Formulation and evaluation of nanocapsules for colon targeted drug delivery

Sanjay J. Kshirsagar*, Naresh C. Bingi, Jaydeep N. Dusane

Department of Pharmaceutics, AISSMS College of Pharmacy, University of Pune, Kennedy road, Near R.T.O., Pune 411001, Maharashtra, India

*For correspondence
Dr. Sanjay J. Kshirsagar,
Associate Professor,
AISSMS College of Pharmacy,
University of Pune, Kennedy road, Near R.T.O., Pune 411001, Maharashtra, India. Email: sanjayjkshirsagar@gmail.com

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ABSTRACT

Objective: Inflammatory bowel disease (IBD) ulcerative colitis (UC) and Crohn’s disease (CD) if not treated leads to colon cancer. Nanocapsules have shown potential for specific accumulation in inflamed tissue. It was to optimize nanocapsule formulation which will release drug specifically at colon.

Methods: Nanocapsules were prepared by modified nanoprecipitation method using probe sonication which produces lower particle size as compared to conventional magnetic stirring.

Results: Enteric polymer Eudragit S100 was used for formulation and optimization was done by 3² full factorial designs. The optimization batches showed particle size in the range of 480-780 nm with entrapment efficiency ranging between 78-90% and lag time of 3.5 to 4.5 hrs. Polymer concentration affected the particle size and lag time. At 100 mg of polymer concentration, lag time obtained was 4.5 hrs corresponding to colonic arrival time which ensure specific drug release in colon. Inverted sac method shows specific accumulation of drug in the colonic tissue. Lower MPO activity in test group as compared to standard and control group indicates decreased inflammation which was further proved by histopathology results.

Conclusions: Higher accumulation of nanocapsular drug in inflammatory area and specific release by enteric polymer, provide better option for treatment of colonic disease.

Keywords: Budesonide, Myeloperoxidase, Optimization, Site specific, Ulcerative colitis

Introduction

Specific drug delivery to the colon is highly desirable for local treatment of a variety of bowel diseases such as ulcerative colitis, crohn’s disease and systemic delivery of protein and peptide drugs. The colon specific drug delivery system (CSDDS) should be capable of protecting the drug en route to the colon.¹ The goal of any drug delivery system is to provide a therapeutic amount of drug to a proper site in body so that the desired drug concentration can
be achieved promptly and then maintained. Targeted drug delivery implies selective and effective localization of drug into the target at therapeutic concentrations with limited access to non-target sites. A targeted drug delivery system is preferred in drugs having instability, low solubility and short half-life, large volume of distribution, poor absorption, low specificity and low therapeutic index. Targeted drug delivery may provide maximum therapeutic activity by preventing drug degradation or inactivation during transit to the target site. Meanwhile, it can protect the body from adverse effects because of inappropriate disposition and minimize toxicity of potent drugs by reducing dose. The preparation of the delivery system must be reasonably simple, reproducible and cost-effective.\(^2,3\)

Solid formulation intended for targeted drug release into the lower gastrointestinal GI tract are beneficial for the localized treatment of several colonic diseases and conditions, mainly inflammatory bowel diseases, irritable bowel syndrome and colon cancer. Research interest in the area of colonic drug delivery has been fuelled by the need to better treatment of pathologies related to colon that range in seriousness from constipation and diarrhoea to the debilitating inflammatory bowel diseases through to colon carcinoma, the third most prevalent form of cancer in both men and women.\(^4\)

Most of the conventional drug delivery systems for treating the colon disorders such as IBD (e.g. irritable bowel syndrome, Ulcerative colitis, Crohn’s disease), infectious diseases (e.g. amoebiasis) and colon cancer are failing as the drugs do not reach the site of action in appropriate concentrations. Thus, an effective and safe therapy of these colonic disorders, using site specific drug delivery systems is a challenging task to pharmaceutical technologist. The use of nanoparticulate systems for the delivery of therapeutic agents in inflammatory diseases is receiving considerable attention for medical and pharmaceutical applications. This increasing interest results from the fact that these systems can target more or less selectively inflamed tissue, mainly on a cellular level. The disruption of the intestinal barrier function could allow for the accumulation of the particulate delivery system at the site of inflammation.

The objective of the present study was to prepare a with Eudragit S100 polymer containing budesonide, spray dried and filled in capsules providing immediate release at the ileo-cecal site, the most affected area in Crohn’s disease. In this study, the novel ex vivo model was developed to ensure the entrapment of nanocapsules in the inflamed areas. Budesonide was used as a model drug because of its therapeutic potential for Crohn’s disease. The manufacture and the in vitro release characteristics of the nanocapsules were described; especially, the in vivo performance in rat was evaluated by examining myeloperoxidase (MPO) enzyme in the inflamed tissue and the histopathology scoring of the same.\(^5,6\)

**Materials and Methods**

Budesonide was obtained as gift sample from CIPLA Pvt. Ltd. Patalganga, Raigad, India, Eudragit S100 was obtained from the Degussa India Pvt. Ltd. Mumbai, as a gift sample. Benzyl benzoate and all other chemicals were of analytical grade, used without further purification and were obtained from Vijay Chemicals, Pune (India).

Animals - Pathogen-free, male wistar rats weighing 300-400 g were used in accordance with a protocol approved by the Institutional Animal Ethical Committee (AISSMS College of pharmacy)

Nanocapsules were prepared using modified nanoprecipitation method using probe sonication. Briefly, required amount of budesonide was weighed properly and dissolved in oil. Polymer and surfactant were dissolved in acetone separately. The drug in oil is added to acetone phase. The mixture was then was added to water under probe sonication for 5min. The nanosuspension was then subjected to rotavapor for removal of free acetone.\(^7\) For nanosuspension; drug encapsulation efficiency was measured followed by spectrophotometric
analysis of both free and nanocapsule-encapsulated drug, using established methods.\(^8\)

Nanocapsule size was determined using a Malvern Mastersizer 2000 MS (Malvern Instruments, Worcestershire, UK). Nanocapsules were used in animal studies as prepared, without separation of free drug, to maintain the original total drug concentration.

**Pre-optimization studies for formulation of nanocapsules**

*Comparative study of effect of Magnetic Stirring And Probe Sonication On Particle Size Of Nanocapsules:*\(^9,13\)

Nanoprecipitation method for formulation of nanocapsules requires magnetic stirring during the addition of acetone phase to aqueous phase. Probe Sonication can also be used for this purpose. A study to compare the effect of magnetic stirring and probe Sonication on particle size of nanocapsules was performed.

**Preliminary study of nanocapsules**

Preliminary studies were done with different concentrations of polymer and surfactant. These studies were used for selection of optimum polymer and surfactant concentrations.

*Study of Effect of Sonication time on particle size of nanocapsules:*\(^11,12\)

In order to optimize the Sonication time, (Batch B 4) the sampling of nanocapsules were carried out at interval of 1 min from 3 min to 8 min and the particle size of each batch was measured.

