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ORIGINAL ARTICLE

Development And Characterization of a Drug Delivery System Targeting the Colon using Polysaccharide-based Nanoparticles

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ABSTRACT
Targeted delivery of medications to the colon offers significant benefits for treating disorders specific to the colon, such as Inflammatory Bowel Disease, diverticulosis, polyps, and colorectal cancer. A highly accurate medication delivery platform that specifically targets the colon is anticipated to decrease the adverse effects of the drugs and enhance the therapeutic response at the location of the disease. This work aims to examine the impact of sodium alginate coating on the surface properties of solid lipid nanoparticles (SLNs), as well as their potential to target ulcerative colons and control drug release. The characteristics of mesalamine loaded SLNs were assessed, including particle morphology, particle size, particle surface charge, drug encapsulation effectiveness, in-vitro drug release, and in-vivo histopathological analysis. These assessments were conducted for the purpose of treating ulcerative colitis. The analysis revealed that the particles had a spherical morphology, with an average size distribution ranging from 125±50 nm. Additionally, the encapsulation efficiency ranged from 61% to 68%. The drug release of alginate coated nanoparticles (F2) in rat caecal media after 24 hours was determined to be 91.9%. The present in-vitro release investigation illustrates that the use of sodium alginate coated nanoparticles effectively inhibited the rapid release of substances in an acidic environment. The therapeutic capacity and effectiveness of the nanoparticles were assessed in an experimental model of colitis produced by acetic acid (AA). The findings of our study indicate that nano-hybrid particles coated with polysaccharide (sodium alginate) are a very efficient oral delivery system specifically designed to target the colon for the treatment of colitis.
Keywords*: Colon, Targeted Delivery, Mesalamine, Nanoparticles*

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INTRODUCTION

Oral lipid-based drug delivery systems have become prominent therapeutic carriers, making a substantial impact in the field of modern medicine. It provides biopharmaceutical benefits in terms of improved compatibility with living organisms, more reliable absorption into the bloodstream for the effective transportation of medicinal substances to specific illness locations in the gastrointestinal system. The oral route of administration is the preferred method for controlled release systems due to the physiological characteristics of the gastrointestinal tract (GIT), which enables for a greater variety of dosage forms to be designed compared to other routes [1-3]. Furthermore, the oral route is the most adaptable and frequently used method for achieving systemic effects, because to its simplicity of application, drug assimilation, patient adherence, and formulation flexibility. Thus, it seems that oral colon-targeted dosage forms, such as time-based [4-6]. pH-dependent [7,8], prodrugs approach [9, 10], and polysaccharides-based (microflora triggered) colonic drug delivery [11, 12] with a suitable release pattern, could be highly beneficial in delivering effective treatment for colonic diseases.

The targeted delivery of different medications to the colon has proven to be highly advantageous for numerous pharmacotherapies, including the treatment of inflammatory bowel illnesses, Crohn's disease, ulcerative colitis, diverticulosis, anaerobic bacterial infections, and primarily colon cancer. Inflammatory bowel diseases (IBD), such as Crohn's disease (CD) and ulcerative colitis (UC), are long-lasting inflammatory conditions that affect the gastrointestinal system. CD and UC have unique pathological and

clinical features. Despite significant research efforts over the past decades, the underlying causes of these conditions are still not well understood [13, 16].

Colon targeted drug delivery presents advantages in treating IBD orally. However, researchers face challenges due to its location in the distal portion of the alimentary canal, enzymatic degradation, and the wide range of pH environments (pH 1.2 to 8) throughout the canal. When administered orally, the dosage form must pass through several pH levels and enzymes before reaching the intended site, which further complicates its reliability and efficacy of delivery [17, 18].

Out of all the systems mentioned (pH, time-dependent, pro-drug, microflora triggered), the microflora activated delivery systems have been proven to be the most effective. This is because the sudden increase in bacteria population and enzyme activity in the ascending colon is a distinct event that is not influenced by the time it takes for food to pass through the gastrointestinal tract or the pH levels.[18, 19] The primary method of microflora-triggered delivery systems is a sequence of polysaccharides that resist enzymatic breakdown in the small intestine and are mostly metabolised by colon bacteria. These polysaccharides include guar gum, alginates, dextrans, and pectins. These polysaccharides are not broken down in the stomach and small intestine, but are instead broken down by the anaerobic microorganisms in the colon (namely bacteroides, bifidobacteria species, and eubacteria) into smaller monosaccharides. These monosaccharides are subsequently used by the bacteria as an energy source. These carriers function as prebiotics, which are indigestible food elements that can be fermented by the microorganisms in the gastrointestinal tract. They are involved in the mechanisms of medication release [20, 21].

In this study, we have endeavoured to apply a sodium alginate polymer coating on lipid-based carriers to achieve a targeted medication delivery system specifically for the colon. Solid lipid nanoparticulate systems (SLNs) are a type of sophisticated nano lipid carrier system, which is distinct from typical systems including liposomes, lipid emulsions, and polymeric nanoparticles [22]. SLNs exhibit sufficient tolerability, scalability, capability to incorporate hydrophobic or hydrophilic medicines, and enhanced stability of encapsulated pharmaceuticals. SLNs are superior in the way they combine the benefits of conventional nanoparticles while eliminating some of their drawbacks [22, 23]. The objective of this study is to construct a solid lipid drug delivery system containing mesalamine, with the goal of addressing formulation and treatment challenges by creating controlled drug delivery systems.

