RESEARCH ARTICLE

Design and Characterization of Mesalamine Loaded Nanoparticles for Controlled Delivery System

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ABSTRACT

Objective(s): Nanoparticles (NPs) are known for their specific accumulation in the inflamed tissues of the colon and thus allow a selective delivery to the site of inflammation with minimum adverse effects. The main objective of this work is to attain mesalamine loaded chitosan nanoparticles as a carrier for oral delivery.

Methods: In this study, mesalamine loaded chitosan nanoparticles were prepared using an ionic gelation method. Experimental design Box-Behnken response surface methodology was used for the optimization of the nanoparticles. The nanoparticles size and gelation process of the polymeric nano-drug controlled release system depends on several variables including the concentration ratio of chitosan-TPP, concentration of mesalamine, concentration of chitosan solution and pH of the solution with optimum conditions of 2.3, 0.02 mg/ml, 0.1 mg/ml and 4.5, respectively. Moreover, the morphology of the prepared nanoparticles was observed by scanning electron microscopy (SEM). Also, characterization of the chitosan-mesalamine nanoparticles was performed by FT-IR spectrophotometer for specifying the chemical structure of nanoparticles molecules and differential scanning calorimetry (DSC) for studying thermal behavior. Drug release profile and the amount of the loaded drug were also monitored by UV-Vis spectroscopy.

Results: The mean particle size of the synthesized nanoparticles was ranging from 53.9 to 322.8 nm using a dynamic light scattering (DLS) technique. SEM image shows segregated and non-aggregated nanoparticles with sub-spherical smooth morphology. An in-vitro release study of the prepared nanoparticles illustrated that the percentage of mesalamine released from the nanoparticles was 90.17 ± 2.45% within 48 hrs.

Conclusion: Drug released showed that the release profile of mesalamine loaded nanoparticles was in a slow manner and no initial rapid release (burst effect) was illustrated.

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INTRODUCTION

Mesalamine is a bowel-specific aminosalicylate drug that is used in the treatment of ulcerative colitis (UC) disease which is recurrent idiopathic inflammatory disorder that causes bloody stools and abdominal pain. There are a number of oral mesalamines that are commercially available, including azo-bond pro-drugs such as sulfasalazine, olsalazine, and balsalazide. Delayed and controlled release dosage forms of mesalamine still appear to be insufficiently selective [1]. This is due to the fact that drug release mechanism is based on physiological parameters that are not related to the inflammation and barely to its location. Controlled release preparations are designed to achieve optimum delivery of the biologically active mesalamine to the colon and to minimize the

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systemic absorption. In the case of ulcerative colitis disease, poor adherence of mesalamine has shown to be an important barrier in successful therapy and therefore, carrier systems such as nanoparticles can exclusively deliver the drug to the inflamed regions after oral administration for a prolonged period. These systems have several advantages over the conventional chemical inflammatory compounds including minor side effects.

In the recent years several studies have been reported on the application of natural polymers for colon delivery. Chitosan is the N-deacetylated product of chitin, which is the second most abundant polysaccharide in nature. It has attracted great attention in the field of colon delivery [2-4]. The difference between chitin and chitosan is the functional group which is situated at C_2 of the monomeric unit. Due to the presence of the amino group in chitosan it can be indicated that chitosan, has higher solubility in comparison with chitin [5]. In addition chitosan has received great attention in the medical, biological, and pharmaceutical area due to its unique characteristics including biodegradability and biocompatibility [6-8]. It has been demonstrated that this natural polymer is non-toxic which makes it an excellent candidate for drug delivery systems. Microspheres, microgels, and nanoparticles are various forms of chitosan-based drug delivery systems that have been studied extensively in the recent years [6].

Chitosan nanoparticles have the potential to be used as hydrophilic carrier systems since they can deliver drugs to specific sites and control the drug release rate. Different techniques can be employed to produce chitosan nanoparticles including ionic gelation, coacervation, emulsion coacervation, and reverse micellar [9]. The most common method for this purpose is the ionic gelation which has several advantages over other methods. This method has mild conditions without applying harmful organic solvents and heat that can damage sensitive proteins. This process can be simply performed by sodium tripolyphosphate (TPP) as a cross-linking agent. The mechanism of nanoparticle formation is based on electrostatic interaction between the positively charged amino group of chitosan and the negatively charged group of TPP. TPP is a favorable cross-linker for the ionic gelation process due to its non-toxicity and quick gelling ability [10-11]. The prepared nanoparticles possess a positive surface charge which makes them quit suitable for mucosal adhesion applications [9].

