Ind. J. Pharm. Edu. Res., 2027; XX(XX):1-11.
https://www.ijper.org

Research Article

Title: Development and Therapeutic Evaluation of Mesalamine-Loaded Nanoemulsion in DSS-Induced Colitis

Shamim Shamim, Pallavi Chandel, Shadab Ali*

[1]Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, Ganga Nagar, Meerut, Uttar Pradesh, India

ABSTRACT

Background: Inflammatory bowel disease requires effective delivery of anti-inflammatory drugs to the affected intestinal region. The present study aimed to develop and optimise a Mesalamine-loaded nanoemulsion and evaluate its therapeutic potential in experimental colitis. **Methods:** Nanoemulsions were prepared using castor oil, Tween 80, and Poloxamer 188 with different formulation compositions. The morphology, in vitro drug release, and physicochemical properties of the formulations were assessed. The optimised A4 formulation was further evaluated in C57BL/6 mice with DSS-induced colitis. Body weight, disease activity index, colon length, Myeloperoxidase activity, inflammatory cytokines, and histopathological changes were assessed. **Results:** A4 demonstrated a droplet size of 83.54 nm, zeta potential of −68 mV, viscosity of 22.2 cP, and spherical, well-dispersed morphology. It showed the highest cumulative Mesalamine release of 93.24 ± 0.39% at 8 h. In DSS-induced colitis, A4 improved body-weight maintenance, reduced disease activity, restored colon length to 8.5 ± 0.3 cm, and decreased MPO activity to 2.0 ± 0.3 U/g tissue. Tumour Necrosis Factor-alpha, Interleukin-6, and Interleukin-1β levels were reduced to 48 ± 7, 39 ± 5, and 32 ± 5 pg/mg, respectively. Histopathology demonstrated improved mucosal architecture and crypt preservation. **Conclusion:** The optimised A4 Mesalamine-loaded nanoemulsion demonstrated enhanced protective effects against DSS-induced colonic injury compared with Free Mesalamine and may represent a promising approach for improved Mesalamine delivery.

Keywords: Drug delivery, DSS-induced colitis, Histopathology, Inflammatory bowel disease, Mesalamine, Nanoemulsion

Correspondence:
Dr. Shadab Ali
Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, Ganga Nagar, Meerut, Uttar Pradesh, India.
Email: shadabali_pharma@iimtindia.net

www.ijper.org

Mesalamine nanoemulsion development and evaluation

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two main forms of inflammatory bowel disease (IBD), a chronic, recurrent inflammatory illness of the gastrointestinal (GI) system. The illness is linked to chronic intestinal inflammation[1,2] and may entail changes in the intestinal microenvironment, immune response, and mucosal barrier. Because the inflammatory process is localised within the GI tract but can be recurrent and progressive, effective delivery of therapeutic agents to the affected intestinal regions remains an important consideration in IBD management. While pharmacologic treatment may offer both symptomatic relief and anti-inflammatory effects, it becomes very difficult to attain the proper level and sustain the adequate drug concentration in areas affected by the inflammation.[3]

Mesalamine, also known as 5-aminosalicylic acid, is an already well-known anti-inflammatory drug used mainly in treatment of inflammation of the colon. The efficacy of this medication is significantly reliant on obtaining an appropriate level of concentration of the drug in the intestinal mucosa. Physicochemical and pH-related properties of Mesalamine affect its dissolution and release in the gastro-intestinal tract. Variations in the release properties of Mesalamine-based drugs under different pH levels show that formulation is an important part of proper intestinal delivery of the drug. Thus, there may be problems with conventional formulations of Mesalamine concerning release and distribution of the drug.[4]

Various colon-targeted drug delivery systems have been investigated to improve the therapeutic performance of Mesalamine, including pH-sensitive systems, mucoadhesive formulations, microspheres, pellets, nanoparticles, and other nanocarrier-based approaches. Nanotechnology-based delivery systems can provide opportunities to modify drug dispersion, release behaviour, surface characteristics, and interaction with biological tissues.[5] In particular, nanoemulsions (NEs) are attractive

DOI: 10.5530/ijper.20270384

Copyright Information :
Copyright Author (s) 2027 Distributed under Creative Commons CC-BY 4.0

Publishing Partner : Manuscript Technomedia. [www.mstechnomedia.com]

colloidal delivery systems characterised by nanometric dispersed droplets and a large interfacial area. Their formulation can be tailored through appropriate selection of oil, surfactant, and co-surfactant components, potentially improving drug incorporation, dispersion, physical stability, and drug-release characteristics.[6]

Despite the development of several Mesalamine delivery systems, there remains a need for simple and optimised formulations capable of providing favourable physicochemical characteristics together with improved drug-release behaviour and demonstrable therapeutic benefit in experimental colitis. Previous work has explored different approaches for colon-specific Mesalamine delivery; however, systematic optimisation of NE composition followed by evaluation of the optimised formulation in an established experimental model of colitis remains relevant. In this context, the selection and optimisation of formulation components may play an important role in controlling droplet size, colloidal stability, viscosity, and drug-release behaviour.[7]

Therefore, the goal of the current work was to create and optimise mesalamine-loaded NEs utilising Tween 80, Poloxamer 188, and castor oil as formulation ingredients. In vitro drug-release assessment was conducted after several formulations were methodically created and assessed for their physicochemical properties, such as pH, droplet size, polydispersity index (PDI), zeta potential, viscosity, and shape. Formulation A4 was chosen as the ideal NE based on these features and drug-release performance. The improved formulation was then assessed in an experimental model of colitis in mice produced by dextran sulfate sodium (DSS). Body weight changes, the disease activity index, colon length, inflammatory biomarkers, and histological changes were used to evaluate the effectiveness of the treatment. The study was undertaken to determine whether optimisation of Mesalamine delivery through a NE platform could provide improved protection against DSS-associated colonic injury compared with Free Mesalamine.[8]

Study Rationale

Conventional use of mesalamine has various limitations, including the inability to deliver the medication consistently to the affected part of the intestine, inconsistency in bioavailability, and the necessity to keep the patient under prolonged treatment. The development of a new delivery system for IBD is justified by these limitations of the conventional medication therapy. NEs can be used as a delivery tool due to their properties.[9] In the present study, Mesalamine-loaded NEs were developed using castor oil, Tween 80, and Poloxamer 188 and systematically optimised based on their physicochemical characteristics and in vitro drug-release behaviour. Among the developed formulations, A4 demonstrated the most favourable characteristics, including a small droplet size, suitable viscosity, high negative zeta potential, and higher cumulative Mesalamine release. Therefore, A4 was selected as the optimised formulation for further evaluation. To determine whether the improved pharmaceutical characteristics translate into therapeutic benefit, the optimised formulation was further evaluated in an experimental model of colitis. The in vivo assessment focused on body-weight changes, disease activity index, colon length, and histopathological alterations, providing a preliminary assessment of the therapeutic potential of Mesalamine-loaded NE for IBD management.[10]

MATERIALS AND METHODS

Materials

The Mesalamine (API) was bought from Dhamtec Pharma and Consultant (Invoice No: 2504035): Poloxamer 188 and Polysorbate 80 were purchased from SM Pharma and Chemicals (Invoice No: 250412). The rest of the excipients were collected from IIMT University, Meerut.

