In the present work, lornoxicam (LOR) loaded guar gum microspheres were prepared and characterized for local release of drug in the colon, for the effective treatment of ulcerative colitis. For the preparation of guar gum microspheres emulsification method was used and glutaraldehyde used as a cross-linking agent. Scanning electron microscopy was used for surface morphological characteristics investigation of microspheres. Particle size, shape, and surface morphology were significantly affected by guar gum concentration, glutaraldehyde concentration and stirring rate. Lornoxicam loaded microspheres showed high entrapment efficiency (76.58%). The in vitro drug release was carried out in different media (pH 1.2, 4.5 and 7.4 buffer solutions, with and without rat cecal contents), results showed that it was affected by a change to the guar gum concentration and glutaraldehyde concentration. The drug release in pH 1.2, 4.5 and 7.4 followed a similar release pattern and had a similar drug release rate, while a significant increase in percent cumulative drug release (86.51%) was observed in the dissolution medium containing rat cecal content. Guar gum microspheres showed adequate potential of colon targeting in achieving local release of drug in colon.

**Keywords:** Guar gum, Microspheres, Lornoxicam, Ulcerative colitis, Glutaraldehyde, Rat cecal content.

**INTRODUCTION**

Ulcerative colitis is an inflammation of the lining of the large bowel (colon and rectum). Symptoms of ulcerative colitis are; rectal bleeding, diarrhea, abdominal cramps, weight loss, and fevers. In addition, patients who have had extensive ulcerative colitis for many years are at an increased risk to develop large bowel cancer. Antibiotics and anti-inflammatory medications are used for initial treatment of ulcerative colitis. If these fail, prednisone is used for a short period of time; but long-term use can be associated with significant side effects. To date, oral delivery is still the preferred route of drug administration, especially for chronic therapies where repeated administration is required. Oral administration offers patients less pain, greater convenience, higher likelihood of compliance, and reduced risk of cross infection and needle stick injuries. Thus, formulations of oral drug delivery continue to dominate more than half of the drug delivery market share. Despite these advantages, the oral route is not amenable to the administration of most drugs available today, due to their high susceptibility to digestive enzymes in the gastrointestinal tract (GIT). Most of the drug delivery systems for treating colonic disorders, colonic infectious and colonic cancer are failing as the drug do not reach the site of action in appropriate concentration. Thus, an effective and safe therapy of the colonic diseases with the help of the site specific drug delivery system is a challenge to the pharmaceutical technologists. As a result, new strategies of drug delivery have been developed to overcome obstacles encountered by oral delivery. Among these strategies from the last two decades, colon-specific delivery has been extensively studied.

The colonic region of the GIT is one area that would benefit from the development and use of such modified release technologies. The longer residence time, less peptidase activity, natural absorptive characteristics and high response to absorption enhancers make the colon a promising site for the delivery of protein and peptide drugs for systemic absorption. For successful colonic drug delivery, many physiologic barriers have to be overcome. Absorption or degradation of the active ingredient in the upper part of the GIT is the major obstacle and must be circumvented. An oral colon delivery system prevents drug release in the upper GIT but allow complete release in the colon. Four primary approaches for colon targeted delivery used namely; prodrugs, pH dependent system, time dependent systems and colonic micro flora activated systems.

Several approaches have been investigated to targeting drug to colon.

Biodegradable polysaccharides are used for targeting of drugs to the colon following oral administration. A biodegradable polymer is a polymer in which the degradation results from the action of naturally occurring microorganisms such as bacteria, algae or fungi. The inability of GIT enzymes to digest certain plant polysaccharides (pectin, guar gum, okara gum) is taken as an advantage to develop colon specific drug delivery systems. The anaerobic bacteria of colon only produce the polysaccharide enzyme which degrade the carrier polysaccharides and release the contents for localized or systemic absorption through colon. Various biodegradable polysaccharides such as; gum gum, locust bean gum, inulin, pectin, amylose, chondroitin sulfate, cyclodextrin, chitosan, dextran, alginate, xanthan gum etc are used for colon targeting.

With the increase in importance of natural polysaccharides, it has become necessary to explore the newer sources of polysaccharides to meet the industrial demands. With this belief, the aim of the present work was to prepare, optimize and evaluate the colon targeted microspheres for the treatment of ulcerative colitis using microbially triggered drug delivery systems.
system. Lornoxicam, a non-steroidal anti inflammatory drug is used for the local treatment of ulcerative colitis. Guar gum, a polysaccharide that degrades only by the enzymatic action of the metabolic enzymes of colonic bacteria is employed for preparation of colon targeted microsphere. Colon targeted microspheres prepared using biodegradable polymers which overcome the inherent drawbacks of existing dosage forms and had the following advantages; increased bioavailability, delivery of drugs in its intact form as close as possible, less local irritation, ability to cut down the conventional dose and reduced risk of systemic toxicity.

