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In-Vitro Evidences for the Formulation, Development, and Evaluation of Budesonide Oral Nano-sponges

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ABSTRACT

Background and Objective: Idiopathic inflammatory bowel disease is quite common, with ulcerative colitis and Crohn's disease being the most common forms. Due to its high receptor affinity and quick diversion, the anti-inflammatory effects of the glucocorticoid budesonide are limited, but it is nevertheless useful in some situations. A unique BUD nano-sponges was developed by employing quasi-solvent diffusion and Eudragit S-100 as the polymer to address the problem of low efficacy and accessibility.

Material and Methods: Drug release profile and percentage of drug entrapment in BUD Nano sponges were also measured and analyzed. Clinical activity score, colon/body weight ratio, and macroscopic ulceration activity were among the criteria used in an in vivo investigation of the formulation in male Wistar rats. Finally, histological investigation was done on colon tissue samples.

Results: When compared to other BUD formulations on the

market, this one performed exceptionally well, suggesting that the designed nanosponges are highly effective. The Wistar rats' clinical activity score was reduced when treated with the formed nanosponges. When compared to the placebo group, those who took this supplement saw a dramatic decrease in their colon weight ratio. Nanosponge colon histology revealed healthy colon anatomy and architecture.

Conclusion: The findings of this study have substantiated the efficacy of BUD nano-sponges as innovative carriers in the treatment of inflammatory bowel disease.

Keywords: Inflammatory bowel illness, colon tissue, budesonide nano-sponges, and quasi-emulsion solvent.

INTRODUCTION

Inflammatory bowel disease (IBD) is the umbrella term used to refer to the conditions ulcerative colitis (UC) and Crohn's disease. Crohn's disease has the potential to impact several segments of the gastrointestinal tract, with a particular predilection for the colon and distal ileum [1, 2]. However, it should be noted that Crohn's disease only affects the colon. The two distinct categories of inflammatory bowel disease are characterized by intensified and unregulated inflammation in the intestines, leading to a diminished quality of life for affected individuals. This condition need prolonged medical or surgical interventions. In Europe, the prevalence of inflammatory bowel disease exceeds 2.5 million individuals, and its incidence is steadily increasing in Asia and developing countries. The prevalence of inflammatory bowel disease is increasing, particularly in developing countries [3, 4].

Despite the incomplete understanding of the etiology of inflammatory illnesses, there is a growing body of evidence suggesting a genetic predisposition to the morbidity of inflammatory bowel disease. The potential contributing components could be associated with dysregulation of the immune system and imbalances in the intestinal microbiota, as evidenced by studies conducted on mice models of inflammatory bowel disease. The intricate interactions described above have significant implications for the disruption of intestinal homeostasis and the dysregulation of the inflammatory microflora [5, 6]. Crohn's disease is distinguished by a form of inflammation that affects the entire thickness of the gastrointestinal wall, perhaps impacting multiple layers. In contrast, ulcerative colitis is characterized by inflammation that is limited to the mucosal layer and confined to the colon.

There is a growing interest in the utilization of multi-particulate modified delivery systems, specifically for the purpose of targeting specific sites inside the gastrointestinal tract. The systems of modified release are characterized by their intricate nature, necessitating a wide range of skills and new advancements for their large-scale assembly [7, 8]. Nano-sponges have emerged as intriguing dosage forms in terms of their economic process development and scale-up prospective, among the numerous types of unit dosage forms. Nano-sponges possess a colloidal structure characterized by the inclusion of minute solid particles within a network of voids and mesh-like systems. This unique architecture enables the encapsulation of diverse compounds such as anti-cancer agents, proteins, DNA, and others. Nano-sponges can be described as three-dimensional structures composed of a naturally occurring polymer with an extended molecular chain [9, 10].

The nano-sponges are synthesized through the cross-linking of polyesters and peptides,

distinguishing them from existing nano-scale drug delivery systems. Nanosponges possess a lipid composition and exhibit the ability to disperse within an aqueous medium for transportation purposes. One potential application of these substances is their ability to mitigate the unpleasant taste associated with pharmaceutical compounds [11]. The modification of cross-linker to polymer proportions can alter the release of medication from the nano-sponge system. The nano-sponge exhibits the ability to adhere to the surface of the intended location during its circulation within the body, subsequently releasing the medication in a regulated and predictable manner [12].