Animals

Experimental animals were procured following Institutional Animal Ethics Committee (IAEC) approval (Ref. No. IAEC/2024/58; Committee for Control and Supervision of Experiments on Animals (CPCSEA) Registration No. 977/PO/Re/S/04/CPCSEA). The study was approved during the IAEC meeting held on 20 September 2024 (Resolution No. 2015/837ac/M.Pharm./07). Preclinical mouse investigations were carried out in compliance with the relevant CPCSEA rules and the authorised IAEC procedure. Prior to research, the animals were acclimated and kept in typical laboratory settings with unrestricted access to water and a pellet diet.

METHODS

Preformulation Studies

Organoleptic Properties

Preformulation organoleptic evaluation was used to determine the appearance, odor, taste, and texture of the formulation, as this will satisfy the product quality and the acceptability by the patient. The state of stability or discomfort was assessed with parameters such as color, clarity, smell, and taste.[11]

Melting Point Determination

The capillary technique was used to determine the melting point. The sample was ground into a powder and then put in a capillary tube that was sealed and heated at a predetermined rate.[12] The temperature at which melting occurs between the partial melting and the total melting point was absolutely determined and analyzed.[13]

www.ijper.org

Solubility study

A test of solubility was performed on the preformulation of the drug to ascertain the drug's solubility in various solvents. More than enough of the powdered drug was added to 1 mL of solvent in a sealed vial and shaken for 12 hours. The supernatant was filtered after it had settled, and UV-Visible spectrophotometry was used to analyse it. The classification of solubility was done according to the Amount of the volume of the solvent used to dissolve one part of the drug.[14]

FT-IR Spectroscopic Determination

Preformulation involved the application of Fourier Transform Infrared Spectroscopy (FT-IR) spectroscopy to determine the existence of functional groups and compatibility between API and excipients. The characteristic absorption peaks were measured in samples of the pure drug, the excipients, and the mixtures.[15] Spectral changes of peak shifts or disappearance indicated possible interactions. This fast, non-destructive technique also assisted in ensuring the formulation stability and selection of suitable excipients.[16]

Calibration curve of Mesalamine

Stock solutions of Mesalamine (100 µg/mL) were prepared in simulated gastric fluids at pH 6.8 and 7.4, in hydrochloric acid, and phosphate buffer at pH 1.2. Aliquots of 0.2 to 2.0 mL were aliquoted into the same buffer, with each stock producing a final volume of 10 mL to neutralise standard solutions with a concentration of 2 to 20 µg/mL. The filters were Whatman filter paper No. 41, and the solutions were analysed on a UV-visible spectrophotometer at 220 nm wavelength.[17]

Formulation Development

The selection of excipients was based on solubilising Mesalamine in a stable NE. The oil phase was castor oil, Poloxamer 188 and Tween 80 were incorporated as the surfactant and co-surfactant components, respectively, to facilitate emulsification and improve the formation and physical stability of the nanoemulsion. Different ratios of these components were evaluated to determine their influence on formulation characteristics. Castor oil containing Mesalamine was added to Poloxamer 188 and Tween 80, and a coarse emulsion was formed by the addition of distilled water in drops with continuous stirring. This was sonicated for 15 minutes to obtain a stable NE, which was adjusted to 100 mL in water. The formulations were tested based on clarity, stability, and lack of phase separation; the most stable of them was used in further investigation.[18]

Post Formulation Studies

pH

A calibrated digital pH meter was used to determine the pH of Mesalamine-loaded NE. The sample (10-20 mL) was added to the clean beaker, and the electrode was dipped without contact with the vessel. After stabilising, the pH was read. This has been done triplicate, and the mean pH was determined.[19]

Droplet Size and Polydispersity Index

Dynamic light scattering was carried out at room temperature via a particle size analyzer to determine the PDI of the Mesalamine NE. Distilled water was added to the sample to dilute it, and the sample was deposited into an exceptionally clean cuvette and analysed at 25 °C. The mean droplet size and PDI were measured, and smaller values of PDI demonstrated a better homogeneity in the distribution of the droplets.[20] Measurements were performed in triplicate, and the results were expressed as mean values.

Zeta Potential Analysis

The static surface charge and NE stability were determined by using the zeta potential analysis. In a zeta potential analyzer at 25 °C, a diluted solution is put in a cuvette where zeta potential has been determined. Having increased absolute values of the zeta potential proved that the electrostatic stability of the formulation had improved.[21] Measurements were performed in triplicate, and the results were expressed as mean values.

Viscosity Measurement

A Brookfield viscometer at room temperature determined the viscosity of the Mesalamine NE. An appropriate spindle was selected, and the sample was inserted into the holder. Frequency was kept constant as the spindle was rotated, and the measurement of the viscosity was in centipoise (cP).[22]

Transmission Electron Microscopy

The morphology of the optimised A4 nanoemulsion droplets was examined using transmission electron microscopy (TEM). A diluted sample of the optimised A4 nanoemulsion was placed as a drop onto a carbon-coated copper grid and allowed to air-dry. The sample was then negatively stained with phosphotungstic acid and examined under TEM. The resulting micrographs were used to evaluate the morphology, shape, surface characteristics, and approximate size distribution of the nanoemulsion droplets.[23]

In Vitro Studies

The released drug Mesalamine, present in NE *in vitro,* was measured with a Franz diffusion cell. A dialysis membrane was used as a barrier, which was pre-soaked in the donor and receptor compartments.[24] The NE was placed in the donor compartment, while the receptor compartment was filled with phosphate buffer (pH 6.8 or 7.4) and maintained at 37 ± 0.5 °C under continuous

www.ijper.org

stirring. Samples of 1–2 mL were withdrawn at predetermined time intervals for a maximum period of 8 h and immediately replaced with an equal volume of fresh receptor medium to maintain sink conditions. The release study was conducted for 8 h to characterise the cumulative release behaviour of Mesalamine from the developed nanoemulsions over the selected experimental period. The collected samples were filtered, and the absorbance was measured at 220 nm to determine the cumulative amount of Mesalamine released.[25]

Experimental Design and Animal Grouping

Following acclimatization, healthy male C57BL/6 mice aged 8-10 weeks and weighing 20-25 g were randomly divided into five experimental groups, with six animals in each group (n = 6).[26] Group I served as the normal control and received normal drinking water without DSS induction along with the vehicle. Experimental colitis was induced in Groups II-V by administration of 3% (w/v) DSS in drinking water for 7 consecutive days. Group II served as the DSS-induced colitis control and received the vehicle only. Group III received Free Mesalamine at 100 mg/kg/day, whereas Group IV received the optimised Mesalamine-loaded NE (A4) at an equivalent Mesalamine dose of 100 mg/kg/day. Group V received the corresponding blank NE without Mesalamine. All treatments were administered once daily by oral gavage for 7 consecutive days.[27] The body weight and disease status of the animals were measured during the entire experiment period. Once the experiment was completed, the animals were sacrificed using the standard procedure, and the colon tissues were harvested for macroscopic and histopathologic examinations. The DSS was fed to the animals ad libitum using the drinking water, whereas the test drugs were administered through oral gavage.[28]