**MATERIALS AND METHODS**

Lornoxicam, guar gum, span 80, castor oil and glutaraldehyde were supplied as a gift sample by Corel Pharm Chem, Ahmedabad, India. Sulphuric acid and sodium hydroxide were purchased from Chemdyes Corporation, Rajkot, India. Potassium dihydrogen phosphate was purchased from Merck Specialities Pvt. Ltd., Mumbai, India. Methanol was purchased from Ranbaxy Fine Chemicals Ltd., New Delhi, India.

**Preparation of lornoxicam colon targeted microspheres**

Drug-loaded guar gum microspheres were prepared by the emulsification method. An aqueous dispersion of guar gum containing 4% W/W of guar gum (an accurately weighed amount of gum was dispersed in a specified volume of 0.1 N NaOH solution containing the drug, and allowed to swell for 2 hours) was dispersed in 100 g of castor oil containing 3 g of Span 80 using a mechanical stirrer at 4000 rpm. After complete mixing, 0.2 mL of concentrated sulfuric acid and 2/3 mL of glutaraldehyde were added to the dispersion, followed by stirring at a constant speed of 4000/5000 rpm for 4 hours at 50°C. The microspheres formed were collected by sedimentation followed by decantation of oil, then washed with several fractions of isopropyl alcohol. The final preparation was a free-flowing powder consisting of spherical micron-sized particles. Two ratios of drug to guar gum are also chosen, which are 1:1 and 1:2. All the different formulations prepared by varying the parameters are enlisted in table 1. The formed microspheres are then subjected to various characterization tests.

**Evaluation of prepared lornoxicam microspheres**

**Determination of surface morphology**

The shape and surface morphology of the guar gum microspheres was characterized using scanning electron microscope (Philips, XL30 ESEM with EDAX model). Small amounts of microspheres were spread on double adhesive tape which was kept on a stub. The stub containing sample was then placed in the microscope chamber. The scanning electron photomicrographs were taken at the acceleration voltage of 30 kV at chamber pressure of 0.7 torr.

**Particle size analysis**

Particle size was determined by using a laser diffraction particle size analyzer (Sympatec, Helos-BF, Germany). Microspheres were suspended in the chamber of the particle size analyzer containing distilled water, and the particle size was determined using the software provided by the manufacturer.

**Practical yield**

Once the microspheres are obtained, its practical yield is calculated. It can be found by dividing the weight of microspheres obtained by the total weight of all the dry material added in the process. The result obtained is multiplied by 100 to obtain the percentage practical yield obtained by the process for each formulation.

**Degree of swelling**

A preweighed 100 mg amount of microsphere was placed in simulated intestinal fluid (SIF) (pH 7.4) and allowed to swell up to a constant weight. The microspheres were removed and blotted with filter paper, and their changes in weight were measured. The degree of swelling (α) was then calculated from the following formula:

$$\alpha = (\omega_f - \omega_0)/\omega_0 \quad \text{……………… (1)}$$

Where, $\alpha$ = degree of swelling, $\omega_f$ = initial weight of microspheres, $\omega_0$ = final weight of microspheres.

**Drug loading and encapsulation efficiency**

Loading capacity is the maximum amount of drug that can be incorporated in the microspheres. Loading capacity can be determined to be the maximum amount of LOR found in 100 mg of microspheres. Encapsulation efficiency is the amount of added drug (in percent) that is encapsulated in the formulation of microspheres. Encapsulation efficiency can be calculated using the ratio of drug in the final formulation to the amount of added drug. The microspheres will be triturated and the sample equivalent to 10 mg of Lornoxicam will be dissolved in 30 ml of 0.1 N NaOH by using sonicator at 37°C for 10 min. and the volume adjusted up to 100 ml with pH 6.8 phosphate buffer. The solution has to be filtered through Whatman filter paper to obtain stock solution A. The Stock solution A (10 ml) is diluted to 100ml to obtain the stock solution B. The absorbance of the resulting solution is observed by using the U.V spectrophotometer at 378 nm.