MATERIAL AND METHODS

The Active Pharmaceutical Ingredient mesalamine, and Sodium alginate were procured from Sigma-Aldrich, India. The compounds Triglyceride monostearate, Stearic acid, and Polysorbate 80 were acquired from Molychem Manufacturers (P) Ltd, located in Mumbai, India. All the chemicals and solvents used in the research were of high purity and were acquired from SD fine Chemicals, Mumbai, India.

Factors study by 2^3 full factorial design

The 2^3 Full Factorial Design was implemented to assess the factors which contributed significantly in the study design. While performing the study, Design Expert 11.0 V was adopted. Three independent variables such as stearic acid, triglycerol monostearate and polysorbate 80 selected. The responses selected are entrapment efficiency, particle size and percentage drug release in 16h. The quanity of factors included in the study was based on the literature review. A total 8 formulation developed separately with triglycerol monostearate and stearic acid and data are analyzed and reported. Half normal plot, interaction plot, interaction plots as well as 3D response surface plot was developed. The data were interpretated by ANOVA which highlighted the significant factors involved in the SLN formulation study. The p value fixed at a level of 0.05.[24]

Preparation of Mesalamine loaded solid lipid nanoparticles (MES-SLNs)

SLNs were produced using the heat homogenization process, as previously reported, with minor modifications [22]. In summary, a measured amount of triglyceride monostearate (TGMS) and stearic acid (SA) from Table 1 were heated until they melted. Then, mesalamine (10 mg) was added to the hot mixture for each group. TGM and SA functioned as lipids that served as a framework for trapping Mesalamine. The lipid and medication mixture were thoroughly mixed using a vortex mixer and sonicator. A total of 20 µl of polysorbate 80, serving as an emulsifier, was introduced into the mixture to create a uniform blend of lipid and aqueous components. This was then followed by the process of homogenization.

In the case of polymer containing SLNs, a sodium alginate solution with varying concentrations was slowly added drop by drop into a heated mixture consisting of TGMS, SA, Mesalamine, and polysorbate 80. The mixture was then constantly stirred using a vortex mixer. The emulsion was subsequently homogenised using a microtipped probe-sonicator.

Table 1: Composition of sodium alginate coated SLNs containing Mesalamine

Formulation code	Mesalamine (mg)	Stearic acid (mg)	Trygylcerol monostearate (mg)	Polysorbate 80 (%)	Milli-Q water (µl)	Rotation speed (rpm)
Effect of Lipids: Fatty acid ratio						
F1	10	80	40	1	1980	15000
F2	10	60	60	1	1980	15000
F3	10	40	80	1	1980	15000
Effect of Surfactant concentration						
F4	10	60	60	0.75	1980	15000
F2	10	60	60	1	1980	15000
F5	10	60	60	2.0	1980	15000
Effect of rotation speed						
F6	10	60	60	1	1980	10000
F2	10	60	60	1	1980	15000
F7	10	60	60	1	1980	16000

Abbreviations: SA, Stearic acid; TGMS, triglyceride monostearate; D.water, Distilled water

Characterization of solid lipid nanoparticles

The SLNs that were created underwent evaluation for several criteria, such as particle size, shape, surface morphology, size distribution, polydispersity index, and surface charge (zeta potential) of the particles. Additionally, the encapsulation efficiency, loading efficiency, temperature analysis, diffraction investigations, and in vitro drug release were conducted.

Particles size, morphology, charge analysis

The MES-SLNs were visualised using a scanning electron microscope (SEM- Jeol, JSM-6100). The samples were created by applying a small amount of the formulation onto a double-sided adhesive tape, which was then attached to an aluminium stub. The particle size, polydispersity index, and surface charge of the particles were determined by dispersing the formulation in milli-Q water and analysing them using the Malvern Zetasizer (Nano series ZS90).

Drug loading and encapsulation efficiency of particles

Encapsulation efficiency refers to the percentage of the medicine that is enclosed within the formulation. The drug encapsulation efficiency of SLNs was evaluated using the dialysis bag method, as previously described with minor adjustments.[24] The SLNs were placed inside dialysis bags with a molecular weight cutoff of 3.5 kDa and submerged in 20 ml of PBS with a pH of 6.8. The samples were agitated for duration of 15 minutes each, followed by a 5-minute interval of rest. The samples were subjected to a 10-hour period of equilibration at room temperature. Once the equilibrium was achieved, the mixture was subjected to centrifugation at a speed of 3000 revolutions per minute for duration of 10 minutes. The mesalamine concentration in the bag was measured using RP-HPLC (Shimadzu LC-20 AD liquid chromatography) at a wavelength of 232 nm.