Budesonide is a corticosteroid that acts locally and exhibits a high affinity for glucocorticoid receptors, resulting in potent anti-inflammatory effects. It presents numerous advantages in comparison to previous iterations of steroids. BUD exhibits a topical potency that is 200 times greater than hydrocortisone, while demonstrating a systemic bioavailability that is only 10%. According to the analysis, BUD exhibited a lower incidence of systemic side effects compared to prednisone. Budesonide is a pharmacological agent that has favorable characteristics for the localized treatment of inflammatory bowel disease. These characteristics include a limited oral bioavailability, rapid elimination, and the presence of potentially hazardous metabolites. BUD is commercially accessible in several formulations, including ileal release formulations, pills, and enemas, among others [13, 14].

The purpose of this research was to investigate and evaluate the potential benefits of using nano-sponges as a drug delivery system for the treatment of inflammatory bowel disease. This study aimed to address the limitations of traditional topical drug delivery methods and the lack of available nano-sponge-based drug delivery systems.

MATERIALS AND METHODS

The BUD sample was acquired from industry, while Dibutyl phthalate and PVA were procured from Central Drug House Pvt. Ltd in Mumbai, India. The sample of Eudragit S-100 used in this study was obtained from Evonik Pharma, located in Mumbai, India. Hualien's Fine Chemicals is located in Mumbai, India. Demineralized water and fresh distilled water were prepared as necessary. This investigation utilized analytical grade materials and reagents. The necessary materials were obtained from several sources.

Characterization of BUD

The medication BUD underwent a comprehensive analysis to determine its purity, with the following key characterizations being outlined below.

UV- Spectrophotometry Study

A quantity of 10 milligrams of BUD was measured and thereafter introduced into separate 10 milliliter volumetric flasks containing a 7.4 pH buffer solution and 0.1 Normal hydrochloric acid (HCl). The solution was prepared with a concentration of 1000 µg/mL. From this solution, 1 mL was transferred to a 10 mL volumetric flask and diluted with buffer solution and 0.1 N HCl separately. A necessary dilution was performed in order to achieve a concentration range of 5-25 µg/mL for both solvents. The solution was observed within the ultraviolet (UV) light spectrum, namely in the range of 200-400 nm. A graph depicting the relationship between concentration and absorbance was constructed, and subsequently, a calibration curve was generated [15, 16].

FT-IR Study

The drug's infrared spectrum was obtained by the use of the KBr technique and Fourier transform infrared. The baseline correction was performed by utilizing a dried pellet of

potassium bromide. The potassium bromide pellet was prepared by subjecting a physical blend of medicine and KBr to hydraulic pressure, wherein 3-5 mg of the blend was crushed. The pellet that was acquired was placed within the infrared (IR) compartment and subjected to measurement at wavelengths ranging from 4000 cm-1 to 400 cm [16, 17].

DSC of BUD

The thermographic image of the pharmaceutical compound was obtained through the utilization of a Differential Scanning Calorimeter (DSC). The drug sample was measured by weighing it using hermetically sealed aluminum pans. The specimen underwent heating in a nitrogen environment at a consistent rate of 10°C per minute, ranging from 50 to 400°C. Alumina was utilized as the reference standard in this experiment [18, 19].

Preparation of nano-sponges
Preparation Method

The formulation of nano-sponges containing BUD was achieved using a technique known as quasi-emulsion solvent diffusion. The precise quantity of polymethyl-methacrylate, namely Eudragit S-100, was carefully measured and combined with varying proportions of dibutyl phthalate. This mixture was then dissolved in a solution consisting of 10 mL of dichloromethane and methanol. The addition of dibutyl phthalate was employed to enhance the pliability of the polymer. The dissolution of BUD occurred in the aforementioned combination. Subsequently, a dispersion media was generated by diluting distilled water with a concentration of 0.5-1.5% w/v. The polymer-drug solution that had been generated beforehand was incrementally introduced into the PVA solution, while maintaining a consistent stirring rate for a duration of 2 hours. Following the thorough evaporation of the solvent from the polymer droplets, the resultant nano-sponges were subjected to centrifugation at a speed of 4000 revolutions per minute in order to facilitate their collection. Subsequently, the collected nano-sponges underwent a washing process, which was repeated three times. The solvent was gradually extracted in order to generate the nano-sponges. The nanosponge suspension in water was subjected to lyophilization and thereafter stored in an airtight container for future examination. Table 1 presents the optimization of the formulation of nano-sponges loaded with BUD [19, 20].

