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Research Article

Budesonide-Loaded Eudragit S 100 Nanocapsules for the Treatment of Acetic Acid-Induced Colitis in Animal Model

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Abstract. Nanoparticles for colon-drug delivery were designed and evaluated to solve many discrepancy issues as insufficient drug amount at diseased regions, high adverse effects of released drugs, and unintentionally premature drug release to noninflamed gastrointestinal regions. Herein, the prepared budesonide-loaded Eudragit S 100/Capryol 90 nanocapsules for the treatment of inflammatory bowel disease. Nanocapsules were prepared efficiently by nanoprecipitation technique and composed mainly of the pH-sensitive Eudragit S 100 polymeric coat with a semisynthetic Capryol 90 oily core. Full $3^1 \times 2^1$ factorial design was applied to obtain optimized nanocapsules. Optimal nanocapsules showed mean particle size of 171 nm with lower polydispersity index indicating the production of monodispersed system and negative zeta-potential of − 37.6 mV. Optimized nanocapsules showed high encapsulation efficiency of 83.4% with lower initial rapid release of 10% for first 2 h and higher rapid cumulative release of 72% after 6 h. The therapeutic activity of the prepared budesonide-loaded nanocapsules was evaluated using a rat colitis model. Disease activity score, macroscopical examination, blood glucose level, and histopathological assessment showed marked improvements over that free drug suspension. Obtained results demonstrate that the budesonide-loaded Eudragit S 100 nanocapsules are an effective colon-targeting nanosystem for the treatment of inflammatory bowel disease. Capryol 90 was found to be a successful, and even preferred, alternative to benzyl benzoate, which is commonly employed as the oil core of such nanocapsules.

KEY WORDS: Budesonide; Nanocapsules; Inflammatory bowel disease; Capryol 90; Acetic-acid induced colitis; Eudragit S 100.

INTRODUCTION

Colon drug delivery systems (CDDS) are an example of drug targeting systems which have promising developments in the area of local and systemic treatment. At the same time, CDDS face various challenges as reaching the distal part of the GIT presents significant physiological difficulties and environmental barriers (1). Targeting drug to the colon is highly valuable for local treatment of numerous diseases such as ulcerative colitis, Cohn's disease, and colon cancer (2).

Inflammatory bowel disease (IBD) is a relapsing, progressive, chronic inflammatory disease of bowel mucosa (3), that is more localized to the colon (4), and characterized by both long-term and short-term inflammation (5). The "IBD" term is used to describe both ulcerative colitis (UC) and Crohn's disease (CD) (6–8). Both diseases are thought to be a result of dysregulated mucosal response in the bowel function

(9). Both UC and CD usually extend over many years and it is sometimes impossible to differentiate between them (7). Treatment lines for IBD are more complex and include many categories of drugs such as 5-aminosalicylates, antibiotics, thiopurines, methotrexate, biological treatment as TNF-α antibodies, and especially corticosteroids which have more effect on the active phase (7). In contrast, systemic absorption of corticosteroids is usually associated with higher levels of adverse effects and complications on long-term treatment. Prednisolone is the most commonly applied corticosteroid member for the treatment of IBD but higher dose is required for this purpose (up to 40–60 mg/day), and many side effects are reported at this dose (10). In addition, the use of traditional dosage forms has many limitations such as extensive first pass metabolism, side effects due to drug absorption from upper gastrointestinal tract (GIT), and only small amounts of the active drug reach the inflamed areas of the colon. This results in lower therapeutic efficiency and higher side effects (1,11).

Budesonide (BSD) is a potent non-halogenated corticosteroid and it was approved by FDA for the management of ulcerative colitis on January 14, 2013 (12). BSD offers many

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advantages over prednisolone and solve many problematic issues related to corticosteroid therapy as the drug possesses potent efficacy, being given at a lower dose of 9 mg/day as a single dose, lower systemic absorption, and fewer side effects (8). Classically, BSD is mainly formulated for the treatment of many respiratory disorders like asthma and chronic obstructive pulmonary disease, either as a single therapy or in combination with bronchodilators (12). Recent trends are evolving towards reformulating the drug for the treatment of IBD. A successful BSD-based formulation for the treatment of IBD should exhibit relatively high selectivity for the colon area, an acceptable biocompatibility profile, and high drug loading.

Drug delivery to the inflamed bowel is of an increased interest as drug delivery systems in the form of nanoparticles (NP) have the ability to protect drug against the environmental conditions of GIT. Also, in this case, NP are able to passively target inflamed area, increase drug deposition at the diseased site, prolong the desired pharmacological drug effect, and lower the side effects of the drugs used in cases of cancer therapy and treatment of IBD (13–16).

Nanocapsules (NC) were prepared efficiently using nanoprecipitation technique as described previously by Fessi et al. study (17). Nanoprecipitation or solvent-displacement technique is a simple, rapid, economical, and power-saving technique used for both nanospheres and nanocapsules fabrication (18). Polymeric NC are composed of an oily core surrounded by a polymeric wall. Polymers used to prepare these NC should be biocompatible and biodegradable for biomedical applications. The polymer Eudragit S100 is commonly used in controlled-release preparations for drugs, it is negatively charged, due to the presence of terminal free carboxylic groups. Eudragit S 100 is commonly used for colon-targeted preparations as polymer molecules dissolves at higher pH of above 7 which is corresponding to the pH of ileum and colon (19).

The objective of this work was to prepare and optimize pH-sensitive NC loaded with BSD for the treatment of IBD. The optimized formula would be evaluated for its ability to minimize burst drug release in the upper GIT and to achieve controlled drug release at the inflamed area. In addition, we provide more in-depth information about the validity and effectiveness of BSD-loaded polymeric NC by *in vivo* evaluation using a relevant mouse model and histopathological examination of the resected colon tissues.

MATERIALS AND METHODS

Materials

Budesonide was generously provided by MUP (Medical Union Pharmaceuticals Company, Egypt). Eudragit S 100 was generously provided by EVONIK Röhm GmbH, Germany. Capryol 90, Labrafac CC, and Peceol were generously provided by GATTEFOSSÉ, France. Miglyol 840 was generously provided by CREMER Oleo division, GmbH, Germany. Pluronic F 68 (poloxamer 188), Span 80, Benzyl Benzoate, Acetone HPLC, and acetic acid were purchased from Sigma-Aldrich chemicals Co. All other chemicals, buffer salts, and solvents were of analytical grade and used without further purification steps.

Methods

Budesonide Oil Solubility Studies

For the preparation of polymeric NC, the most suitable oil core should have the highest possible drug solubility, the absence of polymer dissolution in the oil, absence of toxicity, and lower oil solubility in polymer (20). The solubility studies of BSD were carried out in the following oils (Labrafac CC, Capryol 90, benzyl benzoate (BnB), Miglyol 840, and Peceol). Excess amounts of BSD were added to 10 mL of each oil, the samples were vortexed (DRAGON lab, MX-S, Japan) for 15 min and then sonicated (BranSonic 220, Germany) for 30 min at 25°C. Then, they were shaken using a thermally controlled water bath (Julabo Labrotechnik, Germany) at 37°C for 48 h. The suspensions were allowed to stand for 24 h without shaking. Then, suspensions were subsequently centrifuged (HERMLE-18K, Germany, 3000 rpm) and filtered through 0.45-μm membrane filter (CHEMILAB., Spain). Finally, the samples were diluted using suitable solvent and analyzed using UV-VIS Spectrophotometer (Spectronic Genesys-2PC, USA) at maximum wavelength of 244 nm against suitable controls (21).

Polymer-Oil Compatibility: Swelling and Dissolution Studies

Polymeric films of Eudragit S 100 were obtained by dissolving 2 g polymer in acetone followed by the evaporation of the organic solvent using rotary evaporator (Stuart, RE300, Germany) (40°C, for 30 min). Slices of these films were accurately weighed (22 mg ± 2.1 mg and 75 mg ± 4.4 mg) using calibrated digital balance and then immersed separately in glass containers containing selected oils of higher BSD solubility. All containers should be tightly closed, kept away from light and stored at room temperature. The films were withdrawn from the containers, dried with smooth tissues, and accurately weighed using digital balance after a predetermined time interval (0, 3, 7, 10, 20, 30, 40, 50, and 60 days). All experiments were carried out in triplicate ($n = 3$); average weight ± SD was calculated (22).

Preparation of BSD-Loaded Eudragit S 100 NC

BSD-loaded Eudragit S 100 NC was prepared by nanoprecipitation technique according to the method described previously by Fessi et al. (17). Briefly, For the preparation of organic phase, Eudragit S 100 (1% *w/v*) and Span 80 (0.3% *w/v*) were dissolved in 25 mL of acetone at 45°C (23), then appropriate volume of Capryol 90 or BnB containing 10 mg of BSD added to the solvent phase. Finally, the organic phase was sonicated (BranSonic, Zurich, Germany) for 15 min to ensure complete solubilization of all components; then, it was added to 50 mL aqueous phase containing Pluronic F 68 (0.3% *w/v*) as a stabilizer under magnetic stirrer (250–300 rpm). The solution turned instantaneously into milky white with bluish tinge due to the formation of NC (23,24). The acetone was removed by rotary evaporator (Stuart, RE300 Germany) under reduced pressure (40°C, 30 min) and the colloidal dispersion was concentrated to the required volume. Blank formulations were prepared in the same manner, without BSD. Batches containing Capryol 90 were coded (A1–A3) while those formulated with BnB were coded (A4–A6).

