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Research Article

PREPARATION AND CHARACTERIZATION OF MESELAMINE LOADED PLGA NANOPARTICLES

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ABSTRACT

Nanoparticles (NP's) of poly (d,l-lactic-co-glycolic acid) (PLGA), containing mesalamine were prepared using modified spontaneous emulsification solvent diffusion method (MSESD). NPs were characterized in terms of surface morphology, particle size and distribution, zeta potential, encapsulation efficiency and drug release profile. X-ray diffraction (XRD) and differential scanning colorimetry (DSC) were employed to determine any interactions between drug and polymer. An attempt was made to fit the data to various dissolution kinetics models, including the zero order, first order, square root of time kinetics and biphasic models. NPs having mean diameter of 135±3.4 nm with low polydispersity index of 0.259 and zeta potential value -45.2±1.7 were obtained. By imaging with scanning electron microscopy (SEM), NPs having discrete, spherical morphology with smooth surface and low porosity was observed. *In vitro* drug release demonstrated the mixed order kinetics reveled by the initial first order burst release followed by slow zero order release. The highest correlation coefficients were obtained for the Higuchi model, suggesting a diffusion mechanism for the drug release. DSC and XRD studies revealed that mesalamine was molecularly dispersed within the PLGA nanoparticles during the production process. Also studies revealed no chemical interaction between the drug and the polymer. The results demonstrated that mesalamine NPs encapsulated with PLGA could be a possible alternative for the targeted delivery system for the long-term treatment of ulcerative colitis.

Keywords: Nanoparticles, Mesalamine, PLGA, Ulcerative Colitis, Modified SESD.

INTRODUCTION

NPs have been extensively investigated in biomedical and biotechnological areas and, especially, in drug delivery systems for drug targeting [7, 8, 9] because their particle size that may range from 10 to 1000 nm. The advantages of targeted drug delivery to the specific site of body are in the therapy of several disease states such as anticancer treatment, gene therapy, viral disease, and bacterial infection in the specific body sites [10, 11 12]. In recent years, biodegradable polymeric NPs have attracted considerable attention as potential drug delivery devices in view of their applications in the control release (CR) of drugs, their ability to target particular organs / tissues. Various biodegradable polymers such as poly (L-lactide) or poly(DL-lactide) (PLA), poly(glycolide) (PGA), and their copolymers such as poly(DL-lactide-co-glycolide) (PLGA), have been used in drug delivery research as they can effectively deliver the drug to a target site and thus increase the therapeutic benefit, while minimizing side effects.

Mesalamine (2.4-4.8 g/day) remain the mainstay treatment in the treatment of ulcerative colitis (UC). Three methods have been widely used for targeting of mesalamine to the colon: a pro-drug concept, enteric coating and/or prolonged release of the drug through semi permeable membrane [1, 2, 3]. A number of oral mesalamine agents are commercially available, including azo-bond pro-drugs such as sulfasalazine (2-4 g/day), olsalazine (1-3 g/day) and balsalazide (6.75 g/day), and delayed- and controlled-release forms of mesalamine. Controlled release preparations are specifically designed to minimize systemic absorption and to achieve optimum delivery of the biologically active mesalamine to the distal small intestine and the colon. But, the effectiveness of oral therapy relies on good compliance, which may be adversely affected by frequent daily dosing and a large number of tablets. Furthermore, poor adherence has been shown to be an important barrier to successful management of patients with ulcerative colitis. Thus, a carrier system like NPs that can deliver the drug specifically and exclusively to the inflamed regions after oral administration for a prolonged period would be desirable. Such a system could reduce side effects significantly in the case of conventional chemical anti-inflammatory compounds. An enhanced uptake of administered particles by neutrophils, natural killer cells, mast cells and regulatory T cells in the inflamed tissue was observed [4]. It has been also reported that NPs can be efficiently taken up by macrophages allowing accumulation of the particulate carrier systems in the inflamed tissues in the colon and may therefore allow a selective delivery to the site of inflammation including a reduction of adverse effects [5, 6].

