Research Article

pH-Sensitive Nanoparticles of Mesalamine and Curcumin for the Treatment of Ulcerative Colitis

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ABSTRACT

Inflammatory bowel diseases, especially ulcerative colitis (UC) and Crohn's disease, have become a global threat due to the ineffectiveness of current treatments for all patients. This requires identifying other therapeutic molecules or their combinations of them that may well as first-line or treatment. In this study, both mesalamine, an anti-inflammatory drug and curcumin, an anti-inflammatory drug, were found to help reduce UC. Furthermore, studies on their combination have shown synergy between the two drugs. In this study, we developed pH-sensitive nanoparticles combining curcumin with mesalamine for the treatment of UC. The combination of drug combination, effective delivery of nanocarriers and pH sensitivity of the polymer combined the desire to reduce the total toxicity and total dose of mesalamine and increased the effectiveness in reducing UC. pH-sensitive nanoparticles of mixed drugs have been shown to be more effective than nanoparticles of drugs or drug suspension.

Keywords: Colonic drug delivery; drug targeting; nanoparticles; natural and synthetic drugs; oral drug delivery; polymeric drug carrier; site-specific delivery.

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory disease affecting the colon mucosa and is an important offshoot of inflammatory bowel disease (IBD), which is increasingly becoming a global threat.[1,2] Current therapies for the treatment of this disease include aminosalicylates, corticosteroids, immunosuppressants, and molecules (biological and synthetic) that target tumor necrosis factor-alpha (TNFα), which is involved in the pathogenesis of the disease.[3,4] However, most of these medications are effective in only half of the patients, while the other half are at risk of relapse and need longterm pain management. Identification of drug molecules or combinations thereof that may be first-line treatment or curative therapy for UC.[4,5]

In this case, mesalazine is a salicylate and its treatment does not appear to be associated with cyclooxygenase inhibition, the effectiveness of which works is to inhibit the production of IL1 and TNFα and inhibit the lipoxygenase pathway, removing the free one radicals and oxidants that inhibit NF-κB. The specific action drug has not yet been determined.

Therefore, selective inhibition of this enzyme has been shown to reduce intestinal inflammation and bleeding. Preliminary studies demonstrate the safety and effectiveness of mesalazine in the short-term treatment of IBD patients.[6]

Another molecule that has attracted the attention of researchers for the treatment of IBD is curcumin, an antiinflammatory compound of the herbal medicine turmeric. The anti-inflammatory effect is due to its ability to inhibit COX_2 and inflammatory cytokines such as interleukins1 beta and 6 and TNFalpha. Due to this effect, curcumin has been reported to reduce mucosal damage and has been shown to be effective in some studies in IBD.[7,8] This will be essential for the treatment of IBD.[9]

In addition to studies showing the ability of mesalamine and curcumin alone, researchers have also investigated the combination of the two molecules due to their different mechanisms of inhibiting COX, and result tests have been conducted on diseases that require long term study IBD. Important another advantage of using this combination is that curcumin inhibits 5-lipoxygenase (5-LOX) and COX.[10] In addition, curcumin is a gastroprotective agent with proven antioxidant and antiapoptotic effects that strengthens the gastric mucosa by reducing acid secretion.[11] Negative effects on the stomach. However, to date, no studies have investigated the combination of these two drugs in the treatment of IBD. Despite early evidence of their effectiveness in treating IBD, many molecules have recently failed due to various reasons, including low solubility, poor bioavailability, lack of nasal and side effects, poor absorption and rapid elimination, and poor drug handling. However, most of these problems have been solved by encapsulating drugs into nanoparticle carriers.[9,12] Regarding curcumin specifically, our research group has previously reported nanoparticles made using pH sensitive enteric polymer (Eudragit S100), a nontoxic polymer. This polymer dissolves at pH 7.0 through ionization of the carboxyl functional group and is therefore used to target drugs to the colonic region of the gastrointestinal tract (GIT). In cell analysis, these nanoparticles were found to be more effective than the unencapsulated drug.[9] Polymer nanoparticles hold promise for delivering drugs to specific sites for the treatment of various diseases. Polymer nanoparticles with nanometer range length protect the material from

degradation *in vitro* and *in vivo*, release drugs in a controlled manner and enable medical treatment. to get better. This can be combined with optimal uptake influenced by nanoparticle size, selective targeting to the intestine, and integration of the encapsulated drug.[13] Additionally, with the improved drug bioavailability provided by nanocarriers, this combination can reduce the total mesalamine dose. They can work as drug carriers due to active drug loading, small size, high capture efficiency, amphiphilicity and stability.[14]

MATERIALS AND METHODS

Materials: Curcumin (Cur; assay: 95%) and mesalamine were gift samples from NR Lifecare, Ahmedabad, Shreena Enterprise, Ahmedabad, respectively. Eudragit®S100 was provided by Research -Lab Fine Chem Industries, Mumbai. Poly vinyl pyrrolidone (PVP K-90/D) was procured from Research -Lab Fine Chem Industries, Mumbai HPLC grade ethanol and dichloromethane and AR grade acetone were purchased from Rankem (Mumbai, India). Trehalose was purchased from Gangwal Chemicals Pvt. Ltd. (Mumbai, India). Sucrose was purchased from S.D. Fine Chemicals (Mumbai, India).