Characterization of the Prepared NC

Particle Size and Polydispersity Index

The size of prepared polymeric NC was determined by Malvern-Zetasizer (nanotechnology ZS, Malvern-UK), based on the dynamic light scattering (DLS) technique. The effective average particle size of the prepared NC was obtained from three runs for each sample after suitable dilutions with Milli-Q water. The system was equipped with Helium/Neon laser type, samples were measured with non-invasive backscattering technique at angle of detection of 173°, and at a temperature of 25°C. The polydispersity index (PDI) was measured by the same instrument, PDI value up to 0.2 is considered a good NC formulation (25).

Zeta-Potential Analysis

Zeta-potential of the prepared polymeric NC were measured using Malvern-Zetasizer, after adequate dilutions of the prepared samples in water, samples were measured using the principle of electrophoretic mobility under an electric field. The average of three readings was calculated. The higher the value of zeta-potential, the more stable the prepared NC suspension is. According to literature, zeta-potential values (ζ-values) should fall within the range of -25 and -30 mV (26).

pH Measurements

The pH values were measured in undiluted suspensions of the prepared NC using a calibrated pH meter (JENWAY, UK) (22).

Encapsulation Efficiency Measurements

A specific volume of the prepared NC were centrifuged to separate the non-entrapped drug or free BSD from the loaded nanocapsules by ultra-centrifugation at 22,000×g using a cooling centrifuge at 4°C (HERMLE, 30-K) for 1 h, a free drug was determined in supernatant by diluting it with methanol: phosphate buffer (PBS) pH 6.8 as a blank and measured spectrophotometrically at 244 nm. Samples were prepared in triplicate ($n = 3$). The drug content and encapsulation efficiency were measured indirectly according to the following equations.

$$\text{Encapsulation efficiency (EE\%)} = \frac{\textbf{Total drug (mg)} - \textbf{free drug (mg)}}{\textbf{total drug (mg)}} \times 100 \quad (1)$$

$$\text{Drug content (LE)} = \frac{\textbf{amount of entrapped drug (mg)}}{\textbf{volume of nanocapsules (ml)}} \quad \text{mg/mL} \quad (2)$$

Optimization Using Factorial Design

Based on previous experimental trials, Eudragit S 100, Capryol 90, and BnB were selected for the preparation of BSD-loaded NC. For systematic optimization of BSD-loaded NC batches, full $3^1 \times 2^1$ factorial design methodology was performed using Design-Expert software (Version 11, Stat-Ease Inc., MN) to investigate the effect of formulation variables on BSD-loaded NC. Two main independent variables were investigated which were amount of polymer (X_1) and type of oil carrier (X_2). Mean particle size (Y_1: PS), polydispersity index (Y_2: PDI), zeta-potential (Y_3: ZP), encapsulation efficiency (Y_4: EE%), and percent cumulative BSD release at pH 7.4 (Y_5) were selected as dependent variables (Table I). Optimized formula was selected based upon desirability calculation.

In Vitro Drug Release Studies

A specific volume of BSD-loaded polymeric NC or free drug suspension equivalent to 1 mg of BSD was suspended in 50 mL of the release medium and incubated in a thermally controlled shaking water bath (Julabo labrotechnik, GMBH, Germany) (60 rpm, 37°C) (27). For the first 2 h, pH was maintained at 1.2 using USP simulated gastric fluid without enzymes (0.1 N HCL, 2 g NaCl, 1 L distilled water); then, the pH was raised to 6.5 for 4 h using a solution of 190.4 mg potassium dihydrogen orthophosphate, 230.7 mg disodium hydrogen orthophosphate, and pH adjusted using 1 N HCL or 2 M NaOH. Finally, the pH was raised to 7.4 till the end of the test using 1 N HCL or 2 M NaOH corresponding to the pH and transit time of ileum and colon (28,29). The release experiments were carried out under sink conditions. Aliquots of the release medium were withdrawn at predetermined time intervals, replaced with fresh release media, and centrifuged at 22,000×g for 1 h using cooling centrifuge, 4°C (HERMLE, 30-K). Samples from the supernatant containing free BSD released from the nanocapsules were measured spectrophotometrically at 244 nm.

Table I. Full Factorial Design of BSD-Loaded Polymeric NC for the Selection of Optimal Formula

Independent variables (studied factors)		Levels	
X_1: Polymer amount (mg)	150	200	250
X_2: Oil type	Capryol 90		BnB
Dependent variables (responses)		Goals	
Y_1: Particle size (nm)		Minimize	
Y_2: Polydispersity index		Minimize	
Y_3: Zeta-potential (mV)		Maximize	
Y_4: Encapsulation efficiency % (%)		Maximize	
Y_5: Percent cumulative BSD release at pH 7.4 (%)		Maximize	

All experiments were performed in triplicate ($n = 3$). The percent of cumulative BSD released was plotted *vs.* time (23,28).

In Vitro Drug Release Kinetics Modeling

Release kinetics modeling was used to analyze the mechanism of the BSD release from the prepared NC, the data obtained from the *in vitro* drug release study was analyzed using the linear regression method by the aid of Microsoft Excel software. Release data was fitted with zero-order, first-order, Higuchi diffusion, Korsmeyer-Peppas, and Weibull-model equations. The best model was selected on the basis of regression coefficient (R^2). Release model which had the highest R^2 value was considered as best fit model (19).

Surface Morphology

The morphology and the external surface characteristics of the polymeric NC were examined using scanning electron microscopy (SEM). The suspended NC were mounted on a carbon double-adhesive layer on a metal holder and coated with a thin layer of gold. The NC were then scanned at an accelerating voltage of 15 kV (Jeol JSM-5400 LV, Jeol, Tokyo, Japan) (23).

Short-Term Stability Studies

The *in vitro* stability studies of the prepared BSD-loaded polymeric NC were assessed by studying the effect of storage conditions. The prepared NC aqueous dispersion was stored at 8°C in tightly closed containers and away from light for 2 months. Samples were withdrawn and tested for particle size, PDI, pH value, zeta-potential, visual appearance, and encapsulation efficiency (26,30,31).
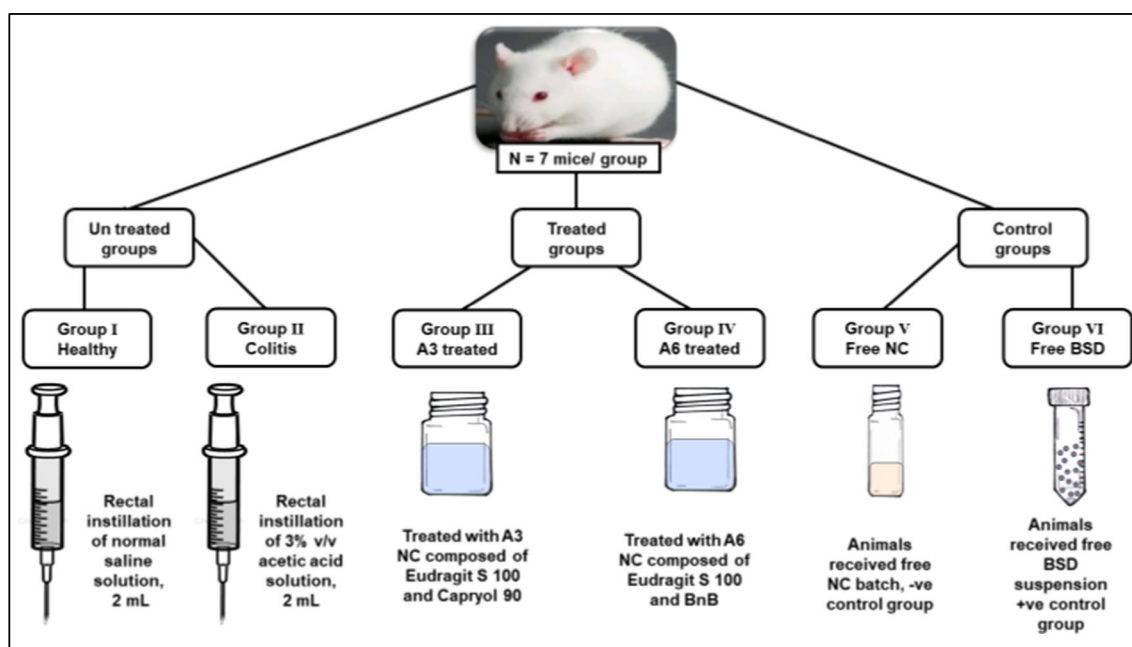
In Vivo Evaluation of BSD-Loaded Eudragit S 100 NC

Animals

All animal experiments in this study were performed under the license and approval of animal ethics committee (AEC) faculty of pharmacy, Minia University. Male Wister albino rats (220 ± 20 g), 10 weeks old, obtained from the animal house of faculty of pharmacy, Assuit University. The animals were housed in separate standard cages in controlled conditions including light-dark cycle (10:14 h.), standard temperature (23–25°C), and at relative humidity of 65% to 85%, with free access to food and water. Rats were fasted for 48 h with free access to water before induction of colitis (32–34). Rats were classified randomly into six groups. Each group contains seven rats as shown in Fig. 1.