Table 1: Optimization batches of the formulation

Batch No.	Polymer	PVA	Budesonide	Stirring Speed
1	0.3	0.4	25	2500
2	0.6	0.4	50	2500
3	0.3	1.0	25	1500
4	0.6	1.0	50	2500
5	0.6	2.0	25	3500
6	0.6	1.5	50	2500
7	0.6	1.5	25	1500
8	0.3	2.0	50	2500

9	0.3	2.0	50	1500

Characterization of Prepared formulation

Particle size and PDI

The polydispersity index and average particle size of the produced nano-sponges were assessed using a zeta sizer instrument. The process of diluting the preparation of nano-sponges was carried out using deionized water, and subsequently assessed for the average size and polydispersity index [21, 22].

SEM Study

The determination of vesicle morphology was conducted using the utilization of scanning electron microscopy. The nano-sponges were affixed onto supports using carbon-based adhesive and subsequently coated with a layer of gold using a gold sputter module within a high-vacuum evaporator. The samples were subjected to visualization using scanning electron microscopy (SEM) at an acceleration voltage of 10 kilovolts (kV) [23, 24].

Drug Content

The investigation of the medication quantity integrated in the nano-sponges was conducted using a UV spectrophotometer. The nano-sponges underwent a 48-hour incubation period in phosphate-buffered saline (PBS). Following the incubation period, the sample underwent centrifugation at a speed of 10,000 revolutions per minute for a duration of 30 minutes. Subsequently, the resulting supernatant was subjected to a tenfold dilution prior to measurement using a UV spectrophotometer system. The absorbance of the diluted sample was measured at a wavelength of 252 nm [25, 26].

Study of In vivo anti-ulcer activity

The male Wistar rats were obtained from the research animal facility. The subjects were accommodated in conditions of standard temperature and relative humidity. All animals were given a standardized pellet meal and had unrestricted access to water [27, 28].

Experimental colitis

The rats were allocated into different experimental cohorts and subjected to light ether anesthesia. A rubber catheter was inserted into the rectum and positioned within the colon, specifically at a distance of 8 cm from the anus, approximately at the location of the splenic flexure. The compound 2, 4, 6-trinitrobenzenesulfonic acid was introduced into the colon lumen using a rubber catheter, with the addition of 50% ethanol as the solvent. The subsequent trials utilized the dosage of TNBZ. Control rats were administered either 0.25 mL of 50% ethanol alone, or 30 mg of TNBZ in 0.9% saline, or 0.9% saline alone [29, 30].

Disease activity index

The evaluation of the disease's clinical activity was conducted through the consistent utilization of a qualitative disease activity index scoring technique. This involved combining

the scores obtained for bodyweight loss, stool consistency, and fecal hemorrhage.

Myeloperoxidase activity

The distal colon sample was sectioned and subsequently treated with 1 mL of hexadecyl trimethyl ammonium bromide buffer while being kept on ice. The sample was then transferred to a test tube and subjected to homogenization. The homogenate underwent centrifugation at a speed of 10,000 revolutions per minute (rpm) for a duration of 15 minutes. The supernatant was subjected to analysis using a UV chamber to measure the activity of myeloperoxidase (MPO). A volume of 0.1 mL of the supernatant was combined with 2.9 mL of a 50 mM phosphate buffer solution containing O-dianisidine hydrochloride and hydrogen peroxide. The estimation was made regarding the alteration in absorbance at a wavelength of 460 nm [31, 32].

Results and Discussion

Study of λmax

The correlation coefficients for BUD exhibited a linear relationship of 0.998 and 0.999 throughout the concentration range of 5-25 µg/mL in phosphate buffer pH 7.4 and 0.1 N HCl, respectively. The absorption maxima of drug BUD were determined to be at a wavelength of 252 nm, indicating the drug's level of purity.