In this study, we investigated the preparation and optimization of mesalamine nanoparticles via the ion gelation method. Box-Behnken response surface methodology has been used for optimization of chitosan-based nanoparticles prepared and also for determination of the effects of mesalamine concentration, chitosan concentration, chitosan to TPP ratio, and pH of solution physicochemical properties of nanoparticles including size, zeta potential, polydispersity index (PdI), and entrapment efficiency (EE%).

MATERIALS AND METHODS

Materials

Chitosan (CS) with molecular weight of 70 kDa and 95% deacetylation was obtained from Primex, Iceland. Mesalamine was received from Sigma (USA). All chemicals and reagents employed in this study are of analytical grade and purchased from Merck (Germany).

Preparation of mesalamine loaded chitosan nanoparticles

Chitosan nanoparticles were prepared via the ionotropic gelation technique. Chitosan was first dissolved in 1 ml of 2.0% (v/v) acetic acid and 2 ml of deionized water and solution was prepared at various concentrations (0.1-0.3 g/100ml). These mixtures were stirred at room temperature for 15 minutes. Mesalamine drug was then dissolved in 2 ml of deionized water and 1 ml of 10% (v/v) HCl. Each solution was then sonicated for 6 min to obtain a homogenous suspension. Finally, solutions of mesalamine were added to the chitosan solution and pH of the resulting solution was then adjusted by NaOH and acetic acid solutions. Chitosan nanoparticles loaded with mesalamine were fabricated through the drop wise addition of TPP solution (0.5 g/100ml) containing Tween (1%, to private aggregation), to 5ml of the final solution under magnetic stirring (400 rpm), resulting in a colloidal solution. The colloidal solution stirred for 30 min to obtain a cross-linked chitosan solution, containing micro and nanoparticles that are formed by means of electrostatic interaction between the positively charged chitosan chains and TPP as a cross-linker. The mesalamine loaded chitosan nanoparticles were separated from the suspension by placing the colloidal solution into microtubes and centrifuging for 5 min (5000 rpm). In this stage, the micro particles separated from nanoparticles. The supernatant was collected and

Table1. Variables and levels used in RSM

Independent variables (factors)	Levels	
	Min	Max
X_1=Concentration of drug (mg/ml)	0.01	0.02
X_2=Concentration of polymer	0.1	0.3
X_3= Concentration ratio of polymer/TPP	2	6
X_4=Polymer solution pH	4.5	5.5
Dependent variables (responses)	**Constrains**	
Y_1=size (nm)	Minimize	
Y_2= PdI	Minimize	
Y_3= zeta potential (mV)	$20<Y_2<30$	

transferred to another microtube and centrifuged for 30 min (15000 rpm), resulting in the formation of white precipitates. Then the precipitate was dispersed via ultra-sonication in 1 ml of deionized water and filtered through a microfilter (450 nm). The resulting solution contained chitosan nanoparticles. Moreover, average particle size and morphology of the synthesized nanoparticles were determined by DLS and SEM method.

Experimental design

The statistical method suggests several profits over the classical method; it is quick and helps to understand the interactions between the parameters at different quantities, and also reduces the total amount of experiments [12]. For this aim, the design-of-experiment (DoE) has been evolved and studied in pharmaceutical researches [13]. Notwithstanding the fact that central composite and D-optimal designs are two main techniques in RSM; computer- generated Box–Behnken design was used in described conditions.

Therefore, Design-Expert® software (V.7.0.0, Stat-Ease, Inc., Minneapolis, USA) with Box-Behnken response surface methodology was used to provide the optimal conditions for the preparation of chitosan nanoparticles and to specify relationships among variables and responses. Four independent variables were chosen as mesalamine concentration (X_1), chitosan concentration (X_2), chitosan to TPP ratio (X_3), and pH of solution (X_4), while the dependent variable were the nanoparticle size (Y_1), polydispersity index (PdI) (Y_2) and zeta potential (Y_3). The symbols and levels are shown in (Table 1). Three replicates at the central point of the designed model were used to determine the particles size and particle distribution.

According to this method, about 26 runs were essential to perform the proper models. Moreover, a second-order model is effective in approximating a contribution of the accurate response surface with parabolic tilt.