Induction of Colitis in Animal Model

Induction of colitis was performed according to the previously reported method described by Varshosaz et al. (32), Aslan et al. (35), and Gorgulu et al. (36). Under light and ether anesthesia, a medical-grade polyurethane tube for enteral feeding (external diameter of 2 mm), with 6–8 cm length, was inserted into the anus, 6–8 cm proximal to the anus verge. About 2 mL of acetic acid solution (3% *v/v*) was instilled into the colon of all groups except healthy one (five groups). The rats were maintained in horizontal position for at least 2 min to avoid leakage of solution. The healthy group (group I) received 2-mL normal saline solution instead of acetic acid solution. All animals were left for 24 h with free access to water and food and without treatment to allow the development of IBD model. All treatment groups received a specific volume of BSD-loaded NC suspensions (groups III and IV) or free drug solution (group VI) once daily for five consecutive days; dose equivalent to 300 μg/kg/day was administrated orally by gavage. The colitis group (group II) and normal group (group I) received 0.5 mL of saline solution instead of NC suspension, while group (V)

Fig. 1. Schematic diagram of *in vivo* evaluation of BSD-loaded Eudragit S 100 NC in acetic acid-induced colitis model

Table II. Scoring System of Disease Activity Index (DAI) Based on Three Main Parameters: Body Weight, Stool Consistency, and Bleeding

Scoring parameter	Score	Score definition
Body weight	0	No weight loss
	1	1–5%
	2	5–10%
	3	10–20%
	4	>20%
Stool consistency	0	Well-formed stool pellets
	2	Pasty and semiformed stool
	4	Liquid stool, diarrhea, sticky to anus
Bleeding	0	No blood
	2	Positive findings
	4	Gross anal bleeding
Total scores (DAI)	12	

received blank NC suspension. The rats were sacrificed either after 24 h or 5 days after the last dose administration, and the distal part of the colon ≈ 6 cm in length was obtained (27).

Assessment of Colonic Inflammation

Disease Activity Index

The clinical activity of colitis model was monitored daily using the clinical activity score system or disease activity index (DAI) consisting of three main clinical parameters: weight loss, anal bleeding, and stool consistency (37) (Table II).

Macroscopic Examination of Colitis

The animals were sacrificed 24 h after the last dose administration and the distal specimens of colon; 6 cm in length was resected. All specimens were opened longitudinally and rinsed from the fecal contents. The weight of colon and colon length were measured and expressed as colon weight/length ratio and colon weight/body weight ratio as an index of colonic inflammation (38).

Blood Glucose Level

Enhanced sugar uptake and higher blood glucose level are a good indicator of the improvement of inflammation and for the potency of nanoparticles for local delivery of BSD to the intestinal mucosa (39). Therefore, all rats were treated with equal doses of budesonide NC suspension or free drug solution, and Gluco-Dr® strips (Allmedicus, South Korea) were used to measure blood glucose level daily. Data of blood glucose level was expressed in mg/dL and compared to untreated animals.

Histopathological Assessment

For the microscopic examination of the colon tissues, tissue samples were harvested from all groups. Rat colons were stored in 4% formalin solution for 24 h to fix the tissue. The tissues were washed with phosphate buffer saline solution to remove excess formalin solution and then transferred into paraffin containing holders and dried in an oven (NS Biotec-ODS 020, Egypt). Sections with a thickness of 20 μm were cut using a microtome (MICROM, Germany) and stained with hematoxylin/eosin (HE) stain. The prepared slides were examined under light microscopy (Olympus, Japan) and imaging. Morphological changes were evaluated using scoring systems for pathological assessment of

Table III. Scoring System for Histopathological Examination Based upon Four Criteria: Inflammation Severity, Inflammation Extent, Crypt Damage, and Percent of Involvement. Total Colitis Score, 10 with the Exclusion of Percent of Involvement Scores

Scoring parameter	Score	Scoring definition
Inflammation severity	0	None
	1	Mild
	2	Moderate
	3	Severe
Inflammation extent	0	None
	1	Mucosa
	2	Mucosa and submucosa
	3	Transmural
Crypt damage	0	None
	1	Basal 1/3 damage
	2	Basal 2/3 damage
	3	Crypt lost, surface epithelium present
	4	Crypt lost, surface epithelium lost
Percent of involvement	0	0%
	1	1–25%
	2	26–50%
	3	51–75%
	4	76–100%
Total score	10	

colitis. Scoring systems are consisting of four main different parameters: inflammation severity, inflammation extent, crypt damage, and percent of involvement. The summation of scores of inflammation severity, inflammation extent, and crypt damage represents the total histopathology score (32). Table III shows the scoring system for the pathological assessment of colitis.

Statistical Analysis

All experiments were performed on three independent preparations and the mean value was calculated. Statistical analysis was performed using GraphPad inStat software, version 3.0.5. The P value of < 0.05 was considered statistically significant ($* < 0.05$, $** < 0.01$, $*** < 0.001$).

RESULTS

BSD Oil Solubility Studies

There is a wide range of oils that are used for NC preparation including natural, synthetic, and semisynthetic oils. It is very important to clarify that the selected oils must have three main characteristics: the ability of oil to dissolve higher amounts of drug, higher oil/ polymer compatibility (lower dissolution or swelling behaviors), and the absence of oil toxicity (26). BSD showed higher solubility in the semisynthetic oil Capryol 90, ~ 19 mg/mL followed by BnB, ~ 17 mg/mL, while it showed lower solubility—around 9 mg/mL—in the other oils such as Labrafac CC, Miglyol 840, and Peceol (Table IV).

Polymer-Oil Compatibility: Swelling and Dissolution Studies

Formation of suitable polymeric NC required two main components, the first is the oil core which **contains** the active substance. The second is the polymeric shell which confined the oil core within it and must be well consistent coat in order to avoid drug leakage and rapid drug release. For this reason, polymeric films from Eudragit S 100 were studied for 60 days in both selected oils. Slices were immersed in either oils (Capryol 90 and BnB) showed slight increase in weight (~ 0.7%) of the starting weight. On the first day, Eudragit S 100 slices weighted 22 ± 2.1 mg and 75 ± 4.4 mg and immersed in Capryol 90 and BnB, respectively. After 60 days, immersed slices showed an insignificant increase in weight of 22.7 ± 1.7 mg and 75.5 ± 3.6 mg, respectively.

Table IV. Budesonide Solubility (mg/mL) in Different Oils, Capryol 90 and BnB Were Selected for Further Investigations

Selected oils	BSD solubility (mg/mL) (mg/mL ± SD)
Capryol 90	19.1 ± 0.47
Benzyl benzoate	17.3 ± 0.25
Labrafac CC	9.4 ± 0.11
Peceol	9.21 ± 0.12
Miglyol 840	8.3 ± 1.7

Formulation of BSD-Loaded Eudragit S 100 NC

BSD-loaded Eudragit S 100 NC was prepared by nanoprecipitation technique (17). All prepared batches showed homogenous milky white appearance with bluish tinge and free from any aggregates. Preparation of colon targeting NC mainly based on the following hypothesis: during nanoprecipitation, BSD can be encapsulated using Capryol 90 or BnB as an oily carrier due to its higher solubility. This oily core droplets are emulsified and stabilized by the aid of Span 80. Finally, the pH-dependent Eudragit S 100 can be instantaneously deposited and forming polymer coat by the aid of Pluronic F 68 as a stabilizer. Eudragit S 100 is dissolved at higher pH values above 7 providing a good polymeric coat which resists dissolution at the upper GIT.

Characterization of BSD-NC

Particle Size, Polydispersity Index, Zeta-Potential, pH, and Encapsulation Efficiency

Data of particle size, PDI, zeta-potential, and EE% of the prepared colon targeted NC batches are shown in Table V. The mean particle size of the prepared NC ranges from 140 to 304 nm. Factorial analysis of the results obtained showed a significant effect of polymer concentration (X_1) and oil type (X_2) on the mean particle size ($p = 0.0089$). Capryol 90 containing batches showed lower mean particle size than those prepared with BnB at different polymer concentrations (Fig. 2a). Moreover, both polymer amount and oil type had insignificant effect on PDI values. Smaller PDI values of all prepared batches indicate the production of monodispersed system. In addition, lower PDI values were obtained with batches containing Capryol 90 (0.127) when compared to BnB containing batches (0.504) at higher polymer amounts (Fig. 2b).

All prepared formulae showed negative zeta-potential ranges from $- 38.1$ to $- 36.1$ mV (Fig. 2c). Increasing polymer amount showed statistically non-significant effect on zeta-potential ($p = 0.0800$), while oil type showed a significant effect on the magnitude of zeta-potential ($p = 0.0111$). Finally, the prepared BSD-loaded Eudragit S 100 NC had EE% ranges from 33 to 84% (Fig. 2d). Both polymer amount (X_1) and oil type (X_2) showed significant effect on the EE% of the prepared formulae ($p = 0.0150$ and $p = 0.0153$, respectively). In addition, higher EE% was observed with Capryol 90 containing formulae than those BnB containing formulae (84% and 53% at higher polymer amount, respectively). All batches showed that acidic pH values range from 3.6 to 4.2. More acidic pH values were obtained with higher polymer amounts and BnB containing formulae, 3.6 when compared to Capryol 90 formulae, 4.1.