**Angle of repose**

Angle of repose was determined by using funnel method. Powder was poured from a funnel that can be raised vertically until a maximum cone height, h, was obtained. Diameter of heap, D, was measured. The angle of repose, $\theta$, was calculated by formula:

$$\theta = \tan^{-1}\left(\frac{D}{h}\right)$$
In vitro dissolution study without rat cecal content

The release studies of LOR from guar gum microspheres can be performed in USP dissolution apparatus 1, at 50 rpm in 900 ml of 0.1N HCl, pH 4.5 Phosphate Buffer and pH 7.4 phosphate buffer, and in a medium containing rat cecal content (with and without enzyme induction) \(^7\). Simulation of gastrointestinal transit conditions are achieved by using different dissolution media\(^6\). The drug release studies will be conducted in pH 1.2 buffer solution for the first 2 hours to mimic the condition in the stomach and in pH 4.5 phosphate buffer for 3 hours. The dissolution medium is then replaced with pH 7.4 phosphate buffer and tested for the next 8 hours. At the specified time, 10 ml of the dissolution fluid is withdrawn and it is replaced with fresh buffer solution maintained at 37°C to maintain constant test volume throughout the experiment. Dissolution studies were performed in triplicate \((n=3)\), and calculated mean values of cumulative drug release were used while plotting the release curves\(^7\).

In vitro dissolution study with rat cecal content

It is essential to carry out in vitro dissolution studies of the formulation in simulated colonic fluids to study the release pattern in presence of rat cecal content and check the change in the release pattern in comparison to dissolution medium without rat cecal content. The in vitro drug release studies are carried out using USP dissolution apparatus (Apparatus 1, 50 rpm, 37°C) with slight modifications\(^6\). A beaker (capacity 200 ml) containing 100 ml of 4% rat cecal content medium is immersed in the phosphate buffer pH 7.4 maintained in 1000-ml vessel, which in turn, is in the water bath of the apparatus. The swollen formulation after completing the dissolution studies in 0.1N HCl (2 hr) and Phosphate buffer pH 4.5 (3 hr) are placed in the basket of the apparatus and immersed in the rat cecal content medium contained in 200 ml beaker. As the cecum is naturally anaerobic, the experiment is carried out with continuous supply of carbon-dioxide into the beaker. At the end of the time periods 5 ml sample are withdrawn, centrifuged, diluted and analyzed for percentage of drug release by UV spectrophotometer\(^5,6,9\).

RESULT AND DISCUSSION

Surface morphology

The external surface morphology of the microspheres was studied by scanning electron microscopy. The microspheres were observed under a scanning electron microscope. The results of the selected formulation F5 showed spherical shape as shown in figure 1. No cracks were observed even on higher magnification, which indicated that the drug release from the microspheres occurred only after the degradation of the guar gum matrix and no drug is released from the crack formation.

Particle size analysis

Guar gum microspheres were prepared by the emulsification method. Glutaraldehyde as well as temperature-induced cross-linking was used for hardening of microspheres. The particle size of the microspheres was determined using a particle size analyzer. The mean diameter of glutaraldehyde cross-linked guar gum microspheres increased with increasing polymer concentration from 1% to 2% wt/wt. In the present investigation a 2% guar gum concentration was found to be optimal, ensuring the optimal size of microspheres as shown in table 2. The average particle size of microspheres increased with increasing polymer concentration, since at higher concentrations the polymer solution dispersed into larger droplets. At concentrations lower than the optimum the solution became less viscous and dispersed into numerous fine droplets that easily coalesced, resulting in larger microspheres\(^5\). The mean particle size of microspheres decreased from with increasing mixer rotational speed, from 4000 rpm to 5000 rpm. Results shown in table 2 revealed that the average diameter of microspheres was controlled by rotational speed. The ultimate mean diameter of microspheres was determined by the size of dispersion of the polymer solution, which decreased with increasing mixer rotational speed. Results also suggested that there was a mixing rate limit for a particular polymer concentration. A higher mixing rate did not further reduce the mean diameter. The mixing speed of 4000 rpm was found to be optimal for guar gum microspheres. The effect of stirring time at a particular rotational speed was also observed, and it was recorded that stirring time influenced the shape as well as the size distribution of microspheres, possibly because of variable shear force experienced by the particulate system\(^7\).

Practical yield

The percentage practical yield of all the formulations was calculated form the amount of initial weight of dry powders added for the preparation of the microspheres. The theoretical yield of the formulations was 80% as describe in table 2. In accordance with that formulation F5 showed to have the nearest result to the theoretical yield.

