Original Research Article

Mesalamine-loaded mucoadhesive microsphere for colon drug delivery system: Effect of process variables and *in vitro* characterization

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Abstract

Objective: The main objective of this study is to formulate mucoadhesive microspheres for colon drug delivery with sodium alginate (ALG) core enriched with drug.

Methods: The core microspheres of ALG were prepared by modified emulsification method followed by cross-linking with different concentration of $CaCl_2$ at different stirring speed with constant drug-to-polymer ratio (1:3). The core microspheres were further coated with Eudragit S-100 using the solvent evaporation technique.

Results: The microspheres (core and coated) were characterized by shape, size, surface morphology, size distribution, entrapment efficiency, and *in vitro* drug release studies. *In vitro* drug release showed that the optimized batch of core microsphere and coated microspheres exhibited 99.53% ± 0.39% and 89.22% ± 0.26%, respectively. The drug release from all formulations of mesalamine microsphere followed Higuchian Kinetics. Moreover, drug release from core and Eudragit S-100-coated microspheres followed Korsmeyer–Peppas equation with anomalous and Fickian kinetics mechanism, respectively. Stability study suggests that the degradation rate constant of mesalamine from Eudragit S-100-coated microsphere was found to be minimum 2 years shelf life of the formulation. On the basis of scanning electron microscopy, the core microspheres were formed slightly irregular in shape due to surface-attached crystals of the drug and coated mesalamine microspheres showed smooth surface and a smaller number of pores due to coating.

Conclusions: It can be concluded that the appropriate combination of a pH-dependent polymer (Eudragit S-100) with a pH-independent polymer sodium ALG) was suitably adequately sustained the drug release from mesalamine microspheres.

Keywords: Eudragit S-100, Higuchian, mesalamine, microspheres, mucoadhesive

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INTRODUCTION

Various drug delivery strategies have been employed to trigger the release of drug to the large intestine, but they do not reach at the site of action in appropriate concentrations. Thus, to ensure an effective and safe therapy for the large bowel diseases, colon-specific drug delivery system is considered to be the preferable approach.[1] In the treatment

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of colon diseases, various attempts have been made to achieve maximum concentrations of drug at active site. Some of the attempts, the scientist have been employed to trigger the release of drugs to the large intestine; however, they do not reach the site of action in appropriate concentrations. So considering the above assumption in mind, it is ensured for effective and safe therapy in case of large intestine infection like large bowel disease, the colon targeted drug delivery system is a promising technique.[2]

The various techniques that have been used previously for colon-specific drug delivery system through the oral route include the pH-sensitive polymers,[3,4] time-dependent delivery and pressure-dependent system.[5] Out of these all novel approaches used for colon-targeted drug delivery system, microspheres possess more advantages as compare to conventional dosage forms such as tablet and capsule. by providing more consistent and reproducible transits through the gastrointestinal tract.[6]

Mesalamine (5-aminosalicylic acid) is the active component of sulfasalazine, belongs to the category of nonsteroidal anti-inflammatory drugs. Inhibition of prostaglandin synthesis (via inhibition of cyclo-oxygenase), inhibition of chemotactic leukotriene synthesis (via inhibition of lipoxygenase), and direct inhibition of leukocyte motility contribute to made of action more recent data, suggesting that the activity of mesalamine is based on a scavenging of oxygen-free radicals and the mesalamine acts as biological antioxidant.[7]

Mucoadhesive microsphere for the treatment of colon diseases is recently wide in use. Bioadhesive delivery could benefit the controlled release of drugs with narrow absorption window. Pectin-based microspheres for the colon-specific delivery of vancomycin have been developed.[8]

Sodium alginate (ALG) being a nontoxic, biocompatible, and biodegradable polymer comes under the group of natural polysaccharides present in the seaweed.[9] ALG is composed of monomers of β-D-mannuronic acid and α-L-guluronic acid residues joined together by (1–4) glycoside linkages. It is a biopolymer widely used for dietetic, biotechnology, cosmetic, and pharmaceutical industry.[10,11] In contact with acidic pH, ALG cross-links and forms swollen polymer matrix which acts as a reservoir enabling sustained drug release.[12,13] In addition, ALG is characterized by the ability to reduce body weight and to control glycemia in diabetic individuals by reduction after meal fluctuations of glucose concentrations, insulin secretion, decreasing of food intake, and delaying gastric emptying.[14,15]

On basis above information, the aim of the present investigation was to prepare and evaluate the sodium ALG-based mesalamine-loaded microspheres and study the effect of different formulations variables such as effect of stirring speed and amount of calcium chloride on the particle size of microspheres.

EXPERIMENTAL

Materials

Mesalamine and Eudragit S-100 were received as gift samples from Evonik Industries (Mumbai, India). Sodium ALG and Span 80 were received from Loba Chemie Pvt. Ltd (Mumbai, India). All polymers and chemicals used were of analytical grade.