Induction of Experimental Colitis

DSS was used to experimentally produce colitis in male C57BL/6 mice. Before colitis was induced, the mice were given time to get used to the lab environment. For seven days, a 3% (w/v) DSS solution was added to drinking water at will in order to induce colitis. To guarantee continuous exposure to DSS, fresh DSS solutions were created and provided during the induction process. Drinking water free of DSS was given to the typical control group. The mice were monitored every day for changes in body weight, faecal blood, stool consistency, and physical appearance during the induction of colitis. The disease activity index (DAI) was used to assess the induction of colitis based on rectal bleeding, stool consistency, and body weight change. Following the DSS injection procedure, colon tissues were removed for examination and the mice were put through the predefined endpoint analysis.[29]

Evaluation of Therapeutic Efficacy

Body-Weight Change

Body weight was measured on an individual basis for all animals prior to the onset of colitis, as well as at regular time intervals during the experiment. Body weight changes were calculated as the percent change relative to the baseline body weight obtained before DSS administration. Loss of body weight was used as a marker of disease progression, while body weight retention was a marker of successful therapy.[30]

Disease Activity Index (DAI)

The Disease Activity Index (DAI), which is based on three clinical criteria such as percentage body-weight loss, stool consistency, and the presence of blood in stools, was used to quantify the illness severity on a daily basis. The DAI of each criterion was measured using a predetermined scale and then summed up to give the final DAI score of the animal. Variation in the DAI value over the period of experimentation was used as a measure of the disease progression and effectiveness of treatment with Free Mesalamine and A4 NE.[31]

Colon Length and Macroscopic Assessment

The animals were sacrificed at the end of the experiment, and the whole colon was removed and isolated from other organs followed by washing with physiological saline. The length of the colon was measured from the cecocolic junction to the distal part and noted down for each animal. The excised colon was also observed macroscopically for any signs of pathology, such as mucosal damage, oedema, hyperemia, ulcers, and hemorrhages. The difference in the macroscopic findings of the colon was observed between different groups to assess the effectiveness of the treatment against DSS-induced injuries.[32]

Histopathological Evaluation and Histological Scoring

Colon tissue specimens were obtained, fixed in 10% neutral buffered formalin solution and subjected to histopathological study. Tissue specimens were fixed through routine paraffin embedding method, sectioning, and staining with haematoxylin-eosin (H&E). The H&E stained tissues were observed using light microscopy for any pathological changes related to colitis, such as epithelial injury, crypt disruption, inflammatory cell infiltration, mucosal ulcerations, and tissue oedema. The scoring was done based on an appropriate semi-quantitative scoring system in the normal control, DSS control, Free Mesalamine, A4 NE, and blank NE groups.[33]

www.ijper.org

RESULTS AND DISCUSSION

Preformulation Studies

Organoleptic properties

The organoleptic characteristics of Mesalamine loaded NEs were analysed. The NE was observed to be transparent and had no signs of phase separation, turbidity, or creaming. It had a clear colour and was in the form of a slightly oily liquid; it had no smell and taste. This shows that the NE possessed satisfactory organoleptic characteristics that were acceptable for oral use.

Melting Point

Mesalamine had a melting point of 283 °C, which indicated that it was highly pure and a good crystalline compound.

Solubility Study

It can be seen from the solubility profile of Mesalamine that there are variations in the solubility characteristics of this compound in different solvents. The solubility of Mesalamine in water is fairly high, and is about 1-2%. This is the reason why water is the best solvent for Mesalamine. On the other hand, the solubility of this compound in alcoholic solvents is somewhat low. Mesalamine is sparingly soluble in ethanol (96%), methanol, and isopropyl alcohol (it is considered to be very slightly soluble in the latter).

FT-IR Spectroscopic Determination

The FT-IR analysis was conducted to ascertain the occurrence of typical functional groups in mesalamine through the observation of various characteristic bands of absorption that were unique to the functional moieties on mesalamine that include hydroxyl, amine, aromatic, and carboxylic acid groups.

The FT-IR spectrum of mesalamine confirmed the presence of its characteristic functional groups. A wide absorption band was observed around **3300-3500 cm^{-1},** and this represents O-H and N-H stretching frequencies. Peaks at **1320-1450 cm^{-1}** indicated C-N stretching of amines, and peaks found near **1620–1660 cm^{-1}** were thought to be attributable to aromatic C=C stretching. The absorption that was found to be between **1000-1300 cm^{-1}** was caused by C-O stretching vibrations, which implies the existence of carboxylic and phenolic acid functional groups in the structure.

Calibration Curve of Mesalamine

The absorbance of Mesalamine was measured at various sample concentrations at 220 nm using UV spectroscopy. The linearity between absorbance and concentration proves that the method could be used in quantitative analysis.

The absorbance at 220 nm increased linearly with increasing sample concentration, indicating a direct relationship between concentration and absorbance. As the concentration was increased, the absorbance level gradually increased, reaching a level of around 1.2 at 20µg/mL. Such findings indicated that UV spectroscopy is appropriate for use in the quantitative analysis of the compound.

A linear relationship was observed between Mesalamine concentration and absorbance, confirming compliance with Beer-Lambert's law and the suitability of the UV spectrophotometric method for quantitative analysis.

Nanoemulsion Formulation and Optimisation

Various NE formulations were prepared using different ratios of S-Mix (Tween 80 188), namely 1:1, 1.5:1, and 2:1. All formulations (A1 to C4) were prepared with a fixed initial Mesalamine concentration of 5% w/v and a total volume of 100 mL, while the proportions of the surfactant, co-surfactant, water, and oil were varied. The surfactant blend and the composition of the aqueous and oily phases were systematically varied to optimise the characteristics of the NE.

On the basis of initial stability and clarity and differing S-Mix ratios, Formulations A2, A4, B2, B4, C2, and C4 were chosen to undergo extensive analysis, and formulations were compared to each other. The characterisation and *in vitro* drug release of these formulations were then done to determine the most optimised NE system to deliver Mesalamine.

Formulation A4 was selected as the optimised nanoemulsion based on its overall physicochemical and *in vitro* release characteristics. Among the evaluated formulations, A4 exhibited the smallest droplet size (83.54 nm), lowest viscosity (22.2 cP), highest absolute zeta potential (−68 mV), and highest cumulative drug release (93.24% at 8 h). Therefore, A4 was considered the most favourable formulation for further *in vivo* evaluation.

Characterisation Results

pH

The pH of A4 was 6.1, indicating its suitability for oral administration based on the measured formulation pH.

