. Tamoxifen citrate, competitively binds to estrogens receptors (ERs) present on colorectal tumors [13]. Anticancer agents with narrow therapeutic indexes are difficult candidates for targeted drug delivery. The purpose of the present study was to prepare colon targeted tamoxifen tablets using by coating tablets with polymer complexes formed between CH and CMF or CMG and CH.

2. Materials and methods

2.1. Materials

Tamoxifen citrate was received as a gift sample from Cadila pharmaceuticals Ltd., Ahmedabad (India). Fenugreek gum was received as a gift sample from Gunasper, USA and Guar gum was purchased from S.D fine chemicals, Mumbai (India). Chitosan was purchased from Central Institute of Fisheries Technology, India, Lactose monohydrate and magnesium stearate were received from Loba Chem Pvt. Ltd., Mumbai. Insoluble starch was received from S.D. Fine Chemicals Ltd., Mumbai. Other reagents and chemicals used were of analytical grade and used as such.

2.2. Studies on CMF, CMG and CH

2.2.1 Carboxymethylation of FG and GG

20g of FG or GG were added to a mixture of 126 mL of ethanol and 110.8 mL of toluene. To this 44.8g, w/v of NaOH was added gradually and mixed thoroughly. This mixture was kept at room temperature for 30 min. After this 24g of monochloroacetic acid was gradually added with agitation and kept overnight. The excess alkali was neutralized with glacial acetic acid using phenolphthalein indicator. The product was filtered and washed with ethanol and dried [16]. The degree of substitution was determined according to the method as described earlier [17].

2.2.2. Preparation of IPC films

CMF–CH or CMG–CH IPC films (50:50) were formed by mixing solutions of CH (prepared by dissolving 450 mg of CH in 20 mL of 1.5%, w/v acetic acid solution and adding 5 mL of ammonium acetate (5 M) solution) and CMF or CMG solution prepared by separately dissolving 450 mg of CMF or CMG in 20 mL water. This mixture was poured in petri plates and dried at 50 °C for 48 hrs. Films with a total polymer content of 2%, w/v containing 60:40, 50:50 and 40:60 ratio of CMF:CH or CMG:CH were prepared using this method. The dried films were stored in desiccator till use.

2.2.3. Characterization of IPC films by FTIR studies

CH, CMF, CMG and IPC films formed by drying admixtures containing different ratios of CMF:CH and CMG:CH were subjected to FTIR analysis (Perkin Elmer, RXI, USA)

2.2.4 Swelling Index Measurement

The swelling index of the IPC films after exposure to different pH was determined by sequentially immersing the films in pH 1.2 for 2 hrs and pH 7.4 for 22 hrs. The swelling index was calculated according to the formula

2.3. Preparation of Tamoxifen Core Tablets

Matrix tablets (average weight 25 mg) containing tamoxifen citrate (5mg) were prepared by wet granulation technique using 7.5%, w/v starch paste as binder. The wet mass obtained, was passed through a sieve no. 22, and retained on sieve no. 44. The wet granules were dried at 50°C for 1 hour. 10%, w/w fines were added and 1%, w/w magnesium stearate was added as lubricant. Tablets were compressed using biconvex punches in a six station rotary tablet compression machine (AK Industries, M207, Nakodar, India). These tablets were tested for dimensions (axial and radial diameters), hardness, friability and weight variation.

2.3.1. Testing of uncoated tablets

The axial and radial diameters of ten compressed tablets of each batch were determined by using electronic digital vernier calipers. Hardness of ten tablets was determined with the help of Pfizer hardness tester (Campbell Electronics, Mumbai). The weight variation test and disintegration test were performed in accordance with the method prescribed in USP.