METHODS

Preparation and optimization of mesalamine mucoadhesive microspheres

The preparation of mucoadhesive microspheres of mesalamine consisted of two steps, that is, in step 1, sodium ALG microspheres were prepared, and in step 2, the optimized formulations from step 1 were coated with Eudragit S-100 polymer to prevent the drug release in the stomach and small intestine. The procedure in step 1 and step 2 were as follows:

Step 1-Preparation of sodium alginate microspheres

Mucoadhesive microspheres of mesalamine were prepared by modified emulsification method (maintaining the temperature at 70°C), keeping the same drug-to-polymer ratios and varying calcium chloride concentration (2.5%, 5%, 7.5% w/v) and stirring speed as shown in Table 1. The weighed amount of sodium ALG was dissolved in warm water and then the drug was dispersed in this aqueous solution. Subsequently, the dispersion was emulsified in light liquid paraffin containing Span 80 (1% v/v), with the help of a mechanical stirrer (propeller type) (REMI Instrument Ltd, Mumbai, India) at 1000 or 1200 or 1400 rpm for 1 h. A solution of calcium chloride was added dropwise to the emulsion at a rate of 1 ml/min, to harden the formed microspheres and continued stirring for 10–15 min more to ensure efficient cross-linking. Then, the temperature was reduced to 15°C and added specific quantity of acetone. Microspheres obtained were collected by filtration and washed thrice with petroleum ether to remove the residual liquid paraffin. Microspheres were frozen for 10 h and then kept in vacuum desiccators for 12 h.

Step 2-Coating of microspheres with Eudragit S-100

The optimized batch (MM 7) of sodium ALG microspheres was coated with two different concentrations of Eudragit

Table 1: Composition and processes variables with levels used in mesalamine microspheres

Formulation code	Variable level (A)*	Variable level (B)*	Drug: polymer ratio (% w/w)	Calcium chloride concentration (%w/v)	Stirring speed (rpm)
MM1	−1	−1	1:3	2.5	1000
MM2	0	0	1:3	5.0	1200
MM3	1	1	1:3	7.5	1400
MM4	−1	−1	1:3	2.5	1000
MM5	0	0	1:3	5.0	1200
MM6	1	1	1:3	7.5	1400
MM7	−1	−1	1:3	2.5	1000
MM8	0	0	1:3	5.0	1200
MM9	1	1	1:3	7.5	1400

Formulation code	Coated mesalamine microspheres Core: Coat ratio	Calcium chloride	Stirring speed (rpm)
MM10	1:2.5	2.5	1000
MM11	1:5.0	2.5	1000

*Details of variables used in microspheres

Variables	Low (−1)	Medium (0)	High (+1)
$CaCl_2$ (% w/v)	2.5	5.0	7.5
Stirring speed (rpm)	1000	1200	1400

S-100 as shown in Table 1. Core microspheres were dispersed in Eudragit S-100 solution (2.5% and 5.0% w/v) in acetone and isopropyl alcohol solution at room temperature followed by the emulsification in light liquid paraffin containing Span 80 (1% v/v) in a beaker with the help of mechanical stirrer (propeller type) at 1400 rpm. The system was agitated for 3 h at room temperature to allow evaporation of the solvent. Finally, encapsulated microspheres (MM10, MM11) were filtered and washed with petroleum ether to remove traces of oil and dried in vacuum desiccators for 24 h.

Characterization of mesalamine microspheres

Fourier transform infrared spectroscopy

The Fourier transform infrared (FTIR) spectra of pure drug (mesalamine), sodium ALG, Eudragit S-100, and coated microsphere were recorded with an Attenuated total infrared reflection (ATIR) FTIR spectrophotometer (Mode spectrum RX 1, Alpha E, Bruker, Japan) using the potassium bromide disc method, in the range of 4000–400 cm^{-1}.

X-ray diffraction analysis

X-ray diffraction analysis (XRD) analysis investigates the effect of microencapsulation on the crystallinity of the drug as well as drug-loaded microspheres. X-ray diffractograms of mesalamine, sodium ALG, Eudragit S-100, physical mixture (mesalamine and sodium ALG), core microspheres, physical mixture (mesalamine, sodium ALG, and Eudragit S-100), and coated microsphere were recorded with an X-ray diffractometer (XPERT-PRO, PAN analytical, The Netherlands) using the positioning reference signals measurement program using Ni-filtered, CuKa radiation with a voltage of 45 kV and a current of 40 mA. The instrument was operated at continuous scanning speed over 2θ range of 5°–49°.