**Statistical design for optimization of nanocapsules**

Full factorial design with 2 factors, 3 levels, and 9 runs was selected for the optimization study. The experimental design consists of a set of points lying at the midpoint of each edge and the replicated centre point of the multidimensional cube. Independent and dependent variables are listed in (Table 1). The polynomial equation generated by this experimental design (Design expert 7.1.6) is given below. Nine coefficients (B1 to B9) were calculated with B0 as the intercept.

\[
Y = B_0 + B_1X_1 + B_2X_2 + B_3X_3 + B_4X_1X_2 + B_5X_1X_3 + B_6X_2X_3 + B_7X_21 + B_8X_22 + B_9X_23 \quad (10)
\]

**Variables for experimental designs**

**Independent Variables**

\[X_1 = \text{Polymer concentration} \]
\[X_2 = \text{Surfactant concentration} \]

**Dependent Variables**

\[Y_1 = \text{Average Particle Size (nm)} \]
\[Y_2 = \text{Entrapment Efficiency (％)} \]
\[Y_3 = \text{Lag time (hrs)} \]

**Table 1: Variables and their levels.**

<table>
<thead>
<tr>
<th>Independent variables</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low</td>
</tr>
<tr>
<td>Polymer (X1)</td>
<td>50mg</td>
</tr>
<tr>
<td>Surfactant (X2)</td>
<td>50mg</td>
</tr>
<tr>
<td>Transformed values</td>
<td>-1</td>
</tr>
</tbody>
</table>

**Table 2: 3\(^2\) Full factorial design layout, experimental runs and their combinations.**

<table>
<thead>
<tr>
<th>Standard run</th>
<th>Polymer (mg)</th>
<th>Surfactant (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O 1</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>O 2</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>O 3</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>O 4</td>
<td>75</td>
<td>87.5</td>
</tr>
<tr>
<td>O 5</td>
<td>75</td>
<td>87.5</td>
</tr>
<tr>
<td>O 6</td>
<td>75</td>
<td>87.5</td>
</tr>
<tr>
<td>O 7</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>O 8</td>
<td>100</td>
<td>125</td>
</tr>
<tr>
<td>O 9</td>
<td>100</td>
<td>125</td>
</tr>
</tbody>
</table>

The amount of Budesonide was kept constant and concentration of polymer and surfactant was decided by pre-optimization studies. All the batches were prepared according to the experimental design (Table 2). The ingredients
used and their levels taken for further study on formulation optimization of nanocapsules is shown in Table 1.

**Optimization data analysis**

Optimization of preparation of nanocapsules was done by Design Expert Software (Version 8.0.7.1., Stat-Ease Inc. and Minneapolis, MN). The nanocapsules were prepared with different independent variable with different levels and responses average particle size, % entrapment efficiency and lag time were obtained. The data was inputted to design expert software and polynomial equation was obtained.

The polynomial regression results were demonstrated using 3-D graphs through proper ANOVA model. Finally, the prognosis of optimum formulations was conducted significantly. Three optimum formulations were picked by the critical evaluation of the significant ANOVA model.

**Validation of the Response Surface Methodology**

Total three optimized formulations were selected as check points to validate RSM. The nanocapsules were formulated using the chosen optimal composition and evaluated for particle size, entrapment efficiency and lag time. Plots between predicted and observed responses were critically compared, and the percent error calculated with respect to the observed responses. Correlation plots were also constructed separately for three optimized formulations.¹³,¹¹

**Evaluation of optimized Nanocapsule dispersion**

**Determination of Particle Size and Zeta potential**

The particle size analysis of the formulations was performed using Malvern Mastersizer 2000 MS (Malvern Instruments, Worcestershire, UK). The average particle size and size distribution of each nanocapsular dispersion was recorded.

**Percent Entrapment efficiency**

Drug entrapment efficiency in the nanocapsules, expressed as percent of the added drug actually entrapped into nanocapsules. The drug nanocapsules were separated from dispersion by ultracentrifugation at about 10000RPM for 1 hour in which nanocapsules settled at base in pellet form. While free drug remain in supernatant liquid. Entrapment efficiency was calculated according to following equation.

\[
EE\% = \frac{The \ amount \ of \ entrapped \ drug \ in \ Nanocapsules}{The \ total \ amount \ of \ drug \ included \ in \ preparation} \times 100
\]

**Estimation of budesonide entrapped in nanocapsules**

Accurately measured 0.1 gm of nanocapsule suspension was transferred to clean and dry 10ml volumetric flask, it was dissolved in 10ml chloroform. Chloroform layer was separated and evaporated to dryness, to it 10 ml methanol was added and sonicated for 5min. This was filtered through whatmann filter paper, resulting solution was diluted suitably with methanol and the drug content was analysed using the UV photometrically at 242nm.

**Estimation of budesonide unentrapped in nanocapsules**

Free drug means, a percent of the added drug remain unentrapped in the nanocapsules and present in the supernatant liquid, after separation. Accurately measured 1ml of supernatant liquid was transferred to clean and dry 10ml volumetric flask, and volume was made up to 10ml with methanol. The resulting solution was diluted suitably with methanol and the drug content was analysed using the UV photometrically at 242nm.

**Spray drying of nanocapsules dispersion**

**Preparation of Spray dried powder**

To the suspension of nanocapsules was added 5% (w/w) of Aerosil 200, and the mixture was fed into a spray drier Labultima (LU 222) with a two component nozzle and concurrent flow. The inlet temperature at the drying chamber was
The outlet temperature was at 90°C±4°C. The residual water content of each spray dried powder was determined by loss of product.

**Determination of drug content to spray dried nanocapsules**

The spray dried powders were dispersed in methanol under magnetic stirring for 60 min at room temperature. The dispersion was filtered through hydrophilic membrane (0.45 um millipore), and the Budesonide was assayed by UV at 242 nm. The recovery of Budesonide in spray dried powders was estimated by correlation of theoretical and practical concentrations.

**Moisture content determination**

The residual moisture content of spray dried powders was measured by Karl fischer titration in dry methanol using Karl Fisher reagent. Measurements were performed in triplicates.

**Process yield determination**

The weights of spray dried powders collected were corrected according to their moisture content. The yield was calculated by dividing these quantities by the total mass introduced in the preparation submitted to drying.

**Morphological analysis**

The morphological examination of the spray dried powders was performed by scanning electron microscopy (SEM) (JEOL JSM-6360). The powder sample was spread on a double adhesive tape previously adhered to SEM aluminium stubs, and then sputtered with platinum in an ion sputter for 300s. Images were collected at an acceleration voltage of 15Kv using a back scattered electron detector on JEOL JSM-6360 SEM. Analysis was performed at 25±2°C.