Because NPs can be designed to control drug release after oral administration, the development of such multiparticulate drug delivery proves to be promising primarily to reduce the dosage frequency. In addition, the successful oral administration of drugs with strong adverse effects pertaining to the high doses such as mesalamine may be a new medical approach. Nanoparticulate delivery systems based on PLGA polymers, have been studied extensively in the recent past. PLGA polymers have the advantage of being well characterized and already commercially used for microparticulate drug delivery systems. PLGA polymers are biocompatible, biodegradable, and bio-resorbable [12]. Considering the above mentioned strategy and advantages, mesalamine loaded PLGA nanoparticles were prepared by MSESD with an objective of reducing the high dose and dosing frequency of mesalamine used in the treatment of ulcerative colitis. The prepared NPs were evaluated for physicochemical characterization, including particle size and distribution, zeta potential, drug content, encapsulation efficiency, surface morphology, polymers distribution, drug–polymers interaction and drug release was performed as a function of the preparation procedure.

MATERIALS AND METHOD

The biodegradable polymer Poly (d,l-lactic-co-glycolic acid) i.e. PLGA 50:50 (Molecular weight 10,000 Daltons) was purchased from Boehringer Ingelheim, Germany. Poly (vinyl- alcohol (PVA) (87-89% hydrolyzed, MW 30,000-70,000) and mesalamine (5-aminosalicylic acid) was purchased from Hi-media, Mumbai. All other chemicals and reagents are of analytical grade purchased from Sigma Chemicals, Mumbai and Merck Chemicals, Mumbai.

Preparation of biodegradable nanoparticles

PLGA nanoparticles loaded with mesalamine were prepared by MSESD method [13, 14]. The mixture of two water-miscible organic solvents, such as ethanol/acetone or methanol/acetone was employed, instead of using the mixture of dichloromethane (DCM) and acetone, used in spontaneous emulsification solvent diffusion. This alteration prevented the aggregation of particles even at a high

fed amount of polymeric solution, resulting in improvement in yield as acceptable for industrial purposes. This alteration also provided some additional advantages; for instance, the use of a toxic solvent such as DCM could be avoided in the preparation process; the recovering and purifying process could be simplified by using ultafiltration technique to omit the solvent-evaporation process; and uniform NPs dispersion could always be attained by even mild agitation. Typically, fix amounts of poly (lactic-co-glycolic acid) (125 mg) and mesalamine (50 mg) were dissolved in the solvent blend consisting of 5ml methanol/ethanol, 5ml DMSO (as a cosolvent), 15 ml acetone. This solution was slowly poured into 50 ml of 2% w/v PVA prepared in demineralized water with continuous stirring at about 400 rpm with propeller mixer for 10-15 min. After evaporation of the solvent, NPs were recovered by centrifugation at 55,000g for 30 min and washed with distilled water. The washing step was repeated once before they were resuspended in distilled water and lyophilized overnight. Variation in the process parameters such as concentration of PLGA and PVA was tried to optimize the nanoparticles.

Surface morphology (SEM)

The morphology of the prepared NPs was observed by means of a scanning electron microscope (JSM-T330A, Nihon Denshi, Japan) The samples were prepared on aluminum stubs and coated with gold prior to examination. A drop of the nanoparticle suspension was placed on a graphite surface. Observation was performed at 25 kV.

Particle size

NPs size was determined using photon correlation spectroscopy (PCS) (Malvern S4700 PCS System, Malvern Instruments, Ltd, Malvern, UK). The analysis was performed at a scattering angle of 90^0 and at a temperature of 25^0C using samples appropriately diluted with filtered water (0.2 mm filter, Minisart, Germany). For each sample, values reported are the mean, diameter± standard deviation for two replicate samples.