Differential scanning calorimetry

Thermal analyses of mesalamine, sodium ALG, Eudragit S-100, mixture of drug and sodium ALG, core microspheres, physical mixture (mesalamine and sodium ALGand Eudragit S-100), and coated microspheres were performed using a differential scanning calorimeter (Shimadzu, differential scanning calorimetry [DSC] 60, Japan) to study the thermal behavior of samples. All samples were heated in hermetically sealed aluminum pans at a constant scanning rate of 10°C min^{-1} from 40°C to 260°C under air atmosphere (50 ml/min) by applying the minimum possible pressure. An empty aluminum pan was used as a reference.

Surface morphology

Shape and surface morphology of both core and coated microspheres was observed using scanning electron microscopy (SEM) (JSM 6100 JEOL, Japan). Samples mounted on an aluminum stub were sputter coated with gold under reduced pressure and a thick gold coat was applied using a JFC 1100 (Japan) sputter coater. The sample assembly was placed in the microscope and vacuum was applied. The microspheres were observed under SEM.

Particle size analysis

The particle size distribution of core as well as coated microspheres was determined. Freeze-dried microspheres were dispersed in 20 ml isopropyl alcohol and sonicated for 5 min to bring about disaggregation of the microspheres. Microspheres were sized using a particle size analyzer (Malvern Instruments, Mastersizer 2000, UK).

Drug loading and drug loading efficiency

To determine the drug content in microspheres, an accurately weighed quantity of microspheres equivalent to

20 mg of the drug was crushed and dissolved in 100 ml phosphate buffer pH 7.4 in a volumetric flask and stirred for 12 h. After stirring, the solution was filtered through Whatman filter paper; the filtrate was diluted using phosphate buffer pH 7.4 and absorbance was measured for the determination of entrapped drug at 334 nm using an ultraviolet (UV)/visible spectrophotometer (Systronics, Mumbai, India). Observations were taken in triplicate to calculate drug loading and drug loading efficiency.

$$\% \text{ Drug loading} = \frac{\text{Drug content in Microspheres}}{\text{Weight of microspheres}} \times 100$$

$$\% \text{ Drug loading efficiency} = \frac{\text{Drug loaded}}{\text{Theoratical drug content}} \times 100$$

In vitro drug release study

Core microspheres

Microspheres equivalent to 2 mg of mesalamine were weighed accurately and suspended in 20 ml of phosphate buffer pH 7.4 containing 1% (m/v) sodium dodecyl sulfate to maintain the sink condition for the drug. The mixture was stirred at 37°C using a magnetic stirrer at a stirring speed of 50 rpm for 3 h. At specified time intervals, samples were withdrawn (2 ml) and replaced with the same volume of fresh media. The withdrawn samples were centrifuged at 3000 rpm for 10 min and were then filtered and diluted with phosphate buffer pH 7.4. The drug content was measured by taking supernatant absorbance using a UV/visible spectrophotometer (Systronics, Mumbai, India).

Coated microspheres

Microspheres equivalent to 2 mg of mesalamine were weighed accurately and suspended in 20 ml of 0.1 N HCl. The mixture was stirred on a magnetic stirrer at 37°C at a stirring speed of 50 rpm for 2 h. Samples were withdrawn at specified intervals and an equivalent amount of fresh medium was added. Collected samples were centrifuged, filtered through a membrane filter (0.45 µm) and analyzed for drug content using a spectrophotometer. The coated microspheres equivalent to 2 mg of the drug were placed in beaker and stirred magnetically at 50 rpm. The pH of the medium was maintained at 6.8 for 2 h, and then it was slowly increased to 7.4 by the addition of disodium hydrogen phosphate till the end of the study. Two milliliters of aliquots was withdrawn at predetermined intervals with the replacement of the same volume of fresh medium. The samples were centrifuged, filtered, and analyzed for drug content at 334 nm using spectrophotometry.

Drug release kinetics

The *in vitro* drug release patterns were fitted to various release kinetic models, zero order, first order, Higuchi model, and Korsmeyer–Peppas power law equation for core and coated microspheres.

In vitro mucoadhesion study

The *in vitro* mucoadhesion study of the optimized batch of microspheres and coated microsphere batch was carried out using the *in vitro* wash-off test reported by Lehr *et al*.[16] Proximal portion of a freshly slaughtered goat's large intestine was cut to expose the mucosal surface and washed with distilled water and phosphate buffer pH 7.4. A 2 cm ×2 cm serosal side was attached through a thread onto a glass slide. Coated microspheres (5 mg) were spread over the exposed mucosal surface and rinsed with phosphate buffer pH 7.4. The assembly was then kept in a humidity chamber (Thermo tech, India, Model TH-7004) at 37°C/90% RH for 30 min. In the above pretreatment, Eudragit S-100 coat got dissolved, exposing sodium ALG core microspheres. Mucoadhesiveness of the microspheres was measured by mounting the complete assembly onto a disintegration apparatus (EI Products, Panchkula, India) with the help of a clamp and a thread. The apparatus was operated in such a manner that the tissue was allowed to move in reciprocating motion at a frequency of 28–32 cycles per min while immersed in phosphate buffer pH 7.4 contained in a 1000 ml beaker. The time taken by the tissue to completely wash off the microsphere was considered the mucoadhesion time.