Methods:

Preparation of Curcumin-, Mesalamine-, and Curcumin–Mesalamine-Loaded Eudragit®S100 Nanoparticles

All the nanoparticles were prepared using solvent emulsion evaporation technique.[9]

For preparing curcumin-loaded nanoparticles (CurNPs), curcumin (10 mg) and Eudragit®S100 (10 mg) were dissolved in acetone (2 ml). The organic solution was emulsified with aqueous solution (20 ml) containing PVP (0.075%, w/v) using Ultraturrax R T25 (Janke and Kunkel, IKA Labortechnik, Staufen, Germany) at 17,500 rpm for 5 min. The organic phase was subsequently evaporated at 27◦C, using a blade-type stirrer (Remi, Mumbai, India), at 2000 rpm for 8 h to formulate the nanoparticles. In case of mesalamine (Mes) loaded nanoparticles (MesNPs), the organic phase consisted of a mixture of mesalamine (10 mg) dissolved in methanol (1 ml) and Eudragit® S100 (15 mg) dissolved in acetone (3 ml). The remaining procedure was identical to that followed for CurNPs. Nanoparticles of combination of Cur–Mes were also prepared by the same technique. However, in this case, the organic phase comprised a mixture of curcumin (10 mg) and Eudragit® S100 (10 mg) dissolved in acetone (2 ml) and mesalamine (1 mg) dissolved in methanol (1 mL). Although the surface-active agents used in aqueous phase (20 mL) consisted of PVP (0.150%, w/v).

Nanoparticle Characterization:

Fourier transform infrared spectroscopy

FTIR emission spectrometer (Shimadzu, Japan) was used to record the FTIR spectrum of the drugs, Eudragit S100, Physical mixture of drug and polymer and formulation of nanoparticle were recorded with an attenuated total infrared reflection FTIR spectrophotometer using the potassium bromide disc method in the range of 400 to 4000 cm^{-1}.

Particle Size and Surface Charge

Average particle size, combined polydispersity index (PI) and surface value of zeta potential were determined using a Zetasizer NanoZS (Malvern Instruments, Worcestershire, United Kingdom) equipped with a 4 mW HeNe laser. Measurements were performed in triplicate at a temperature of 25°C, a wavelength of 633 nm and an angle of 173°. All samples were diluted sufficiently with MilliQ water to ensure that the light intensity was within the sensitivity range of the instrument.

Encapsulation Efficiency

The encapsulation efficiency (EE) of a nanoparticle represents the amount of drug in the nanoparticle compared to the total amount of drug added to the formulation. The EE of the whole nanoparticle suspension was determined by ultracentrifugation of the preparations (2 ml) at 70,000 rpm (450000 × g) for 40 min at room temperature. The amount of unencapsulated drug in the supernatant is analyzed by a validated HPLC method.

The EE (%) was calculated by the following equation:

$$EE(\%) = M_{initial\ drug} - M_{free\ drug}\, M_{initial\ drug} \times 100 / M_{initial\ drug}$$

where “Minitial drug” is the mass of total drug used for the formulation and “Mfree drug” is the mass of unencapsulated drug analyzed in the supernatant after centrifugation.

Freeze-Drying of Nanoparticles

Frozen nanoparticle preparations were freezedried (40°C, 12 h) using a vacuum dryer (Freezone[12], Labconco, Kansas City, MO). The cryoprotectants used in each formulation consisted of a mixture of trehalose and poloxamer 188 (20% each, w/w) (CurNP), sucrose (5%, w/v) (MesNP), and sucrose (3%, w/v). mixture. Primary drying was done at ~15°C for 6 hours followed by 6 hours at 0°C, and secondary drying was done at 15°C for 6 hours followed by 4 hours at 30°C. Ambient temperature was maintained at ≤52°C throughout the entire procedure. Lyophilized preparations were stored at 4°C until further analysis.