Droplet Size

A4 exhibited the smallest droplet size among the evaluated formulations, measuring 83.54 nm. This reduction in droplet size indicators improved dispersion, a larger surface area, and possible improvement in bioavailability.

Viscosity

The A4 formulation showed the lowest viscosity of 22.2, indicating a comfortable flow property and administration.

www.ijper.org

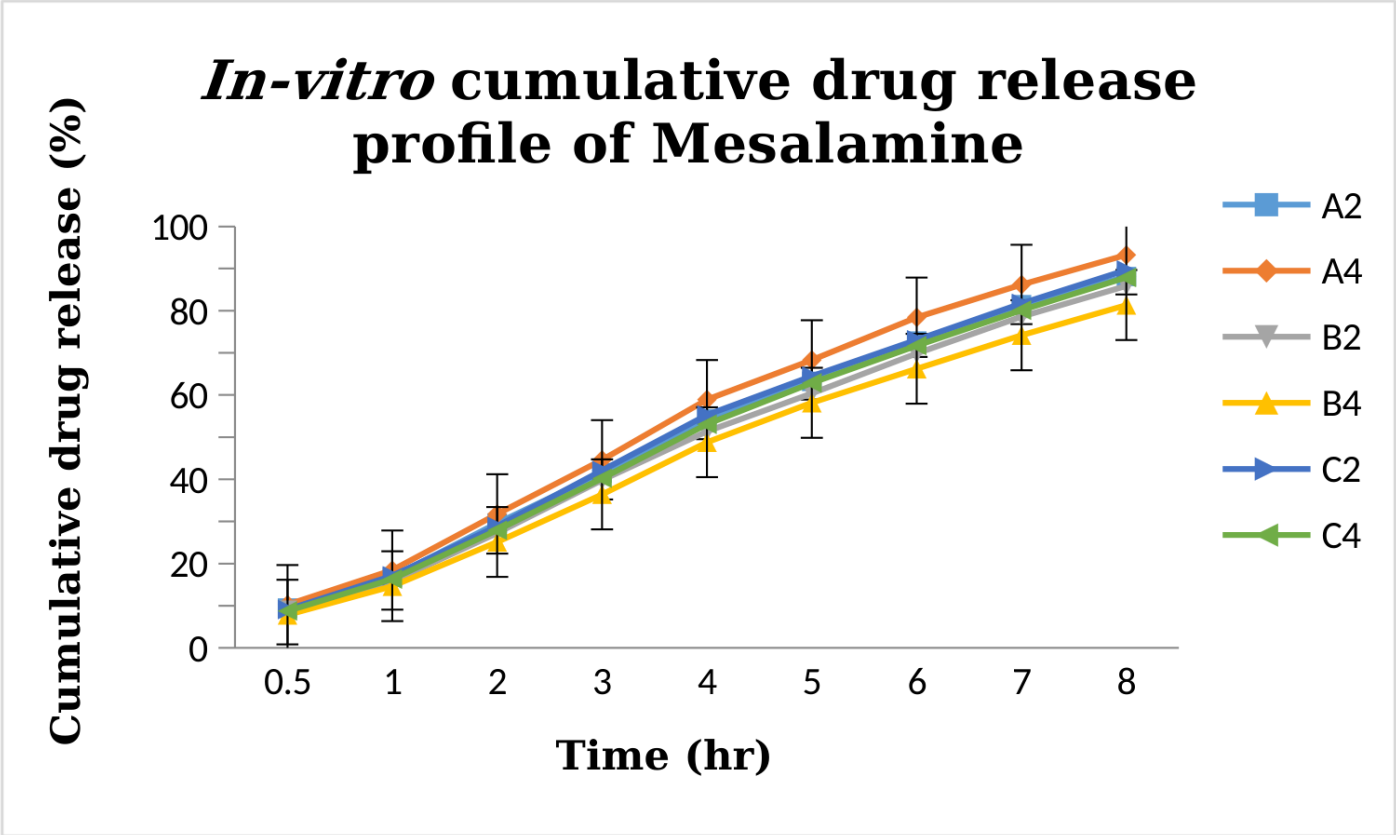


Figure 1: *In vitro* drug release profiles of Mesalamine NEs.

Zeta Potential

A4 indicated the lowest value of zeta potential in terms of negativity (-68 mV), a factor that indicates a high potential of electrostatic repulsion among the particles as well as a high degree of colloidal stability, which means minimal aggregation with time.

The six NE formulations of Mesalamine have a summary of their physicochemical properties are summarised in Table 1. A4 presents the most favourable profile due to the minimum droplet size, lowest viscosity, and maximum zeta potential, referring to greater stability. A higher droplet size and a comparatively lower zeta potential of formulation B4 make the formula less stable. The pH of all formulations is neutral with a slightly acidic aspect, and the PDI values indicated a relatively broad droplet-size distribution among the tested formulations and should therefore be interpreted together with the droplet-size and zeta-potential results.

Transmission Electron Microscopy

TEM analysis of A4 showed predominantly spherical and well-dispersed droplets with no prominent aggregation under the examined conditions.

In vitro Drug Release

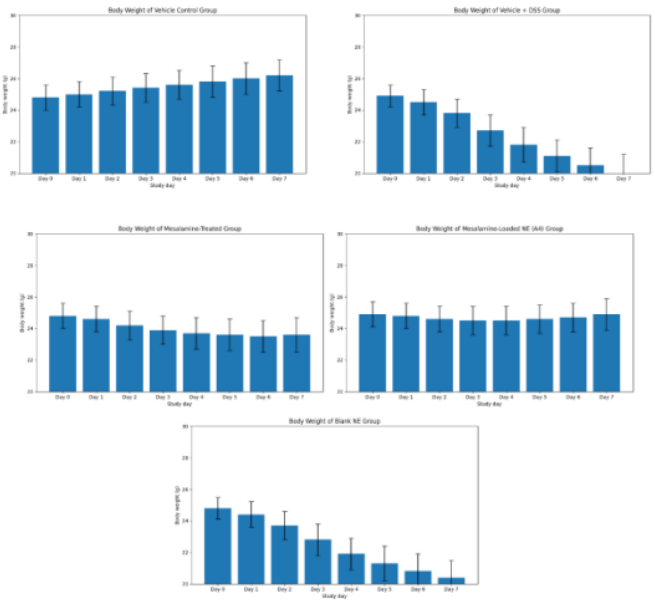


Figure 2: Changes in body weight of mice across the 7-day experimental period in different treatment groups.

www.ijper.org

Table 1: Physicochemical Characterisation of Mesalamine-Loaded NE Formulations

Formulation	A2	A4	B2	B4	C2	C4
pH	5.8	6.1	5.9	5.5	6.0	5.7
Droplet Size (nm)	120.3	83.54	105.4	135.7	98.2	110.5
Viscosity (cP)	28.5	22.2	25.1	31.8	24.0	26.3
PDI	0.89	1.00	0.95	1.10	0.88	0.92
Zeta Potential (mV)	–45	–68	–60	–38	–54	–50

From Table 2, it is seen that NE formulations lead to a release of the cumulative Amount.