Stability studies

Stability studies were carried out for all formulation of mesalamine microspheres by storing the microspheres in sealed airtight cellophane packets at room temperature studies and 40°C ± 2°C/75% ± 5% RH (elevated temperature studies) for 6 months as per the ICH guidelines. The microspheres were analyzed after 0 days, 3 weeks, 6 weeks, 3 months, and 6 months.

Uncoated and coated microspheres were put into hard gelatin capsules wrapped in aluminum foil laminated on the inside with polyethylene. The samples were kept at room temperature and under accelerated conditions in stability chamber (Stability Oven, Nirmal Instruments, India). Real-time stability studies were performed by periodical testing of the drug content at intervals of 0, 3 weeks, 6 weeks, 3 months, and 6 months. The samples were evaluated for their physical characteristics (color) and drug content. The degradation rate constant (K_{cal}), shelf life (t_{90}), and initial drug concentrations providing 2 years shelf life (Int_{cal}) were determined.

RESULTS

Drug loading and drug loading efficiency

The percentage drug entrapment was found to be in the range from 81.09% to 92.59% for sodium alginatemicrospheres (core microspheres). The highest drug loading efficiency was found to be 93.05% for MM7 at the drug-to-polymer ratio 1:3 and calcium chloride concentration of 2.5%. The results revealed that as the concentration of crosslinker increased and also as the stirring speed increased, the drug loading efficiency also increased.[17] On the other hand, coated microspheres showed drug loading efficiency of 92.26% and 98.50% for formulations MM10 and MM11, respectively [Table 2].

Characterization of mesalamine microspheres

ATIR- Fourier transform infrared spectroscopy

FTIR spectra of mesalamine, sodium ALG, Eudragit S-100, and coated microspheres are depicted in Figure 1. The drug sample showed characteristics peaks at 1648 cm^{-1}, 1447 cm^{-1}, and 1137 cm^{-1} for functional Group C = C aromatic bending, C-C aromatic stretching, and (C-O stretching), respectively. In case of sodium ALG, spectrum showed characteristic absorption band for C = O stretch and O-H stretch at 1740 cm^{-1}, 1728 cm^{-1}, and 3362 cm^{-1}, respectively. The major peaks of the drug were also observed in the coated microspheres at 1447 cm^{-1} due to C-C aromatic stretching. For sodium ALG and Eudragit S-100, IR spectrum showed characteristic absorption band for C = O stretch and C-C aromatic stretching at 1728 cm^{-1} and 1460 cm^{-1}, respectively, in the coated microspheres.

X-ray diffraction analysis

To study the changes occurred in crystalline nature of drug, during microspheres (core and coated) process, XRD patterns of mesalamine, physical mixture of mesalamine with excipients, and core microspheres and coated microspheres were obtained [Figure 2]. XRD pattern of pure mesalamine indicated intense peak at 5°and 15° and 16.5° which were characteristics peaks for mesalamine. XRD of physical mixture retained intense peak of mesalamine, and there was no such peak of sodium ALG and Eudragit S-100 due to the dilution effect of the amorphous polymers.

Differential scanning calorimeter

To observe the changes occurred in mesalamine during microspheres process, DSC study was conducted. Figure 3 indicates DSC of mesalamine, sodium ALG, Eudragit S-100, mesalamine + sodium ALG, core microspheres, physical mixture of mesalamine + sodium ALG + Eudragit RS 100, and coated microspheres. A sharp endothermic peak was observed at 288.20°C corresponding to its melting point in the crystalline form of mesalamine. Eudragit S-100 exhibited two endothermic peaks at 98.59°C and 232.44°C. DSC scan of the uncoated microsphere of sodium ALG showed a sharp endothermic peak was observed around 285.00°C

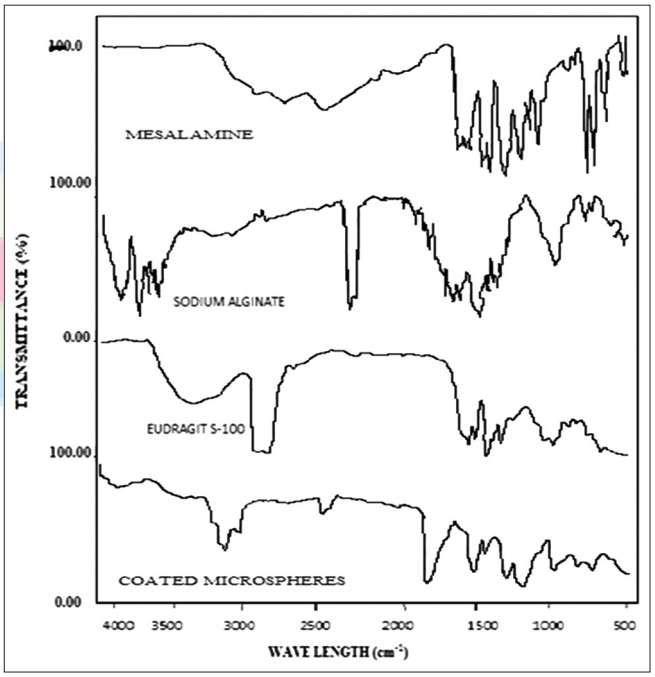

Figure 1: Layout of Fourier transform infrared spectra of drug, polymers, and coated microspheres