Zeta potential (ζ)

The zeta potential of the particles was determined by laser Doppler anemometry (Malvern Zetasizer IV, Malvern Instruments Ltd, Malvern, UK). All analyses were performed on samples appropriately diluted with 1 mM HEPES buffer (adjusted to pH 7.4 with 1 M HCl) in order to maintain a constant ionic strength. Values reported are the mean value ± standard deviation for two replicate samples.

Polydispersity index (PI)

Polydispersity was determined according to the equation [15],

Polydispersity = D (0.9)-D (0.1)/ D (0.5)

Where,

D (0.9) corresponds to particle size immediately above 90% of the sample.

D (0.5) corresponds to particle size immediately above 50% of the sample.

D (0.1) corresponds to particle size immediately above 10% of the sample.

Drug incorporation efficiency

For the measurement of drug incorporation efficiency, freeze dried sample of mesalamine loaded PLGA nanoparticles were suspended in phosphate buffer (PBS, pH 7.4), vigorously stirred for 3 h and sonicated for 15 min. Resulting solution was centrifuged with 15000 × g for 20 min and supernatant was taken for the measurement of concentration using UV spectrophotometer (Schimandzu 1601, Japan) at 330 nm[16]. Both drug Content (% w/w), also referred as drug loading in the literature, and drug entrapment (%); represented by equations (1) and (2) respectively. The individual values for two replicate determinations and their mean values are reported.

$$\text{Drug Content (\% w/w)} = \frac{\text{Mass of drug in nanoparticles} \times 100}{\text{Mass of nanoparticles recovered}} \quad (1)$$

$$\text{Drug Entrapment (\%)} = \frac{\text{Mass of drug in nanoparticles} \times 100}{\text{Mass of drug used in formulation}} \quad (2)$$

Evaluation of polymer-drug interaction

Stability of PLGA entrapped mesalamine NPs revealed by polymer-drug interaction was evaluated by using DSC and XRD study. These techniques not only evaluate the polymer-drug interaction but also the physical state of polymer and drug in nanoparticles.

Differential scanning colorimetry *(DSC)*

DSC studies were performed using a DSC Mettler Toledo model 30TC 15 (Mettler, Zurich, Switzerland). The samples (2–5 mg) were scanned in sealed aluminum pans under nitrogen atmosphere. DCS thermograms were scanned in the first heating run at a constant rate of 10^0C/min and a temperature range of 0–325^0C. DSC thermograms of pure substances, their physical mixtures and drug-loaded nanoparticles were recorded.

X-ray diffraction study

The XRD study was carried out with the intention to assess the physical nature of NPs sample and further to confirm the stability attributed to polymer-drug interaction. X-ray diffraction pattern was recorded on a Philips PW1710 model (Holland), Cu Kα1 radiation, λ = 1.54056 A°, slit width of 1, in the 2 θ range from 10^0 to 100^0, in steps of 0.02^0 at 0.500 s per step, while diffraction patterns for the physical mixtures of polymer with mesalamine and nanoparticles loaded with mesalamine were recorded in steps of 0.05^0 at 15 s per step.

Drug release study

The drug release study from nanoparticles was determined by the method described as below [17, 18]. The 100 mg of drug (Mesalamine) loaded PLGA nanoparticles were added to 200 ml Phosphate Buffer (PBS, pH 7.4) in a conical flask and incubated into a bath at 37^0C under magnetic stirring at 250 rpm. At different time intervals, a sample volume of 1.5 ml was withdrawn and filtered through a Millipore 0.1 µm filter. 1.5 ml fresh PBS buffer was added to the remaining volume in the flask. The amount of mesalamine released in the medium was determined by spectrophotometrically at 330 nm using UV Schimandzu 1601 spectrometer, Japan. Drug release constants were determined by different kinetic models like Higuchi, zero and first order kinetics[19]. Microsoft excel based PCP Disso Version 2.08 was used to deduce the mechanism of drug release from nanoparticles.