Analysis of Factorial Design

Full $3^1 \times 2^1$ factorial design created and statistically analyzed using Design-Expert® software (version 11). Polynomial analysis was applied for all responses and the linear model is the most fitted one for all responses except for PDI. The model selected for the analysis of PDI is the mean model. Three main statistical parameters were used for the

Table V. Observed Responses Data Used for the Analysis of the Design

Formula	X_1	X_2	Y_1: PS (nm)	Y_2: PDI	Y_3: ZP (mV)	Y_4: EE%	Y_5: pH 7.4 release %
A1	150	Capryol 90	140 ± 0.802	0.076 ± 0.008	− 38.1 ± 1.7	52.6 ± 1.2	52 ± 7.1
A2	200		147.5 ± 0.624	0.139 ± 0.013	− 38.1 ± 3.2	63.8 ± 1.2	60 ± 7.4
A3	250		171.4 ± 1.058	0.127 ± 0.028	− 37.6 ± 4.9	84 ± 2.9	72 ± 4
A4	150	BnB	228 ± 3.25	0.076 ± 0.011	− 37 ± 2.3	33.1 ± 3.2	39 ± 3.2
A5	200		244 ± 6.54	0.311 ± 0.023	− 37 ± 4.1	50 ± 4.2	47 ± 5.1
A6	250		304.6 ± 24.99	0.504 ± 0.057	− 37.1 ± 6.2	53 ± 2.6	51 ± 6.1

analysis of obtained data including adequate precision, adjusted R^2, and predicted R^2. Adequate precision measures the signal to noise ratio. A ratio greater than 4 is desirable for the selection of the model and can be used to navigate the design space (40). All responses showed ratio greater than the desirable value (Table VI) except for PDI. On the other hand, adjusted R^2 measures the amount of variation around the mean explained by the model, while predicted R^2 measures amount of variation in new data explained by the model and used to predict how the model predicts a specific response value (41,42). The adjusted R^2 and predicted R^2 should be within 0.20 and not greater than this value to be in a reasonable agreement (43). It is important to clarify that the predicted R^2 and the adjusted R^2 are within the accepted value and in a reasonable agreement except for PDI response. As mentioned previously, PDI values were not affected by polymer amount (X_1) or oil type (X_2) and the

selected model for PDI analysis is the mean model not the linear model.

Optimization technique was performed to select the optimum batch for further investigations. It is very difficult to achieve all desired responses simultaneously. In many cases, the optimum condition reached in one response may interfere with another response or may have an opposite effect on it (44,45). For this reason, the software calculates desirability function which mathematically combines all the responses into one variable and predicts the optimum levels for the studied factors. The desirability was calculated based on target goals for optimized batch with minimized mean particle size, minimized PDI value, maximized zeta-potential, maximized EE%, and maximized cumulative BSD release at pH 7.4. The highest desirability value was 0.867 for batch A3 (composed of 250 mg Eudragit S 100 and Capryol 90) as shown in Table VI. Hence, batch A3 was selected for further
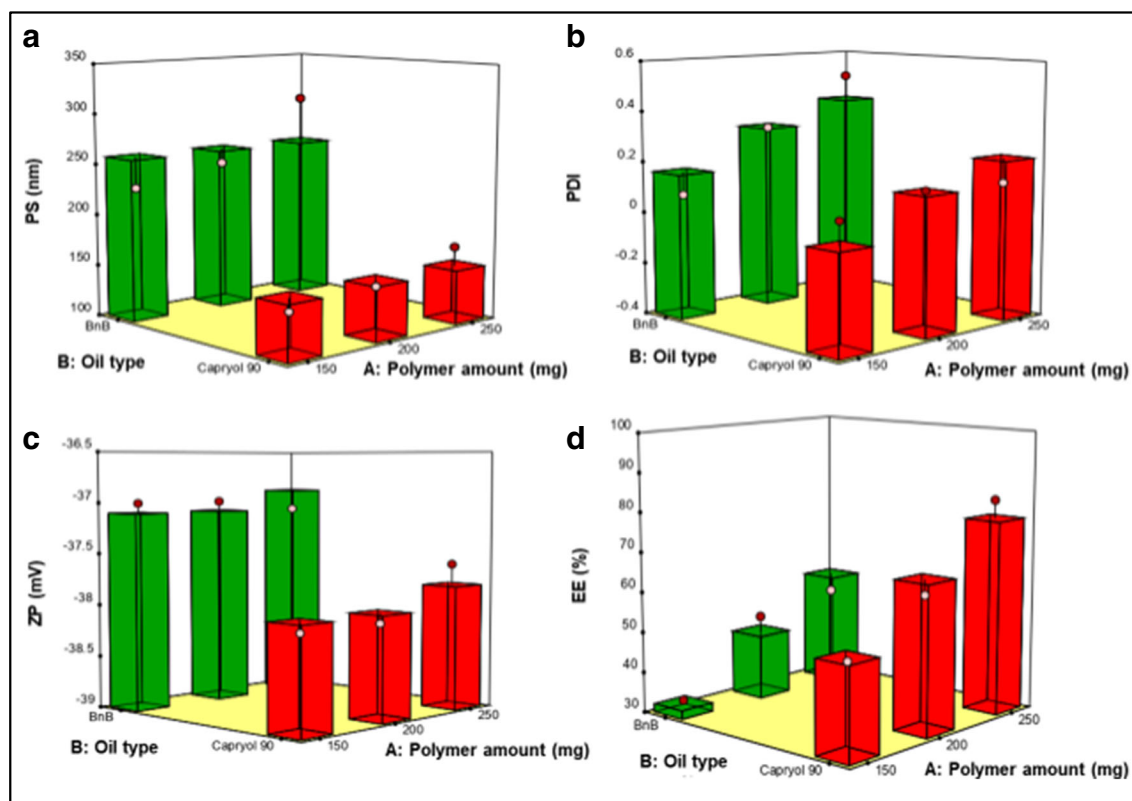
Fig. 2. Response 3D graphs for the effects of polymer amount (A) and oil type (B) on the mean particle size (**a**), polydispersity index (**b**), zeta-potential (**c**), and encapsulation efficiency % (**d**)

Table VI. Full Factorial Design Analysis of BSD-Loaded Polymeric NC

Responses	PS (nm)	PDI	ZP (mV)	EE% (%)	Release % (%)
Analysis	Polynomial	Polynomial	Polynomial	Polynomial	Polynomial
Model	Linear	Mean	Linear	Linear	Linear
Maximum	304.6	0.504	− 36.1	83	72
Minimum	140	0.076	− 38.1	33	39
Ratio	2.17	6.6	1.05	2.5	1.8
Transformation	Inverse	None	None	None	None
Adequate precision	13.15	–	10.17	12.9	16.7
Adjusted R^2	0.9283	–	0.8792	0.9069	0.9449
Predicted R^2	0.7950	− 0.440	0.7207	0.7571	0.8290
Significant factors	X_1 and X_2	None	X_1 and X_2	X_1 and X_2	X_1 and X_2
Optimized formula (A3)					
Observed responses	171.4	0.127	− 37.6	84	72
Predicted responses	179.9	0.140	− 37.1	78.7	69.3
Desirability	0.867				

in vivo studies and batch A6 was selected for comparison purpose only.
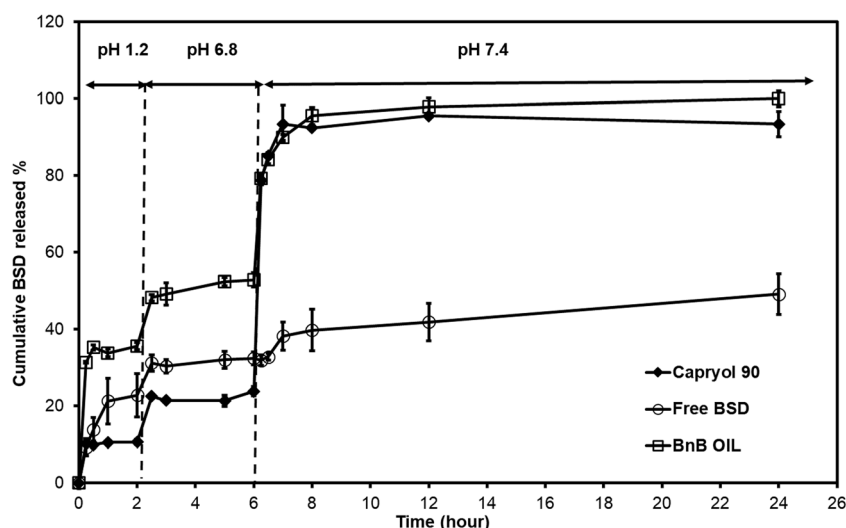
In Vitro Drug Release

Release profiles of BSD-loaded NC batches A3, A6, and the free drug were performed in various pH systems corresponding to different regions of GIT (Fig. 3). Firstly, the release was initiated for 2 h at pH 1.2 simulated to stomach transient time and pH. Secondly, the pH increased to 6.8 for 4 h corresponding to small intestine. Finally, the pH was raised to 7.4 corresponding to end of ileum and colon. In general, colon-targeted formulations should not release the drug at upper GIT to decrease systemic absorption and minimize side effects. The optimal batch A3 showed lowest initial drug release of ~ 10.6 ± 1.1% and 12 ± 2.4% for the first 2 h and second 4 h, respectively, with highest cumulative drug release at pH 7.4 of ~ 72 ± 1.9% within a short time of ~

15 min. On the other hand, batch A6 showed desirable cumulative release at pH 7.4 of ~ 51 ± 1.8%. Both A3 and A6 showed complete drug release of 93.4 ± 5.7% and 100 ± 3.6%, respectively. Finally, free BSD suspension showed initial release at first 2 h of ~ 20% and total release of 50%. It is important to clarify that the visual observation of batches A3 and A6 showed intact NC at acidic pH values as the milky white appearance not disappeared until the pH raised to 7.4 and the media instantaneously turned into a clear solution.