Table 2: Particle size, entrapment efficiency, and percentage cumulative release of mesalamine microspheres

Formulation code	Calcium chloride concentration (%w/v)	Stirring speed (rpm)	Particle size (μm)	Entrapment efficiency (%)	Cumulative release (%)
MM1	2.5	1000	423	92.17	98.50
MM2	5.0	1200	339	86.23	88.62
MM3	7.5	1400	310	80.97	86.62
MM4	2.5	1000	401	89.12	95.20
MM5	5.0	1200	382	88.25	94.42
MM6	7.5	1400	314	82.75	88.27
MM7	2.5	1000	445	93.04	99.54
MM8	5.0	1200	376	88.00	92.25
MM9	7.5	1400	312	82.98	88.61
MM10	2.5	1000	458	95.26	89.12
MM11	5.0	1000	432	98.50	83.06

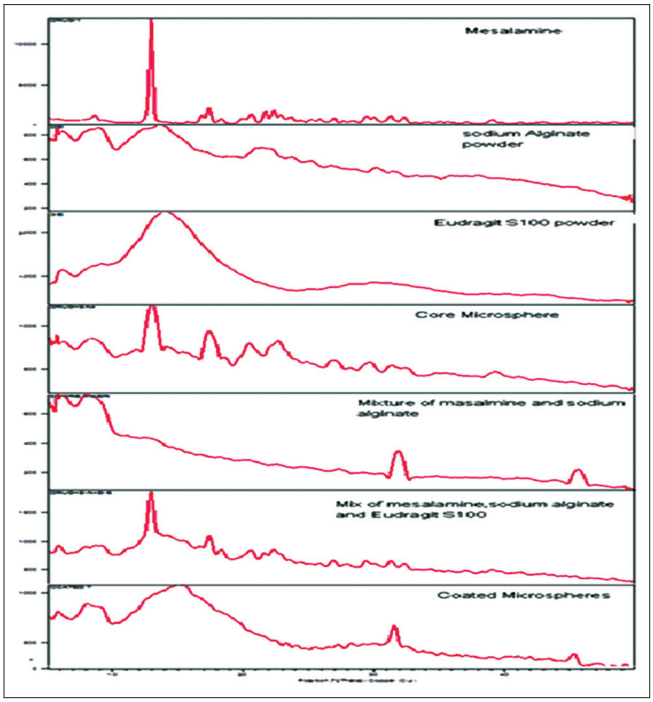

Figure 2: Layout of X-ray diffraction analysis spectra of Mesalamine, sodium alginate, Eudragit S-100, drug + sodium alginate, core microspheres, physical mixture (mesalamine + sodiumalginate + Eudragit S-100), and coated microspheres

and a broad exothermic peak at 143.00°C. DSC of coated microspheres indicated endothermic peak at 124.00°C.

Scanning electron microscopy

Figure 4 suggested that, the surface morphology of core and coated microspheres. SEM of core microspheres revealed slightly irregular in shape. On the other hand, SEM images of coated microspheres showed smooth surface and a smaller number of pores due to coating [Figure 4].

Particle size analysis

The particle size of all formulations (core and coated microspheres) was determined. The particle size of microspheres ranged from 310 to 458 to μm and were found to decrease in size at higher stirring speed, that is, 1400 rpm. The particle size of MN10 and MN11 (coated microspheres) were found to be 458 μm and 432 μm, respectively. In case of coated microspheres, the concentration of calcium chloride was increased the result decrease in particle size of microspheres.

In vitro release study

Core microspheres

The drug release from core microspheres in phosphate buffer, pH 7.4 showed up to 10 hrs.. Formulation (MM7) of mesalamine microspheres showed maximal drug release of 99.54% ± 0.39% within 10 h and its