Mesalamine increases over 8 hours *in vitro*. The A4 presented the highest drug release (93.24 ± 0.39% at 8 hours), among all the formulations, followed by A2 and C2. The ideal ratio of S-Mix (1.5:1) and the smallest droplet size may be attributed to the increased release that A4 can obtain as a result of more effective diffusion of the drugs. One-way ANOVA and its follow-up test through Tukey post hoc analysis results showed that the drug release of A4 was significantly better. $p< 0.05$ to$^{***}p < 0.001$) at different time points than other formulations.

As shown in Figure 1, the *in vitro* drug release profiles in eight hours can be plotted for six optimised mesalamine-loaded NE formulations. The difference is that the cumulative release of formulation A4 is the highest (93.24 ± 0.39%), and at various time points is significant. This improved delivery is credited to the improved formulation, with an improvement in the content of water and the optimal ratio of a surfactant, whereby the size of the smallest droplet, as well as extremely low zeta potential, is observed. Such results indicate that formulation parameters play an essential role in the release of drugs in NEs.

In-vivo Studies

Effect of Mesalamine-Loaded Nanoemulsion on Body-Weight Changes

A progressive and treatment-dependent change in body weight was observed across the experimental groups during the 7-day study period. The vehicle control group showed a gradual increase in body weight from 24.8 ± 0.8 g on Day 0 to 26.2 ± 1.0 g on Day 7, indicating normal growth and absence of appreciable disease-associated stress. In contrast, DSS administration produced a marked and progressive reduction in body weight, with the DSS control group declining from 24.9 ± 0.7 g to 20.0 ± 1.2 g by Day 7. A comparable reduction was observed in the blank NE group, which decreased from 24.8 ± 0.7 g to 20.4 ± 1.1 g, indicating that the blank formulation did not substantially prevent DSS-associated weight loss (Table 3).

Treatment with Free Mesalamine attenuated the reduction in body weight, with values decreasing moderately from 24.8 ± 0.8 g on Day 0 to 23.6 ± 1.1 g on Day 7. Notably, the optimised Mesalamine-loaded NE (A4) produced the most pronounced protective effect. Body weight in the A4-treated group remained relatively stable throughout the study, changing from 24.9 ± 0.8 g at baseline to 24.9 ± 1.0 g on Day 7 (Table 3).

Graphical results confirmed the tabulated data, showing a considerable retention of body weight among the A4 group mice when compared to DSS group and blank NE group mice (Figure 2). Generally, these results indicate that the optimised A4 NE offered more protection against body weight loss caused by DSS when compared to Free Mesalamine.

Effect on Disease Severity and Inflammatory Response

The DSS-induced colitis group showed marked alterations in all evaluated disease and inflammatory parameters compared with the normal control. Colon length was substantially reduced from 9.2 ± 0.4 cm in the normal group to 6.1 ± 0.5 cm in the DSS group, while the DAI score increased from 0.2 ± 0.1 to 3.8 ± 0.4. Free Mesalamine partially improved these parameters, with colon length increasing to 7.6 ± 0.4 cm and DAI decreasing to 2.0 ± 0.3. The A4 NE produced a greater improvement, with colon length restored to 8.5 ± 0.3 cm and DAI reduced to 1.0 ± 0.2 as shown in Table 4. Similarly, Myeloperoxidase (MPO) activity was reduced from 4.8 ± 0.5 U/g tissue in the DSS group to 2.0 ± 0.3 U/g tissue following A4 treatment. A4 also markedly reduced Tumour Necrosis Factor-alpha (TNF-α), Interleukin-6 (IL-6), and Interleukin-1beta (IL-1β) levels to 48 ± 7, 39 ± 5, and 32 ± 5 pg/mg, respectively, compared with 118 ± 12, 96 ± 10, and 82 ± 9 pg/mg in the DSS group. The blank NE showed comparatively limited improvement in the evaluated parameters (Table 4).

The graphical results showed a clear DSS-induced increase in DAI, MPO activity, TNF-α, IL-6, and IL-1β, together with a marked reduction in colon length. Free Mesalamine partially reversed these disease-associated changes, whereas the A4 NE produced a more pronounced improvement across all evaluated parameters. The A4-treated group showed values approaching those of the normal control, while the blank NE exhibited limited protective effects (Figure 3).

Histopathological Evaluation of Colonic Tissue

Histopathological examination of the colonic sections revealed distinct differences among the experimental groups. The normal control group (I) showed well-preserved colonic mucosal architecture with an intact epithelial lining and regularly arranged crypts, with no prominent pathological alterations. In contrast, the DSS-treated group (II) exhibited marked disruption of the normal mucosal architecture, characterised by epithelial damage, crypt distortion, mucosal disruption, and prominent

www.ijper.org

Table 2: *In vitro* cumulative drug release profile of Mesalamine from different NE formulations over 8 hours

Time (Hours)	A2	A4	B2	B4	C2	C4
0.5	9.45 ± 0.38	10.25 ± 0.42	8.95 ± 0.36	7.85 ± 0.41	9.10 ± 0.39	8.78 ± 0.35
1.0	16.82 ± 0.50	18.45 ± 0.61	15.73 ± 0.48	14.65 ± 0.56	17.12 ± 0.53	16.33 ± 0.49
2.0	29.63 ± 0.61	31.78 ± 0.73	27.25 ± 0.58	25.12 ± 0.64	28.85 ± 0.62	27.93 ± 0.60
3.0	41.52 ± 0.54	44.63 ± 0.58	39.80 ± 0.57	36.44 ± 0.61	42.11 ± 0.55	40.24 ± 0.52
4.0	54.33 ± 0.48	58.91 ± 0.49	51.36 ± 0.50	48.77 ± 0.59*	55.27 ± 0.53	53.06 ± 0.50
5.0	63.22 ± 0.55	68.33 ± 0.66	60.40 ± 0.61	58.15 ± 0.63*	64.50 ± 0.58	62.88 ± 0.56
6.0	72.80 ± 0.51	78.45 ± 0.52	69.85 ± 0.54	66.23 ± 0.57*	73.10 ± 0.52	71.74 ± 0.50
7.0	81.45 ± 0.47	86.22 ± 0.47	78.60 ± 0.49	74.18 ± 0.50**	81.73 ± 0.48	80.12 ± 0.46
8.0	88.10 ± 0.43	93.24 ± 0.39	85.91 ± 0.46*	81.34 ± 0.48***	89.66 ± 0.45	87.89 ± 0.42

**p < 0.05, **p < 0.01, ***p < 0.001 versus A4; statistical significance was determined using one-way ANOVA followed by Tukey's multiple comparisons test.*

Table 3: Experimental grouping and treatment schedule for the evaluation of mesalamine-loaded NE in DSS-induced colitis