RESULTS

PLGA nanoparticles prepared by MSESD were characterized in terms of particle size, surface potential, surface morphology, drug content, encapsulation efficiency, polymer drug interaction and drug release. Also, effect of concentration of PLGA and PVA on the drug loading, particle size was evaluated. Discrete spherical NPs having a diameter of less than 140 nm with low standard deviations were produced (Table 1).

Table 1: **Characteristics of PLGA nanoparticles**

Solvent Composition	Particle size (nm)	Polydispersity Index (PI)	Zeta Potential (mV)	Drug Content (%w/w)	%Drug Entrapment
Ethanol/ acetone (5/5)	147.1±5.1	0.226	-42.4±1.2	28.8 (28.9; 28.7)	44.7 (45.6; 43.9)
Methanol/acetone (5/5)	135.0±3.4	0.259	-45.2±1.7	22.4 (22.6; 22.2)	37.7 (36.8; 38.7)

The small polydispersity index suggested that the size distribution of the products is fairly monomodal. By imaging with SEM, not only an acceptable spherical morphology was observed (Figure 1), but also discrete particles were seen. The surface appeared to be smooth with low porosity. The yields of NPs as the function of PLGA concentration has shown in figure 2. Although a satisfactory yield was obtained at PLGA concentrations lower than 0.2%, the value decreased with the increase in the PLGA concentration.

The effect of the PVA concentration in the aqueous phase on the yield of nanoparticles is shown in figure 3 indicates a maximum yield at PVA concentrations within a range of about 2-4%. On the other hand, the precipitation of PLGA around the propeller used in agitation was immediately observed when the PLGA solution was poured into solutions containing more than 5% PVA.