In Vitro Drug Release Kinetics Modeling

Mathematical release kinetics modeling was applied to all batches in order to elucidate drug release mechanisms and release kinetics. The calculated regression coefficient (R^2) for all models showed non-ideal results (075–0.98). Based on the results obtained, it is expected that the prepared BSD-loaded NC have more than one mechanism for the drug release. In

Fig. 3. *In vitro* drug release profile of Capryol 90 containing formulae (A3), BnB containing formulae (A6), and free budesonide in various pH system. Drug release initiated in pH 1.2 corresponding to stomach then increased to 6.8 corresponding to early intestine and finally raised to 7.4 corresponding to ileum and colon. All experiments were performed in triplicate ($n = 3$), mean percent cumulative BSD release was calculated ± SD

order to confirm results, the obtained data about BSD release profile was fitted to two kinetic models. The first is Korsmeyer-Peppas equation, where 60% of release data was included, to determine the exponent (*n*) value in order to assess the release mechanism (19). The (*n*) value for all formulations and especially for the optimal A3 batch was > 0.85, indicating the release mechanism is super case II behavior. The second model is the Weibull model which is an empirical model used to describe release mechanisms from nanoparticles and seems to be applicable for any release mechanism including diffusion, dissolution, and mixed types (46). The Weibull model showed the highest R^2 of 0.98.

Surface Morphology

Scanning electron microscopy (SEM) of the prepared batches showed spherical particles in the nanoscale range, smooth surface, uniform size, and free from drug crystals as shown in Fig. 4. In addition, the mean particle size obtained by SEM was in good agreement with those obtained by DLS analysis.

Short-Term Stability Studies

Prepared NC showed good stability behavior for 2 months of storage with insignificant change in mean particle size, EE%, and pH values. Batch A3 showed minimal increase in mean particle size from 171 to 196 nm and minimal decrease in EE% from 83 to 79%. Samples were visually inspected and showed no aggregate formation.

In Vivo Evaluation of BSD-Loaded Eudragit S 100 NC

Induction of Colitis in Animal Model

One day after experimental colitis induction, all the animals except healthy group (group I), presented by weight loss, loosely stool, bloody diarrhea, weakness, and decreased food intake, which indicated that the all animals present ulcerative colitis model. All examined animals treated for 5 successive days with BSD-loaded NC or free drug suspension. Disease activity index (DAI) was calculated and represented for each group.

Disease Activity Index

The activity of the acetic acid-induced colitis was evaluated and confirmed using clinical DAI system taking in consideration three main parameters: body weight, stool consistency, and rectal bleeding. DAI calculated by the summation of observed scores for each group and presented graphically (Fig. 5a). On the first day of treatment, group III which treated with (A3) optimized formula showed statistically non-significant difference ($p < 0.005$) from colitis group scores, but from the third day, treatment produces significant difference in the total disease activity score. Finally, after 5 days of treatment, animals in group III showed highly significant ($p < 0.0001$) lower DAI scores of ~ 3 when compared to colitis group (II) which showed DAI of ~ 8.3, the results obtained confirmed by histopathological examination and blood glucose level. On the other hand, group IV treated with BnB containing formulations showed to some
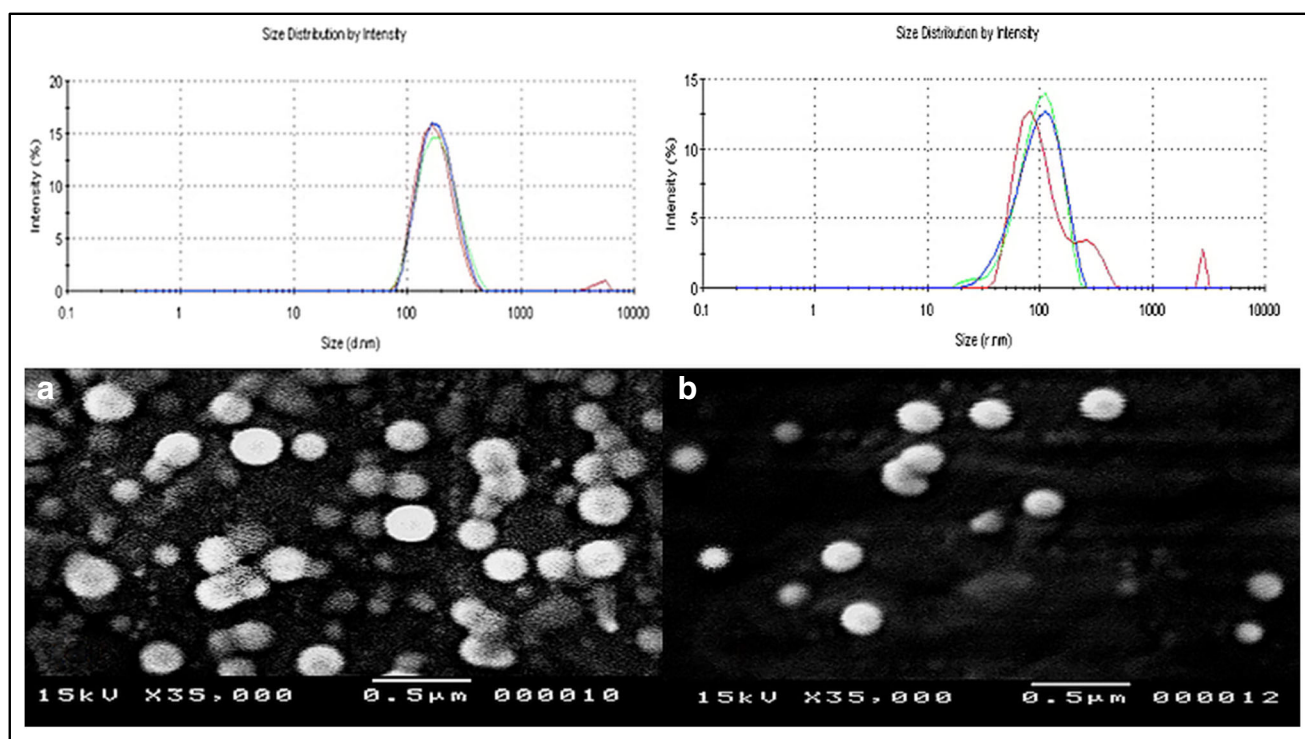
Fig. 4. Scanning electron microscopy of Capryol 90 containing formulae (**a**) and BnB containing formulae (**b**). The upper part of the figure shows particle size analysis of the prepared batches. N. B: particle size analysis determined using dynamic light scattering (DLS) technique, particle size of BnB batch measured in radius (r. nm)
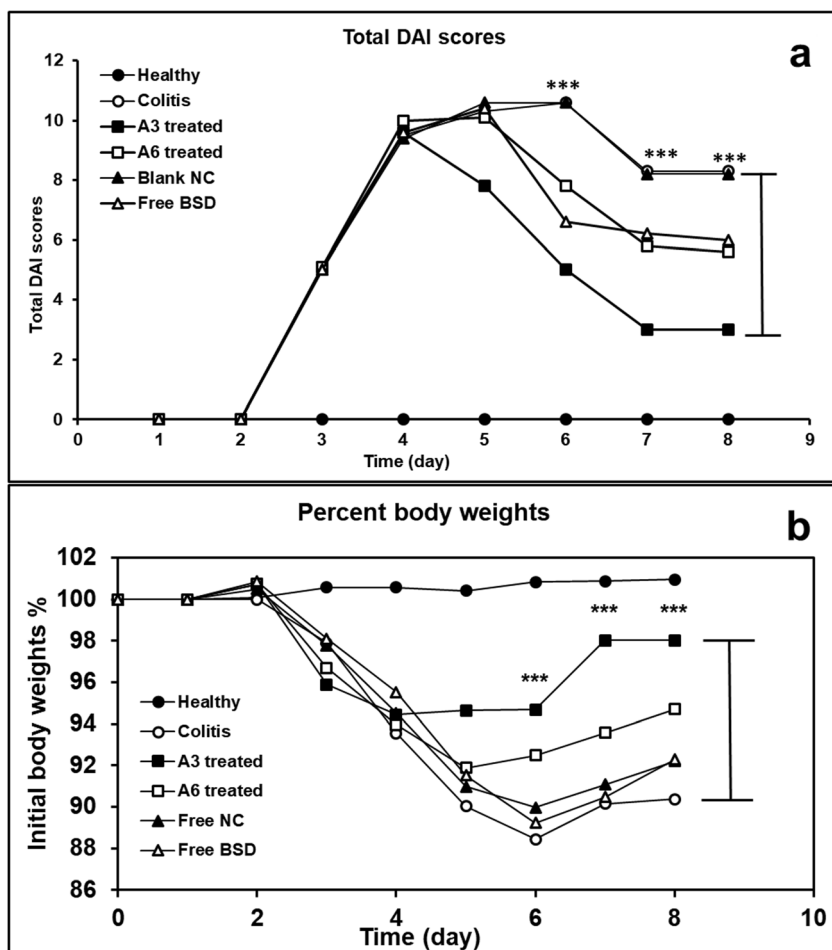
Fig. 5. Total DAI scores and body weight changes of healthy, colitis, A3 treated, A6 treated, free NC, free BSD groups. **a** Total DAI scores, **b** percent body weight changes from initial body weights. Error bars are not shown for clarity reasons, $n = 7$ rats/group. Statistical significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) compared with colitis group