**Measurement of particle size and Zeta potential of nanocapsules**

The volume particle size distribution of the spray dried powders was determined by photon correlation spectroscopy (PCS) using a Malvern Zetasizer Nano ZS (Malvern Instruments, UK). Prior to measurement samples were diluted 10 times with distilled water. DLS or photon correlation spectroscopy (PCS) is based on the measurement of the Brownian motion of the particle. The Brownian motion is random movement of particles in suspension. The smaller the particle, the faster the Brownian motion will be. When the incident laser beam reaches the sample, light is scattered in such a way, depending on the Brownian motion, and then detected by a photomultiplier positioned at a determined angle. Fluctuations in the intensity of the scattered light are converted into output current, which is passed to an auto corrector. In this way a correlation function is generated and analysed by software. The computer can provide the mean size and the distribution width of the nanoparticles in the batch.

**Density determination**

The particulate density of the spray dried powders was determined by calculating weight of powder and volume occupied.

**Differential scanning colorimetry study**

Differential Scanning Calorimetry (DSC) measurements were carried out on pure budesonide and spray dried nanocapsules on Mettler-Toledo DSC 821e (Columbus, OH) instrument. DSC scans were recorded at heating rate of 10°C/min in temperature range 30°-300°C. An empty standard aluminium pan was used as reference.

**Angle of repose**

In this study, the angle of repose is measured by passing the powder from a funnel fixed to a stand and measuring the radius of the heap of powder formed.

**Bulk density**

Bulk density is defined as a ratio of weight of powder mass to volume occupied by the same when it is poured. The bulk density was calculated in g/ml.
Tapped density

Tapped Density is defined as a ratio of a weight of a powder to a volume occupied by the same after sufficient tapping has been done. The tapped density was calculated in g/ml.

Compressibility Index

Compressibility index is defined as a 100 times the ratio of the difference between the tapped density and a bulk density to the tapped density. The bulk density and tapped density was measured and compressibility index was calculated.

Drug release study of spray dried nanocapsule powder filled in capsules (Lag time determination).

The dissolution studies of Budesonide nanocapsules were carried out in a USP XXIII dissolution apparatus II (DA 6D Veego, TDT 08L Electrolab) at a rotation speed of 50 rpm in a 900 ml medium at 37°C. The capsules (n=3) are transferred to the dissolution medium and samples were taken at selected time intervals, filtered through 0.1 um membrane filter (Millipore) and analysed by UV spectrophotometer (V-530 Jasco) at 247nm. The continuous dissolution method USP XXIII was used by simulating conditions of the GI tract. In this study capsules were added in 700 ml of 0.1 N HCl (pH 1.2) for 2 h. At the end of 2 h 233.3 ml of tribasic sodium phosphate was added to all the dissolution vessels and the pH was adjusted to 6.5 (1hr), 6.8 (2 hr) and 7.2 (till end of test) by using 2 M NaOH or 2 M HCl.

Stability study

To investigate the stability of the prepared nanocapsules, the change in particle size and % E.E of the nanocapsule dispersion kept at 2-8 °C and 25 ± 2 °C/60 % RH for a period of 3 months was examined.

Animal studies

All the animal studies were carried out following the guidelines of CPCSEA. Prior permission was taken for animal experiments from institutional ethical committee.

Inverted Sac method

Intestinal deposition of budesonide was evaluated based on comparison between entrapments of nanocapsular budesonide as compared to pure budesonide using inflamed colon tissue. This experiment was conducted using male adult wistar rats weighing 270 ± 20g. The animals (approx 300 gm) were fasted for 16 hours with access to water ad libitum. Each rat was sedated by brief respiration of ether. A flexible polyethylene tube (outside diameter 2 mm) was inserted into the colon to 8 cm and 2 ml of acetic acid (3% v/v in 0.9% saline) or saline alone (control animals) infused into the colon. The acetic acid/saline was retained in the colon for 30 seconds, after which fluid was withdrawn. The rats were killed after 24 hours by cervical dislocation. The intestine was dissected and immediately inverted with the aid of a cylindrical flexible rod, for exposure of the mucosal surface. The inversion of the segment of intestine was performed carefully to avoid morphological damage. Due to the absence of blood and nerves these tissues have low viability. However, when kept at 37°C in Krebs solution, these segments were morphologically intact and metabolically active for about 2 hours. An intestinal segment of about 8.0 cm in length was gently washed with Krebs solution. The intestinal segment thus obtained was closed at one end with the aid of cotton thread, and filled with Krebs solution. The other end was closed forming an inverted intestinal sac, which was immediately incubated in a previously oxygenated Krebs solution, with the addition of nanocapsular budesonide/ pure budesonide at a concentration of 9 mg/40 mL. The incubation medium was kept under mild agitation while oxygenation was maintained by an oxygen pump. The quantity of budesonide absorbed was determined after 120 min. of incubation. After this time, the intestinal segment was removed from the incubation medium and thoroughly
was washed in the physiologic solution. The contents of the intestinal sac and organ tube were filtered in a 0.22 mm pore membrane separately. The concentration of budesonide in the medium was determined by spectrometry using a modified procedure. The tissue was homogenized using 5 ml methanol and the content was centrifuged at 2000 RPM for 15 minutes. The entire content was filtered using 0.45 micron membrane filter and the obtained solution was added to the contents of intestinal sac and the volume was made up to 10 ml using methanol. Methanol is used for extraction of drug from tissue because drug is highly soluble in methanol and UV method is also developed so that the drug content could be found out by scanning the obtained solution after proper dilution. Similar solution was prepared using another colitis induced intestinal tissue without addition of drug and it was used as blank to nullify any hindrance due to tissue remnants during absorbance.

*Myeloperoxidase (MPO) activity*:

MPO is an enzyme present in neutrophils, macrophages and monocytes at a much lower concentration. It has been demonstrated that the level of MPO activity is directly proportional to the amount of neutrophils infiltrated in the inflamed tissue. Thus the amount of MPO was determined in the colonic tissue of inflammation induced animals and normal animals. The animals were grouped in four groups; test group, standard group, test control group and normal control group each containing 6 animals. All animal experiments were performed in compliance with the guidelines of ethics committee. Except normal control group all the three groups were induced by colitis 0.3% using acetic acid method as described in the previous section. For the next 5 days test and standard group received dosing of 0.9 mg of nanocapsular budesonide and pure budesonide respectively while both the control groups received normal saline. On the fourth day the animals were kept for fasting except for water ad libitum and dosing. At the end of fifth day the animals were sacrificed and the colon was isolated for further procedure.

The colonic tissue (~8 cm) was isolated and washed with the phosphate buffer pH 6.0, then it was minced finely and homogenized in 3.0 ml of ice cold 50 mM potassium phosphate buffer containing 0.5% HTAB. The homogenate was freeze thawed three times. Then the suspension was removed and the homogenizer washed twice with 1 ml HTAB, the washes being added to the suspension then the suspension was centrifuged at 11000 RPM for 20 min at 4°C. The supernate was collected and reserved and the pellet was extracted 2 additional times with 5 ml HTAB and the extract added to the supernate and assayed.