In-vitro drug release studies

Mesalamine incorporation efficiency

In order to determine the amount of mesalamine loaded on nanoparticles, UV-Vis spectroscopy was used for plotting a standard curve. For this purpose, a solution of mesalamine (2 ml of water, and 1 ml of HCl) and 2% (v/v) acetic acid was prepared as a blank. Then, blank and mesalamine drug were located in separate cells and absorption spectra were recorded in the range of 200-600 nm. It was demonstrated that maximum absorbance for mesalamine drug was 230 nm. In addition, specific concentrations of chitosan were prepared and their absorptions were recorded at 230 nm, indicating that no interaction between the polymer and drug was observed.

In order to plot the standard curve, 10 mg of mesalamine was weighed into a 100 ml volumetric flask. Water, hydrochloric acid, and acetic acid were then added to flask. Concentrations of 5, 10, 20, and 50 µg/ml of this stock solution were diluted with a proper amount of solvent and their absorptions were recorded at 230 nm.

The mesalamine loading efficiency (LE %) of the nanoparticles was calculated indirectly by use of the following equation (Eq. 1).

$$\text{LE (\%)} = \frac{\text{Total mass of drug} - \text{Released drug}}{\text{Total mass of drug}} \times 100 \qquad (1)$$

Also, the nanoparticles production yield was calculated by gravimetry. Fixed volumes of nanoparticles suspensions were centrifuged and sediments were dried.

Table 2. Box-Behnken experimental design

Run	Mesalamine%	Cs%	Cs/TPP	pH	Size (nm)	PDI	Zeta (mv)
1	0.01	0.1	2	5.5	378.3	0.46	22.5
2	0.01	0.1	6	5	297.1	0.28	22.3
3	0.02	0.3	6	5.5	266.0	0.39	20.5
4	0.02	0.2	4	4.5	168.0	0.49	23.9
5	0.02	0.1	4	5	142.0	0.37	22.9
6	0.02	0.2	4	4.5	284.0	0.38	27.6
7	0.02	0.3	2	4.5	215.0	0.48	18.4
8	0.02	0.1	2	4.5	120.0	0.29	27.3
9	0.02	0.2	2	5.5	220.0	0.31	19.9
10	0.02	0.1	4	5.5	212.0	0.39	19.7
11	0.01	0.2	4	5.5	203.9	0.43	18.8
12	0.02	0.1	4	4.5	122.0	0.35	28.2
13	0.02	0.2	4	5	228.0	0.42	20.5
14	0.01	0.2	2	5	282.0	0.45	22.9
15	0.02	0.1	2	5.4	53.9	0.34	19.7
16	0.02	0.3	2	5	190.0	0.37	17.5
17	0.02	0.1	4	5	61.3	0.20	23.2
18	0.01	0.3	4	4.5	96.7	0.33	24.3
19	0.01	0.2	4	5.5	233.0	0.27	20.2
20	0.01	0.2	6	4.5	250.7	0.45	15.2
21	0.01	0.2	6	5.5	322.8	0.41	15.7
22	0.02	0.1	4	5	58.8	0.18	21.1
23	0.01	0.2	6	4.5	294.0	0.29	18.3
24	0.02	0.3	4	5	204.0	0.28	22.0
25	0.01	0.3	6	5	321.5	0.49	16.4
26	0.01	0.2	4	5	190.2	0.19	19.6

The process yield (PY) was calculated as follows (Eq. 2):

$$PY(\%) = \frac{\text{Nanoparticles weight}}{\text{Total solid (CS + TPP + Mesalamine)}} \times 100 \quad (2)$$

In vitro drug release

Drug release studies were carried out via a dialysis method. For this purpose, samples of mesalamine loaded chitosan nanoparticles from freeze dried drug-polymer nanoparticles that supply sink conditions were added to phosphste buffer (PBS) in a dialysis bag with a molecular cut-off of 12 KDa (Sigma) and suspended in specific concentration of phosphate buffer into a shaker incubator at 37 ± 0.5°C. Then, at different time intervals (1, 2, 4, 6, 8, 24 and 48 hours.), a sample volume of 2 ml was withdrawn and replaced by 2 ml fresh PBS buffer. Then, the amount of drug release was determined by UV-Visible spectrophotometry (OPTIZEN 2120UV plus, Korea) at 230 nm to define the amount of drug released. All the experiments were accomplished in triplicate under similar condition. The percentage of drug released at each time point was measured according to Eq. (3).