DSC and Powdered XRD analysis

The DSC analysis validated the physical composition of the medication within the nanoparticles. The experiment was conducted using a Shimadzu DSC-60 differential scanning calorimeter, manufactured by Shimadzu Corporation in Kyoto, Japan. A heating rate of 10°C per minute was employed, with the temperature range spanning from 20°C to 400°C. A vacant aluminium pan was utilised as a point of comparison. The solid state of lipid and drug in SLN was analysed using X-ray diffraction measurements conducted with a Rigaku Rint/2000 Model X-ray diffractometer.[25, 26]

The crystalline or amorphous nature of the chemical was determined definitively using an X-ray diffractometer by X-ray diffraction.

Drug release studies of SLNs in different simulated gastrointestinal media

The drug release tests were conducted utilising the dialysis bag method in various media, including pH buffer media and rat caecal content (biorelevant) media.

Preparation of fresh rat cecal content medium

The study protocol for in vivo research received official approval from the Institutional Animal Ethics Committee. One animal was euthanized thirty minutes before to the start of the medication release trials. The abdominal cavity was surgically incised and the contents of the cecum were separated, tied off at both ends, dissected, and promptly transferred to a pH 7.4 phosphate-buffered saline solution that had been pre-treated with nitrogen gas. A bag containing cecum was opened, and its contents were weighed separately. The weighted contents were then combined and suspended in a buffer solution that was

continually bubbled with nitrogen. This resulted in a dilution of 4% weight/volume. The aforementioned procedures were conducted in the presence of nitrogen to uphold anaerobic conditions [18, 27].

***In-vitro* drug release studies of mesalamine SLNs**

A drug release study was conducted in vitro utilising pH media and caecal contents media, with a dialysis bag as the experimental setup. Nevertheless, a minor alteration was made to the technique. The dissolution investigation of mesalamine loaded SLNs was conducted in a pH 1.2 hydrochloric acid (HCl) buffer at a rotation speed of 50 revolutions per minute (rpm) at a temperature of 37 ± 0.5 degrees Celsius for duration of 2 hours. Subsequently, the pH of the dissolving fluid was modified to pH 6.8 and the investigation was extended for a maximum duration of 5 hours. After five hours, the media was treated with nitrogen gas to eliminate any remaining oxygen that was not dissolved. This was done to ensure that the medium remained in an anaerobic state for a duration of 15 minutes. A 4% weight/volume concentration of recently prepared cecal solution was introduced into the dissolution media. The study was then conducted for a maximum duration of 24 hours, with a continuous flow of nitrogen maintained during the entire trial. Approximately 1 millilitre of samples was extracted from the dissolving medium at time intervals ranging from 0.5 to 24.0 hours. These samples were then replaced with fresh medium that was kept in an anaerobic environment.

***In vivo* studies**

Experimental animals

The present investigation selected guinea pigs of both sexes.

Induction of colitis and treatment schedule

Subsequently, we examined the therapeutic effectiveness of mesalamine loaded SLNs in a guinea pig model induced with acetic acid. Ulcerative colitis was caused by administering a weak solution of acetic acid, and the animals were rendered unconscious using isoflurane. Colitis was generated by injecting 2, 4, 6-trinitrobenzene sulfonic acid (TNBS) into the colon, with a 24-hour interval for three consecutive days. The animals were divided into groups of three animals each, in a random manner. The allocation of groups and dosage regimen is as follows,

Table 2. Treatment and doses given to animals

Groups	Treatments	Dose (p.o)
Group-I	Healthy control	50 ml/kg
Group-II	TNBS	2 ml/kg (intracolonic)
Group-III	Mesalamine solution + TNBS	320mg/kg/d+2ml/kg
Group-IV	Mesalamine SLNs (F5) + TNBS	320mg/kg/d + 2ml/kg
Group-V	Blank SLNs+ TNBS	Blank+ 2ml/kg

Abbreviations: po- per oral route; kg - kilogram; d- day

Assessment of colitis

The different groups, as shown in table 2, were given orally to the animals, and the Guinea pigs were evaluated for characteristics such as determining body weight, assessing diarrhoea and scoring its activity, evaluating the ratio of colon weight to length, and conducting histological examination of colonic inflammation.

Diarrhoea assessment and clinical activity scoring

Following the administration of 2 ml of TNBS to induce colitis, the animals were placed individually in cages with a clean white sheet at the bottom. They were then monitored for a period of 4 hours to observe the onset of diarrhoea, the number of faecal matter discharges, the consistency of faecal content, the presence of faecal bleeding, and the assessment of diarrhoea severity based on the consistency of the faeces. In addition, as part of the pharmacological treatment, faecal matter from each group was analysed every morning to evaluate the presence of diarrhoea. The recordings were completed and subsequently ranked in accordance with their respective criteria. The clinical activity score for weight loss, stool consistency, and rectal bleeding was computed using the previously reported method. [28] The level of inflammation was assessed using a scale ranging from 0 to 4.

