Preparation and Characterization of Monensin Loaded PLGA Nanoparticles: In Vitro Anti-Malarial Activity Against Plasmodium Falciparum

Ranu Surolia, Manendra Pachauri, and Prahlad Chandra Ghosh*
Department of Biochemistry, University of Delhi South Campus, Benito Juarez Road, New Delhi 110021, India

PLGA nanoparticles loaded with monensin (carboxylic ionophore) were prepared by emulsion solvent evaporation method using PLGA of molecular weight (Mw.) 19 000 and 110 000 Da. The nanoparticles were characterized by applying dynamic light scattering (DLS), atomic force microscopy (AFM), differential scanning calorimetry (DSC) and Fourier transformed infrared spectroscopy (FTIR). Negatively charged and spherical smooth surfaced nanoparticles of size range between 147–167 nm were obtained. The nanoparticles of monensin-PLGA showed no chemical interaction between monensin and the polymer molecules. The release kinetics in vitro studies exhibited biphasic release profile characterized by an initial fast release followed by a slower release. The antimalarial efficacy of monensin-PLGA nanoparticles was also examined. Monensin loaded in nanoparticles was 10-fold more effective in inhibiting the growth of P. falciparum in vitro as compared to free monensin. The antimalarial efficacy of monensin-PLGA nanoparticles was significantly dependent on the Mw. of the polymer.

Keywords: Malaria, Monensin, PLGA Nanoparticles, PLGA Molecular Weight, In Vitro Release, Antimalarial Efficacy.

1. INTRODUCTION

Malaria is the most important endemic parasitic disease transmitted by female Anopheles mosquitoes. About 3.3 billion people; half of the world’s population is at risk of malaria. Every year, this leads to about 250 million malaria cases and nearly one million deaths. People living in the poorest countries are the most vulnerable. One in five (20%) of all childhood deaths in Africa are due to malaria. It is estimated that an African child has on average between 1.6 and 5.4 episodes of malaria fever each year and Every 30 seconds a child dies from malaria in Africa.1

Malaria is caused by protozoan parasites belonging to the genus Plasmodium. Four species account for almost all human infections (P. falciparum, P. vivax, P. malariae, and P. ovale). P. falciparum causes the majority of infections. The parasite is transmitted to people when they are bitten by an infected mosquito. In the human body, the parasites reproduce in the liver before invading red blood cells. Here, they multiply again before bursting out and infecting more red blood cells (erythrocytic phase) as well as causing a high fever and sometimes damaging vital organs. The two most frequent presentations of severe malaria in African children are severe anaemia and cerebral malaria.

For decades, drug resistance has been one of the main obstacles in the fight against malaria. Drug resistance results in a delay in or failure to clear asexual parasites from the blood, which allows production of the gametocytes that are responsible for transmission of the resistant genotype. After the parasite resistance to first-line treatment drugs (chloroquine, sulfadoxine–pyrimethamine and mefloquine), new drug artemisinin-based therapy is recommended by WHO from year 2001. However, the emerging resistance against artemisinin has also been reported in recent years.2-6 In this scenario, to tackle the aforementioned problems; novel delivery systems are needed to be developed in order to improve the efficacy of existing and novel drugs.7

Monensin, a carboxylic ionophore, exchanges specifically Na⁺ for H⁺ resulting in elevated cytosolic Na⁺ concentration and pH.8 Monensin isolated from Spreptomyces cinnamonensis, is well-known as veterinarian sciences
antibiotic. Its synthetic and semi-synthetic derivatives are reported to have antimalarial activities that are executed by disruption of ionic gradients in the malaria parasite.9–12 In recent reports by Mahmoudi et al.13 and Leitao et al.14 monensin has been shown active against the hepatocytic stages of malaria parasites.13,14 Despite its strong potential as an antimalarial its extreme hydrophobic nature hinders its application in therapy. Being lipophilic in nature, it has a short half-life and therefore needs to be formulated in a suitable drug delivery system to improve its applicability in antimalarial therapy. Nanoencapsulation of drugs using polymer for drug delivery prolong the systemic circulation time,15 obviate their side effects16 and enhance drug activity.17,18 All of which together improve their therapeutic index. A number of polymers have been exploited for formulating the nanoparticulate carrier system among them poly-lactic (PLA), poly-glycolic acid (PGA) and their copolymers poly(DL-lactide-co-glycolide) (PLGA) have been extensively employed because of their biodegradability, bioocompatibility and versatile degradation kinetics.19 The physical properties and the FDA approval of the products containing these biodegradable polymers have made them very popular.20 Depending on the desired mode of administration, the size of the carriers should be optimized. In general, the smaller the particle size, the better the performance of the nanoparticles. Size under 1 μm enables an intravenous injection (the diameter of the smallest blood capillaries is 5–6 μm) and it is also desirable for intramuscular and subcutaneous administration, by minimizing the possible irritant reactions.20,21