Group	Treatment and dose	Day 0	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
I	Vehicle, equivalent volume	24.8 ± 0.8	25.0 ± 0.8	25.2 ± 0.9	25.4 ± 0.9	25.6 ± 0.9	25.8 ± 1.0	26.0 ± 1.0	26.2 ± 1.0
II	Vehicle + DSS	24.9 ± 0.7	24.5 ± 0.8	23.8 ± 0.9	22.7 ± 1.0	21.8 ± 1.1	21.1 ± 1.0	20.5 ± 1.1	20.0 ± 1.2
III	Mesalamine, 100 mg/kg/day	24.8 ± 0.8	24.6 ± 0.8	24.2 ± 0.9	23.9 ± 0.9	23.7 ± 1.0	23.6 ± 1.0	23.5 ± 1.0	23.6 ± 1.1
IV	Mesalamine-loaded NE (A4), 100 mg/kg/day mesalamine equivalent	24.9 ± 0.8	24.8 ± 0.8	24.6 ± 0.8	24.5 ± 0.9	24.5 ± 0.9	24.6 ± 0.9	24.7 ± 0.9	24.9 ± 1.0
V	Blank NE, equivalent formulation volume	24.8 ± 0.7	24.4 ± 0.8	23.7 ± 0.9	22.8 ± 1.0	21.9 ± 1.0	21.3 ± 1.1	20.8 ± 1.1	20.4 ± 1.1

Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using two-way repeated-measures ANOVA followed by Tukey's multiple comparisons test; p < 0.05 was considered significant.

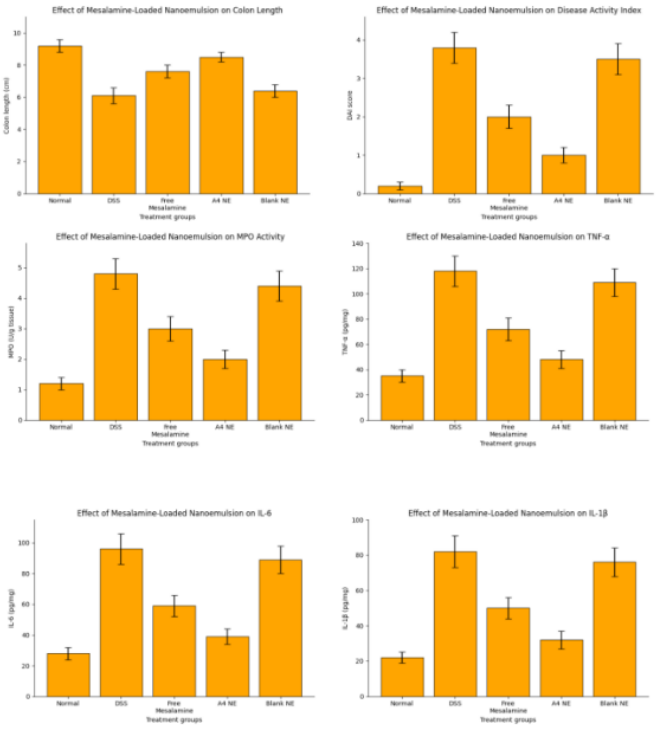


Figure 3: Effects of mesalamine-loaded NE on colon length, DAI, MPO, and inflammatory cytokines in DSS-induced colitis.

Table 4: Effects of mesalamine-loaded NE on disease severity and inflammatory biomarkers in DSS-induced colitis

Parameter	Normal	DSS	Free Mesalamine	A4 NE	Blank NE
Colon length (cm)	9.2 ± 0.4	6.1 ± 0.5	7.6 ± 0.4	8.5 ± 0.3	6.4 ± 0.4
DAI score	0.2 ± 0.1	3.8 ± 0.4	2.0 ± 0.3	1.0 ± 0.2	3.5 ± 0.4
MPO (U/g tissue)	1.2 ± 0.2	4.8 ± 0.5	3.0 ± 0.4	2.0 ± 0.3	4.4 ± 0.5
TNF-α (pg/mg)	35 ± 5	118 ± 12	72 ± 9	48 ± 7	109 ± 11
IL-6 (pg/mg)	28 ± 4	96 ± 10	59 ± 7	39 ± 5	89 ± 9
IL-1β (pg/mg)	22 ± 3	82 ± 9	50 ± 6	32 ± 5	76 ± 8

Values are expressed as mean ± SD (n = 3). Statistical analysis was performed using one-way ANOVA followed by Tukey's multiple comparisons test; p < 0.05 was considered significant.

tissue alterations, indicating successful induction of colitis. The Free Mesalamine-treated group (III) showed partial restoration of the colonic architecture compared with the DSS group, with comparatively improved epithelial integrity and reduced structural distortion. The A4 mesalamine-loaded NE group (IV) demonstrated the most pronounced preservation of colonic tissue architecture, with relatively intact crypt organisation and improved mucosal integrity compared with the DSS-treated group. The blank NE group (V) showed comparatively less

www.ijper.org

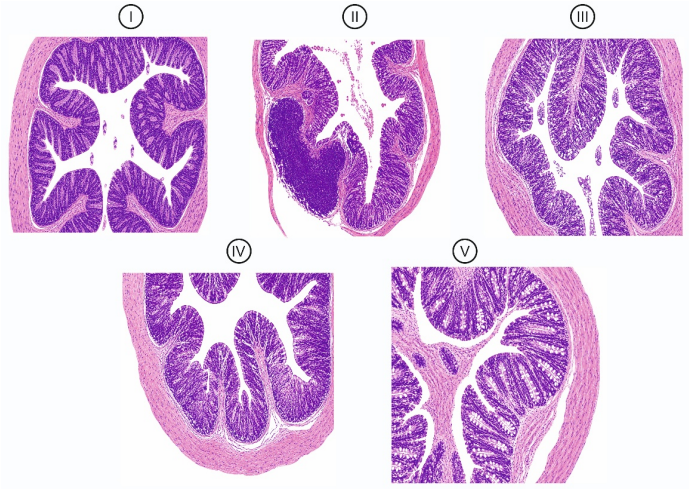


Figure 4: Representative H&E-stained colonic sections showing histopathological changes in normal, DSS, Free Mesalamine, A4 NE, and Blank NE groups.

pronounced structural preservation than the A4 formulation, although some maintenance of crypt architecture was evident. Overall, the histopathological findings demonstrated that the optimised A4 mesalamine-loaded NE provided greater protection against DSS-induced colonic tissue injury than Free Mesalamine and the blank NE.