Colon weight to length ratio assessment

The ratio of colon weight to length is a useful parameter for assessing the severity of colitis. Colitis causes the colon to become shorter. Colon length measurements were assessed to evaluate colitis. The Guinea pigs were euthanized using isoflurane on the 7th day. Their colons were then removed and measured, after washing out the contents. The weight of the colons was divided by their length to calculate the colon weight/colon length ratio. This ratio is regarded as a reliable and responsive indication of the intensity and extent of the inflammatory response in colitis. [29]

Table 3: Ranking of diarrhoea

Rank	Diarrhoea Score	Visible Fecal Blood	Type of diarrhoea
0	Normal pellets	Normal	No diarrhoea
1	Slightly loose feces	Slightly bloody	Mild diarrhoea (staining to anus)
2	Loose feces	Bloody	Moderate diarrhoea (staining over top of the legs and lower abdomen)
3	Watery diarrhoea	Blood in whole colon	Severe diarrhoea (staining over legs and greater abdomen, often with continual anal leakage)

RESULTS AND DISCUSSION

Formulation Development

SLNs are solid lipid matrices that entrap the drug in their crystal structure. These are extensively used in drug delivery systems. They are targeted to colon as they are biodegradable, provide adequate stability and importantly show low cytotoxicity due to the absence of organic solvents. Mesalamine was selected as a model drug in the advancement of SLNs.

Factors screening and analysis by 2^3 full factorial design

SLN are developed as per 2^3 full factorial design and was to assess the significant factor and variables which could impact on SLN development.

Response surface plots were developed to show the relationship between variables and responses. ANOVA study analyses significant factors. Particle size: 112-171 nm. The highest particle size observed in formulation "R8" with high quantities of stearic acid and Triglycerol monostearate. EE is higher in "R8" due to the presence of stearic acid and Triglycerol monostearate. Polysorbate 80 significantly impacts EE by solubilizing the drug and leading to low EE. The observation can be seen in "F5" with an EE of 85.23% and "F8" with a higher EE of 88.47%. *In-vitro* drug release was performed in different pH media using the dialysis bag method with different buffers. The study showed that drug release was dependent on excipient concentration. The information in figure ."R3" had faster drug release in 16h compared to F8, which had 58.18% drug release. The data concluded that particle size and EE are dependent on stearic acid and Triglycerol monostearate. Polysorbate 80 showed emulsifying property and EE decreased. High Polysorbate 80 led to faster drug release.

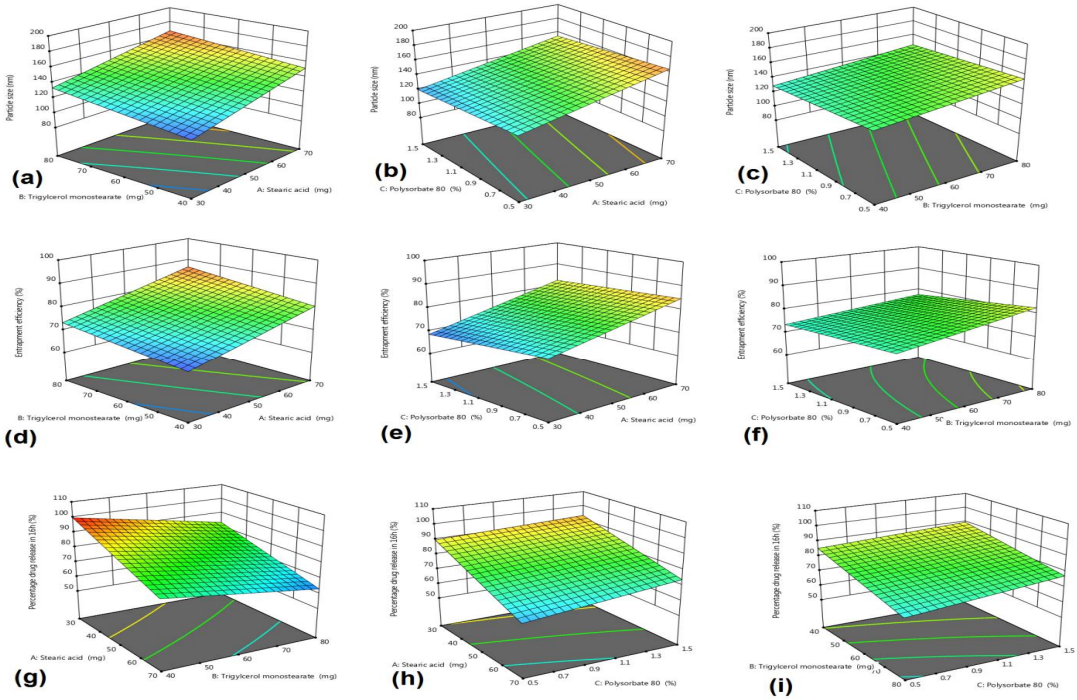

Figure 1: response surface plotted for (a) Particle size: Triglycerol monostearate Vs stearic acid (b) Particle size: Stearic acid Vs polysorbate 80 (c) Particle size: Triglycerol monostearate Vs polysorbate 80; (d) EE: Triglycerol monostearate Vs stearic acid (e) EE: Stearic acid Vs polysorbate 80 (f) EE: Triglycerol monostearate Vs polysorbate 80; (g) percentage drug release: Triglycerol monostearate Vs stearic acid

(h) percentage drug release: Stearic acid Vs polysorbate 80 (i) percentage drug release: Triglycerol monostearate Vs polysorbate 80

Pareto chart highlight information on significant variable with the help of t-value as well as banferroni limit. In figure , it can be seen Stearic acid have crossed t-value for Response-1, similarly in Response-2 Stearic acid once again found to cross t-value. Whereas, in Response-3, both Stearic acid and Trigylcerol monostearate crossed t-value. Hence it can be ascertained that, in current study while developing the nanoparticles Stearic acid and Trigylcerol monostearate both have significant impact.