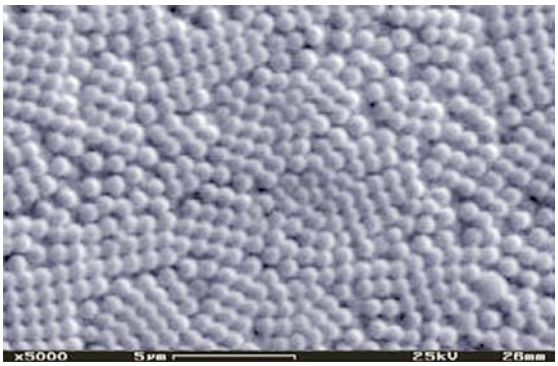

Fig. 1: SEM image of PLGA nanoparticles

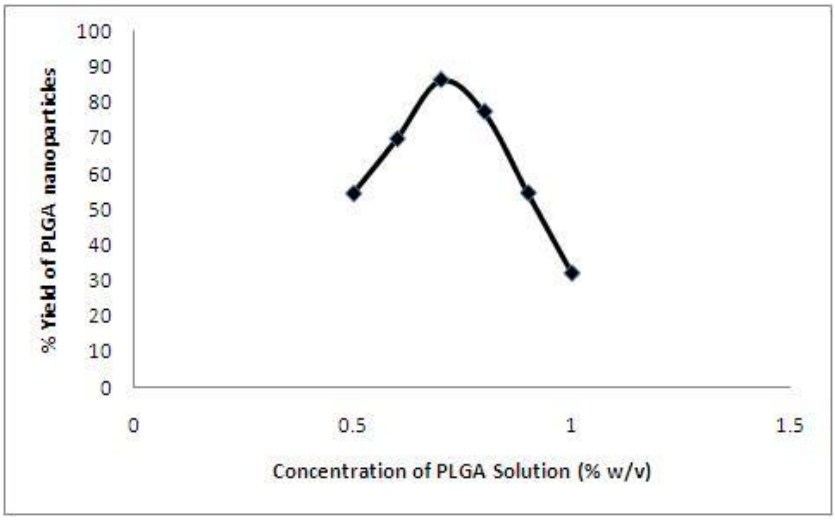

Fig. 2: Effect of concentration of PLGA on yield of nanoparticles

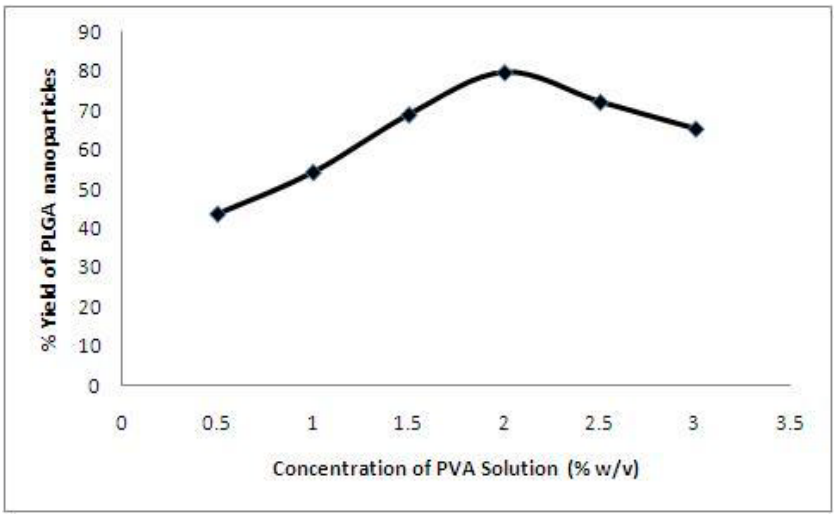

Fig. 3: Effect of concentration of PVA on yield of nanoparticles

Differential scanning colorimetry (DSC)

In the colorimetric study, thermogram of physical mixture of drug and polymer shows broad endothermic peak at about 50^{0}c and sharp endothermic peak at about 240^{0}c (Figure 4.1) which corresponds to Tg values of PLGA (50:50) and mesalamine respectively. The first peak is attributed to dehydration process followed by decomposition of polymer and the second peak denotes the degradation of drug i.e. mesalamine, at about 240^{0}c. In the thermogram of mesalamine loaded NPs (Figure 4.2) the single broad peak at 50^{0}c was only shown and the second peak of mesalamine was not observed.