There are earlier reports of liposomal monensin and monensin PLGA nanoparticles,22,23 which have been shown to have immunotoxins potentiation properties, but none of them have been studied for their antimalarial activity. PLGA nanoparticles are drug delivery systems in an attempt to eliminate the shortcomings observed with free monensin. In this communication we have prepared small sized and less polydispersed monensin PLGA nanoparticles by emulsification solvent evaporation method. The degradation rate of PLGA depends on the molecular weight of polymer, the degree of crystallinity, and glass transition temperature of the polymer ($T_g$). By manipulating the molecular weight, the degradation time of PLGA and subsequently, release profile can be varied accordingly.24,25 Therefore, the present study reports the influence of polymer molecular weight on the release behavior of monensin in vitro as well as its effect on the inhibition of the growth of *P. falciparum* in culture.

We have also studied the effect of polymer molecular weight on the encapsulation efficiency, size and polydispersity of the particles. Further DLS and FTIR studies are done for their physical characterization and then their antimalarial activity is evaluated in *P. falciparum* culture in human erythrocytes.

2. MATERIALS AND METHODS

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA) 50:50 DL (molecular weight, 19 kDa and 110 kDa was purchased from Lactel, USA. Polyvinyl alcohol (PVA) (MW 30000–70000, 88% hydrolyzed) and Monensin were purchased from Sigma, USA. Dichloromethane (DCM) was purchased from Fisher Scientific. [3H] Monensin and [1H] Hypoxanthine was purchased from ARC, Saint Louis, USA.

2.2. Methods

2.2.1. Preparation of Monensin PLGA Nanoparticles by Solvent Emulsification Evaporation Method

The PLGA nanoparticles with or without monensin, were prepared by an emulsion–solvent evaporation method. Typically, 50 mg of PLGA in 4 ml of Methylene Chloride (DCM) with or without monensin (10% w/w), was mixed with 16 ml of 5% PVA aqueous solution. This mixture was sonicated using a microtip probe sonicator set at 55 W of energy output (Misonix Sonicator®) for 3 min to produce the oil-in-water emulsion. The nanoparticles were formed from nanodroplets by evaporating highly volatile organic solvent at room temperature with constant stirring on magnetic stirrer at 300 rpm for 4 h. The nanoparticles were recovered by centrifugation at 15,000 rpm for 25 min. The nanoparticles were washed twice with double distilled water in order to remove the adsorbed monensin and PVA on the surface of nanoparticles. Purified nanoparticles were freeze-dried. The final product was stored in a vacuum desiccatior at 4 °C.

For *in vitro* release studies, radiolabelled monensin-PLGA nanoparticles were prepared by adding [3H] monensin (20 μCi) during the preparation of nanoparticle.

2.2.2. Determination of Nanoparticle Size, Size Distribution and Zeta Potential

The nanoparticles size and size distribution was determined in double distilled water at 25 °C by Dynamic Light Scattering using a Zetasizer Nano ZS (ZEN 3600, Malvern Instruments, Worcestershire, UK). For the measurements, 100 μl of the nanoparticles suspension was dispersed in 1 ml of distilled water and sonicated during 1 min. The analyses were performed at a scattering angle of 90° and at a temperature of 25 °C. For each sample, the mean diameter and the standard deviation of ten determinations were calculated using multimodal analysis.

The zeta potential was measured by a combination of Laser Doppler Velocimetry and Phase Analysis Light Scattering (M3-PALS) technique at 25 °C using Zetasizer Nano ZS (ZEN 3600, Malvern Instruments, Worcestershire, UK). All samples were analyzed in triplicate.
2.2.3. Evaluation of Morphology of Nanoparticles

2.2.3.1. By Transmission Electron Microscopy (TEM).
A drop of nanoparticle suspension in distilled water was placed on carbon coated copper grid (Polysciences, Warrington, PA) and coating with 1% uranyl acetate was done. TEM analysis (Philips, Holland) was done and images were obtained by using digital imaging software—AMT image capture engine (version 5.42.391).

2.2.3.2. By Atomic Force microscopy (AFM). The samples for AFM were prepared by placing a drop of the diluted suspension of nanoparticles in water over a freshly cleaved mica sheet and subsequent drying of the samples in a vacuum desiccator overnight. The images of nanoparticles were taken with AFM (Agilent AFM 5500). The images were scanned by contact mode using a cantilever.