**Table 3: Composition of nanocapsule dispersion for detailed studies.**

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Composition (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Budesonide</td>
<td>10 mg</td>
</tr>
<tr>
<td>Benzyl benzoate</td>
<td>2 ml</td>
</tr>
<tr>
<td>Eudragit S 100</td>
<td>100 mg</td>
</tr>
<tr>
<td>Pluronic F 68</td>
<td>125 mg</td>
</tr>
<tr>
<td>Acetone</td>
<td>25 ml</td>
</tr>
<tr>
<td>Water</td>
<td>50 ml</td>
</tr>
</tbody>
</table>

**Table 4: Scoring system for histopathological assessment of induced colitis.**

<table>
<thead>
<tr>
<th>Scoring parameter</th>
<th>Score definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td>severity</td>
<td>0: None</td>
</tr>
<tr>
<td></td>
<td>1: Mild</td>
</tr>
<tr>
<td></td>
<td>2: Moderate</td>
</tr>
<tr>
<td></td>
<td>3: Severe</td>
</tr>
<tr>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td>extent</td>
<td>0: None</td>
</tr>
<tr>
<td></td>
<td>1: Mucosa</td>
</tr>
<tr>
<td></td>
<td>2: Mucosa and submucosa</td>
</tr>
<tr>
<td></td>
<td>3: Transmural</td>
</tr>
<tr>
<td>Crypt damage</td>
<td>0: None</td>
</tr>
<tr>
<td></td>
<td>1: Basal 1/3 damaged</td>
</tr>
<tr>
<td></td>
<td>2: Basal 2/3 damaged</td>
</tr>
<tr>
<td></td>
<td>3: Crypts lost, surface epithelium present</td>
</tr>
<tr>
<td></td>
<td>4: Crypts lost, surface epithelium lost</td>
</tr>
</tbody>
</table>

MPO in the supernate was assayed spectrophotometrically; 0.1 ml of material to be measured was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0 containing 0.167
mg/ml o-dinisidine dihydrochloride and 0.0005% hydrogen peroxide. These conditions were employed because they confer increased sensitivity to the assay. The change in absorbance at 460 nm for 2 minutes was measured using spectrophotometer. One unit of MPO activity was defined as that degrading one micromole of peroxide per minute at 25°C.

Histopathology study.20,21

Rats were euthanized 24 hrs after the treatment for 5 days as described in the previous section. Using the distal portion of the colon (8 cm), microscopic damage score was calculated. For this, full thickness biopsy specimens were fixed in 10% buffered formalin solution, embedded in paraffin, stained with haematoxylin and eosin (H & E) and subjected to the histopathological studies. Microscopic evaluation was performed by a pathologist unaware of the study design. The histological scoring was carried out according to the criteria shown in Table 4.

Results and Discussion

Preoptimization Studies

Comparative study of effect of Magnetic Stirring and Probe Sonication on Particle Size of Nanocapsules

The formulations F1 and F2 have shown the particle size of 2993nm and 1674nm which were prepared by magnetic stirring and probe sonication respectively. This indicates that Probe Sonication reduces the particle size of Nanocapsules to a higher extent as compared to magnetic stirring. The mechanism behind the reduction of particle size by Sonication is due to creation of bubbles (cavitation) followed by collapse which releases shock waves along with temperature and pressure changes for nucleation. Ultrasonic waves were found to cause i) faster and more uniform nucleation through the sonicate volume, leading to smaller and more uniform-sized particles; and ii) reduction of agglomeration by reducing contact between particles and controlling the number of nuclei.9,10

Preliminary Trials

The objective of the study was to prepare smallest nanocapsules for colon specific drug delivery. Polymer and Surfactant concentration affect particle size, entrapment efficiency and lag time for colon specific drug delivery. Therefore preliminary study was done to set the level of these factors (polymer and surfactant) for subsequent optimization by design of expert.

Study of Effect of Sonication time on particle size of nanocapsules(Batch B4) :14,12

As sonication time was increased from 3 min to 8min the particle size first decreases up to 5min and then goes on increasing further. Minimum particle size was obtained at 5min. The reason behind the increase in particle size after 5min is that, as particle size decreases, the forces of attraction between particles like Van der Waals, electrostatic, and capillary forces become dominant as compared to gravitational force. If we neglect electrostatic force due to the absence of charge on particle and the capillary force due to hydrophobicity of particles, Van der Waals forces will be mainly responsible for the agglomeration of nanoparticles. Hence sonication time was optimized to 5min for further studies14,12.

Statistical Design for Optimization of Nanocapsules22,23,24

The experiments were designed using 3² full factorial design to study the effect of two independent variables at three levels on particle size and percent drug entrapment efficiency and lag time.

Experimental Design

Nanocapsules were prepared using nanoprecipitation method. Factorial designs with 2 factors, 3 levels, and 9 runs was selected for the optimization study.

The formulations were prepared by 3² full factorial design and the responses obtained are shown in Table 5.
Table 5: $3^2$ full factorial experimental design with measured responses.

<table>
<thead>
<tr>
<th>Std run</th>
<th>Polymer (mg)</th>
<th>Surfactant (mg)</th>
<th>Particle size (nm)</th>
<th>% EE</th>
<th>Lag time (hrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>O 1</td>
<td>50</td>
<td>50</td>
<td>690</td>
<td>78</td>
<td>3hrs 30min</td>
</tr>
<tr>
<td>O 2</td>
<td>50</td>
<td>87.5</td>
<td>500</td>
<td>79</td>
<td>3hrs 30min</td>
</tr>
<tr>
<td>O 3</td>
<td>50</td>
<td>125</td>
<td>480</td>
<td>82</td>
<td>4hrs</td>
</tr>
<tr>
<td>O 4</td>
<td>75</td>
<td>50</td>
<td>780</td>
<td>84</td>
<td>4hrs</td>
</tr>
<tr>
<td>O 5</td>
<td>75</td>
<td>87.5</td>
<td>690</td>
<td>82</td>
<td>4hrs</td>
</tr>
<tr>
<td>O 6</td>
<td>75</td>
<td>125</td>
<td>660</td>
<td>85</td>
<td>4hrs</td>
</tr>
<tr>
<td>O 7</td>
<td>100</td>
<td>50</td>
<td>740</td>
<td>87</td>
<td>4hrs 30min</td>
</tr>
<tr>
<td>O 8</td>
<td>100</td>
<td>87.5</td>
<td>720</td>
<td>89</td>
<td>4hrs 30min</td>
</tr>
<tr>
<td>O 9</td>
<td>100</td>
<td>125</td>
<td>700</td>
<td>90</td>
<td>4hrs 30min</td>
</tr>
</tbody>
</table>

Table 6: Summary of results of regression analysis for responses Y1, Y2 and Y3.