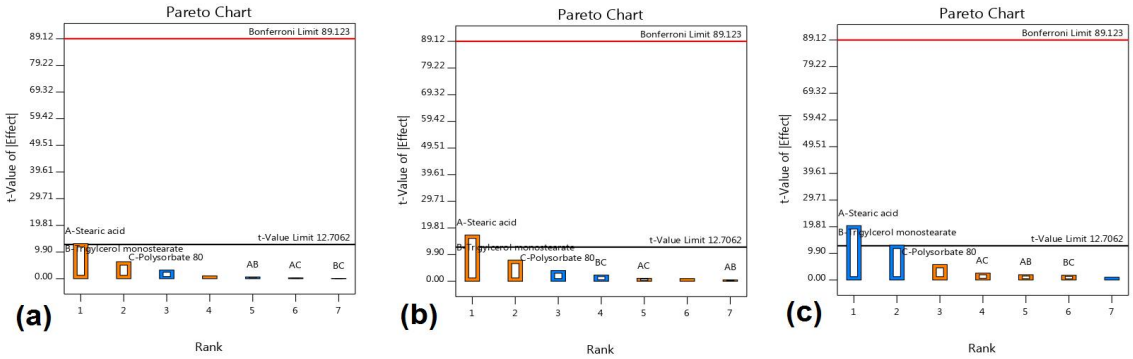

Figure 2: Pareto chart established for (a) Particle size (b) Entrapment efficiency (c) Percentage of drug release in 16h

Characterization of SLNs

The particle analysis of SLNs loaded with mesalamine is presented in Table 4 and Figure 1. The average size of the coated and uncoated particles ranged from roughly 125 to 135 nm. The particles were spherical in form and had a rough surface. All of the batches had a negative zeta potential, however the particles coated with sodium alginate demonstrated an enhanced surface charge. The bigger the negative charge, the stronger the adherence to the inflamed mucosa, which has a positive charge (31-33). As the ratio of surface area (SA) to total grain mass (TGMS) rose, the particle size was shown to increase. This could be attributed to the sluggish solidification of lipids and fatty acids during the high-temperature homogenization procedure.

Table 4. Summary of particles characterization

Formulation Codes	Size (nm)	Zeta potential (mV)	PDI	Encapsulation efficiency (%)
F1	135.1±0.7	-8.3±0.02	0.223±0.04	62.26±0.5
F2	125.4±0.5	-8.9±0.01	0.166±0.05	68.72±1.2
F3	134.2±0.8	-8.4±0.02	0.221±0.01	63.24±1.1
F4	130.6±0.4	-8.6±0.04	0.241±0.02	61.38±0.7
F5	126.2±0.7	-8.6±0.02	0.212±0.03	63.58±1.0
F6	131.7±0.3	-8.6±0.03	0.253±0.06	63.14±0.6

Abbreviations: F, formulation type; nm, nanometer, mV, milli volts, PDI, polydispersity index; (n = 3; data are expressed as mean±SD).

The data revealed that the particle size of SLNs was significantly smaller in comparison to SLNs containing sodium alginate. The polydispersity index falls within the range of 0.16 to 0.25, indicating a homogenous character. The formulations comprising a sodium alginate concentration of 0.4% w/v for coating exhibited greater entrapment efficiency, specifically 68.72±1.2, compared to the other sodium alginate containing SLNs. The higher concentration of the coated polysaccharide surrounding the mesalamine SLNs is responsible for the increased electrostatic forces of attraction.

***In-vitro* drug release studies in different simulated gastrointestinal media**

The formulations have been designed with a lag period of 5-6 hours to inhibit medication release in the upper gastric tract and facilitate release in the colon at the location of the disease. The dissolution profile of all sodium alginate containing SLNs, as shown in Figure 3C, clearly demonstrated that the proportion of drug release was much greater in rat caecal media compared to buffer media. This could be attributed to the existence of several microorganisms that contribute to the deterioration of sodium alginate. The bacteria Bifidobacterium, Lactobacillus species, and Bacteroides were the dominant microorganisms in

the medium that degraded the sodium alginate coating, leading to effective drug delivery in the rat caecal media.