Degree of swelling

Native guar gum swells 100-120-fold in gastric and intestinal fluids. As a result of cross-linking with glutaraldehyde the overall swelling of polymer decreased significantly as shown in table 2. Cross-linking interferes with free access of water to the guar gum hydroxyl group, which in turn reduces the swelling properties of the cross-linked polymer. The crosslinking of the modified guar gum formulation depended on the glutaraldehyde concentration, but the optimal concentration of the cross-linking agent was a compromise between swellability...
and in vitro digestion of microsphere preparation in the presence of rat cecal content. Formulation F5 shows the least swelling amongst all the formulations, showing that the free hydroxyl groups of the galactose and mannose unit of guar gum have been cross linked efficiently and they are no longer available for linking with the water. This reduces the swelling rate of the microspheres and thus prevents unnecessary loss of drug.

**Drug loading and encapsulation efficiency**

Encapsulation efficiency was calculated as the ratio of the weight of LOR content in the final microspheres (100 mg) and the LOR introduced in the process. Percent encapsulation efficiency increased up to 76.58% ± 0.638% with increasing polymer concentrations up to 2%. The concentration of the cross-linking agent had no significant effect on percent encapsulation efficiency. But the rotational speed had an impact on the drug loading and encapsulation efficiency. With increase in speed above the optimum, cross linking agent could not come in proper contact with the polymer, thus causing less loading. At the same time, lower rotational speeds caused the leaching out of the rug from the cross linked structures before proper hardening of the microspheres. The optimum speed was found to be 4000 rpm. From all the formulations, formulation F5 showed the maximum entrapment efficiency as observed in table 2.

**Flow property (angle of repose)**

The flow properties of all the formulations were carried out and the results obtained are mentioned in the table 2. All the formulations showed to have a good flow property that is within the range for good flowability. Thus any of these formulations could be used for filling in capsules or for compression in the form of a tablet without the need of any externally added aid. Moreover, formulation F5 showed to have excellent flow property from all the formulations. This could be attributed to a better uniformity in its shape and size, which is obtained by proper stirring speeds which resulted into uniform spherical microparticles.

**In vitro dissolution study**

The prepared microspheres are filled in a capsule. The dissolution tests were conducted for all the formulations at pH 1.2, 4.5 and 7.4. The dissolution data obtained are shown in figure 2, showed that all formulations showed no or less drug release in 0.1N HCl and phosphate buffer pH 4.5. In pH 7.4 the drug gets released to some extent. Due to the cross linking of the drug with Guar gum the release of the drug will occur only in the presence of the cecal contents, thus only small amount of drug is released. With 23.21% release being the lowest amount of free drug released into dissolution fluid, formulation F5 shows to have been made with all the optimum parameters that are required for the colon targeted cross linked microspheres. The selected formulation F5 was subjected to in vitro dissolution testing in simulated conditions of the gastro intestinal tract. This testing was carried out pH 7.4 phosphate buffer with rat cecal contents added to it for 12 hours.

The in vitro drug release of LOR from the guar gum microspheres of formulation F5 showed considerable increase in the presence of rat cecal contents than in its absence. It clearly indicated that the release of the drug from the cross linked microspheres occurred mainly due to the enzymatic action of the colonic microflora. In the simulated colonic condition with the cecal contents the drug was released up to 86.51% which showed a previous release of only 23.21% in the absence of colonic contents of the rat as shown in figure 3. This proves that the desired release pattern of the drug can be obtained by selected formulation and no unwanted release occurs in any other region of the GIT.

**CONCLUSION**

The goal of any drug delivery system is to provide a therapeutic amount of drug to the proper site in the body and also to achieve and maintain the desired plasma concentration of the drug for a particular period of time. Treatment could be made more effective in case of ulcerative colitis, if it were possible for drugs to be targeted directly on the colon. In the present study, an attempt was made to prepare, optimize and evaluate colon targeted microspheres of Lornoxicam for the treatment of ulcerative colitis in order to target the drug to the colon. Eight formulations (F1 to F8) were prepared using Lornoxicam and guar gum in the different ratio by emulsification method and using varying rotational speed and amount of cross-linking agent. The experimental results demonstrated that the prepared microspheres of Lornoxicam for colon targeting may reduce the side effects of the drug caused by its absorption from the upper part of GIT when given in conventional dosage forms. Also the dose required could be reduced due to targeting of the drug. The formulation showed very little release in the absence of the colonic microflora, thus indicating that no drug loss would occur in the other parts of the GIT except the colon. Thus, the Glutaraldehyde Cross-linked Lornoxicam Microspheres have the potential to be used as a colon targeted delivery system for the treatment of ulcerative colitis.