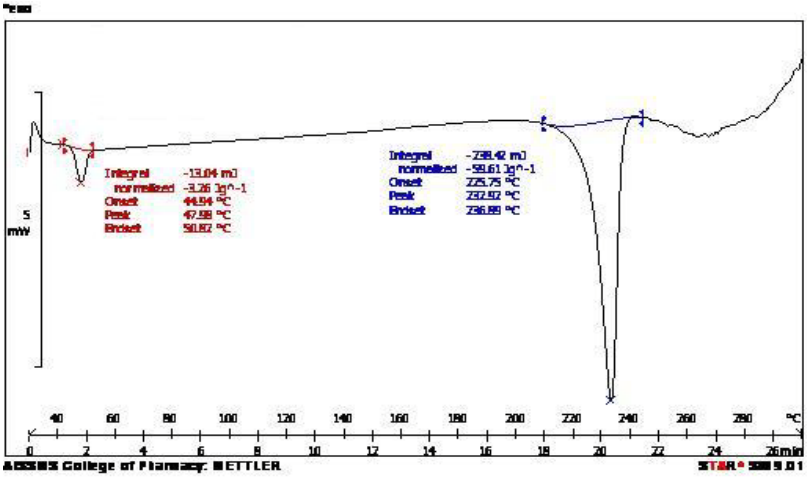

Fig. 4.1: DSC of physical mixture of drug and PLGA

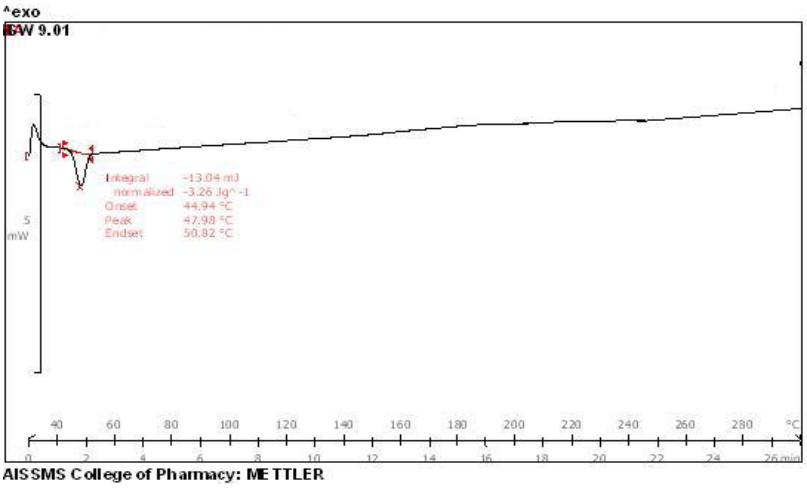

Fig. 4.2: DSC of mesalamine encapsulated PLGA nanoparticles

X-ray diffraction study (XRD)

XRD was employed with an intension to confirm the characteristic of mesalamine loaded PLGA nanoparticles. The characteristics sharp and intense peaks of around 15θ and 18θ were observed in the XRD pattern of mesalamine (Figure 5.1). PLGA (50:50) showed low intensity peaks over the range of 15 to 25 θ forming a dome shaped region (Figure 5.2). The intense and sharp peaks of drug were not seen in the XRD pattern of drug loaded nanoparticles (Figure 5.3). It actually resembles the pattern of PLGA showing the low intensity peaks. As the area under the peaks is very small it was concluded that the obtained sample was mostly amorphous rather than crystalline nature.