<table>
<thead>
<tr>
<th>Model</th>
<th>R2</th>
<th>SD</th>
<th>%CV</th>
<th>Remark</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response Y1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quadratic</td>
<td>0.9</td>
<td>28.7</td>
<td>4.35</td>
<td>Suggested</td>
</tr>
<tr>
<td></td>
<td>710</td>
<td>9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Response Y2</td>
<td></td>
<td></td>
<td></td>
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Regression equations of the fitted model:

\[
\begin{align*}
Y_1 &= +684.44+81.67*A-61.67*B+42.50*A*B-71.67*A^2+38.33*B^2 \ldots (2) \\
Y_2 &= +83.00+4.50*A+1.33*B-0.25 *A*B+0.50*A^2+1.00*B^2 \ldots (3) \\
Y_3 &= +3.94+0.42*A+0.083*B-0.13*A*B+0.083*A^2+0.083*B^2 \ldots (4) 
\end{align*}
\]

Analysis of Experimental results

Analysis of experimental results was done by using the Stat-Ease Design Expert. After filling the data in the design, quadratic model were suggested to run the design. F-values, P-value and model F-value for average particle size, %EE and lag time was obtained from ANOVA. The selection of model and polynomial equations generated for average particle size, %EE and lag time of nanocapsule dispersion listed in Table 6. Nine coefficients (B1 to B9) were calculated with B0 as the intercept.

\[
Y = B_0 + B_1A + B_2B + B_3AB + B_4A^2 + B_5B^2 \ldots \ldots (1)
\]

The equation can be used to obtain estimates of the responses.

For particle size response, the Model F-value of 20.10 implies the model is significant. There is only a 1.63% chance that a "Model F-Value" this large could occur due to noise. P values were found to be 0.0500, less than 0.100 indicate model terms are significant. "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. The obtained ratio of 13.579 indicates an adequate signal. This model can be used to navigate the design space. The value of \( r^2 \) for particle size was found to be 0.9710

For %EE response, the Model F-value of 15.92 implies the model is significant. There is only a 2.27% chance that a "Model F-Value" this large could occur due to noise. P values were found to be 0.0227, less than 0.0500 indicate model terms are significant. "Adeq Precision" measures the signal to noise ratio. The ratio of 10.977 indicates an adequate signal. The value of \( r^2 \) for EE was found to be 0.963.

For Lag time response, the Model F-value of 14.49 implies the model is significant. There is only a 2.60% chance that a "Model F-Value" this large could occur due to noise. P values were found to be 0.050, less than 0.0500 indicate model terms are significant. "Adeq Precision" measures the signal to noise ratio. The ratio of 10.423 indicates an adequate signal. The value of \( r^2 \) for EE was found to be 0.9602

Since the values of \( r^2 \) are quite good for all responses, i.e., 0.9710 for particle size and 0.963 for %EE and 0.9602 for lag time, the polynomial equations form excellent fits to the experimental data and are highly statistically valid.

The value of the correlation coefficient (\( r^2 \)) of particle size in equation (2) was found to be
0.9710, indicating good fit. The values of average particle size for different runs were ranging from 480nm to 780nm, indicating the influence of selected variables on the average particle size. This is also reflected by the wide range of values for coefficients of the terms of Equation (2). The interaction terms (X_1X_2, X_1X_3, X_2X_3, X_1^2, X_2^2, and X_3^2) show how the average particle size, percent entrapment efficiency and lag time changes when 2 variables are simultaneously changed. The negative coefficients for all 2 independent variables indicate an unfavorable effect on the response variables, while the positive coefficients for the interactions between 2 variables (X_1X_2, X_1X_3, and X_2X_3) indicate a favorable effect while the small coefficients in Equation 2, 3 and 4 indicate that these terms contribute the least in prediction of average particle size, entrapment efficiency and lag time.

The value of the correlation coefficient (r^2) of Equation 3 was found to be 0.963, indicating good fit. Among the independent variables selected and their interactions, only X1 was found to be significant (P < .05), indicating a major contributing effect of X1 on entrapment efficiency. A positive value of the coefficient for X1 (polymer conc.) indicates a favorable effect on entrapment efficiency. High polymer content enhances the entrapment of budesonide in the nanocapsule while a negative value of coefficient for X2 indicated unfavorable effect of surfactant on entrapment efficiency of budesonide in nanocapsules.

The relationship between the independent and dependent variables was further elucidated by constructing contour and 3-D response surface plots. The effects of X1 and X2 on response variables are shown in Figure 1-3.
Search for optimum formulations

Among the various formulations optimum formulations were selected on the basis of Desirability factor (Table 7). Formulations having highest desirability factor were selected among the obtained solutions. Criteria for the selection were primarily based upon the highest possible values of %EE and Lag time, lowest possible values of particle size. Selection criteria were as per below:

For region: Particle size 450-750nm
%EE 85-90%,
Lag time 3.5-4.5hrs.

Figure 1: 3-D response surface plot showing the influence of polymer and surfactant concentration on particle size of nanocapsules

Figure 2: 3-D response surface plot showing the influence of polymer and surfactant concentration on %EE

Figure 3: 3-D response surface plot showing the influence of polymer and surfactant concentration on Lag time

The response surface diagrams, known to facilitate an understanding of the contribution of the variables and their interactions, and the respective contour figures and 3 D response surface plots (Figure 1-3) were shown for effect of variables on responses such as when the surfactant concentration increases particle size is not affected much, but when polymer concentration increases it has been seen that the particle size increases. It is evident that both the factors alone are not capable of increasing particle size, but when both are increased simultaneously there is rapid increase in the size of nanocapsules. Also it is very noticeable that when polymer concentration is less and surfactant concentration is more there is not much increase in the particle size due to less rigidity. It is also observed that there is very less effect of surfactant on the particle size. Reason behind increase in particle size only after increase in polymer must be attributed to its rigidising property and the moment more amount of surfactant is added it rigidises it in the same state to form large nanocapsules, when polymer in low concentration and high amount of surfactant smaller nanocapsules observed due to less rigidity of structure. For less amount of polymer no matter how much surfactant is present its evident to form small nanocapsules due to low polymer content as exhibited by formulations O1, O2, O3.

Similarly it is very noticeable that when polymer concentration is less and surfactant concentration is more there is not much increase
in the entrapment efficiency. It is also observed that there is very less effect of surfactant on the entrapment efficiency. Reason behind increase in entrapment efficiency only after increase in polymer must be attributed to its rigidising property and the moment more amount of surfactant is added it rigidises it in the same state. While when polymer in low concentration added high amount of surfactant less increase in entrapment efficiency due to less rigidity of structure. For less amount of polymer no matter how much surfactant is present its evident to form nanocapsules with less entrapment efficiency due to low polymer content.