$$\text{Drug Release (\%)} = \frac{\text{Drug in solution } (\mu g/ml)}{\text{Initial drug in nanoparticles } (\mu g/ml)} \times 100 \quad (3)$$

Characterization of nanoparticles

Size, zeta potential and polydispersity index of nanoparticles (DLS)

The size, zeta potential and polydispersity index of the mesalamine nanoparticles were analyzed by dynamic light scattering using a Malvern Zeta Sizer, UK. The sample of nanoparticles was set in the analyzer cell and readings were taken at 25°C with the 90 degree of discoverd angle. Each example was evaluated three times and the outcomes were shown as mean ± standard deviations (SD) [14]. The particle size distribution was represented as a polydispersity index (PdI) with the range from 0 to 1. Contents close to zero illustrated a homogenous dispersion and data bigger than 0.5 displayed high heterogeneity [15].

Fourier transform infra-red spectroscopy (ftir)

The FTIR spectra of mesalamine nanoparticles were recorded as wave numbers (cm^{-1}) using a Thermo Nicolet Nexus 870 FT-IR spectrometer.

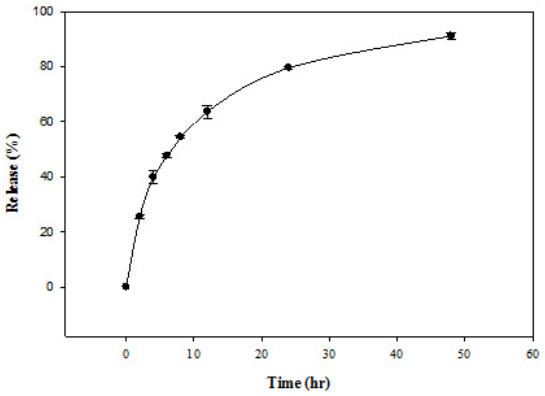

Fig. 1. % Drug release of Mesalamine loaded chitosan.

The tablets were provided by the blending of sample and potassium bromide at a pressure of 5000 atm and scanned at 500 to 4000 cm^{-1}. Then, the spectra of the nanoparticles were compared with pure drug [16, 17].

Differential scanning calorimetry (DSC)

Differential scanning calorimetry was carried out using a Rheometric Scientific model STA-1500 scanning calorimeters with crucible as a sample pan made of alumina to define the thermal stability of mesalamine nanoparticles. The experimental parameters included the temperature range 0-450 °C, the rate of heating scanning 10 °C/min and gas the atmosphere (nitrogen) 25-35 ml/min.

X-ray diffractometry (xrd)

The crystal structure of the nanoparticles was defined by X-ray diffraction using Siemens D5000 X-ray diffractometer, Munich, Germany, with a horizontal protractor at room temprature. XRD is used for knowing the atomic and molecular formation of a crystal, in which the crystalline atoms make a beam of dependent X-rays to diffract into many particular directions.

For this purpose the samples were put in the sample holder and scanned from 0° to 90° at the rate of 1°/min.

Scanning electron microcopy (sem)

The scanning electron microscopy was used to characterize the surface morphology of nanoparticles using Philips XL model, Holland. The nanoparticles were placed on metal plates using double-sided tape and coated with a thin layer of gold. After that, electrons which are emitted from the sample were discovered [18].

RESULTS AND DISCUSSION

Experimental design

Box-Behnken experimental design was carried out for testing the accuracy of the proposed quadratic model [and] effects of the influential variables on the chitosan nanoparticle responses. The values of complete variables and the relevant experimental data (26 indicated formulations) based on Box-Behnken design have been summed up in (Table 2).

The mean particle size of the formulations ranging from 53.9 to 378.3 nm is shown in Table 2. The high zeta potential value of nanoparticles (15.2 to 28.2 mv) illustrated in Table 2, was because of the amino groups of chitosan due to being of cationic groups [19]. This is incured due to the high amount of electrical double-layer thickness, which in turn impeded aggregation [12]. PDI of nanoparticles was 0.18 to 0.49 (smaller than 0.5) which illustrating a comparative homogeneous dispersion.

In-vitro drug release studies

Investigation of drug loaded chitosan nanoparticles

The release behavior of the mesalamine from chitosan nanoparticles was observed by UV-Vis spectroscopy. In order to determine the drug release profile, the wavelength was set at 230 nm, indicating that mesalamine has the maximum absorption in this wavelenght. Different concentrations of drug were prepared and their abosorbances were recorded at 230 nm.