Within the uncoated SLNs labelled as F1 to F3, over 86-91% of the drug was released within a time frame of 2 hours, as shown in Figure 4A. Drugs that need to be released at a specific spot or specifically in the colon cannot utilise this form of burst release. The drug release profiles of sodium alginate coated SLNs exhibited a biphasic pattern, characterised by an initial delay in release time. This delay can be attributed to the presence of a viscous protective covering made of polysaccharide. When the coating level is increased, the rate at which the drug is released from SLNs is slower compared to SLNs that are not coated. Experiments were conducted for 24 hours in an anaerobic medium containing 4% w/v of rat cecal contents to investigate the drug release based on sodium alginate concentration. The *in-vitro* drug release investigation of the mesalamine loaded SLNs shown that only 0.5-1.5% of the drug was released when exposed to simulated stomach and intestinal fluids. After duration of 5 hours, when the contents of the cecum were injected, the cumulative release of mesalamine from SLNs after 24 hours was found to be 88.7% for F5 and 88.4% for F6.

Among of all the formulations using sodium alginate, F2 (91.9) showed effective release in the specific location of the colon that was targeted. After duration of 5 hours, the drug release in rat caecal media is only 15.85%. The stiffness of sodium alginate during this period may be the cause. However, a notably larger drug release of 88.0% was seen after 24 hours in rat caecal media. This phenomenon can be explained by the pliability of sodium alginate, which allows it to be readily absorbed by the microbiota. This leads to the highest possible release of medication at the site of inflammation, where the therapeutic effects are prolonged.

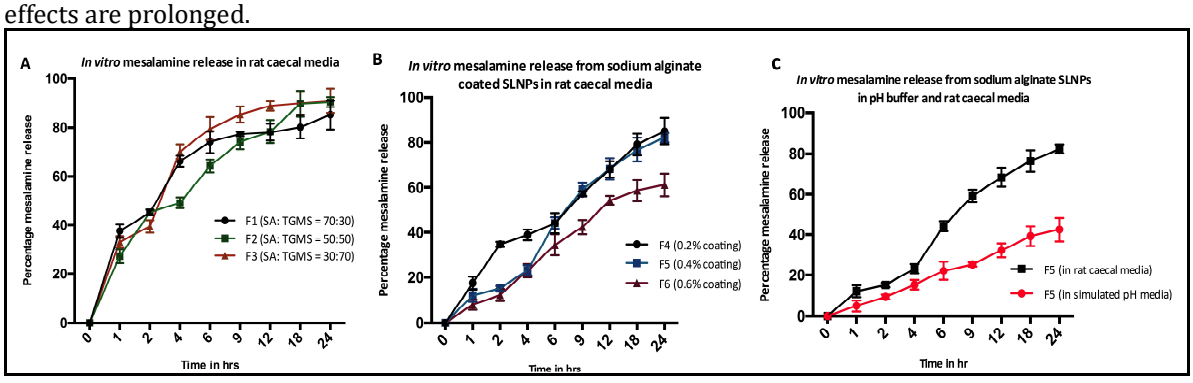

Figure 3. *In-vitro* drug release studies of mesalamine SLNs. Uncoated mesalamine SLNs in rat caecal media (3A). Sodium alginate coated SLNs release in rat caecal media (3B). Comparision of mesalamine release (F3) in simulated buffer media vs rat caecal media (3C).

DSC and Powdered XRD analysis

Figure 4 displays the DSC thermogram of both pure mesalamine and lyophilized samples of mesalamine SLNs. The DSC thermogram clearly showed exothermic peaks occurring at temperatures of 279°C and 257°C, respectively. The decrease in the melting peaks of mesalamine in lyophilized samples indicates a potential decrease in the crystalline structure of mesalamine in nanoparticles. The drop in melting enthalpy has a direct correlation with larger particles, which necessitate more energy to overcome lattice force.

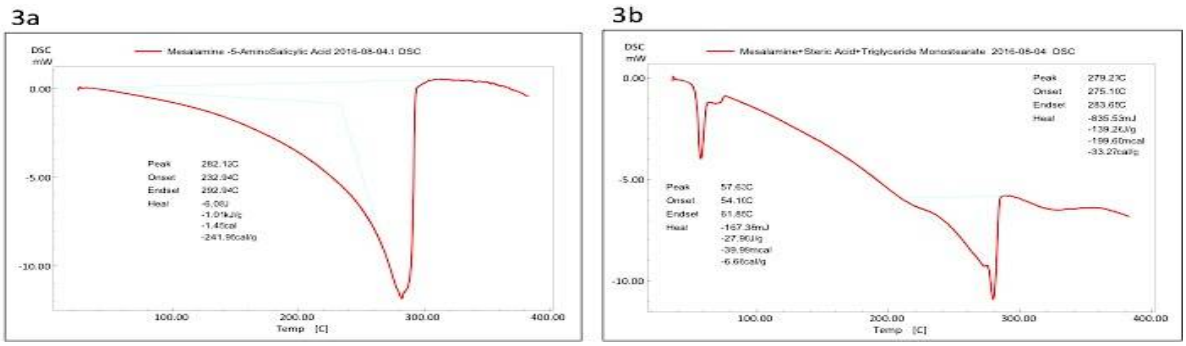

Figure 4. DSC thermogram of pure mesalamine powder (3a); and lyophilized sample of mesalamine SLNs (3b).

The lyophilized SLNs formulation was examined using PXRD (powder X-ray diffraction) experiments, and the findings are presented in Figure 5. The X-ray diffraction pattern of the lyophilized SLNs dispersion was compared to that of the pure medication.