Characterization of Freeze-Dried Products

Particle Size and Surface Charge

Freeze dried preparations were rehydrated with MilliQ water and the resulting aqueous nanoparticle suspensions were analyzed for average size and surface charge as described previously.

Drug Content

The actual weight of freeze-dried nanoparticles equivalent to 1 mg drug/combination was dissolved in distilled water (1 ml) and methanol (9 ml). The solutions were diluted appropriately and the amount of single drug was determined using the UV method.[15]

In Vitro Drug Release

In vitro drug release from nanoparticle formulations was evaluated according to the illustrated method with slight modifications in environments with different pH values (pH 1.2, 4.5 and 7.2). Cur, Mes, and CurMes were applied to membrane filters (Himmedia Laboratories, Mumbai, Indiacutoff size 1214 kDa). The release of individual compounds from all three samples was measured at specific times using the early UV technique.[16]

Scanning Electron Microscopy

Morphological studies were carried out using scanning electron microscopy (SEM). Place a drop of freshly prepared nanoparticle solution onto the stub and tap the solution with filter paper. Particle morphology was analyzed using scanning electron microscopy (SEM) JSM6390 (JEOL, Japan). Samples and an appropriate amount of pure chemical were fixed onto the SEM stub using double tape and coated with platinum for 6 min at 50 mA with a sputtering machine (KYKY SBC12, Beijing, China). Digital images of the samples were obtained using a scanning electron microscope with a secondary voltage detector at an acceleration of 20 kV.

RESULTS AND DISCUSSION

Preparation of Curcumin, Mesalamine, and Curcumin–Mesalamine-Loaded Eudragit® S100 nanoparticles. All the nanoparticles were prepared using solvent emulsion evaporation technique.

- The FT-IR spectrum of pure drug, Eudragit®S100 and nanoparticle formulation were shown in fig. In below FTIR there was no found any incompatibility and not found any other additional functional group.

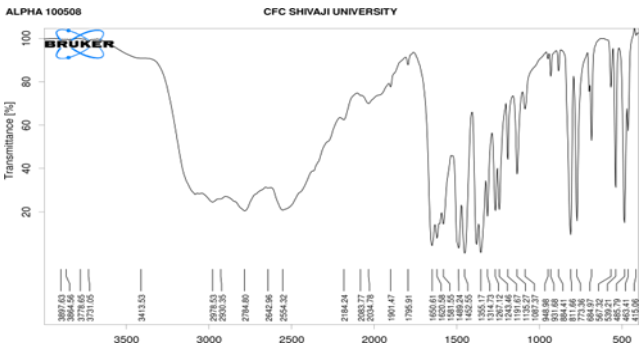

Figure 1: FTIR Spectrum of Mesalamine

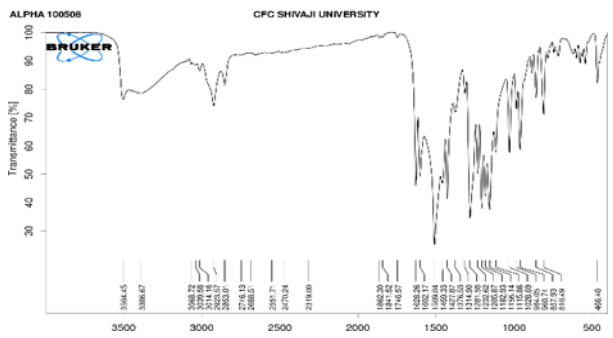

Figure 2: FTIR Spectrum of Curcumin

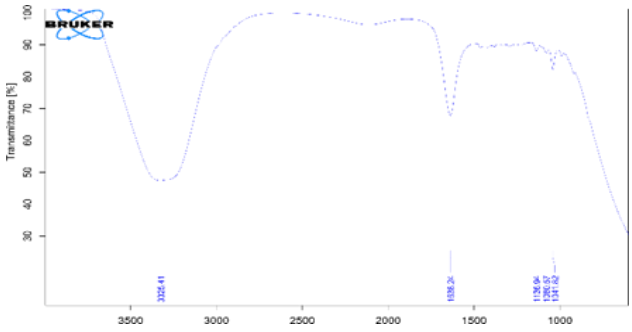

Figure 3: FTIR Spectrum of nanoparticle formulation XRD analysis

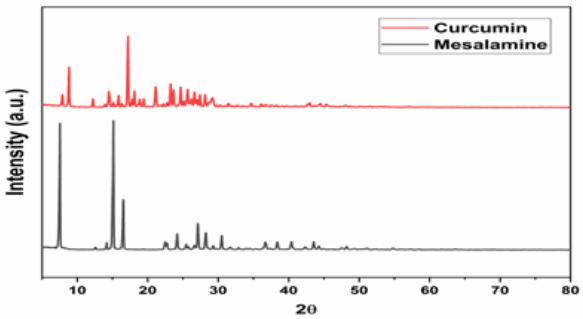

Figure 4: XRD peak of Curcumin and mesalamine

a) In the curcumin sample, the XRD peaks were observed at 10.28°,16.30°, 18.20°, 19.40°, 22.29°, 24.71°, 25.70°, 26.44° and 29.55°.

b) The mesalamine shows the XRD peaks at 07.20°,15.00°, 16.20°, 18.40°, 22.32°, 26.71°, 30.71°.