**REFERENCE**


**Figure 1:** SEM Images of the Microspheres in Different Magnifications; [A] Magnification = 154X, [B] Magnification = 400X, [C] Magnification = 200X and [D] Magnification = 149X

**Figure 2:** *In vitro* dissolution of lornoxicam microspheres without rat caecal content

**Figure 3:** *In vitro* dissolution of lornoxicam microspheres F5 in presence of rat caecal content

**Table 1:** Various formulations for guar gum based lornoxicam microspheres

<table>
<thead>
<tr>
<th>Formulation Name</th>
<th>Drug: Polymer Ratio</th>
<th>Amount of Gluteraldehyde (ml)</th>
<th>Rotational Speed</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>1:1</td>
<td>2</td>
<td>4000</td>
</tr>
<tr>
<td>F2</td>
<td>1:1</td>
<td>2</td>
<td>5000</td>
</tr>
<tr>
<td>F3</td>
<td>1:1</td>
<td>3</td>
<td>4000</td>
</tr>
<tr>
<td>F4</td>
<td>1:1</td>
<td>3</td>
<td>5000</td>
</tr>
<tr>
<td>F5</td>
<td>1:2</td>
<td>2</td>
<td>4000</td>
</tr>
<tr>
<td>F6</td>
<td>1:2</td>
<td>2</td>
<td>5000</td>
</tr>
<tr>
<td>F7</td>
<td>1:2</td>
<td>3</td>
<td>4000</td>
</tr>
<tr>
<td>F8</td>
<td>1:2</td>
<td>3</td>
<td>5000</td>
</tr>
</tbody>
</table>
Table 2: Mean particle size, mean % practical yield, degree of swelling, mean drug loading, mean % encapsulation efficiency and angle of repose of the Prepared Microspheres

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean Particle Size (µm)</th>
<th>Mean Percentage Practical Yield</th>
<th>Degree of Swelling</th>
<th>Mean Drug Loading</th>
<th>Mean % Encapsulation Efficiency</th>
<th>Angle of Repose</th>
</tr>
</thead>
<tbody>
<tr>
<td>F1</td>
<td>33.15±1.04</td>
<td>76.27±0.16</td>
<td>1.27±0.002</td>
<td>33.23±0.30</td>
<td>66.47±0.60</td>
<td>26.56±0.004</td>
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<tr>
<td>F2</td>
<td>35.39±1.25</td>
<td>77.03±0.22</td>
<td>1.19±0.008</td>
<td>35.20±0.36</td>
<td>70.41±0.73</td>
<td>30.46±0.008</td>
</tr>
<tr>
<td>F3</td>
<td>41.12±1.23</td>
<td>78.66±0.32</td>
<td>1.36±0.015</td>
<td>34.65±0.08</td>
<td>69.30±0.17</td>
<td>27.75±0.001</td>
</tr>
<tr>
<td>F4</td>
<td>32.63±1.57</td>
<td>75.33±0.41</td>
<td>1.57±0.002</td>
<td>36.71±0.51</td>
<td>73.42±1.03</td>
<td>33.69±0.008</td>
</tr>
<tr>
<td>F5</td>
<td>29.14±1.02</td>
<td>79.25±0.49</td>
<td>1.03±0.002</td>
<td>38.29±0.31</td>
<td>76.58±0.63</td>
<td>29.05±0.009</td>
</tr>
<tr>
<td>F6</td>
<td>35.57±1.22</td>
<td>75.83±0.15</td>
<td>1.38±0.004</td>
<td>37.03±0.27</td>
<td>74.06±0.54</td>
<td>23.49±0.007</td>
</tr>
<tr>
<td>F7</td>
<td>31.43±2.02</td>
<td>78.09±0.20</td>
<td>1.45±0.006</td>
<td>36.26±0.64</td>
<td>72.52±1.28</td>
<td>25.46±0.002</td>
</tr>
<tr>
<td>F8</td>
<td>33.7±1.74</td>
<td>76.23±0.15</td>
<td>1.29±0.005</td>
<td>37.25±0.13</td>
<td>74.51±0.27</td>
<td>37.56±0.004</td>
</tr>
</tbody>
</table>

Values are Mean ± SD, n = 3

For Correspondence:
Rajesh A. Keraliya
Kalol Institute of Pharmacy, Department of Pharmaceutics,
Kalol – 382721, Gujarat, India
E-mail: rajeshmpharm21@gmail.com