FT-IR Study

The compound BUD exhibited a distinct peak at a wavenumber of 3738.45 cm-1, which can be attributed to the stretching of the hydroxyl (OH) group. Additionally, another peak was observed at 2836 cm-1, which corresponds to the stretching vibration of the carbon-hydrogen (CH) bonds. The C=O stretching vibration was observed at a wavenumber of 1672.49 cm-1, while the peak at 1413.05 cm-1 was attributed to C-H bending. No further summit was detected, hence validating the veracity of the specimen depicted in Figure 1.

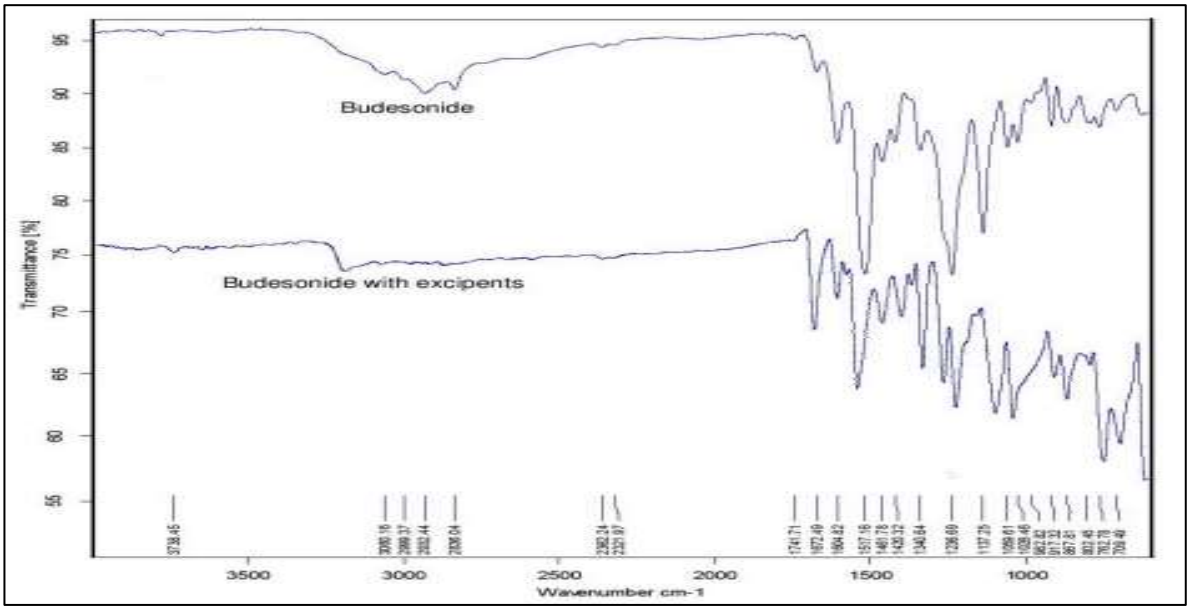

Figure 1: FTIR of Budesonide and Mixture

DSC of BUD

Figure 2 displays an observable endothermic peak at a temperature of 225°C, which pertains to the melting point of the medication. The compatibility between BUD and the excipients was established. The method employed for determination was differential scanning calorimetry (DSC). The differential scanning calorimetry (DSC) thermogram of BUD revealed a distinct melting point observed at a temperature of 225°C. The medication and cholesterol mixture was exposed to an accelerated setting of 40°C/75% relative humidity for a duration of 30 days, after which it underwent DSC analysis.