2.3.2. Coating of Tamoxifen Core Tablets

The formulated tamoxifen tablets containing starch paste as binder were coated with aqueous solutions containing different CH:CMF or CH:CMG ratios (60:40, 50:50 or 40:60) to obtain a weight gain of 10%, w/w. The total polymer concentration was kept constant at 2%, w/v. The coating solution was sprayed at a rate of 5 mL/min with the help of peristaltic pump using a spray gun of 1 mm nozzle (Electrolab, PP201V, Mumbai, India) in a coating pan (12” diameter) being rotated at 18 rev/min (AK Industries, M1107, Nakodar, India). Compressed air was introduced at a pressure of 1.5 kg/cm2. The inlet air temperature was maintained at 60°C. The inner surface of coating pan was modified by attaching inert tubes (8 mm diameter) from the centre to the periphery for easy rolling of tablets thereby ensuring efficient mass transfer of polymer. The coated tablets were also evaluated for weight variation, disintegration time. Further, the axial and radial diameters were measured as described above.
2.4. Release studies of tamoxifen

2.4.1. In vitro release kinetics of tamoxifen

In vitro drug release studies were carried out using USP XXX–NF XXV (Apparatus I–basket method) dissolution apparatus utilizing temperature of 37 ± 0.5°C with constant stirring rate of 50 rpm. The uncoated tablets as well as those coated with different ratios of CMF:CH and CMG:CH solutions were evaluated for in vitro drug release. The dissolution studies were carried out in a pH progression media. The tablets were sequentially exposed to buffer pH 1.2 (900 mL) for 2 hrs (gastrointestinal residence period), buffer pH 7.4 (900 mL) for 3 hrs (small intestinal residence period) and buffer pH 6.8 (900 mL) for a further period of 19 hrs. A sample of 5 mL was withdrawn from each dissolution vessel at various intervals and replaced with equal volume of respective fresh dissolution medium. The samples were filtered and at the end of the study, the tablets were crushed in pestle and mortar to determine the residual drug content of tamoxifen in the tablet. Amount of drug released was determined using UV Spectrophotometer. All the dissolution studies were carried out in triplicate and standard deviation was applied.

2.4.2. In vitro dissolution studies in the presence of rat cecal contents

The formulated tablets were also evaluated for the amount of tamoxifen released in the presence of rat cecal contents. The tablets after sequential exposure to buffer L.P. pH 1.2 (2 hrs) and phosphate buffer L.P. pH 7.4 (3 hrs) were placed in phosphate buffer L.P. pH 6.8 containing 2%, w/v rat cecal contents. Drug release studies were carried out under continuous supply of CO2 at 37 ± 0.2°C with a constant stirring rate of 50 rev./min. Sample (1 mL) was withdrawn at various intervals and replaced with fresh dissolution medium containing 2%, w/v rat cecal content maintained under continuous supply of CO2. [18]. This sample was diluted suitably with buffer pH 6.8 and amount of tamoxifen released was determined using UV Spectrophotometer. All the dissolution studies were carried out in triplicate.

2.5. Stability studies

The tablets coated with 40:60 or 50:50 ratio of CMF:CH or CMG:CH were sealed in glass vials and stored under 45°C/75% RH for 6 month. Tablets were taken out after every 15 days and evaluated for weight variation, disintegration time, and in vitro drug release. The dissolution data of stored tablets was compared with that of freshly prepared tablets by f1 (dissimilarity) and f2 (similarity) analysis.