Preparation and Characterization of Cur, Mes, and Cur–Mes-Loaded Eudragit®S100 Nanoparticles

Cur-loaded Eudragit® S100 nanoparticles having a median particle size of 479 nm and PI of 0.802 have already been reported by our research group. These nanoparticles exhibited 72.32% encapsulation of curcumin and demonstrated nearly double inhibition of colon cancer cells as compared with unencapsulated curcumin.[9] In the present investigation, we loaded Eudragit®S100 nanoparticles with Mes and Cur–Mes combination. Mes NPs possessed median particle size of 165 nm and PI of 0.13, whereas Cur–MesNPs had a median particle size of 86 nm and PI of 0.304 nm. The EE for MesNPs was found to be 72.32%, whereas for the combination, nanoparticles showed 82.36% encapsulation of Cur and 81.24% encapsulation of Mes.

Freeze-Drying of Nanoparticles and Characterization of Freeze-Dried Products

Three nanoparticle formulations were freezedried using different concentrations of different cryoprotectants or their combination with stabilizers. This indicates that the nanoparticles can completely redisperse to their original form. The content of the freezedried solution ranged from 99% to 103%; This shows that the freeze-dried process had no effect on the preparation.

The in vitro drug release curve of this preparation (Figure 6) shows that the release of the drug is less than 4% at pH 1.2 and 4.5 conditions; This indicates that this arrangement can control drug release from above. Part of pH 1.2 and 4.5. path. However, at pH 7.4 the sample produced approximately 70% of the drug entering the solution as the polymer dissolved at this pH.

Table 1: Average particle size, Encapsulation efficiency and polydispersity of Nanoparticles

Formulation Codes	Particle size (nm)	Encapsulation efficiency	Polydispersity Index	Zeta Potential (mv)
Mes	165	72.32	0.13	-1.6
Cur	479	71.37	0.802	-0.2
MesCur	86.5	81.24 & 82.36	0.304	-36.0

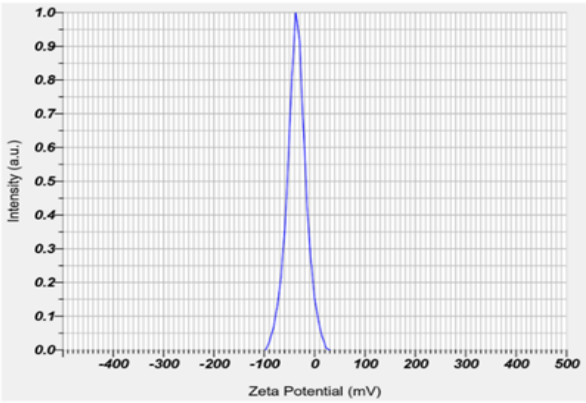

Figure 5: Zeta potential of Mes & Cur NP

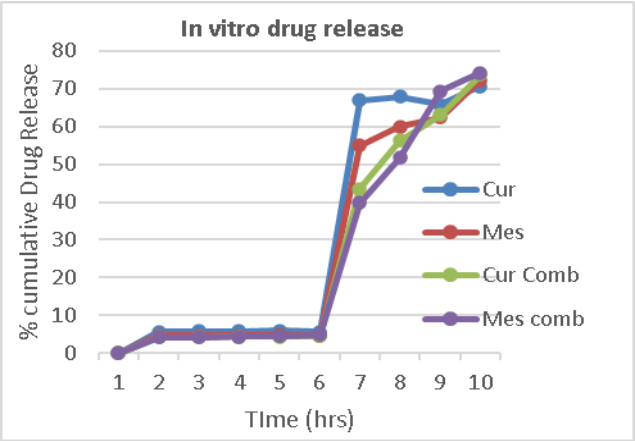

Figure 6: *In vitro* % cumulative drug release of formulation Mesalamine and Curcumin NP