Loading efficiency was acquired by the equation (1), as described before. According to this equation, the amount of the loaded nanoparticles was equal to 89% which affirms the high amount of mesalamine loaded into the chitosan nanoparticles. Moreover, nanoparticles production yield was calculated by equation (2) and resulted to 85%.

In-vitro release of mesalamine from chitosan nanoparticles

The *in-vitro* release profile of mesalamine from chitosan nanoparticles was carried out within 48 hours in PBS as shown in Fig. 1. Experiments were done in triplicates and data are shown as the mean ±SD and the drug released from nanoparticles calculated by Eq. (3) at about 90.17 ± 2.45%.

It is concluded that release rate increased with time. between chitosan and TPP. Also, rapid release (burst effect) of mesalamine was not observed due to the slow initial release of mesalamine during the first few hours indicating suitable interaction

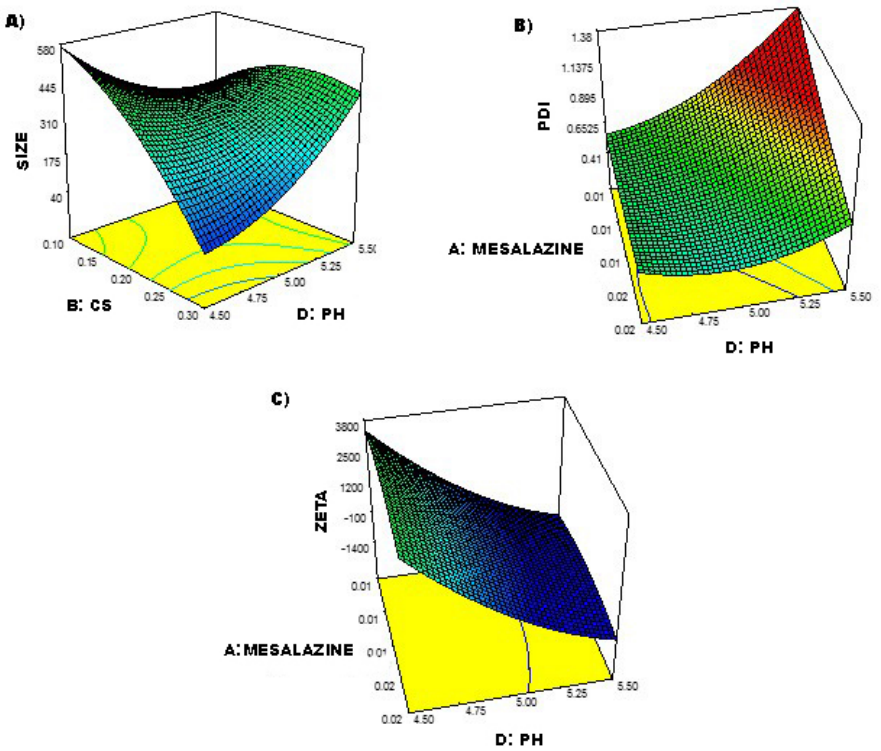

Fig. 2. Response surface plots showing effect of factorial variables on (A) particle size, (B) poly dispersity index and (C) zeta potential