2.2.4. DSC Thermograms of Monensin-PLGA Nanoparticle Formulations

Drug crystalline state in the polymer matrix was evaluated in the different molecular weight nanoparticles (freeze-dried) by Differential Scanning Calorimetry (DSC). Thermograms were taken using Perkin Elmer (DSC-7) instrument in a standard aluminum pan. Nitrogen was the sweeping gas, and the heating rate was 10 °C/min. The initial and end temperatures were 50 °C and 300 °C, respectively.

2.2.5. Fourier Transform Infra-Red spectroscopy (FTIR) Study

It has been even shown that drug–polymer interaction can be so intense that it becomes the predominant feature in drug release. Therefore, the properties of drug compound, which cause the interactions, should be determined because these interactions can crucially affect the in vitro behavior. To determine any chemical interaction between monensin and PLGA in monensin loaded PLGA nanoparticles, Fourier Transform Infra-Red spectroscopy (FTIR) study was done. Fourier transform infrared (FTIR) spectroscopy was performed with Bruker (Thermo Electron Co, Newington, NH) attached to an attenuated total reflectance (ATR) accessory. ATR was fitted with a single bounce diamond at 45° internally reflected incident light providing a sample area of 1 mm in diameter with a sampling depth of several microns. A small amount of the sample was directly placed on the diamond disk and scanned for absorbance over the range from 4000 to 1000 cm⁻¹ wave numbers at a resolution of 1 cm⁻¹.

2.2.6. Determination of Monensin Entrapment

Briefly, 1 ml methanol was added to 10 mg of monensin PLGA nanoparticle sample and vortexed for 20 min. This suspension was centrifuged at 12000 rpm for 30 min at room temperature. The concentration of monensin in supernatant was measured as reported earlier. All analyses were performed in triplicate and the average results are reported.

2.2.7. In Vitro Release Study

3H-Monensin-PLGA nanoparticle sample containing about 60 µg of monensin in 3 ml of phosphate-buffered saline (PBS) pH 7.4 was taken in a dialysis bag (MWCO 12-14000; Sigma, USA). The dialysis bag was placed into 100 ml of PBS (pH 7.4) taken in a dissolution vessel at 37 °C and stirred at 50 rev min⁻¹. At periodic intervals, 500 µl samples were taken from the dialysate and then the equal volume of PBS (pH 7.4) was added. The 500 µl sample was taken in scintillation vial and 5 ml of scintillation cocktail (5 ml) was added and vortexed for 2 min and the concentration of monensin was determined by assaying radioactivity using Liquid Scintillation Analyzer (Model Tri-Carb 2900 TR, Perkin Elmer).

2.2.8. Evaluation of Monensin-PLGA Nanoparticle Antimalarial Activity

Antimalarial effects of the various molecular weight PLGA nanoparticles (19,000 and 110,000 Da) containing monensin was monitored by studying the inhibition of incorporation of [³H]-hypoxanthine in the nucleic acid of parasite. In brief, monensin-PLGA nanoparticles preparations were serially diluted and added to Plasmodium falciparum infected erythrocytes suspension (2% final hematocrit and 1% parasitemia) in a 96-well tissue culture plate. After 24 h of incubation at 37 °C, 0.2 µCi of [³H] hypoxanthine was added to each well and cells were harvested 18 h later by using a Skatron Semi-automated cell harvester. [³H] Hypoxanthine incorporation in nucleic acid was measured in a Liquid Scintillation Analyzer (Model Tri-Carb 2900 TR, Perkin Elmer) and inhibition of growth was calculated by comparison with control (Control consists of complete medium in a substitute for the test molecule). All data points were collected in triplicate for each experiment. Concentration of monensin required inhibiting 50% of growth of parasites (IC₅₀) as measured by incorporation of [³H]-hypoxanthine of the data was generated using Origin Cal software (version 3).

2.2.9. Assessment of Hemolytic Activity

Hemolytic assay with uninfected RBC: The hemolytic potentials of monensin, placebo PLGA nanoparticles and monensin PLGA nanoparticle of various molecular weights were assessed after incubation with human RBCs in phosphate-buffered saline (PBS). Heparinized fresh blood was rinsed three times in PBS (by centrifugation at
200 × g for 2 min) and resuspended in PBS at 4% hematocrit. Increasing concentration of nanoparticle preparations was added to uninfected erythrocyte (2% hematocrit) in a 96-well plate for 42 h at 37 °C. PBS alone (for baseline values) and 0.4% Triton X-100 (for 100% hemolysis) were added to attain negative and positive control respectively. After incubation at 37 °C with stirring, the samples were centrifuged and the hemolytic activity was determined by measuring the absorbance at 405 nm.