2.6. Pharmacokinetic studies

Sprague–dawley rats of either sex weighing 200–300 g maintained on normal diet were used for this study. The rats were divided into three groups, each containing six rats. The coated tablets from batches selected as best after in vitro dissolution studies were administered to the rats. The uncoated tablets were also administered to compare the drug release. Blood samples were collected from retro orbital vein at various time intervals and were mixed with 40 μl of heparin to prevent clotting of blood and subjected to centrifugation at 4000 rpm for 15 minutes. Plasma (upper layer) was removed carefully. To 250 μl of plasma 250 μl of internal standard (clomiphene) was added [19]. The mixture was centrifuged at 4000 rpm for 10 minutes. The drug and internal standard were extracted from plasma using 4ml of 2% butanol in hexane. The mixture was vortexed for 1 min and centrifuged for 10 minutes at 1000 g (2500 rpm). The organic layer was transferred to clean glass tube and evaporated to dryness at 37 °C under gentle steam of nitrogen and reconstituted with 2ml of methanol. These samples were processed by waters HPLC system equipped with 515 binary pumps, 2487 dual wavelength ultraviolet (UV) adjusted at 254nm and rheodyne manual injector. Mobile phase consisted of Methanol:Water:Triethylamine (85:14:90.1) pH 4.6. Column used was C18 column (250 mm × 4.6 mm, 5μm). The flow rate of mobile phase was 0.5mL/min and run time of samples was 20 minutes.

3. Results

3.1. Preparation and characterization of CMF, CMG and CH–CMG or CH–CMF IPC films

The Fourier Transform Infra Red (FTIR) spectra of CMF (Figure 1) and CMG (Figure 2) showed characteristic peaks at 1421.2 cm⁻¹ and 1424.1 cm⁻¹. The FTIR spectra of CMF and CH (Figure 1) or CMG and CH (Figure 2) showed peaks at 1563.3 cm⁻¹ and 1413.1 cm⁻¹. The FTIR spectra of CMF and CH (Figure 1) or CMG and CH (Figure 2) showed peaks at 1563.3 cm⁻¹ and 1413.1 cm⁻¹.

![Figure 1. FTIR spectra of FG (A); CH (B); CMF (C); complex formed by interacting CMF and CH in the ratio of 60:40 (D); 50:50 (E) or 40:60 (F)]](image)
3.2.2. Swelling studies

The IPC films were found to exhibit pH sensitive swelling. Figure 3 represents the swelling indices of different IPC films on sequential exposure.

3.3. Physical evaluation of tablets

The average weight of uncoated core tablets was 29.44±1.10 mg. The acceptance value calculated was 11.98%, which was well below the maximum 15% USP tolerance limit. Hence, the tablets passed the weight variation test. Hardness of the tablets was 5.1±0.15 Kg/cm² and friability was found to range from 0.46 to 0.55%, w/w. The axial and radial diameters, respectively, ranged from 1.75 to 1.80 mm and 3.98 to 4.02 mm. The uncoated tablets started showing signs of cracking within 30 min of exposure to 0.1M HCl.

The average weight of coated tablets was 33.20±1.25 mg. The acceptance value calculated was 11.54%. Hence, the tablets passed the weight variation test. The axial and radial diameters of coated tablets, respectively, ranged from 2.02 to 2.08 mm and 4.01 to 4.15 mm. Although, these tablets exhibited swelling, they didn’t soften or crack after exposure to 0.1M HCl for 2 hrs.

3.4. In vitro dissolution studies in the absence and presence of rat cecal contents

Tamoxifen tablets (coated and uncoated) were evaluated for drug release using USP Apparatus I–basket dissolution apparatus. The release of tamoxifen from the uncoated tablets was 100% within 2 hrs of exposure to pH 1.2. The tablets coated with films comprising of CMF–CH or CMG–CH ratio of 40:60 or 50:50, respectively released 0.8% and 0.9% tamoxifen after 2 hrs in pH 1.2 while only 3% and 4% drug was released in pH 7.4 over a period of 5 hrs. The tablets coated with 60:40 or 50:50 CMG:CH or 60:40 or 40:60 CMF:CH were releasing around 8% tamoxifen in pH 1.2 and 13–15% tamoxifen in pH 7.4 (small intestine). The percentage release of drug from tablets coated with CMF:CH or CMG:CH ratio of 60:40, 50:50 or 40:60 in the absence and presence of rat cecal enzymes is shown in Figure 4. The final exposure of coated tablets to pH 6.8 containing rat cecal enzymes for 19 hrs eventually released, approximately 90% tamoxifen.