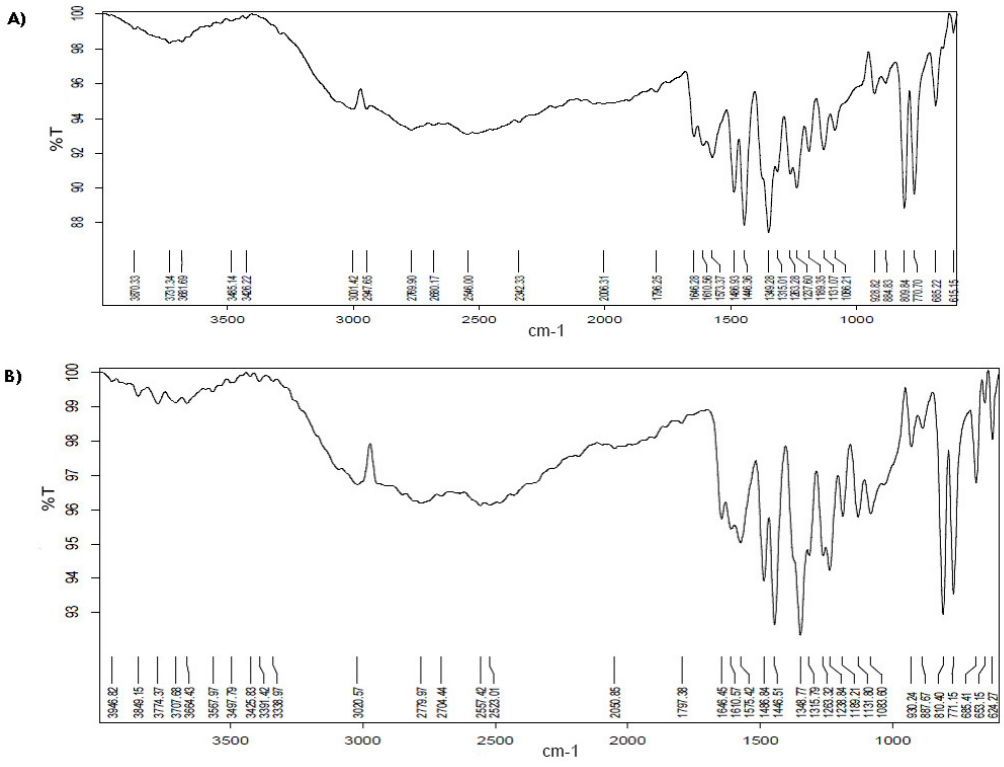

Fig. 3. Comparison of FTIR spectra of (A) mesalamine, (B) mesalamine-chitosan nanoparticles

Table 3. FT-IR data of mesalamine-chitosan nanoparticles

Groups	Wave numbers cm^{-1}
O–H stretch	3489.15
C–H stretch	3020.57
N–H stretch	1610.57
C–C stretch	1446.51
O–H bend	1348.77
C–O stretch	1131.80
In plane bending	1189.21-1263.32
C–H bond out of plane bending	685.41-810.40

between drug and polymer, which is the most important advantage of these nanoparticles.

Characterization of nanoparticles

Size, polydispersity index and zeta potential of nanoparticles (DLS)

In order to demonstrate the relationship among responses and independent variables, 3-D response surface graphs were used. The curves were depicted by the statistically significant model to get a better realization of the interaction of four independent variables.

The response surface graphs showing effect of factorial variables particle size (A), poly dispersity index (B) and zeta potential (C) are deppicted in (Fig. 2).

From the results it can be indicated that each factor can have a major influence on the nanoparticles' size. As shown in Fig. 2A, the particle size of nanoparticles was decreased with the decrease of pH and enhancement of chitosan concentration. As the concentration of chitosan increases, the viscosity of solution increases consequently and the stableness of liquid phase reduced against scattering. Therefore, the resulting nanoparticles are smaller. Zhang *et al.* [20] and also Gan *et al.* [21] have indicated that increasing in the concentration of polymer results in the declining of particle size.

According to 3-D response surface diagram of polydispersity index (Fig. 2B), the PDI of the nanoparticles were at the high point when the pH of polymer solution was maximum and also, concetration of mesalamine has no considerable effect on PDI. When the pH declined, the viscosity of the solution went up due to the high solubility of chitosan and making smaller nanoparticles due to stirring and further decreases in PDI [22].

Fig. 2C., shows the response surface plots showing effect of factorial variables on zeta potential. According to this result, the concentration of mesalamine has no considrable effect on zeta potential; incontrary, with augmentation in pH the zeta potntial was went down. With decreasing in pH, the solubility of chitosan increased and lead to increasing of surface charge of nanoparticles and further increas in zeta potential.

Optimization and model validation

The optimization of nanoparticles was done based on statistical analysis of experimentally obtained data. Box-Behnken response surface method was performed for optimization and prediction of conditions for responses.

For validation and specification of prediction error of the model, the nanoparticles were prepared empirically for 5 times and then characterized. The perceived responses followed by predicted error values was incluiding size of 90 nm and PDI value of 0.34. The calculated prediction errors were below 10% which demonstrated the efficiency, implication, and predictability of models.

Fourier transform infra-red spectroscopy (ftir)

The comparison which has been illustrated in Fig. 3 indicated that mesalamine was loaded on chitosan nanoparticles successfully. The IR spectra of the mesalamine-chitosan nanoparticles are summarized in (Table 3).