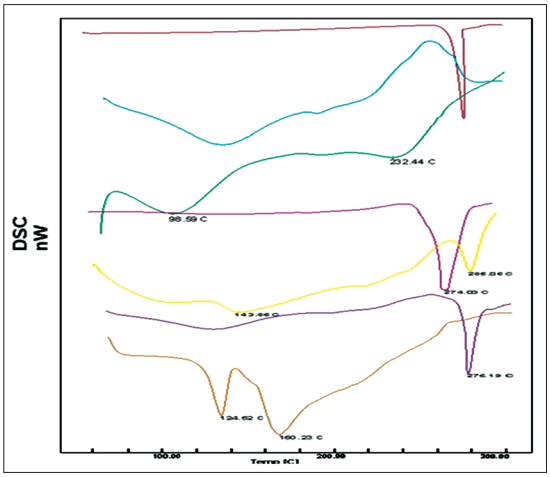

Figure 3: Layout of differential scanning calorimetry thermograms of Mesalamine, sodium alginate, Eudragit S-100, mesalamine + sodium alginate, core microspheres, physical mixture (mesalamine + sodium alginate + Eudragit S-100), coated microspheres

entrapment efficiency was 93.04%. Other formulations MM 1 to MM 6, MM 8, and MM 9 showed lower percentage of drug release, that is, from 88.27% ± 0.75% to 98.50% ± 0.39% due to which they could not be selected for further preparation of coating microspheres [Figure 5].

For this, coated microspheres were prepared in two batches (MM10 and MM11) considering different core: Coat ratios (1:2.5%w/v and 1:5%w/v) using same conditions as that was for optimized batch MM7, that is, 1000 rpm and 2.5% $CaCl_2$. The drug release from coated formulations of optimized batches provide sustained release from microspheres and maximum cumulative release was found to be 89.22% ± 0.26% and 82.96% ± 0.23% for MM10 and MM 11, respectively [Figure 6].

Drug release kinetics

The percentage drug release profiles obtained from *in vitro* release experiments were subjected to different kinetics models to find the drug release mechanism and kinetics. The table data reveal that *in vitro* release from the core microsphere (sodium ALG microsphere) was better explained by the Higuchi equation since the plots provide the highest linearity. The "*n*" value as per Korsmeyer–Peppas (KP) model for sodium ALG microsphere was found to be between 0.45 and 0.89 that indicates anomalous release behavior of drug. The coated microspheres were followed Fickian kinetics and the value of n (<0.45) as per the KP model also compliment the same [Table 3].[18]

Mucoadhesion studies

In the *in vitro* mucoadhesion study, the complete detachment of the optimized formulation of microsphere from

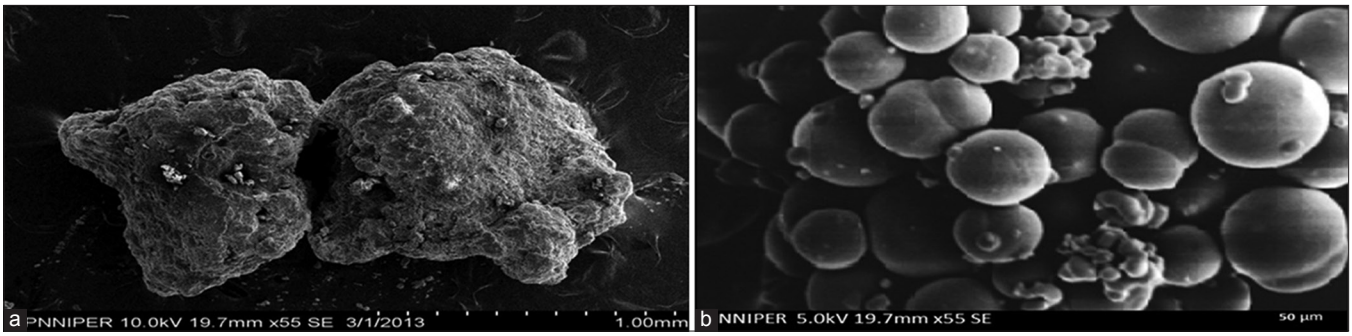

Figure 4: SEM micrographs of (a) core Mesalamine microspheres, (b) coated Mesalamine microspheres

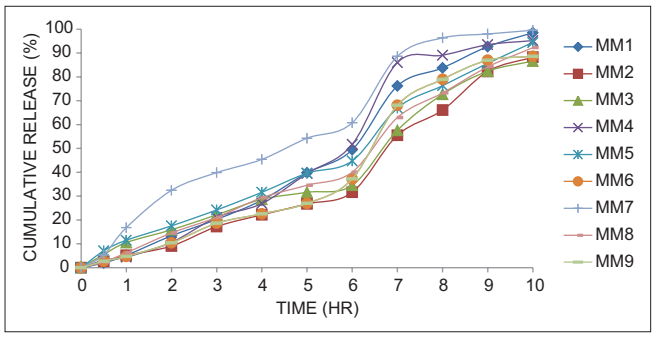

Figure 5: *In vitro* release profile of mesalamine core microspheres in phosphate buffer, pH 7.4 coated microspheres