<table>
<thead>
<tr>
<th></th>
<th>pH 1.2</th>
<th>pH 7.4</th>
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<tbody>
<tr>
<td>CMF:CH (60:40)</td>
<td>1.25</td>
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</tr>
<tr>
<td>CMF:CH (50:50)</td>
<td>0.95</td>
<td>0.84</td>
</tr>
<tr>
<td>CMF:CH (40:60)</td>
<td>0.85</td>
<td>0.73</td>
</tr>
<tr>
<td>CMG:CH (60:40)</td>
<td>2.44</td>
<td>1.54</td>
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<td>CMG:CH (50:50)</td>
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<tr>
<td>CMG:CH (40:60)</td>
<td>2.58</td>
<td>1.43</td>
</tr>
</tbody>
</table>

3.4. In vitro dissolution studies in the absence and presence of rat cecal contents.

Figure 2. FTIR spectra of GG (A); CH (B); CMG (C); complex formed by interacting CMG and CH in the ratio of 40:60 (D); 50:50 (E) or 60:40 (F).

Figure 3. Swelling indices of the formulated CMF–CH or CMG:CH IPC films in pH 1.2 (2h) and pH 7.4 (24hrs). Data is represented as mean±SD.

Figure 4. Percentage tamoxifen released during in vitro dissolution studies in the absence and presence of rat cecal contents. Data is represented as mean±SD.
3.6. Stability Studies

The tablets coated with 40:60 or 50:50 ratio of CMF:CM or CMG:CH respectively were found to exhibit complete physical and chemical stability on storage at 40°C/75% RH. There was no change in the color and weight of the tablets. The value of f1 was found to be 4.98 and that of f2 was found to be 93.63, this indicated that the coated tablets were not affected adversely on storage.

3.7. In vivo studies

The pharmacokinetic profile of tamoxifen uncoated tablets as well as of tablets coated with 40:60 or 50:50 ratio of CMF:CH or CMG:CH, respectively, following oral administration to rats are depicted in Figure 5. The uncoated tablets released tamoxifen within 1 hrs of administration. The plasma concentration of tamoxifen was found to rise quickly after administration of uncoated tablets and Cmax of 18.65 ng/mL was achieved in 2 hrs. However, after administration of coated tablets tamoxifen was detectable in plasma only after 2 hrs of administration.

![Figure 5. Pharmacokinetic profile of uncoated and coated tamoxifen tablets after oral administration to rats (n=6)](image)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Uncoated tablets</th>
<th>CMF:CH (40:60)</th>
<th>CMG:CH (50:50)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% drug release)</td>
<td>(% drug release)</td>
<td>(% drug release)</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>75.1</td>
<td>4.26</td>
<td>1.85</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>6.86</td>
<td>3.26</td>
</tr>
<tr>
<td>3</td>
<td>9.67</td>
<td>7.98</td>
<td>1.21</td>
</tr>
<tr>
<td>5</td>
<td>20.58</td>
<td>10.67</td>
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<td>26.54</td>
<td>14.56</td>
</tr>
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<td>50.93</td>
<td>40.36</td>
<td>26.23</td>
</tr>
<tr>
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<td>65.96</td>
<td>52.36</td>
<td>44.58</td>
</tr>
<tr>
<td>24</td>
<td>74.21</td>
<td>70.23</td>
<td>60.36</td>
</tr>
</tbody>
</table>

4. Discussion

The prepared CMF and CMG were characterized by spectroscopy. The appearance of a peak at 1421.2 cm⁻¹ and 1424.1 cm⁻¹ indicated the presence of –COO⁻ ions (representing C=O stretch of –COO⁻) in the CMF (Figure 1) and CMG (Figure 2), respectively. This peak was absent in FG and GG sample indicating that carboxymethylation had been successfully carried out. The degree of substitution was found to be 0.385 in CMF and 0.271 in CMG. The smooth regions of the mannan backbone (Mannose:Galactose 2:1) present in guar gum come closer and form intrachain hydrogen bonds, which reduces the hydration of the gum [20]. This might be responsible for the greater degree of substitution in fenugreek gum (Galactose: Mannose 1:1) as compared to guar gum (Galactose: Mannose 1:2).