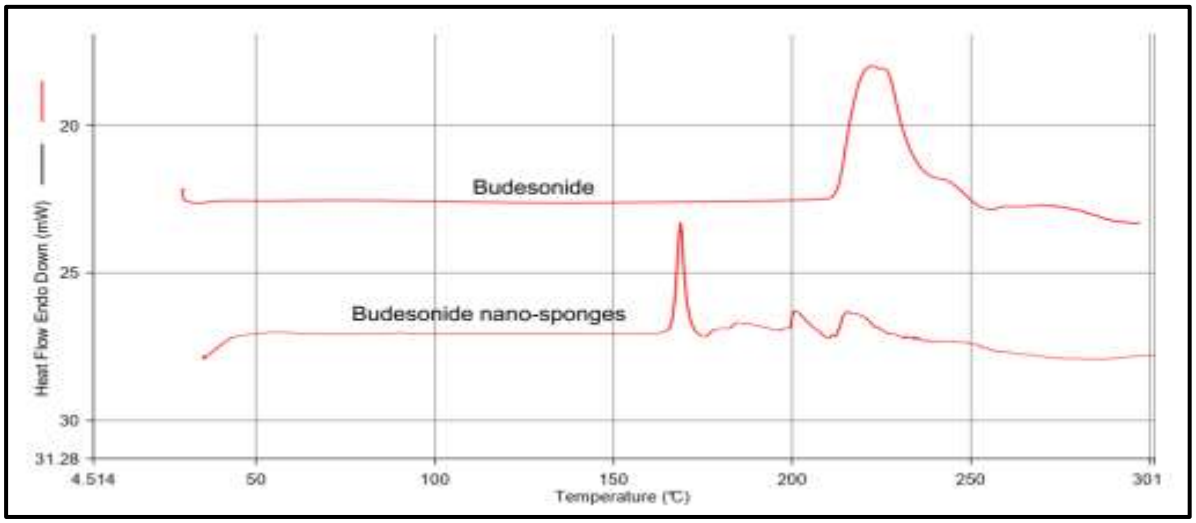

Figure 2. DSC study of Budesonide and Mixture

SEM Study

The surface topography and morphology of nano-sponges were assessed through the utilization of scanning electron microscopy analysis. The scanning electron microscopy images of the nano-sponges that were obtained and documented are presented in Figure 3. The scanning electron microscopy (SEM) images revealed that the nano-sponges had a porous structure. The formation of pores was initiated through the process of solvent diffusion from the surface of nano-sponges. Moreover, previous research has demonstrated that the unique interior composition consists of a spherical hollow.

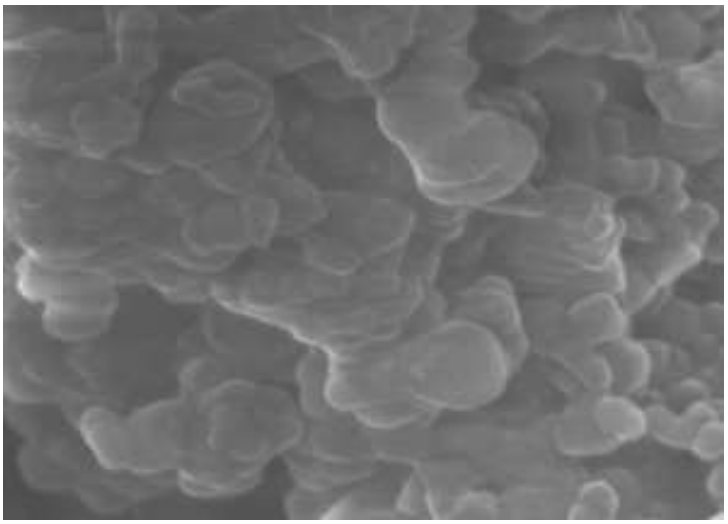

Figure 3. SEM image of the prepared formulation

Drug Content

The determination of the drug quantity in the nano-sponges was conducted through the analysis of drug content percentage in the formulation. The drug content in both batches was determined to be 96.89% and 94.73%, respectively.

In-vivo anti-ulcer activity

The rats were subjected to observation in order to identify symptoms of ulcerative colitis (UC). It was shown that a significant proportion of rats belonging to groups III and IV did not exhibit a thickened colon accompanied by ulcerations, thrombosis, or endothelial growth. The extent of injury in the affected regions shown a significant decrease, with certain animals demonstrating levels comparable to those observed in the control group. The mass percentage of C/B following intracolonic injection of TNBZ exhibited a significantly greater value in comparison to the average. Following the oral administration of the improved formulation, the ratio of C/B exhibited a less pronounced disparity compared to the colitis control group. The decrease in the C/B ratio can be attributed to the anti-inflammatory properties exhibited by the improved formulation [33, 34].