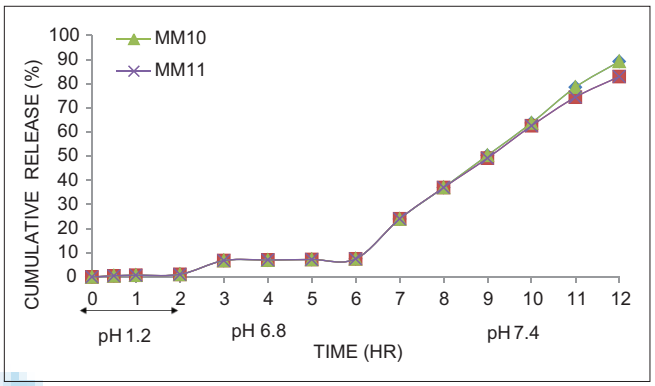

Figure 6: *In vitro* release profile of Eudragit S-100-coated mesalamine microspheres at different dissolution medium

Table 3: Comparison of different dissolution kinetics models of all mesalamine microspheres

Formulation code	Zero-order	First-order	Higuchi	Korsmeyer-Peppas	
	R^2	R^2	R^2	R^2	n
MM1	0.967	0.812	0.976	0.988	0.652
MM2	0.910	0.842	0.993	0.970	0.624
MM3	0.924	0.864	0.945	0.984	0.554
MM4	0.960	0.898	0.979	0.988	0.536
MM5	0.956	0.841	0.989	0.994	0.879
MM6	0.968	0.878	0.997	0.991	0.672
MM7	0.959	0.884	0.963	0.974	0.781
MM8	0.974	0.853	0.993	0.994	0.566
MM9	0.959	0.878	0.979	0.984	0.655
MM10	0.945	0.787	0.953	0.969	0.339
MM11	0.965	0.835	0.988	0.993	0.425

mucosal tissue took 90 min, indicating good mucoadhesive properties of sodium ALG.

Stability studies

The degradation of drug from all formulations followed first-order kinetics and the former could provide 2 years shelf life at room temperature. All the formulations provide 2 year shelf life except MM2, MM9, MM10 and MM11. Hence, the formulations MM2, MM9, MM10, and MM11 need some overage to ensure 2-year shelf life at room temperature. The degradation rate constants (K_{cal}) and shelf life (t90) for all formulations were found to range between 1.09–2.48 days^{-1} and 956–422 days, respectively. The optimized formulation MM7 showed 1 year stability and it further needed 5%–10% overages to ensure a shelf life of 2 years [Tables 4 and 5].

DISCUSSION

In the preparation of mesalamine-loaded microspheres, the different variables were studied such as cross-linking agent, stirring speed, and polymer ratio to understand the influence of these parameters on particle size and *in vitro* drug release. The release behavior of ALG microspheres depends on the valency and size of cations of respective cross-linking agent.[19] Release rate decrease with increased amount of cross-linking agent. More of calcium ions become available for cross-linking glucuronic acid units of sodium ALG which in turn could increase the viscosity of formulation to form larger microspheres. Increasing concentration >2.5% leads to decrease in drug release. Hence, we concluded that the optimum concentration of calcium chloride was 2.5% w/v.[20]

In preliminary batches, the effect of stirring speed was studied at different agitation speed like 1000, 1200 and 1400 rpm. The drug-to-polymer ratio was kept the same, that is,1:3 for all microspheres which may be due to increase in viscosity of the polymer which ultimately resulted in larger emulsion droplets and finally greater microsphere size.[21]

Table 4: Stability profile of mesalamine microspheres (uncoated or coated) under accelerated temperature storage conditions (40°C±2°C, 65% RH±5% RH)

Formulations code	Drug content (%)				
	0 day	3 weeks	6 weeks	3 months	6 months
MM1	100.00±0.006	98.43±0.007	97.38±0.006	96.33±0.003	95.60±0.003
MM2	100.00±0.001	98.87±0.004	97.84±0.006	96.12±0.017	94.39±0.025
MM3	100.00±0.002	98.17±0.001	96.90±0.015	95.94±0.020	94.35±0.028
MM4	100.00±0.002	99.43±0.001	98.95±0.001	96.96±0.012	94.73±0.025
MM5	100.00±0.001	99.30±0.001	98.79±0.000	95.96±0.001	93.99±0.254
MM6	100.00±0.002	99.50±0.002	98.98±0.001	96.39±0.016	94.15±0.027
MM7	100.00±0.001	99.62±0.002	99.31±0.002	97.56±0.008	95.65±0.019
MM8	100.00±0.002	99.48±0.002	97.98±0.002	96.96±0.019	94.38±0.027
MM9	100.00±0.001	99.69±0.002	98.92±0.003	97.62±0.009	94.17±0.028
MM10	100.00±0.001	99.65±0.002	99.04±0.003	97.83±0.007	94.35±0.027
MM11	100.00±0.001	99.69±0.002	97.88±0.009	96.50±0.016	94.00±0.029