According to Fig. 3, peaks of mesalamine (A) (3485.14, 3001.42, 1610.56, 1446.36, 1349.28, 1131.07, 1189.35-1263.28, 685.22-809.84), can be found in the peaks of mesalamine loaded chitosan nanoparticles (B) (3489.15, 3020.57, 1610.57, 1446.51, 1348.77, 1131.80, 1189.21-1263.32 and 685.41-810.40 cm^{-1}). So the results of two figs affirm the presence of unmodified mesalamine in the nanoparticles.

Differential scanning calorimetry (DSC)

DSC is a method used to study the thermal properties of nanoparticles also to gain an understanding of the physicochemical characteristic of the drug in system of nanoparticles. It can be clearly discovered that existance of any peak of the seperate components (polymer or drug) in the nanoparticle DSC, confirms that there is no intraction between drug and polymer. In contrary, appearance of one or two new peaks or shift in their locations demonstrate the interaction between two components [23, 24].

DSC thermograms of mesalamine-chitosan nanoparticles are shown in (Fig. 4).

Pure mesalamine has an endothermic melting

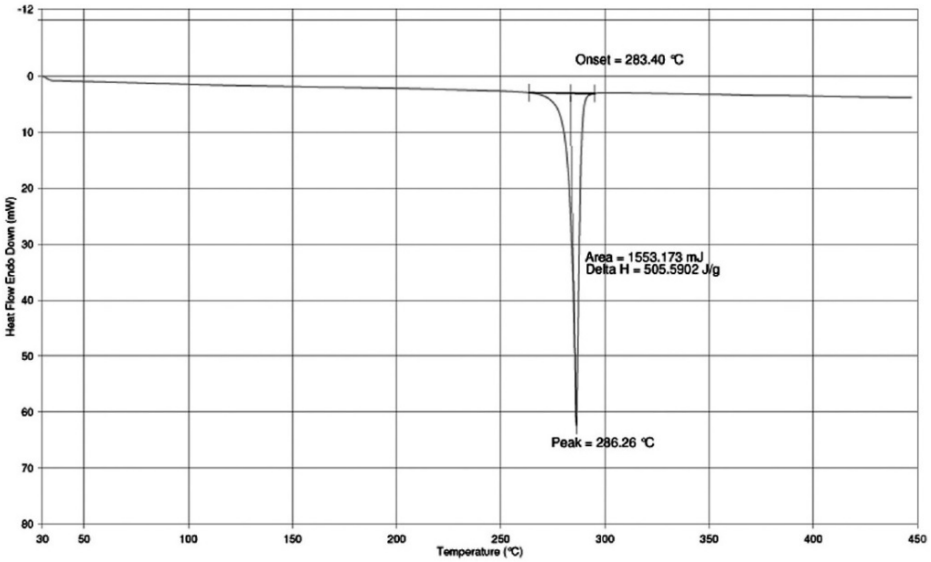

Fig. 4. DSC of mesalamine-chitosan nanoparticles

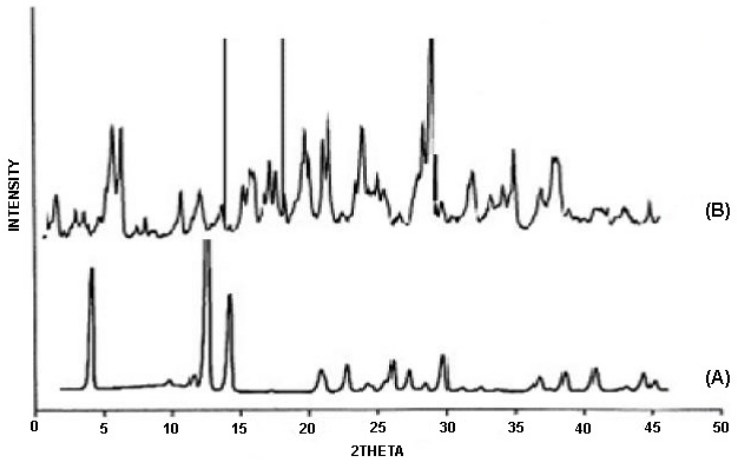

Fig. 5. XRD of (A) mesalamine, (B) mesalamin-chitosan nanoparticles

peak at 286 °C representing its crystalline nature [25]. Moreover, chitosan has a sharp endothermic peak at 320 °C which is related to its crystalline matter and also the amount of water loss which is linked to the hydrophilic groups of the polymer [12]. The calorimetry of nanoparticles demonstrated mesalamine peak shifted from 286 to 283 °C, showing a reduction in its glass-transition temperature (Tg).