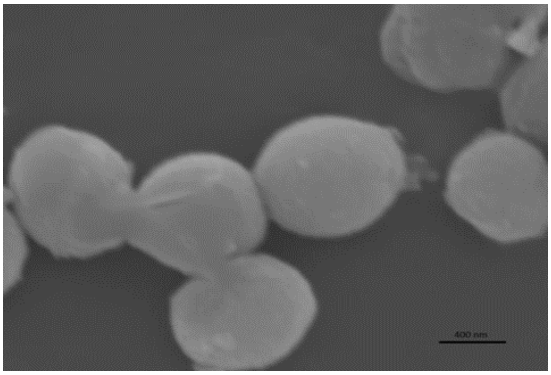

Figure 7: SEM of Mesalamine and Curcumin NP

DISCUSSION

In general, colon diseases, including UC and Crohn's disease, are recurrent idiopathic inflammatory diseases involving the colon mucosa and submucosa. Because intestinal inflammation in IBD is limited to specific mucosal or transmural sites, medications can be delivered to the site of inflammation.[17] It has been shown to be effective in preclinical and clinical studies of IBD.[7,8] In this study, we encapsulated a combination of Cur and Mes in Eudragit® S100 nanoparticles that dissolve at pH 7.0 through polymer structural changes related to ionization of the carboxyl functional group, and the compounds are loaded into the vacuum of the nanoparticles.[19] PH sensitive nanoparticles are designed to target the drug directly to the intestinal region. It is also predicted that nanosized carriers will be better absorbed by the tissue, retained there, and release the drug into the colonic cavity through the breakdown of the polymer. This local application is expected to prevent the nephrotoxicity and cardiotoxicity of mesalamine. To our current knowledge, no studies to date have examined the effectiveness of this combination in reducing IBD. pH sensitive nanoparticles of Cur, Mes, and CurMes were produced by the solvent emulsification evaporation method,[9] which is a simple and industrially applicable technology. The solvent used for CurNP is acetone, and the solvent used for MesNP and Cur-MesNP is methanol. Solvents are selected based on the high solubility of the drug and their ability to form good emulsions with an aqueous phase to form a stable product and disperse the product. This is evidenced by the low particle size and PI, indicating the homogeneity of the formulations. Reports suggest that particles of this size are digested by macrophages and M cells at the site of inflammation. Additionally, disruption of intestinal function during infection may allow nanoparticles to enter the site of inflammation, leading to selective drug release.[20] It has been reported that beneficial proteins are abundant in the inflammatory tissue in IBD. Therefore, it is thought that negative nanoparticles will interact better with intestinal bacteria due to the electrostatic effect. The average EE of nanoparticles can be attributed to their particle size. As other researchers have reported, small particles have a higher surface area to volume ratio, increasing the risk of

medication being lost through contamination in middle school.[21] The amount of drug in the formulation varies between 99% and 102%. These results, including analysis of the dimensions of the samples, clearly show that all drugs not packaged into nanoparticles exist in nanocrystal form and can be expected to contain nanoparticles. The study found that the *in vitro* drug release rate of a pH sensitive nanoparticle was less than 4% in environments at pH 1.2 and 4.5, respectively. This indicates that the nanoparticles have the ability to delay drug release at gastric pH or intestinal pH due to hydrogen bonding between the hydroxyl group of the carboxylic acid moiety and the carbonyl oxygen of the ester group of the Eudragit® S100 polymer. When the pH of the release medium changes to 7.4, the nanoparticles release a drug or their combination; this can be attributed to the combination of deprotonation of the carboxyl functional group and dissolution and swelling of the polymer. Thus, *in vitro* release studies demonstrate the ability of nanoparticles to inhibit drug release in the intestine and intestine, while the two drugs will be released at colonic pH, making them available locally in tissues.

CONCLUSIONS

pH-sensitive nanoparticles of Curcumin, mesalamine, and Curcumin–mesalamine were successfully developed. Inflammatory bowel diseases, which largely comprise ulcerative colitis (UC) and Crohn's disease, are increasingly posing as a global threat because of the incompetence of the current therapy in the entire patient population. This necessitates the identification of alternative therapeutic molecules or their combinations, which may serve as effective first-line or maintenance therapeutics. In this quest, mesalamine anti-inflammatory agent and curcumin, a natural antioxidant and anti-inflammatory agent, have both been found to be useful in alleviating UC. The nanoparticles exhibited the potential to restrain the release of encapsulated agents at pH of stomach and upper intestine and selectively release it at the colon region. Here, nanoparticles of the drug combination were found to be superior to those of either agent alone, because of the synergistic effect mesalamine and curcumin. This synergistic action along with delivery advantages of nanosized carriers, such as improved bioavailability of encapsulated agents and targeting ability of the pH-sensitive polymer may help to reduce the total dose of mesalmine and curcumin.

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