The study developed and optimised Mesalamine-loaded NEs, with A4 showing the most favourable formulation characteristics. Variation in the S-Mix ratio and oil/water composition influenced the physicochemical characteristics of the formulations. The A4 formulation, containing an S-Mix ratio of 1.5:1 with 40% water and 5% oil, showed the smallest droplet size and the highest drug release among the tested formulations. The smaller droplet size may provide a greater interfacial area, which can facilitate drug diffusion and contribute to the observed release profile. A4 exhibited a droplet size of 83.54 nm, zeta potential of −68 mV, viscosity of 22.2 cP, spherical morphology, and the highest 8-h drug release (93.24 ± 0.39%). In the DSS-induced colitis model, DSS produced marked disease-associated changes, including body-weight loss, increased DAI, reduced colon length, elevated MPO and inflammatory cytokines, and histopathological damage. Free Mesalamine partially improved these parameters, whereas A4 produced greater improvement. A4 maintained body weight, increased colon length to 8.5 ± 0.3 cm, reduced DAI to 1.0 ± 0.2 and MPO to 2.0 ± 0.3 U/g tissues. TNF-α, IL-6, and IL-1β were also reduced to 48 ± 7, 39 ± 5, and 32 ± 5 pg/mg, respectively.

Histopathological findings further supported these observations, with A4 showing better preservation of mucosal architecture and crypt organisation than the DSS and Free Mesalamine groups. Overall, the findings suggest that the optimised A4 NE provided greater protective effects against DSS-induced colonic injury than Free Mesalamine and blank NE. These findings indicate that formulation optimisation improved the pharmaceutical and therapeutic performance of Mesalamine. Further pharmacokinetic, safety, mechanistic, and translational studies are required to establish its broader therapeutic potential. The histopathological findings demonstrated that the optimised A4 mesalamine-loaded NE provided greater protection against DSS-induced colonic tissue injury than Free Mesalamine and the blank NE. Representative H&E-stained colonic sections from all experimental groups are shown in Figure 4.

CONCLUSION

The present study successfully developed and optimised Mesalamine-loaded NEs for evaluation against DSS-induced colonic injury. Among the developed formulations, A4 demonstrated the most favourable physicochemical characteristics, including a small droplet size, high negative zeta potential, suitable viscosity, spherical morphology, and the highest cumulative in vitro Mesalamine release. In the experimental colitis model, A4 produced greater improvement in body-weight maintenance, disease activity, colon length, MPO activity, and inflammatory cytokine levels than Free Mesalamine. Histopathological examination further demonstrated comparatively better preservation of colonic mucosal architecture and crypt organisation following A4 treatment. The overall findings indicate that the optimised A4 Mesalamine-loaded NE provided enhanced protective effects against DSS-associated colonic injury compared with Free Mesalamine and the blank NE. Thus, A4 represents a promising NE-based approach for improving the delivery and therapeutic performance of Mesalamine in experimental colitis, although further pharmacokinetic, mechanistic, safety, and translational studies are required before clinical applicability can be established.

ABBREVIATIONS

ANOVA – Analysis of Variance; **API** – Active Pharmaceutical Ingredient; **CD** – Crohn's Disease; **CPCSEA** – Committee for the Purpose of Control and Supervision of Experiments on Animals; **DAI** – Disease Activity Index; **DSS** – Dextran Sulfate Sodium; **FT-IR** – Fourier-Transform Infrared Spectroscopy; **GI** – Gastrointestinal; **H&E** – Haematoxylin and Eosin; **IAEC** – Institutional Animal Ethics Committee; **IBD** – Inflammatory Bowel Disease; **IL** – Interleukin; **MPO** – Myeloperoxidase; **NE** – Nanoemulsion; **PDI** – Polydispersity Index; **S-Mix** – Surfactant Mixture; **SD** – Standard Deviation; **TEM** – Transmission Electron Microscopy; **TNF-α** – Tumour Necrosis Factor-alpha; **UC** – Ulcerative Colitis; **UV** – Ultraviolet.

www.ijper.org

ACKNOWLEDGEMENT

The authors thank and feel obliged to the Department of Pharmacy, IIMT College of Medical Sciences, IIMT University, Meerut, for providing the requisite facility and support to conduct the research work, particularly to all the colleagues and the staff members who have participated in the study.

AUTHOR CONTRIBUTIONS

S.S.: Supervision, Validation. **P.C.:** Methodology, Original Draft Preparation. **S.A.:** Conceptualization, Visualisation.

CONFLICT OF INTERESTS

The authors declare that there is no conflict of interest.

ETHICS APPROVAL

The study was approved by the IAEC (Ref. No. IAEC/2024/58) and conducted according to CPCSEA guidelines.

SOURCE OF FUNDING

There is no funding available to conduct this study.

CLINICAL TRIAL NUMBER

Not Applicable

DATA AVAILABILITY STATEMENT

All the information confirming the results of this work can be found in the article itself or can be sent by the corresponding author.

SUMMARY

In this study, the optimised Mesalamine-loaded NE system especially A4 has been shown to have good physicochemical properties and good in vitro drug release performance. In the induced colitis by DSS model, the protective efficacy of A4 has been shown to be higher than that of Free Mesalamine.