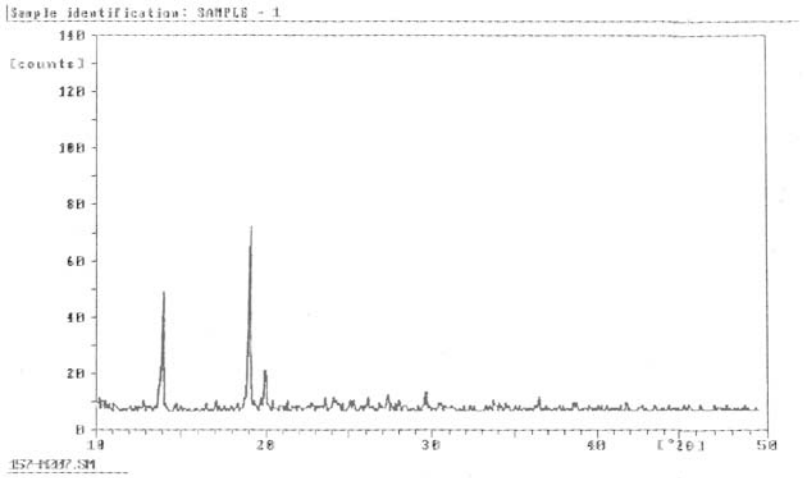

Fig. 5.1: XRD pattern of mesalamine

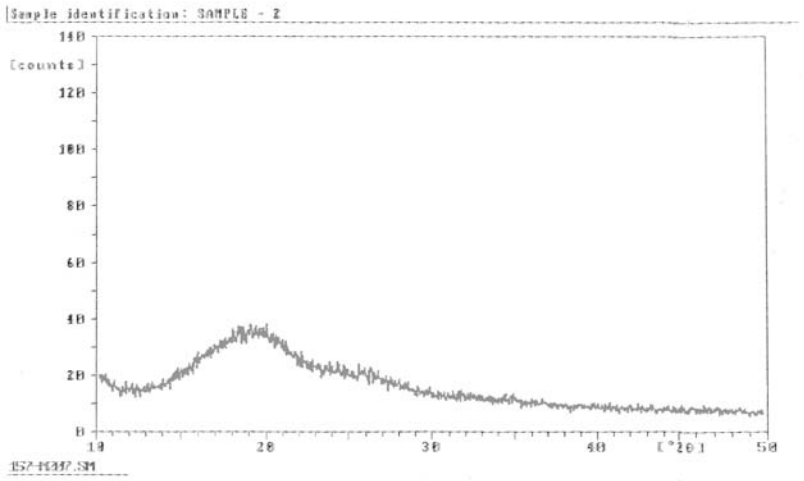

Fig. 5.2: XRD pattern of PLGA 50:50

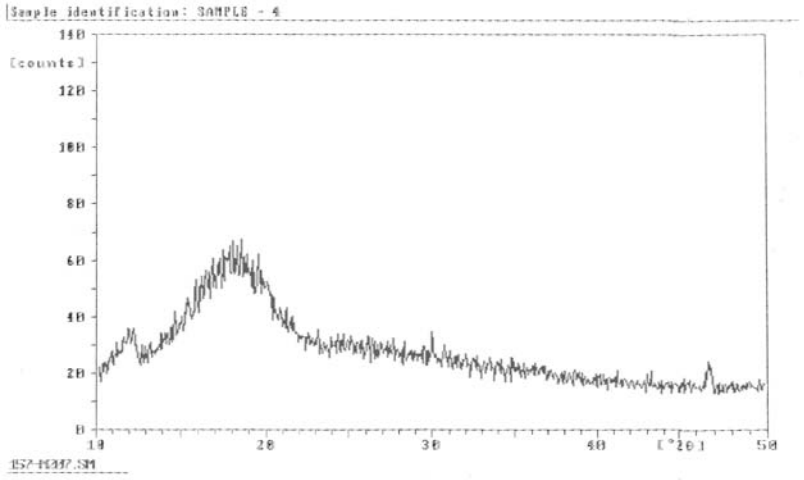

Fig. 5.3: XRD pattern of mesalamine encapsulated PLGA nanoparticles

Drug release study

The release behavior of mesalamine from PLGA nanospheres is illustrated in Figure 6, which indicates the biphasic pattern. Mesalamine, encapsulated within PLGA (RG 503, 50:50, *M*w = 10,000 Da), showed an initial burst of 47.19 ± 0.05% during the first 24 h, followed by a sustained release and until 10thday, when most of the drug has been released. Zero order and Higuchi kinetics models (Table 2), shows highest correlation as evident from the values of regression coefficients (R^2) as 0.97 and 0.89 resp.

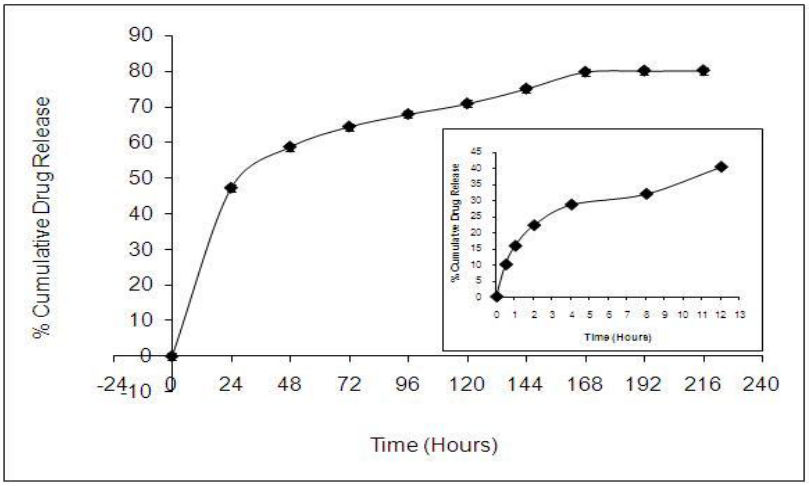

Fig. 6: Drug release profile from PLGA nanoparticles

Table 2: ***In vitro*** **drug release kinetics of PLGA nanoparticles**

Sr. No.	Dissolution Models	Regression Coefficient (R^2)	Slope (m)
1	Zero Order	0.8936	0.3931
2	First Order	0.6195	0.0052
3	Hixon Crowell	0.7708	0.0398
4	Kesmayer Peppas	0.7237	0.3239
5	Higuchi Plot	0.9711	0.1696