Similarly when polymer concentration is less and surfactant concentration is more there is not much increase in the lag time. It is also observed that there is very less effect of surfactant on the lag time. Reason behind increase in lag time only after increase in polymer must be attributed to its rigidising property. For less amount of polymer no matter how much surfactant is present its evident to form nanocapsules with less lag time due to low polymer content.

Same kind of results were obtained by Fessi et al 2006

Response surface analysis

From three dimensional (3D) response surface graphs Figure 1-3, it was observed that, higher polymer concentration causes increase in particle size due to bulkiness of polymer and rigidization due to surfactant at the same time. Surfactant has very less influence on particle size, higher the surfactant concentration, lower the particle size depending on concentration of polymer. Increase in surfactant concentration causes slight decrease in particle size due to reduction in interfacial tension between aqueous and oil phase which lead to the formation of nanocapsules of smaller size thereby stabilize the particles by forming a steric barrier on particle surface and by protecting the particle from coagulation.

Validation of the response surface methodology

The selected optimum formulations were prepared and the particle size, entrapment efficiency and lag time were determined. The validation of optimum design was conducted through comparison of the observed responses with that of the predicted responses along with residual error, for knowing whether the used statistical design is correct or not. All the responses are shown in Table 7. The plots between the observed and the predicted responses, forced through the origin, for the three response variables along with the linear plots found to be highly linear, values of R² were 0.978, 0.979 and 0.988 for particle size, entrapment efficiency and lag time respectively. Hence, the prognostic ability of the experimental design to predict particle size, entrapment efficiency and lag time of prepared nanocapsules was validated.

Evaluation of nanocapsules

Particle size determination

The particle size analysis of the nanocapsule showed particle size in the range between 480nm to 780 nm as shown in Table 34. Particle size of optimized formulations S 1, S 2, S 3 was found to be 712 nm, 710 nm and 713 nm respectively. Particle size distribution curve of optimized sample S 1 is shown in figure 35. The low particle size was attributed to effect of sonication surfactant which imparted charge to the nanocapsules thus preventing the aggregation of Nanocapsules. Polydispersity index (PDI) is a measure of droplet homogeneity and it varies from 0.0 to 1.0. Polydispersity is the ratio of standard deviation to mean droplet size; hence, it indicates the uniformity of droplet size within the formulation. Higher the polydispersity, the lower the uniformity of the droplet size in the formulation. The closer to zero the polydispersity value the more homogenous are the droplets. The polydispersity index was found to be between 0.229 for optimized formulations which indicates homogenous nature.
Zeta (ζ) potential determination

ζ Potential or electrokinetic potential is defined as the difference in the potential between the surface of particle and the electroneutral region of the solution. It governs the degree of repulsion between the adjacent particles. The electrical double layer formed around the particle also moves along with the particle when particle is in motion in the solution. Generally, an increase of electrostatic repulsive forces between nanocapsules prevents the coalescence of particles. On the contrary, a decrease of electrostatic repulsive forces will cause phase separation. In this study we got the zeta-potential of all formulation less than -30 mV. The zeta potential value of optimized formulation was -41.2 mV. The high zeta potential value suggests good long term stability. This is in agreement with the observed stability data during storage.

Percent entrapment efficiency (%EE):

Percent EE of the nanocapsule dispersion was found to be in the range of 78% to 90%. %EE of optimized formulation was found to be 89%. It can be seen that, polymer concentration shows positive influence on entrapment efficiency as samples O7, O8 & O9 show %EE 87%, 89% & 90% respectively since these formulations have more polymer content and hence form rigid nanocapsules. While samples O1, O2 & O3 show less %EE (78%, 79% and 82%) due to less polymer content. Fessi.et al found similar results in the optimization study of nanocapsules.

In vitro dissolution study of spray dried Nanocapsules

Independent of type of formulation, the transit time of the formulation through the small intestine is constant i.e. 3-4 hrs. So the average time required for any formulation to reach colon is considered to be 4-5hrs. As the transit time in the small intestine is relatively constant (approximately 3-5hrs) same as that represented by simulation of small intestine was divided in three parts; proximal part of small intestine with pH 6.5 and residence time 1hr, lower part of small intestine with pH 6.8 and residence time 2hr and finally terminal ileum with pH 7.2 till end of test. Thus the continuous dissolution test was carried out for Nanocapsules with pH 1.2, 6.5, 6.8 and & 7.2 for 2, 1, 2 hrs and till end of study respectively (Figure 8).

Letters a, b and c indicate p < 0.05 compared with the colitis control group, the standard budesonide-treated group, and the normal group respectively.

(n=3)Mean ± SD

1. At pH 1.2 (simulated stomach) and pH 6.5 (proximal part of small intestine) none of the formulation released drug more than 10% except O1, O2 and O3.

2. At pH 6.8 release was observed to be 13-14% in 4hrs except Nanocapsules with formulations O1, O2, O3 & O4. This shows 82% drug release after 4hrs.

3. At pH 7.2 all formulation shows more than 85% of drug release corresponding to 4.5hrs lag time which is approximate time for any formulation to arrive in colon.

4. Drug release from Nanocapsules is attributed to complete dissolution of Eudragit S100 polymer. Since it contains COOH groups which get easily hydrolised only at higher i.e. alkaline pH facilitates release of drug from nanocapsules.

5. It was observed that the lag time at pH 7.2 increased simultaneously with an increase on polymer concentration (O7, O8 & O9) because of increased path length and tortuosity. Polymers used for targeting the drug at the most distal part of the GI tract should withstand the lower pH values of the stomach and proximal part of small intestine and were able to release drug at neutral to alkaline pH of the ileum. The formulation with lower polymer were not selected for subsequent studies because of disadvantage like decreased lag time which may correspond to intestine and initial part of inflamed area not getting covered for local effect of the drug. So O7, O8, O9 were selected as optimum which shows drug release after 4hrs corresponding to coloni arrival.
Evaluation of Spray Dried Nanocapsules

The choice of drying method is usually determined by the ability of the preparation contents to resist against the drying stress as well as the final product functions of use. In the spray drying NC case, these technical problems have to be solved (1) the preservation of the particles integrity and (2) the promotion of dried particles with adequate sizes permitting separation from the drying fluid. NC is characterized by a great specific surface that requires protection from the thermal stress during drying because of the polymer nature of the wall and the oil nature of the core. Thus colloidal silicon dioxide appears as a candidate of choice to cover the NC surface and stabilise them with a solid matrix because it also develops a great specific surface and possesses a good thermal conductivity interesting for water removal. This silica is a biocompatible amorphous carrier largely used as tablets excipients, considered by the FDA and other regulatory agencies as a safe component for non-parenteral administration of drugs. The prepared spray dried NC was characterized with respect to yield determination, shape, entrapment efficiency, lag time, crystallinity, density moisture content.