extent little improvement in the total DAI, as the total DAI was ~ 5.5 at the last day of the treatment. Results obtained from the other two groups V and VI showed that animals treated with free NC presented with non-significant improvement ($p = 0.102$) as the total DAI was ~ 8.2 when compared to the colitis group on the fifth day of the treatment, but animals treated with free BSD suspension showed little improvement as DAI was ~ 6 when compared to colitis group ($p = 0.0018$) at the end of the treatment.
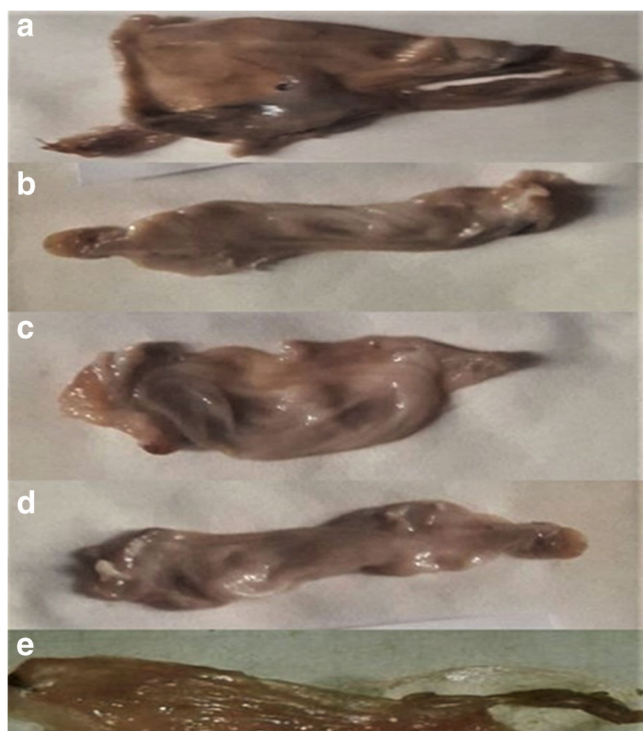
Macroscopical Examination

Mean Body Weight

Body weights of all animals graphically represented as a percent of the initial body weight are shown in Fig. 5b. The results obtained showed that animals treated with the optimized formula (group III) presented with improved body weight after starting treatment compared to the first day of colitis induction. Body weights increased from 94% on the first day to 98% on the fifth day of the treatment. All animals treated with A3 showed highly significant increase in their body weights ($p = 0.0004$) when compared to group II colitis

group after 3 days of starting treatment. On the other hand, colitis group II presented with a rapid decrease in body weight from 98 to 90% at colitis induction and the last day, respectively. Moreover, mean body weight for other groups IV, V, and VI showed little improvement as body weights changed from 96%, 97%, and 98%, respectively, at colitis induction to 94%, 92%, and 92%, respectively, on the last day of treatment.

Gross Examination of Resected Colons

All animals were sacrificed 24 h after the last dose administration; the distal colon specimens were collected and macroscopically examined for inflammation signs. All the animals in colitis group II developed inflamed colons with hyperemia, edema, and ulceration. On the other hand, all the animals treated with BSD-loaded NC (III and IV) showed enhanced colon health with minimum inflamed areas and decreased edema when compared with colitis group. Whatever the specimens were taken from groups V and VI, untreated animals developed marked inflammation with hyperemia (Fig. 6).

Fig. 6. Photographs for macroscopical examination of the distal colon specimens resected from colitis group (**a**), healthy animals (**b**), animals treated with Capryol 90 containing formulae (**c**), animals treated with BnB containing formulae (**d**), and animals treated with free BSD (**e**). Specimens from colitis group presented with severe ulceration and perforation

Colon Length and Colon Weight/Body Weight Ratio

The colons (6 cm lengths, distal colons) of the examined rats were resected, opened, and rinsed from the fecal matter (27). The ratio of the wet weight of the colon to the total body weight was calculated at the last dose administration (Fig. 7a). All resected specimens from healthy animals showed colon length and colon weight of 5.7 cm and 1.3 g, respectively. Other resected specimens converted to a colon weight/colon length ratio as an inflammatory index. The results obtained showed a higher ratio with colitis (group II) of ~ 0.41 while animals treated with optimized (A3) formula showed a ratio of ~ 0.3. On the other hand, resected specimens from groups IV, V, and VI showed a higher ratio when compared with colitis group and significantly differ from group III, results showed ratio of ~ 0.34, ~ 0.38, and ~ 0.37, respectively. Additionally, colitis group showed the highest colon weight/body weight ratio of 0.01 while healthy group showed the lowest ratio of ~ 0.005. animals treated with optimized NC (A1) showed lower ratio of ~ 0.08 when compared to other groups of ~ 0.009, ~ 0.01, and ~ 0.01 for groups IV, V, and VI, respectively (Fig. 7b).

Blood Glucose Level

Blood glucose level (BGL) is an indicator of increased food intake and consequently, health improvement. Glucose levels were measured for all studied animals on the first day, colitis induction day, and after last administered dose. The results obtained showed that healthy animals (group I) presented with normal blood glucose level from the first day till the end of the experiment (~ 99 mg/dL). Regarding to animals treated with A3, blood glucose level declined from ~ 96 mg/dL at the first day to 87 mg/dL at colitis induction then rose after third day of the treatment until reached ~ 95 mg/dL on the last day which is statistically different when compared to colitis group of BGL ~ 56 mg/dL at the last day. Moreover, animals in groups IV, V, and VI showed lowered blood glucose level at the last day of ~ 82, ~ 83, and ~ 71 mg/dL, respectively, as shown in Fig. 8.

Histopathological Assessment

The normal group I showed normal intact mucosa containing colonic glands with goblet cells; submucosa and musculosa were free from inflammation though some specimens showed mild inflammation and limited to mucosa which might be normally detected due to the colonic environment. For colitis group, all layers showed infiltration by acute inflammatory cells in the form of polymorph nuclear cells (neutrophils and macrophages); mucosa showed ulceration at same areas with necrosis. Crypts were totally lost with the loss of surface epithelium in most of the examined specimens. Inflammation in some specimens extended to musculosa (transmural inflammation). For treated group (III), mucosa showed mild inflammation represented by infiltration of acute inflammatory cells in the form of neutrophils. Submucosa, musculosa, and serosa were free from inflammation. In some of the examined specimens, there was crypt damage which was limited to basal 1/3 damage. Animals treated with (A3) dramatically changed all inflammatory scores from ~ 9.2 to ~ 3.5 when compared with colitis group. All animals treated with BSD-loaded nanocapsules (A3) showed reformed epithelium, and a decrease in crypt damage, inflammatory cells infiltration, mucosal edema, and muscle damage when compared to the normal group and colitis-induced group. The treated group showed a very significant difference when compared to colitis group scores ($p = 0.0043$). Animals treated with A6 showed improvement to some extent as the inflammation was limited to mucosa and submucosa with characteristic infiltration of inflammatory cells in the form of neutrophils. Inflammation was characterized by minimal crypt damage when compared to animals with colitis which showed basal crypt damage. Group IV showed total colitis score of ~ 6, which is statistically not quite significant when compared to colitis-induced group ($p = 0.05$). Animals treated with free BSD solution showed inflammation limited to mucosa and submucosa; mucosa showed necrosed glands with partial ulceration in some areas. Inflamed colonic mucosa not totally cured after treatment with free drug solution and this might be attributed to lower colonic delivery for free drug. Group VI showed total colitis score of ~ 7.5, which differ significantly ($p = 0.0018$, $p = 0.0058$) when compared to normal group and treated group III, respectively. Figure 9 shows histopathological scores of all examined animals; Fig. 10 shows a microscopical examination of all examined specimens.

DISCUSSION

First of all, BSD showed higher drug solubility in both Capryol 90 and BnB which could be explained, the Capryol
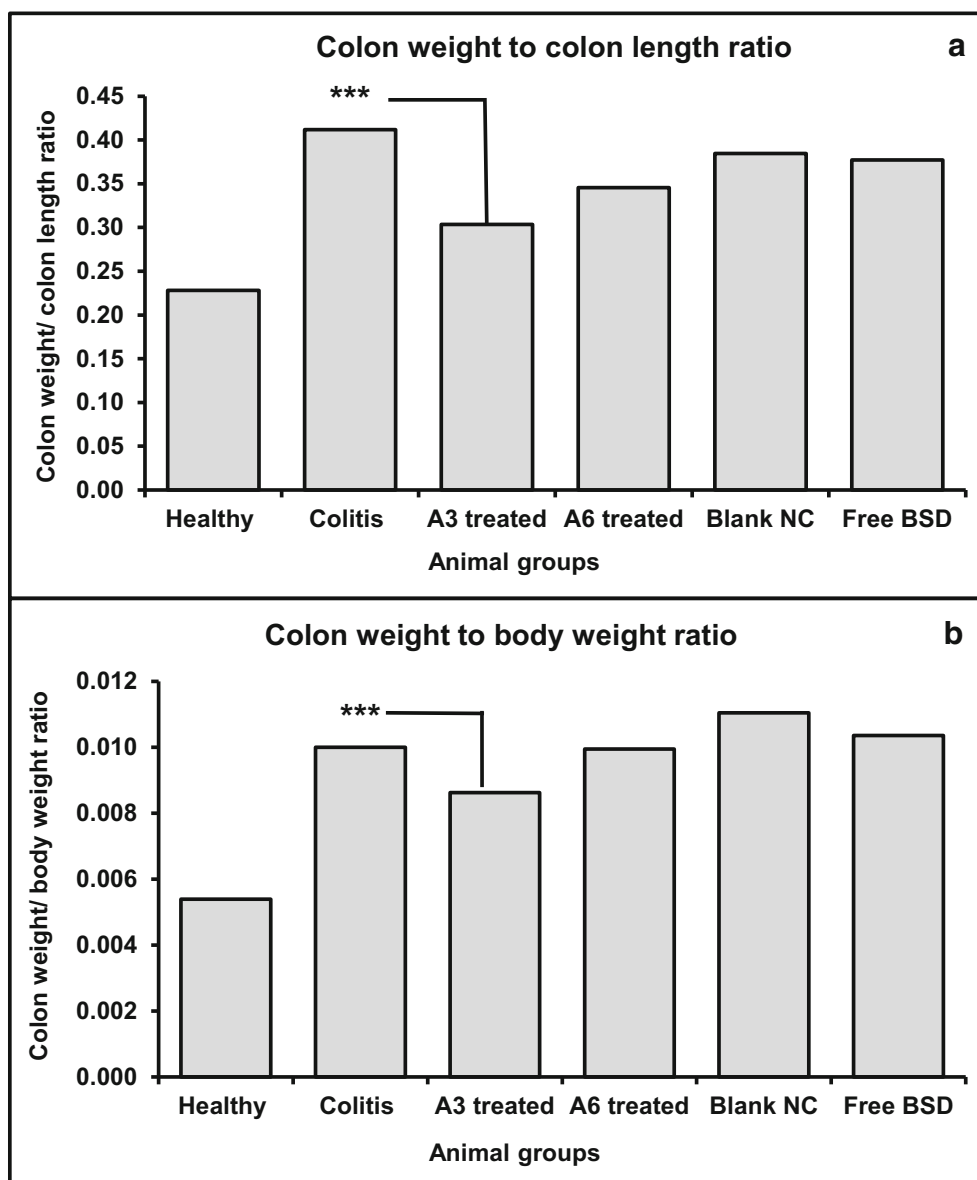
Fig. 7. Colon weight to colon length ratio (**a**) and colon weight to body weight ratio (**b**) as an inflammatory index. Statistical significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) compared with colitis group