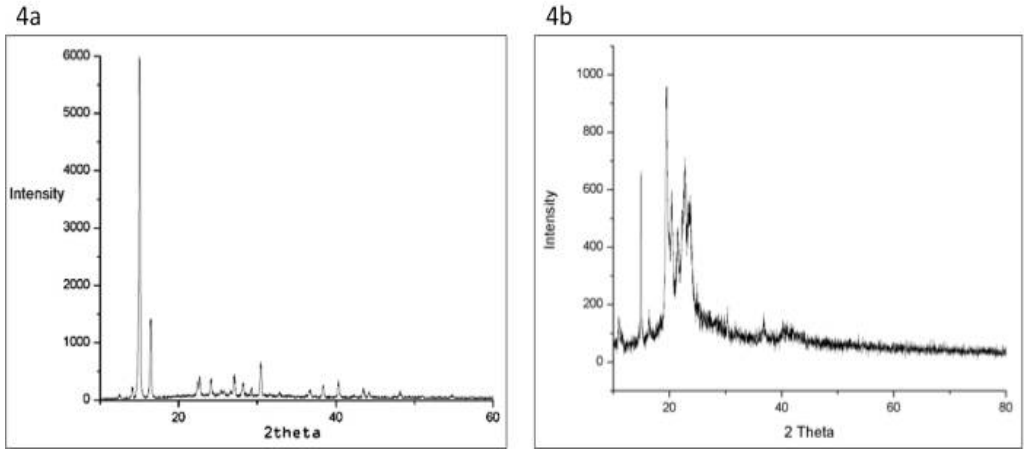

Figure 5. PXRD pattern of pure mesalamine powder (5a). Lyophilized SLNs dispersion (5b)

The presence of distinct and strong peaks indicated that there was no notable transformation from a crystalline state to an amorphous state. The XRD pattern of lyophilized mesalamine loaded SLNs formulation shows that pure mesalamine has distinct crystalline peaks between 12-18 θ. These peaks either remain unaltered or their intensity is lowered. Although the PXRD design indicates a slight amorphous nature, the high likelihood of crystallinity can be inferred due to the significant drug loading into the solid dispersion.

***In vivo* therapeutic efficacy**

Animals with acetic acid-induced colitis were given uncoated SLNs and SLNs coated with sodium alginate orally every day for a period of 6 days. Following the treatment, we evaluated the severity of colitis by assessing diarrhoea, monitoring changes in body weight, measuring the ratio of colon length to weight, and doing histological investigation of colon tissue sections.

Change in body weight and clinical clinical activity score of colitis

The untreated colitis group displayed significant diarrhoea with bleeding and a substantial reduction in body weight compared to the healthy control group (Figure 6). Prior to inducing colitis, there were no disparities in body weight between the groups. The group treated with MES-SLNs exhibited a more rapid restoration of body weight compared to the groups that were not treated or treated with Mes-suspension. The data clearly indicated that there were no stains present on the rectum, legs, or abdomen of the animal in the healthy control group. The untreated animal exhibited severe diarrhoea with pronounced staining on the legs and belly, accompanied by persistent anal leakage. In contrast, the animal treated with mesalamine medication suspension showed only mild staining. The staining in the mice treated with mesalamine SLNs completely disappeared, as confirmed by the clinical data scoring shown in Figure 6.

Colon weight to length ratio assessment

The length of the colon was significantly reduced in animals with colitis caused by acetic acid, which is a prominent characteristic of colitis (Figure 6A and B). The healthy group exhibited a colon length of 57.5±2 cm, whereas the induction of colitis resulted in a significant decrease in colon length to approximately 36 cm. In comparison to the untreated group, mice treated with MES-suspension and blank SLNs showed an augmentation in colon length. Nevertheless, the treatment with MES-SLNs resulted in a substantial improvement in colon length, and a noticeable disparity in colon length was detected.

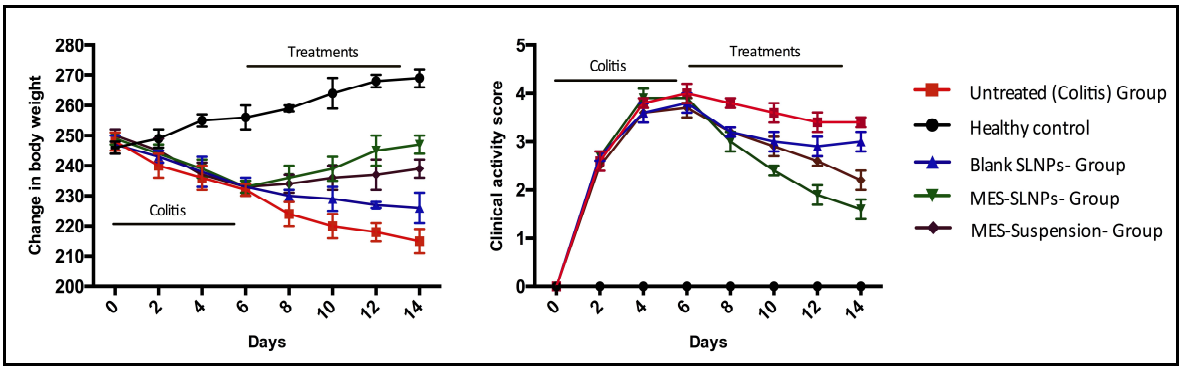

Figure 6. Body weight changes of healthy, untreated, and SLNs-treated groups, clinical activity score throughout the experimental period

Similar to previous findings on weight/length measurement, the colon weight/length ratios were greater after MES-SLNs treatment compared to the colitis group (Figure 7A, 7B).