Disease activity index

The study assessed the impact of varying doses of the commercially available BUD formulation and the improved formulation in comparison to the TNBZ group, focusing on the dose-dependent effect. The evaluation of clinical activity ratings, such as stool consistency, stool blood, and weight loss, was conducted to measure the severity of the condition on day 7. The colitis control group exhibited clinical activity score, indicating a pronounced advancement of acute colitis with a Disease Activity Index (DAI) score of 3.9. On the other hand, the group that received the commercially available formulation exhibited a decrease in Disease Activity Index (DAI) scores, with a mean reduction of 2.6. The group that received the improved formulation exhibited a decrease in Disease Activity Index (DAI) ratings, with an average score of 3.0. Additionally, the control group experienced a decline in body weight and rectal bleeding, whereas the treatment groups maintained weight loss and consistent stool consistency. Additionally, colon tissues obtained from the experimental groups were gathered and assessed in terms of their colon length. The improved formulation exhibited therapeutic efficacy and demonstrated a reduction in colon length, a known indicator of colonic inflammation [35, 36].

Macroscopic Damage Score

The animals that received the BUD optimized formulation, in addition to the standard medication, exhibited a notable improvement in the wasting disease as compared to the animals that were solely treated with TNBZ. This improvement was evaluated through the analysis of macroscopic activity score and the change in body weight of the rats. The colons of TNBZ rats exhibited significant edema, hyperemia, and inflammation, whereas the colons of normal control rats treated solely with saline displayed either no inflammation or a minor degree of inflammation. The administration of therapy, in conjunction with a formulation optimized for bioavailability and uniformity of dosage (BUD), as well as a standard treatment, resulted in a significant reduction in both hyperemia and inflammation in the colons [37].

Histopathological Study

Histological examination was employed to evaluate the extent of colon tissue injury, utilizing Hematoxylin and Eosin staining. The histological evaluation of the colons obtained from the normal control group revealed the presence of intact mucosal epithelial cells, as well as the observation of submucosal glands, without any signs of ulceration or

inflammation. The rats in the control group had preserved epithelium and mucosa, demonstrating intact cellular structure and absence of leukocyte infiltration. The examination of tissue sections obtained from rats that were injected solely with TNBZ demonstrated significant damage to the crypt structure, leading to the loss of goblet cells and disruption of the epithelial layer. Additionally, there was a notable presence of moderate to severe submucosal inflammation, characterized by the infiltration of inflammatory cells. Furthermore, the colon tissue exhibited a substantial infiltration of inflammatory cells, resulting in the formation of cryptic abscesses and severe lesions. These lesions were characterized by the complete loss of colonic epithelial cells and the presence of inflammatory cell infiltration. Nevertheless, the administration of optimal formulations in mice induced with TNBZ did not result in any colon damage. Additionally, there was a reduction in inflammation observed in the colonic tissue, along with the preservation of the epithelial layer and mucosal epithelial cells. The crypt structure remained intact, and the presence of normal goblet cells indicated the absence of inflammation [37, 38].

CONCLUSION

The successful development of a nano-sponge-based BUD system was achieved through the utilization of a quasi-emulsion solvent diffusion approach. This system enables the longer transportation of pharmaceuticals over an extended period, resulting in a reduced frequency of application compared to the standard commercial formulation. Additionally, it enhances both the bioavailability and safety of the drugs. The results of the analytical characterization demonstrated a high level of drug purity. The in vitro drug release exhibited a favorable release profile for the formulated sponge's improved formulation. The results of the in vivo experiment demonstrated that the optimized formulation effectively mitigates the morphological and functional changes observed in the colonic tissues. This is achieved through the enhancement of the redox balance inside the colon. The results have demonstrated a significant reduction in colon to myeloperoxidase activity, clinical activity score, and body weight ratio, indicating the protective efficacy of the intervention. The histopathological analysis further indicated that the improved formulation was effective in providing protection against inflammatory bowel disease. Therefore, the nano-sponges, which are the focus of the delivery system created and evaluated in this study, appear to hold promise in terms of their potential to prevent BUD, as well as other colonic disorders. Furthermore, these nano-sponges show potential for practical application in the pharmaceutical industry.

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None

Conflict of Interest

None

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