The crystallinity of the network increases by addition of nanoparticles, results in decreasing Tg. When the crystal part of network increases, the amorphous part (that is related to Tg) decreases and the terminal (zero shear) viscosities of the systems were always found to decrease upon nanoparticle addition, paralleling the reduction of the Tg [26].

X-ray diffractometry (xrd)

The crystalline nature of mesalamine (A) and mesalamine-chitosan nanoparticles (B) was determined by XRD (Fig. 5). X-ray Diffractometry study of nanoparticles manifests the physical interaction among the drug and polymer.

Fig. 5 shows changes in the number of peaks in XRD of mesalamine-chitosan nanoparticles (B) as compared to spectra of pure mesalamine drug (A), which illustrate a decrease in crystallinity. The reduction of drug crystallite amount can explain

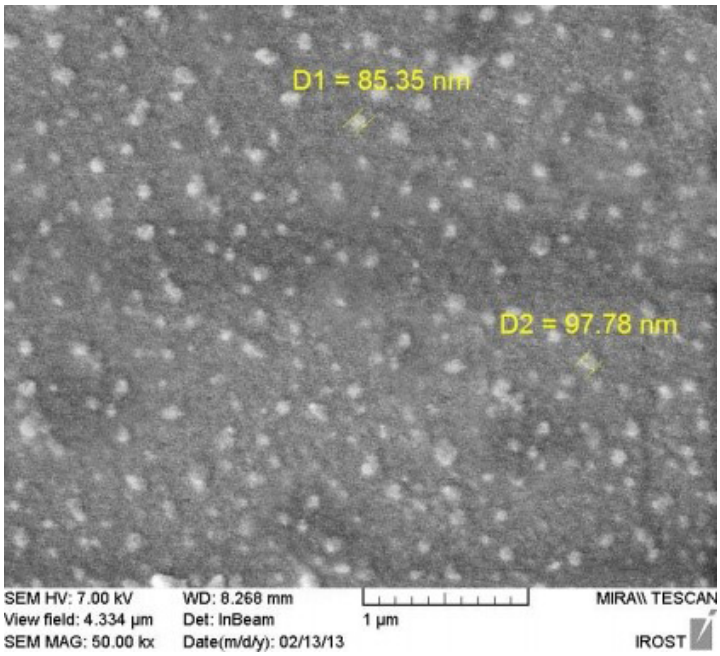

Fig. 6. SEM image of the mesalamine loaded chitosan nanoparticles

the faster dissolution and increase in solubility. Besides, it is demonstrating physical interaction between drug and polymers.

Physiochemical characterization of nanopartiles

The morphology of the prepared nanoparticles was observed by SEM images. It can be shown from the SEM images (Fig. 6) for optimum formulation, that mesalamine loaeded chitosan nanoparticles have the average diameter of 90 nm. The mean particle size of SEM image was defined using CLEMEX® particles image analysis software package. The average particle size was measured for at least 100 particles, resulting to particle size of about 100 nm.

Accorfing to DLS and SEM studies, it can be concluded that the particle size of nanoparticles was in agreement with the obtained optimum particle sizes from the Box-Behnken sofware.

CONCLUSIONS

In this study mesalamine loaded chitosan nanoparticles were synthesized successfully with an ionic gelation method. According to the Box-Behnken design, the optimum conditions for the preparation of the nanoparticles are as follows: chitosan concentration (0.1mg/ml), mesalamine concentration (0.02 mg/ml), chitosan-to-TPP ratio (2.3), and pH of drug loaded chitosan solution (4.5). Under the optimized conditions we have synthesized mesalamine loaded chitosan nanoparticles with the maen size of 90 nm. The PDI value of these nanoparticles was 0.34 which is in an acceptable range.

The mean particle size of the synthesized nanoparticles using a dynamic light scattering technique was ranging from 53.9 to 322.8 nm. Also, the morphology of the prepared nanoparticles was observed by scanning electron microscopy and chemical structure of nanoparticles molecules was specified with FT-IR spectrophotometer. Also, thermal behavior of nanoparticles was studied using differential scanning calorimetry. Drug release profile and the amount of the loaded drug were also monitored by UV-Vis spectroscopy.

The in vitro studies revealed that these nanoparticles can be proper candidates for drug delivery systems due to their slow drug release rate.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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