Table 5: Stability profile of mesalamine microspheres (uncoated or coated) under room temperature storage conditions (30°C±2°C, 65% RH±5% RH)

Formulation code	Drug content (%)					K_{calc} (days^{-1}×10^4)	t_{90} days	Int_{calc} for 2 years
	0 day	3 weeks	6 weeks	3 months	6 months			
MM1	100±0.001	99.68±0.002	98.89±0.002	98.42±0.002	97.71±0.002	1.17	892	104.0
MM2	100±0.006	99.50±0.006	97.92±0.006	98.30±0.003	97.95±0.006	1.22	859	104.4
MM3	100±0.011	99.37±0.001	97.92±0.006	96.55±0.006	96.15±0.005	2.30	455	108.5
MM4	100±0.020	99.67±0.002	99.19±0.001	98.79±0.003	98.08±0.004	1.12	930	104.1
MM5	100±0.006	99.70±0.012	99.09±0.012	98.75±0.003	98.23±0.011	1.22	859	104.4
MM6	100±0.001	99.48±0.000	98.88±0.001	98.45±0.002	97.93±0.012	1.09	956	103.9
MM7	100±0.002	99.54±0.004	99.14±0.040	98.90±0.014	97.87±0.012	1.12	930	104.1
MM8	100±0.001	99.51±0.002	98.91±0.002	98.56±0.002	97.62±0.002	1.22	855	104.4
MM9	100±0.001	99.65±0.001	99.14±0.005	98.62±0.001	97.24±0.002	1.53	682	105.6
MM10	100±0.001	99.60±0.001	99.17±0.002	98.82±0.000	96.56±0.001	1.86	563	106.73
MM11	100±0.001	99.65±0.005	99.14±0.005	98.62±0.001	95.25±0.002	2.48	422	109.24

Increase in level of emulsifier will allow it to stabilize a greater interfacial surface area, thus leading to smaller particle size but no significant reduction in size of microspheres above 1%. This is due to tightening of polymeric network leading to microsphere shrinkage as concentration of emulsifier increases.[22,23]

The main characteristic peaks of mesalamine were unaffected in IR spectra of physical mixture and mesalamine-loaded coated microsphere. Thus, there was no chemical change occurred in the formulation during the agglomeration process. The X-ray diffractogram of core microspheres of mesalamine showed characteristic peaks at 5°, 14.5° 2θ which states that there is no interaction, whereas coated microspheres showed no such characteristic sharp peaks as that of drug which may be attributed to amorphous material devoid of any crystallinity due to the dilution effect of the amorphous polymers. A sharp endotherm of mesalamine indicates crystalline nature of the drug. In the physical mixture, the presence of Eudragit S-100, interaction between Eudragit S-100 and sodium ALG (disappearance of sodium ALG exothermic peak at 283.00°C) can be proposed. The endothermic peak of mesalamine at 288.00°C reduced due to its low percentage in the physical mixture. However, thermograms of coated microspheres suggest that a depressed, broad endothermic peak at 124.00°C could be a contribution of the dilution effect of amorphous polymer. The SEM study suggested that the core microspheres formed were slightly irregular in shape due to surface-attached crystals of the drug. They were discrete, having a rough surface with an increased number of pores causing rapid release of the drug in the medium. Release rate of drug decreased with the increasing amount of cross-linking agent. The release behavior of ALG microspheres depends on the valency and size of cations of respective cross-linking agent. As in case of Ca^{2+} microspheres, the smaller size of Ca^{2+} as compared to other crosslinkers ensure rapid removal of Ca^{2+} from microspheres due to ion exchange process with Na+ of phosphate buffer leads to rapid release.[24] The *in vitro* release study of the core microsphere (sodium ALG microsphere) was better explained by the Higuchi equation since the plots provide the highest linearity. The "*n*" value as per KP model for sodium ALG a microsphere was found to be between 0.45 and 0.89 that indicates anomalous release behavior of the drug. The coated microsphere were followed Fickian kinetics and the value of n (<0.45) as per the KP model also compliment the same. To provide better mucoadhesion, the polymer should show the presence of a strong hydrogen bonding group such as –OH, –COOH, and strong anionic

charges spreading on the mucus. Sodium ALG is in the first-generation mucoadhesive polymer group, which contains higher hydrogen bond forming polymers.[25]

CONCLUSIONS

Controlled release system of mesalamine for colon-specific delivery was developed successfully using modified emulsification method. The drug release from coated formulations was found as sustained manner compared to uncoated formulations. Coated microspheres showed a longer residence time in the colon after removing the Eudragit S-100 coating due to better mucoadhesion properties of sodium ALG. The optimized formulation showed 1 year stability and it further needed 5%–10% overages to ensure a shelf life of 2 years. This study finally concluded that, Eudragit S-100 coated sodium alginate based mesalamine microspheres provides sustained drug release.

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Conflicts of interest
There are no conflicts of interest.

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