90 might affect drug solubility from the point of molar volume and hydrophilic-lipophilic balance (HLB). The higher solubility of Capryol 90 may be attributed to the polarity of poorly soluble drugs which favor solubilization in small molar volume oils such as medium mono, di, or triglycerides (47). Also, Capryol 90 having a hydrophilic head composed of propylene glycol, which adds more impact on their solvent behaviors (48). Finally, short chain length and higher fluidity of the medium chain triglycerides over the long chain triglycerides have good drug solubility (49). Moreover, the higher BSD solubility in BnB might be correlated to solubility parameter (δ) which showed closer solubility parameters of BnB (20.8 (mPa)$^{0.5}$) to the solubility parameter of BSD (25.22 (mPa)$^{0.5}$) (48). Therefore, the Capryol 90 and BnB were selected for the preparation of BSD-loaded polymeric NC suspension. However, other oils such as Labrafac CC, Peceol, and Miglyol 840 were rejected due to their lower capacity to dissolve higher amounts of BSD. The lower BSD solubility

may be attributed to very low HLB value of Peceol, Miglyol 840, and Labrafac CC of 3, 1, and 1, respectively, when compared to HLB value of both Capryol 90 and BnB of 6. In addition, preparations with these oils required the use of higher volumes of the oil and subsequently required higher amounts of surfactants which might increase the instability and toxicity of the system. In the same way, polymer dissolution/swelling studies showed good polymer/oil compatibility with a small increase in polymer weight. Similar results obtained by Katzer et al. and Flores et al. studies (22,50).

Regarding the prepared NC batches, all samples presented with clear milky white appearance with bluish tinge which is correlated to Tyndall effect. Nanocapsules showed negative zeta-potential and acidic pH values which is correlated to the free terminal acidic groups present in Eudragit S 100 (23). The more acidic pH obtained with BnB batches is correlated to the free acid traces present in the central oil (BnB) may increase acidity of the sample (51,52).
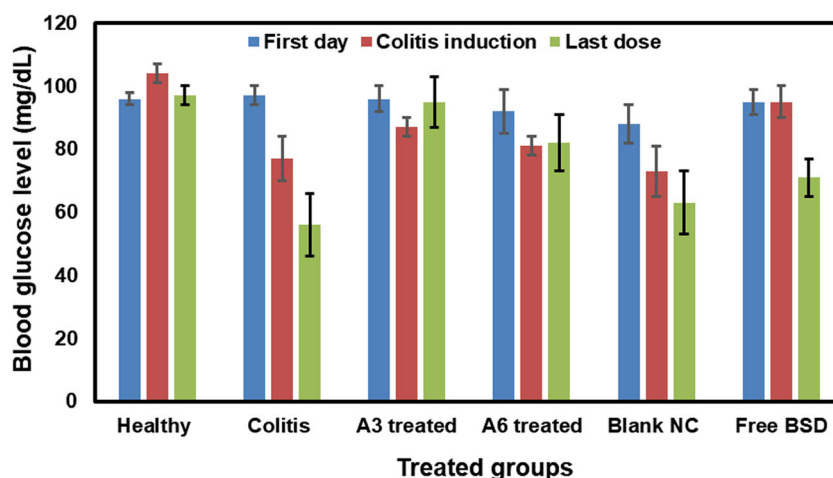
Fig. 8. Blood glucose levels for all animals (mg/dL). Samples were collected at three different days during experiment: first day, colitis induction day, and after the last dose administration

Concerning mean particle size, the results obtained could be explained through mass transfer theory and Gibbs-Marangoni effect, as increasing polymer concentration produced highly viscous organic phase. The higher viscosity leads to increase the resistance to mass transfer which decreases the diffusion rate of the solvent phase into the aqueous phase, and subsequently to production of emulsion droplets with larger size resistant to breakdown and these finally, produce larger particle size (interfacial turbulence theory) (26,53,54). Also, increasing polymer concentration leads to increase polymer chains per unit volume of the solvent which produces larger particle size during polymer precipitation (nucleation-growth theory) (53). Additionally, Capryol 90 formulae showed smaller particle size when compared to BnB formulae which may be related to the interfacial tension property. Oils with lower interfacial tension produce smaller droplets and subsequently NC with smaller particle size. Accordingly, Capryol 90 oil is characterized by the lower interfacial tension, 5.5 mN/m (55), which would produce smallest droplet size when compared to the higher interfacial tension of BnB, 40 mN/m.

Higher EE% was observed with higher polymer concentration and Capryol 90 formulae and this may be attributed to the diffusion mechanism, increasing polymer concentration at constant aqueous phase volume leading to increasing system viscosity. Therefore, increase diffusional resistance to drug molecules from solvent phase to aqueous phase and furthermore, increase drug entrapment (54). In the same way, BSD showed higher solubility in Capryol 90 more than BnB that might have an impact on EE%.

Moreover, the results obtained from *in vitro* release for A3 showed good targeting property and this may be attributed to the nature of polymer and oil type. Firstly, at lower pH 1.2 and 6.5, Eudragit S 100 possesses very low permeability due to hydrogen bonding between the ester group and carboxylic moiety of the polymer. Formation of this bond increases polymer compactness and decreases polymer porosity. At higher pH of the GIT (ileum, above 7), the polymer starts to dissolve due to carboxylic group ionization which induces the electrostatic repulsion forces between the polymer chains, and consequently disrupt the

polymer matrix, and finally increases both polymer swelling and erosion (19,56). Secondly, BSD showed higher solubility and higher oil/water partitioning in Capryol 90 which produce more controlling properties (57,58). It is very important to clarify that optimized formula (A3) showed the most acceptable drug release profile at higher polymer concentration which may be attributed to increased polymer matrix and consequently increased diffusion path length, tortuosity, and produced more consistent polymeric wall. On the other hand, BnB formulae A6 showed rapid initial release at pH 1.2 and 6.8 due to the presence of the low viscosity BnB oil and the more hydrolyzable oil in the intestinal fluid to benzoic acid which may produce unwanted side effects (59). The obtained data from the release kinetic modeling indicates that the release mechanism of BSD from Eudragit S 100 based NC involves different mechanisms. From the Korsmeyer-Peppas model, the super case II release involves many mechanisms such as diffusion, polymer relaxation by swelling, and polymer erosion due to dissolution, but polymer erosion is the main mechanism. From Weibull model, the release data showed that the highest regression coefficient R^2 of 0.98 which indicates that the release mechanism may be diffusion or dissolution or mixed type. The results obtained confirm the fact that the release mechanism from acrylic polymers such as Eudragit S 100 is controlled by both polymer swelling and erosion (19,60). Finally, insignificant changes in pH values and EE% of stored batches indicate good stability behavior with no polymer degradation or drug release (26).

With respect to animal studies, all animals presented with colitis which is related to intra-rectal instillation of 3% diluted acetic acid. Acetic acid-induced colitis is one of the most commonly chemical-induced colitis models. Diluted acetic acid solutions produce inflammation similar to human ulcerative colitis. The effect of acetic acid is not only due to its irritating effect, but acetic acid solutions produce more histopathological changes including edema, cellular inflammation, crypts damage, colon ulcer and necrosis, and increased secretion of inflammatory mediators. This model is simple, rapid, and reproducible (32,36,61).