REFERENCES

1. Sunand K, Tripathi AK, Patel A, Nasa P, Ali S, Shamim S. Mechanistic insights and therapeutic advances in natural bioactive compounds as multi-target agents in rheumatoid arthritis. Curr Rheumatol Rev. 2026;22(5):1-19. doi:10.2174/0115733971409871251206061614.
2. Ali S, Ali SA, Jahan I. An in-depth analysis of arthritis (inflammatory joint diseases): from pathophysiology to diagnosis to treatment. Int J Pharm Sci Nanotechnol. 2026;19(1):8547-8566. doi:10.37285/ijpsn.2026.19.1.9.
3. Saez A, Herrero-Fernandez B, Gomez-Bris R, Sánchez-Martinez H, Gonzalez-Granado JM. Pathophysiology of inflammatory bowel disease: innate immune system. Int J Mol Sci. 2023;24(2):1526. doi:10.3390/ijms24021526.
4. Sutaria R, Mok ZH. Nanoparticle-enhanced mesalazine therapy for inflammatory bowel disease. Pharm Sci Adv. 2023;1(2):100014. doi:10.1016/j.pscia.2023.100014.
5. Zheng B, Wang L, Yi Y, Yin J, Liang A. Design strategies, advances and future perspectives of colon-targeted delivery systems for the treatment of inflammatory bowel disease. Asian J Pharm Sci. 2024;19(4):100943. doi:10.1016/j.ajps.2024.100943.
6. Amidon S, Brown JE, Dave VS. Colon-targeted oral drug delivery systems: design trends and approaches. AAPS PharmSciTech. 2015;16(4):731-741. doi:10.1208/s12249-015-0350-9.
7. Bayan MF, Bayan RF. Recent advances in mesalamine colonic delivery systems. Future J Pharm Sci. 2020;6:43. doi:10.1186/s43094-020-00057-7.
8. Fatima I, Khan A, Rahdar A, Fathi-karkan S, Kharaba Z, Baino F. Optimized mesalamine-loaded polyelectrolyte complex nanoparticles for targeted colon delivery in inflammatory bowel disease treatment: a central composite design approach. Next Mater. 2025;8:100530. doi:10.1016/j.nxmate.2025.100530.
9. Kjeldsen S, Nielsen J, Nørgård BM, Kjeldsen J. Mesalazine in inflammatory bowel disease and COVID-19: hospitalization and adverse in-hospital outcomes based on nationwide data. Inflamm Bowel Dis. 2022;28(10):1513-1519. doi:10.1093/ibd/izab299.
10. Pawar AY, Ahirrao RA. Formulation and evaluation of colon targeted drug delivery system of mesalamine using wet granulation. Res J Pharm Technol. 2014;7(11):1270-1279.
11. Patil A, Bhide S, Bookwala M, Soneta B, Shankar V, Almotairy A, et al. Stability of organoleptic agents in pharmaceuticals and cosmetics. AAPS PharmSciTech. 2018;19(1):36-47. doi:10.1208/s12249-017-0866-2.
12. Dai Z, Wang L, Lu X, Ji X. Melting points of ionic liquids: review and evaluation. Green Energy Environ. 2024;9(12):1802-1811. doi:10.1016/j.gee.2024.01.009.
13. Austermeier LE, Voigt K, Böhme A, Ulrich N. Prediction of melting points of chemicals with a data augmentation-based neural network approach. ACS Omega. 2025;10(23):24296-24306. doi:10.1021/acsomega.5c00.
14. Wyttenbach N, Niederquell A, Kuentz M. Machine estimation of drug melting properties and influence on solubility prediction. Mol Pharm. 2020;17(7):2660-2671. doi:10.1021/acs.molpharmaceut.0c00355.
15. Al-Amin K, Kawsar M, Mamun MTRB, Sahadat Hossain M. Fourier transform infrared spectroscopic technique for analysis of inorganic materials: a review. Nanoscale Adv. 2025;7(21):6677-6702. doi:10.1039/d5na00522a.
16. Pramod K, Suneesh CV, Shanavas S, Ansari SH, Ali J. Unveiling the compatibility of eugenol with formulation excipients by systematic drug-excipient compatibility studies. J Anal Sci Technol. 2015;6:34. doi:10.1186/s40543-015-0073-2.
17. Gaikwad S, Suresh PK. Formulation and characterization of magnetically responsive mesalamine microspheres for colon targeting. J Ravishankar Univ B Sci. 2023;36(1):113. doi:10.52228/JRUB.2023-36-1-11.
18. Chen J, Chen Z, Wang W, Wang L, Zheng J, Wu S, et al. Effects of commonly used surfactants, Poloxamer 188 and Tween 80, on the drug transport capacity of intestinal glucose transporters. AAPS PharmSciTech. 2024;25(6):163. doi:10.1208/s12249-024-02881-z.
19. Jin L, Ding YC, Zhang Y, Xu XQ, Cao Q. A novel pH-enzyme-dependent mesalamine colon-specific delivery system. Drug Des Devel Ther. 2016;10:2021-2028. doi:10.2147/DDDT.S107283.
20. O'Sullivan J, Murray B, Flynn C, Norton I. The effect of ultrasound treatment on the structural, physical and emulsifying properties of animal and vegetable proteins. Food Hydrocoll. 2016;53:141-154. doi:10.1016/j.foodhyd.2015.02.009.
21. Singh A, Mandal UK, Narang RK. Development and in vivo evaluation of pectin based enteric coated microparticles loaded with mesalamine and *Saccharomyces boulardii* for management of ulcerative colitis. Assay Drug Dev Technol. 2022;20(1):22-34. doi:10.1089/adt.2021.052.
22. Shahabadi N, Moradi Fili S, Shahlaei M. Synthesis, characterization and comparative DNA interaction studies of new copper(II) and nickel(II) complexes containing mesalamine drug using molecular modeling and multispectroscopic methods. J Coord Chem. 2015;68(20):3667-3684. doi:10.1080/00958972.2015.1078897.
23. Lu E, Zhang M, Zhao Y, Meng H, Xu Z. Fecal microbiota transplantation in ulcerative colitis: mucosal immune mechanisms and precision microbiota therapy. Front Immunol. 2026;17:1888117. doi:10.3389/fimmu.2026.1888117.
24. Bahekar J, Wadher S. Formulation development of colon targeted mesalamine pellets: in vitro-in vivo release study. Int J Appl Pharm. 2019;11(6):125-132. doi:10.22159/ijap.2019v11i6.34920.
25. Andreas CJ, Chen YC, Markopoulos C, Reppas C, Dressman J. In vitro biorelevant models for evaluating modified release mesalamine products to forecast the effect of formulation and meal intake on drug release. Eur J Pharm Biopharm. 2015;97(Pt A):39-50. doi:10.1016/j.ejpb.2015.09.002.
26. Lu S, Zheng K, Wang J, Xu M, Xie Y, Yuan S, et al. Characterization of *Treponema pallidum* dissemination in C57BL/6 mice. Front Immunol. 2020;11:577129. doi:10.3389/fimmu.2020.577129.
27. Joshi M, Pandey M, Ved A. Assessment of ameliorative effect of *Myrica esculenta* in a DSS-induced murine model against ulcerative colitis. Adv Gut Microbiome Res. 2024;2024(1):6616549. doi:10.1155/2024/6616549.
28. Wang C, Jiang T, Ye L, Du M, Liu F. Optimization of the cholesterol gallstone model in C57BL/6 mice and evaluation of *Lactobacillus* intervention effects. BMC Gastroenterol. 2025;25:56. doi:10.1186/s12876-025-03653-0.

www.ijper.org

29. Reichmann F, Hassan AM, Farzi A, Jain P, Schuligoi R, Holzer P. Dextran sulfate sodium-induced colitis alters stress-associated behaviour and neuropeptide gene expression in the amygdala-hippocampus network of mice. Sci Rep. 2015;5(1):9970. doi:10.1038/srep09970.

30. Kolahi Z, Yaghoubi A, Rezaeian N, Khazaei M. Exercise improves clinical symptoms, pathological changes and oxidative/antioxidative balance in animal model of colitis. Int J Prev Med. 2023;14:46. doi:10.4103/ijpvm.ijpvm_162_22.

31. Yang C, Merlin D. Unveiling colitis: a journey through the dextran sodium sulfate-induced model. Inflamm Bowel Dis. 2024;30(5):844-853. doi:10.1093/ibd/izad312.

32. Song S, Zhang X, Zheng H, Liao Y, Tang P, Liu Y, et al. Intermittent fasting reduces intestinal inflammation in dextran sulfate sodium-induced colitis of mice. Food Sci Nutr. 2025;13(2). doi:10.1002/fsn3.70014.

33. van Deventer BS, du Toit-Prinsloo L, van Niekerk C. Practical tips to using formalin-fixed paraffin-embedded tissue archives for molecular diagnostics in a South African setting. Afr J Lab Med. 2022;11(1):1587. doi:10.4102/ajlm.v11i1.1587.

Cite this article: Shamim S, Chandel P, Ali S. *Title: Development and Therapeutic Evaluation of Mesalamine-Loaded Nanoemulsion in DSS-Induced Colitis*. Indian J of Pharmaceutical Education and Research. 2027;XX(XX):1-11.

www.ijper.org