DISCUSSION

NPs prepared by the modified SESD method had a spherical shape, sub micrometer size, relatively monodispersed and the surface appeared to be a rigid film structure. The ideal spherical morphology and lower values of particle size can be probably attributed to the diffusion of alcohol. The above-mentioned monodispersed size distribution and excellent redispersability of nanoparticles indicate that the surface of PLGA nanoparticles is stabilized by some reasons to prevent aggregation. The most likely reason to explain the findings might be the adsorption of PVA onto the surface of PLGA nanoparticles. With this manufacturing technique, process parameters such as PLGA and PVA concentration were determined to achieve the optimum processing conditions. This was probably caused by the increasing viscosity and hence resulting poor dispersability of the PLGA solution into the aqueous phase. At lower concentrations, decreasing coacervation of PVA on the nanoparticle surface could be responsible for lower yields. This phenomenon could be caused by the worse dispersion of PLGA solution into aqueous phase due to the increase of viscosity of PVA solution.

This process led to formation of uniform population of NPs having mean diameter in the range of 135 ± 3.4 nm to 178.5 ± 7.8 nm and also improved drug loading as evident from 44.7% entrapment efficiency with 22.4% (w/w) drug content. Polydispersability observed to be low in the range of 0.226 to 0.259. The above mentioned monodispersed size distribution and excellent redispersability of NPs indicates the surface of PLGA nanoparticles is stabilized by some reasons to prevent aggregation. The most likely reason to explain the findings might be the absorption of PVA on to the surface of PLGA nanoparticles. DSC and XRD studies were carried out to determine whether the drug was incorporated in the nanoparticulate system or not. The analysis showed that the drug is encapsulated in the polymeric matrix which was evident from the disappearance of endothermic peak of drug on DSC thermograms and intense and sharp peaks of drug in XRD of drug loaded nanoparticles.

Drug release profiles showed a burst release in the first day, followed by a lag phase and then sustained release appeared with the nanospheres with lower-molecular-weight polymer. At a later stage, the drug was released more slowly, the rate of which might be controlled by the degradation speed of the polymer of nanoparticles. It was notable that the degradation of the polymer did not substantially occur, at least for the monitored period in the present system. The initial burst might be due to the rapid release of drugs deposited on the surface and in the water channels in nanospheres because the molecular weight of the polymer did not decrease during this period. At a later stage, the drug was released more slowly, the rate of which was determined by the diffusion of the drug in the rigid matrix structure. The values of dissolution models suggest that drug release occurs mainly through diffusion and erosion processes.

CONCLUSION

The present work has shown that mesalamine containing nanoparticle formation by the modified SESD method. It demonstrates the potential process to control the size of PLGA nanoparticles. The nanoparticle formation process was to be related to the reduction of globule size due to the rapid diffusion of solvent and hence nanoparticles of particle size as low as 135 nm were produced. The significant step for success of this method is the variation in the solvent composition to that of the original SESD process. The stability and the size of droplets formation during the stage are important factor. Preparative variables such as the type and concentrations of stabilizer, polymer concentrations, found to be the crucial factors for the formation of PLGA nanoparticles. Especially, the use of selected solvent such as DMSO used to dissolve the drug, significantly enhance the drug contents and the encapsulation of mesalamine. PLGA encapsulated mesalamine nanoparticles have spherical shapes from the observations of SEM. The analysis of DSC and XRD patterns showed well encapsulated compatible nanoparticles as a drug carrier system. Release kinetics of mesalamine used as a model drug shows sustained biphasic drug release governed by diffusion and erosion. The obtained results indicate the potential use of nanoparticles using PLGA for sustained release of mesalamine. Optimization of formulations with different polymers, concentration and surfactants are the future scope of study.

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