Percent Process Yield

The recovered spray dried particles were the sum of the powder collected in the receptor and that removed by sweeping the cyclone wall with a small brush. The spray-dried particles were of cohesive nature as a result of their very small size and/or their moisture content results in inefficient collection in the receptor.

Yield of optimized formulations S 1, S 2 and S 3 was found to be 49.22%, 49.79% and 49.49% respectively.

Moisture content

The residual moisture content varies from (1.02 to 1.10%) of the nanocapsules. The moisture of batches S1, S2 & S3 were found to be 1.02, 1.10 &1.08 respectively. The higher moisture content values were obtained with formulation S 2. Increasing the additive concentration resulted in slight decrease of the moisture content. The final moisture level of the spray-dried powders is mainly determined by the nature of the material due to its interactions with water molecules. The solubility, hygroscopic character, thermal conductivity, molecule’s size are among the intrinsic properties determining these interactions. Another factor which has a major impact on the moisture level of the powder is the outlet temperature. Generally, lower moisture content is associated to higher outlet temperatures.

Density determination

Density of optimized batches S1, S2 & S3 were found to be 0.456, 0.461 &0.449 respectively. These results show that with increase in polymer concentration density of nanocapsules increases. The density modifications could be explained by two reasons: (i) the Nanocapsule presence in the reservoir system and (ii) the formation of spherical nanocapsules with internal closed space as commonly seen with the spray-drying process. It has been shown that with low viscosity feeds; there is much likelihood of high occluded air content with in the particles. In general the particles density increases as the feed concentration increases. With low concentrated feeds less dry substance will be kept in a droplet after atomization causing the formation of a light particle.
Effect of Spray drying

A spray-dried nanocapsule should have certain desirable characteristics, such as preservation of the primary physical and chemical characteristics of the product provide an acceptable relative humidity, and Long-term stability. The particle size of the three optimized formulations after re-dispersion was 710-713nm which does not shown significant difference (< 5 nm) as compared to nanocapsule dispersion. There was no significant change in particle size of nanocapsules before and after spray drying.

Powder characteristics

The value of angle of repose of powder was found to be in the range of 17.9 to 19.5 while Carr’s index value was between 10.41 to 13.37 which indicates excellent flow property of the powder. All the studies were done in triplicate.

Solid state study

Drug content determination

Drug content of optimized formulations was found in range of 96%. No significant change in drug content was observed.

Scanning electron microscopy (SEM)

The SEM analysis shows that the spray dried powders are spherical particles, a characteristic that usually leads to a free flow and represents an important characteristic for the application of spray dried powders as an intermediary pharmaceutical product. However in this case, the most interesting characteristics observed were related to the spray dried particle surfaces. While the particles of Aerosil 200 obtained from suspensions usually show a rugged surface with the presence of some cavities, the particles prepared from mixture of Aerosil 200 and nanocapsule entrapped with Budesonide do not show the presence of cavities, it was clearly observed in the nanocapsules adsorbed on the surface of silicon dioxide. There was no detectable sign of breaking of the nanocapsules. The micrographs of the surface of the spray dried powder surface showed that nanocapsules remained intact and no change in shape was detected after spray drying process. (Figure 5).

DSC study

DSC thermograms were recorded to study the thermal behaviour of the drug. The DSC curves of budesonide loaded nanocapsules and Budesonide are shown in Figure 6. The appearance of sharp endothermic peak of the drug is due to its crystalline nature, so it is confirmed that Budesonide is crystalline. The thermogram of spray dried Budesonide nanocapsules showed endothermic peak at 236.53°C. Characteristic peak of Budesonide (near its M.P.245-255°C) was not observed in Budesonide nanocapsules, indicating that Budesonide was totally entrapped in nanocapsules. The melting endothermic peak of Budesonide loaded nanocapsules was slightly lower. The intensity of peaks was reduced or peak broadening was observed possibly due to solubilization of the drug in selected excipients. No new endothermic or exothermic peaks were observed in the Budesonide loaded nanocapsules, indicating no incompatibilities. DSC results suggest the absence of any interaction between drug and selected excipients. The melting point depression might be due to the increased lattice defects resulting from drug incorporation, small particle size (nanometer range), their high specific surface area and presence of surfactant. These observations were similar to the observations of PeiRan zhang et al.29

Figure 5: (a) SEM of Budesonide loaded nanocapsules (b) SEM of Aerosil 200
In vitro drug release study of optimized formulations

Drug release study was also carried out for optimized batches obtained using design expert. The release was studied for eight hours. The comparative release patterns of the three solution batches were studied and the standard error of mean for each hour was calculated for each batch which is shown in the figure 7 which demonstrates similar result as per previous batches.

Animal studies

Inverted Sac method

Studies have revealed that nanocapsules have a tendency to accumulate to a greater extent in inflamed tissue. To test this, in this study the intestinal inverted sac method was used to evaluate the effect of nanocapsular budesonide on the entrapment of drug as compared to pure drug. The percent drug entrapped for nanocapsular budesonide and plain budesonide are 21.86 ± 2.52 and 15.90 ± 2.68 respectively. The concentration of drug remaining outside the tissue i.e. in the organ tube was also determined which represents the unentrapped drug and it was found to be 76.48 ± 4.053 and 84.08 ± 2.67 for nanocapsular budesonide and plain budesonide respectively. This shows that the entrapment of nanocapsular drug is much more as compared to the plain drug which proves our hypothesis. The minor difference in the amount of drug added and the sum of entrapped and unentrapped drug can be attributed to the limitations of the extraction process of complete drug from the tissue.

Figure 6: DSC thermograms of (A); Pure Budesonide (B); Budesonide loaded nanocapsules

Figure 7: Drug release from S 1-S 3 batches. (with standard error of mean)

Data shows mean (n = 3). Series1=S 1, Series2=S 2, Series3=S 3

Figure 8: Effect of nanocapsular budesonide on MPO activity in inflamed colon tissue

Myeloperoxidase (MPO) activity

MPO is located within the primary granules of neutrophils. Acetic acid induced inflammation of colon is reported to result in neutrophils infiltration (Bradley et al). The results of MPO activity determined in the colon tissue of different groups. Figure 8; they are in confirmation with the findings of Bradley et al. In this study, the inflammation induced due to acetic acid led to the infiltration of neutrophils which in turn led to MPO release in the inflamed tissue. This is evident from the significant increase in MPO activity of control group as compared to the normal group. Decrease in the activity of MPO in tissue is the sign of repair and healing of the tissue. MPO activity after use
of standard (11.14652 uMPO) and nanocapsular budesonide (5.23164 uMPO) signifies the same when compared with the control group (19.05123 uMPO). At the same time comparative study between standard and test group shows the higher efficiency of test group over standard group, thus we can conclude that the nanocapsules are effective to cure inflammation at a faster rate as compared to plain drug.