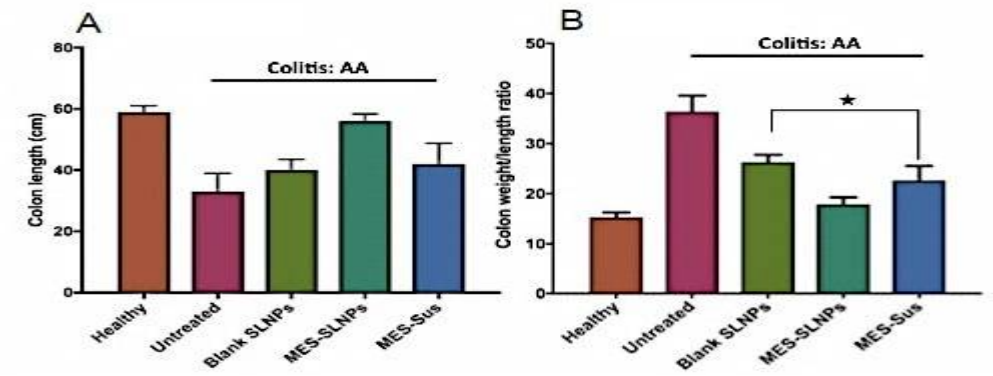

Figure 7. Macroscopic evalaution of colitis. (A). Colon weight to length ratio. (n=6). statistical significance ($^{\star}P$ <0.01) compared with colitis control.

CONCLUSION

The formulations with a lipid to fatty acid ratio of 1:1 and 1% polysorbate 80 were chosen for their favourable sustainability. The formulation creation of SLNs lacking sodium alginate was ineffective in preventing drug release in the upper gastrointestinal tract (GIT). Preformulation studies of mesalamine were conducted to identify the medication, determine its purity, develop calibration curves, and establish an analytical method for assessment. FTIR, DSC, and PXRD analyses confirmed the satisfactory quality of mesalamine for its usage in development. The interactions between drugs and excipients were examined and assessed using Fourier Transform Infrared Spectroscopy (FTIR), Powder X-ray Diffraction (PXRD), and Differential Scanning Calorimetry (DSC). The study indicates that the selection of excipients is crucial and it is important to ensure that there are no interactions between the medicine and the chosen excipients for the formulation composition. The formulations with a balanced ratio of lipids to fatty acids and 1% polysorbate 80 were chosen for their excellent sustainability. The formulation creation of SLNs lacking sodium alginate was ineffective in preventing drug release in the upper gastrointestinal tract (GIT). SLNs containing sodium alginate as a polysaccharide effectively released the medicine at a specific targeted spot in the colon. The SLNs formulations, which contained sodium alginate at a concentration of 0.4%w/v, demonstrated satisfactory release in rat caecal medium and were deemed appropriate for targeting drug delivery to the colon. The synthesised SLNs exhibited a spherical morphology, displaying consistent size and a smooth surface with a homogeneous composition. The SLNs consist of 0.4%w/v sodium alginate and have a particle size that is considered acceptable. They also have high encapsulation efficiency and a greater zeta potential. The SLNs formulated with sodium alginate exhibit a stronger negative charge, resulting in enhanced adherence to the positively charged inflammatory mucous membrane. The in vitro release exhibits a regulated release pattern that allows for the targeted delivery of the medicine. In vivo experiments have determined that SLNs containing sodium alginate (0.4%w/v) as a polysaccharide effectively achieve drug localization in the specific target area of the colon. Furthermore, it shown that the probiotic properties of spirulina augment the effectiveness of mesalamine. The

formulated SLNs composed of 0.4% sodium alginate and a 50:50 ratio of SA: TGMS demonstrate potential as a stable system for delivering poorly soluble medicines to the colon. SLNs including sodium alginate as a polysaccharide effectively released the medicine at a specific targeted spot in the colon. The synthesised SLNs exhibited a spherical morphology, displaying consistent size and a smooth surface with a homogeneous composition. The SLNs consist of 0.4%w/v sodium alginate and have a particle size that is considered acceptable. They also have high encapsulation efficiency and a greater zeta potential. The SLNs formulated with sodium alginate exhibit a higher negative charge, resulting in enhanced adherence to the irritated mucous membrane that possesses a positive charge. In vivo investigations have determined that SLNs containing sodium alginate (0.4%w/v) as a polysaccharide effectively deliver drugs to the intended region in the colon.

DISCLOSURE

The authors report no conflicts of interest in this work.

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