The presence of peak at 1563.3 cm⁻¹ and 1413.1 cm⁻¹ indicated the existence of –NH₃⁺ ions and –COO⁻ ions, respectively, thus indicating the presence of ionic interaction between –NH₃⁺ moieties of CH and –COO⁻ moieties of CMF (Figure1) or CMG (Figure2) in all the ratios of CMF:CH and CMG:CH IPC films.

The IPC films comprising CMF:CH were showing swelling to considerable less extent as compared to CMG:CH films. The intrinsic viscosity (volume per unit mass that a polymer occupies in solution) of guar gum has been reported to be greater than that of fenugreek gum. The intermolecular entanglements between the polymer chains of guar gum increase the intrinsic viscosity of guar gum. The molecular aggregation decreases with the degree of substitution of the mannan backbone by galactosyl residues. The higher degree of galactosyl substitution of fenugreek gum means the molecules have fewer tendencies to form intermolecular interactions leading to decreased intrinsic viscosity of fenugreek [21]. This might be responsible for decreased swelling index of CMF:CH films as compared to CMG:CH films.

The tablets coated with CMF=CH or CMG=CH ratio of 40:60 or 50:50 showed only 4% drug release in pH 7.4 till 5 hrs however, tablets coated with 60:40 or 50:50 CMG:CH or 60:40 or 40:60 CMF:CH released around 13-15% tamoxifen in pH 7.4 till 5 hrs therefore tablets coated with CMF=CH 40:60 or CMG=CH 50:50 were selected for in vitro dissolution studies in the presence of rat cecal contents. It is evident from Figure 4 that the release of tamoxifen increased tremendously in the presence of rat cecal contents. The final exposure of coated tablets to pH 6.8 containing rat cecal contents for 19 hrs eventually released,
approximately 90% tamoxifen. This significant increase in drug release could be attributed to the fact that rat cecal contents contain anaerobic enzymes specifically acting on carbohydrate polymers. These anaerobic enzymes are known to act on these polymers thus weakening the cross linking between CMF–CH or CMG–CH leading to extended and continuous release of tamoxifen. However, complete release of tamoxifen was not observed even after 24 hrs of sequential exposure to different pH media simulating in vivo gastrointestinal transit. This seems to be due to declined activity of polysaccharidases over the long period of time during in vitro testing. The time to achieve Cmax after oral administration was delayed to 10 hrs for coated tablets. This strongly indicated that the IPC films were able to inhibit the release of tamoxifen in gastric pH. The plasma tamoxifen concentration in rats administered with coated tablets (40:60 or 50:50 ratio of CMF:CH or CMG:CH, respectively) rose steadily after 7 hrs and then declined after 10 hrs thus suggesting that the polymers were susceptible to degradation by the polysaccharidases present in the colon.

5. Conclusion

The results of the present investigation indicate that interpolymer complexes between CMF–CH or CMG–CH can be employed for protecting the release of tamoxifen in the upper parts of GIT. The FTIR studies of the IPC films demonstrated interaction between −NH3+ group of CH and −COO− of CMF or CMG. The polysaccharidases produced by the colonic microflora caused the enzymatic breakdown of the polysaccharides thus releasing the drug in the colon. The in vivo pharmacokinetic studies demonstrated that the time required for attaining maximum plasma concentration was delayed indicating that the coated tablets did not release drug in stomach and small intestine. To conclude, the findings suggested great potential of CH–CMF or CH–CMG IPC films for delivering tamoxifen in the colon.

Conflict of interest statement

We declare that we have no conflict of interest.

References