Regarding DAI, animals treated with A3 showed more improvement in total DAI scores above animals treated with

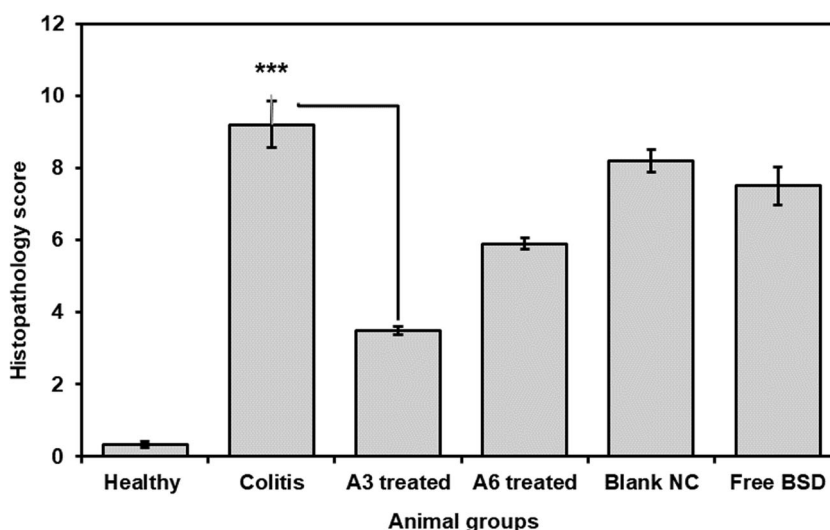| Group	Inflammation severity	Crypt damage	Inflammation extent
I	a	b	c
II	d	e	f
III	g	h	i
IV	j	k	l
V	m	n	o
VI	p	q	r
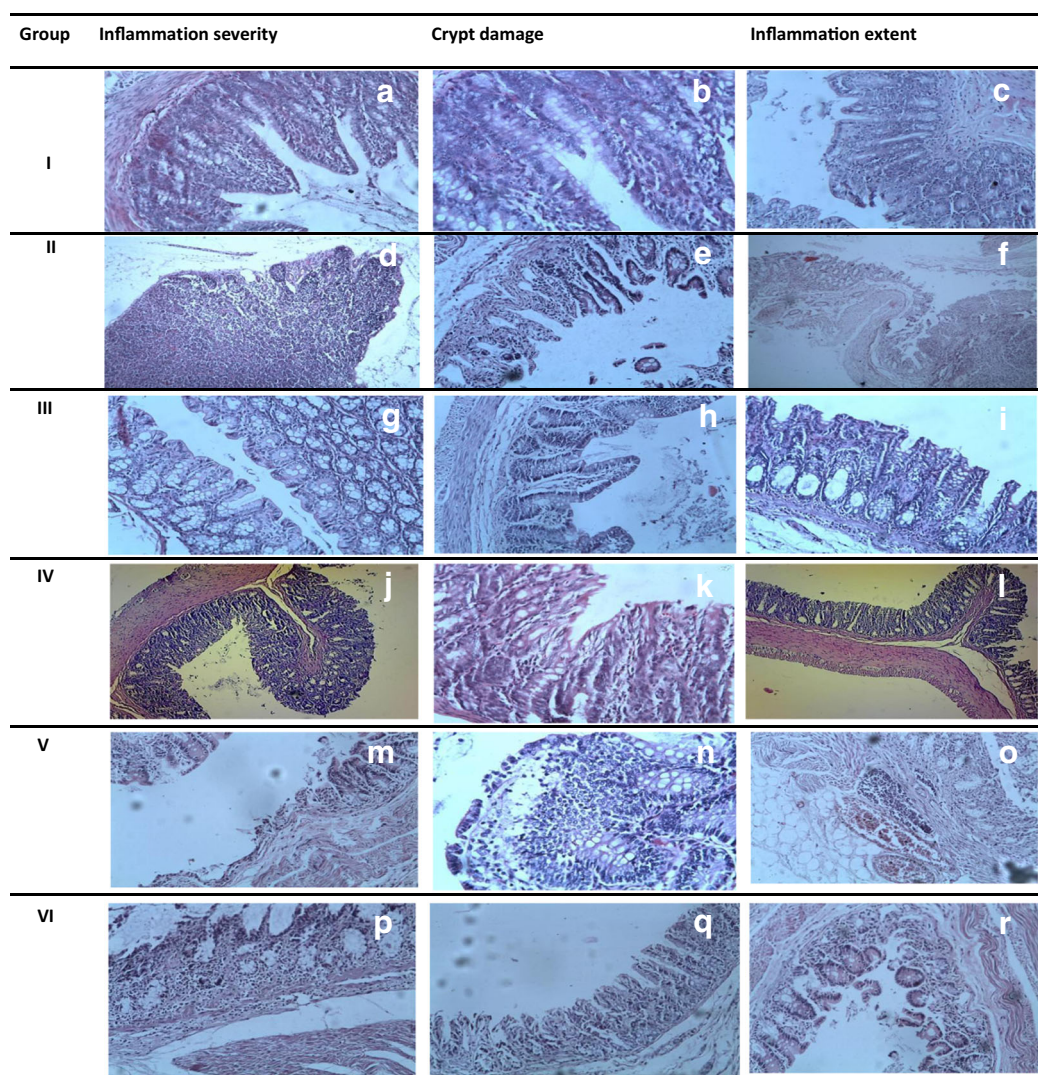
Fig. 9. Histopathological examination of colitis for all animals. For healthy group (I), microscopical examination shows normal colonic structure (**a**), normal crypt structure (**b**), and normal structure of all colonic layers (transmural structure). For colitis group (II), figures show severe inflammation with marked infiltration of inflammatory cells in the form of neutrophils (**d**), ulcerated mucosa with crypt distortion (**e**), and severe inflammation affects musculosa (transmural inflammation) (**f**). For animals treated with Capryol 90 containing formulae (III), figures show intact mucosa with normal colonic layers and lower inflammatory cell infiltration (**g**), normal crypt structure with basal 1/3 damage (**h**), and intact mucosal structure (**i**). For animals treated with BnB containing formulae, microscopical examination shows inflamed mucosa with higher inflammatory cells infiltration (**j**), crypts with mild structural damage in some areas (**k**), and infiltration of inflammatory cells in all colonic layers (**l**). For animals that received free NC, figures show mucosal ulceration with marked inflammatory cell infiltration (**m**), crypt structure lost (**n**), and transmural inflammation (**o**). For animals that received free BSD suspension, figures show inflamed mucosa and submucosa with necrosed glands (**p**), distorted crypt structure (**q**), and ulcerated mucosa (**r**)

(A6) formulae. The results could be explained on the basis of the *in vitro* drug release results, these data showed an initial release of small amounts of BSD (23%) in case of (A3) batches, while the rest of BSD (72%) was released in the colon and exerted its anti-inflammatory effects. Moreover, batches formulated with BnB (A6) showed higher initial release of ~ 50% which is related to lower oil viscosity and hydrolysis of the oil to benzoic acid in gastrointestinal lumen (59). On the other hand, all animals treated with free BSD suspension or free NC showed lower total DAI score which is related to the absence or lower amounts of BSD delivered to the colon.

Additionally, all animals in group II colitis group, animals treated with free NC (V), and animals receiving free BSD suspension (VI) characterized by higher colon weight and lower colon length. The results obtained could be due to ulcerative colitis represented by marked localized inflammation, thickening, and edema of the bowel (27). Animals treated with BSD-loaded NC showed marked decrease in inflammation severity, edema, and inflammatory secretions which is reflected by lower colon weights and higher colon lengths near to normal.

From the point of mean body weight, colitis-induced group II showed higher decline in their body weights on the last day which is

Fig. 10. Histopathological scoring system of all animals. Scores based upon inflammation criteria: inflammation severity, crypt damage, and inflammation extent. Total score is equal to 10. Statistical significance (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$) compared with colitis group

explained based on ulcerative colitis markedly decreases body weight due to lower food intake, weakness, and bloody diarrhea occurred (27). Animals receiving BSD-loaded NC showed increased body weights at the last administrated dose which is related to effect of BSD on the inflamed area. The action of BSD resulted in decreasing bloody diarrhea and increasing food intake and consequently reflected by increased body weights. With respect to blood glucose level, animals receiving BSD-loaded NC (A3) showed higher increase in BGL when compared to colitis group II. The results obtained could be explained on the basis that BSD is a local glucocorticoid known to enhance glucose uptake and intestinal transport (39).

Finally, results from histopathological assessment showed that all animals treated with BSD in the form of nanocapsules presented with reformed epithelium, and a decrease in inflammatory cells infiltration, crypt damage, and underline tissues damage. On the other hand, animals that received BSD in the form of rapid release nanocapsules (A6) or free suspension form presented with lower improvement in inflammation pattern. The results obtained could be explained based on the fact that polymeric NC can readily deliver higher amounts of BSD to the colon as shown in *in vitro* release studies. In this case, higher amounts of BSD are available at the inflamed area and are capable of decreasing inflammation at all grades, while the BSD in the form of free suspension or rapid release forms can be absorbed from the upper gastrointestinal tract and could not be delivered in higher amounts to the colon. In the same way, BSD has high first pass metabolism which resulted in more decrease in BSD amounts delivered to the inflamed colon (32).

CONCLUSION

Based upon the represented findings, Eudragit S 100 nanocapsules loaded with budesonide showed mean particle size in the nanoscale range (~ 171 nm) with good stability (zeta-potential value of − 37 mV). *In vitro* drug release studies showed that the initial rapid and unwanted release

was greatly affected by composition variables like polymer nature, polymer concentration, and oil nature. Optimized formulations showed lower initial release of 10% at first 2 h, and higher rapid release of 72% at the end of ileum and colon. *In vitro* release studies were confirmed by the activity of BSD-loaded nanocapsules in animal studies as the prepared formulations showed higher improvements in disease activity index, significant increase in blood glucose level, increased body weight near to normal rats, and higher therapeutic activity confirmed by histopathological examination that showed treated colons with minimal inflammation. Further human studies are required to prove the ability of nanocapsules to treat inflammatory bowel disease.

COMPLIANCE WITH ETHICAL STANDARDS

Conflict of Interest The authors declare that they have no conflict of interest.

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