MPO activity is a useful index for evaluating granulocyte infiltration in colonic tissues following induction of colitis. There was increased MPO activity in the acetic acid-induced colitis rat model, implying that leukocytes were recruited. These results were in accordance with the prior studies showing an increase in MPO activity in experimental colitis. MPO catalyzes the formation of such potent cytotoxic oxidants as HOCI from H2O2, chloride ions and N-chloramines in neutrophils. Our results showed that the nanocapsular budesonide treatment inhibited MPO activity in rat model of UC, suggesting that nanocapsules can interrupt the feedback loop between upregulation of inflammatory mediators and the recruitment of leukocytes. Meanwhile, nanocapsular budesonide lessened the demolition of the intestinal tract barrier and inflammation aggravation in UC caused by the damage of intestinal mucosa. As a result, nanocapsules can act as an efficient agent for treatment of UC.

All data were expressed as the mean ± SD and analyzed using software GraphPad Instat 3. One-way analysis of variance (ANOVA) was used followed by bonferroni’s test for multiple comparisons for MPO activity, and an unpaired Student’s t-test was used to evaluate the level of statistical significance in the inverted sac study. Differences with a p<0.05 were considered statistically significant.

The activity of MPO in the colitis control group was higher than that in the normal group (p < 0.001). When rat with colitis were treated with nanocapsular formulation of budesonide and plain budesonide (pure drug), the MPO activities were significantly lower than that of the colitis control group (p < 0.001 and p<0.05 respectively). Further MPO activity in the nanocapsules-treated group was significantly lower than that in the standard budesonide-treated group (p < 0.01)

In Vivo Study

Histopathology study

Rats in the colitis control group had pasty-to-liquid grossly bloody stool from day 2 to day 5. In contrast, rat in standard and test groups showed better formed stools with no evident blood. Similar results were found in previous study by Yao J et al.21

Figure 9: Histopathological findings of different groups

Normal: Intact mucosa. Control: Discontinuous mucosa and neutrophil infiltration Standard: Discontinuous mucosa but little neutrophil infiltration still observed. Test: Repaired mucosa and no neutrophil infiltration

Histological features in the acute phase of UC are mucosal erosions, crypt shortening, oedema, and infiltration of neutrophils in the mucosa and lamina propria. Compared to the normal group (Figure 9 A), the colitis control group exhibited marked erosion of the lamina propria, mucosa, disappearance of glandular epithelium, and inflammatory cell infiltration (Figure 9 B).
the standard budesonide-treated group (Figure 9 C) and nanocapsule formulation-treated group (Figure 9 D), erosion, disappearance of glandular epithelium, and inflammatory cell infiltration tended to be less severe and the histological disease scores were significantly lower than those in the colitis control group. Compared to the standard budesonide-treated group, the nanocapsular budesonide treated group had a strikingly lower histological disease score as the haemorrhages was less prominent, upper mucosa was completely repaired and infiltration of neutrophils was completely absent, no mucosal congestion was observed, the glandular cells were also properly organized and no crypt damage was observed. (Figure 9 D), while for standard budesonide treated group there were few haemorrhages observed along with the visible space between mucosa and lamina propria. Colonic mucosa was still not intact, though infiltration of neutrophils was also not observed. Thus comparatively test group rats were recovered fast from acute UC as compared to the standard group animals.

Daily administration of nanocapsular budesonide for 5 days significantly reduced mucosal inflammation and improved symptoms of colitis in rat, as demonstrated by a reduction of indices of colitis at the microscopic levels. Histopathology study showed the better recovery of tissues of test group which was treated with nanocapsular budesonide, the results of MPO activity supports the above observation as there was marked reduction in MPO activity as compared to the control group (19.05123 uMPO). The reason for this phenomenon could be attributed to the higher degree of accumulation of nanocapsular budesonide in the inflammatory area which is evident from the results of inverted sac method. Histological changes, as indicators of the presence of an inflammatory injury, were remarkably ameliorated by nanocapsular treatment, suggesting that nanocapsular budesonide have a fast and efficient repairing effect on colonic tissue. Figure 10.

![Figure 10: Data of histopathological evaluation of colitis in control and treatment groups after 5 days treatment](image)

1=Normal, 2=Control, 3=Standard, 4=Test, IS=Inflammation Severity, IE=Inflammation Extent, CD=Crypt Damage, TMS=Total Microscopic Score.

The overall animal study showed that standard budesonide as the positive control has a similar depressant effect on the inflamed colonic tissue, in consistence with a previous report demonstrating that standard budesonide was a precious anti-inflammatory agent. Nanocapsular budesonide might be more effective as an anti-inflammatory agent due to specific accumulation of nanocapsules in the inflammatory area which is shown by the inverted sac method.

From the data it is evident that the MPO is a marker for tissue neutrophils contents. We propose that the tissue MPO content may be useful as a measure of the neutrophils inflammatory response in a variety of clinical and experimental states. Of course, use of this approach in humans depends upon adaptation of the technique specifically for human cells and tissue.

In conclusion, this short-term pilot study has provided evidence that nanocapsular budesonide has therapeutic potentials for patients suffering from UC, as it may accumulate and target drug molecules by membrane to membrane transfer of drug at the inflammation site (colon cells) in the colon of patients with active disease.

**Stability studies**

In the stability test performed at 25 ± 2 °C/60 % RH. As expected the spray-dried nanocapsules clearly showed enhanced stability compared to
that of nanocapsule dispersion. This increased stability in spray-dried nanocapsules was probably caused by the protection from the moisture that was essentially added in the formulation. In general, drug stability increases in a solid state as compared to a solution state especially a drug is degraded by a hydrolysis mechanism. Thus, from the results obtained so far, it is believed that the spray-dried nanocapsules can be used as its initial nanocapsules dispersion by simple agitation but it offers a considerably increased stability. No significant reduction in the content of the active drug was observed over a period of three months indicating stability of formulation. However, storage temperature not exceeding 25°C and moisture proof packaging are essential to ensure stability of these formulations.

Conclusions

Nanocapsules have shown to be deposited in inflammatory area providing better effect. Experimental findings shown the stable budesonide loaded nanocapsules with suitable physicochemical characteristics with optimized concentration of Eudragit S 100 and Pluronic F 68. The study showed the significance of 3^2 full factorial design for optimization of nanocapsule preparation. In vitro release study shown the drug release after 4.5hrs corresponding to arrival time in initial segment of colon. Ex vivo and in vivo study demonstrated that there is higher drug deposition in colonic inflammatory area from the nanocapsular formulation. Thus nanocapsular formulation is a promising tool for releasing the drug specifically in inflammatory area for effective treatment of IBD, similar formulation with anticancer drug can be used for colonic cancer. Further studies are required to establish the efficacy of nanocapsular budesonide in human beings.

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References