Hemolysis of infected red blood cells: To assess the hemolysis of infected cells, cultures were exposed to increasing concentrations of the monensin, placebo PLGA nanoparticles of different molecular weight and monensin PLGA nanoparticles of various molecular weight for 42 h. The optical density in the supernatant was determined after centrifugation, and the percent lysis compared to the amount of full lysis (by Triton X 100) of the cells present in the culture was calculated. Hemolytic activity data were obtained from at least two independent experiments.

3. RESULTS AND DISCUSSION

3.1. Determination of Nanoparticle Size, Size Distribution and Zeta Potential

There are many factors which influence the particle size; polymer molecular weight is one of them. The particles size increases with the increase in molecular weight (19,000 to 110,000 Da), for blank as well as monensin loaded nanoparticles (Table I). On changing Inherent viscosity/Molecular Weight of PLGA (50/50), there is slight increase in nanoparticle size with increase in molecular weight. The possible reason could be the increase in viscosity of the polymer solution (organic phase) with increasing molecular weight which poses resistance in breaking down the nanodroplets in smaller size on the same energy input. Mittal et al. also have reported similar results for the estradiol PLGA nanoparticles. Slight increase in the encapsulation efficiency is observed with the increase in polymer molecular weight. For lower molecular weight the encapsulation efficiency is ~18% which increases to ~28% for the higher molecular weight polymer formulations. One possible reason could be increase in molecular weight causing the increase viscosity of polymer solution. Thus the escape of drug molecules from denser polymer matrix becomes difficult; thereby the encapsulation efficiency increases in higher molecular weight polymer formulations.

Table I shows the increase in the negative zeta potential values (i.e., −7.4 mV to −11.5 mV) with the decrease in the molecular weight of PLGA. The negative electric charge attributed on the surface of nanoparticles is due to carboxylate end groups of PLGA. At equal weight, the number of carboxylate end group decreases which leads to decreasing of negative zeta potential values with increase in molecular weight of PLGA.

3.2. Evaluation of Morphology of Nanoparticles

Surface morphology was a crucial property for the prepared nanoparticles. The morphology of for monensin-PLGA nanoparticles (19,000 Da and 110,000 Da) prepared was examined by TEM and AFM. The TEM image indicated that the monensin-PLGA nanoparticles of Mw. 19,000 Da (Fig. 1(b)) and 110,000 Da (image not shown) appeared to be round in shape and smooth on the surface. Moreover, the particle size distribution was well-proportioned in good agreement with the result measured by DLS (Fig. 1(a)). The AFM image of monensin PLGA nanoparticles of Mw. 19,000 Da (Fig. 1(c)) and 110,000 Da (figure not shown) revealed the structure at high resolution in two or three dimension on nanometer scale.

3.3. DSC Thermograms of Monensin-PLGA Nanoparticle Formulations

The physical state of both the drug and the polymer was investigated since this would have an influence on the release kinetics of the drug. Different combinations of drug/polymer may coexist in the polymeric carriers, such as (1) amorphous drug in either an amorphous or a crystalline polymer, and (2) crystalline drug in either an amorphous or a crystalline polymer. Also, a drug may be present either as a solid solution or a solid dispersion in an amorphous or crystalline polymer.

DSC is a thermal analytical technique which provides information about the physical properties of products, for example about the crystalline or amorphous nature of the samples. To determine the state of monensin in nanoparticle, the samples were subjected to Differential Scanning calorimeter (DSC). Figure 2 showed the DSC thermograms of pure monensin, the physical mixture of monensin (50%) and PLGA-nanoparticles (50%) samples, various

Table I. Effect of polymer (PLGA 50:50) molecular weight on particle characteristics of blank as well as drug loaded nanoparticles.

<table>
<thead>
<tr>
<th>Mw (kDa)</th>
<th>Blank nanoparticles</th>
<th>Monensin loaded nanoparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PS (nm)</td>
<td>PDI</td>
</tr>
<tr>
<td>19</td>
<td>97 ± 7</td>
<td>0.07 ± 0.009</td>
</tr>
<tr>
<td>110</td>
<td>110 ± 10</td>
<td>0.074 ± 0.019</td>
</tr>
</tbody>
</table>

Notes: Mw: molecular weight, PS: particle size, PDI: polydispersity index, ZP: zeta potential, EE: entrapment efficiency. Values given are mean ± standard deviation (n = 3).

*The zeta potentials reported are in the pH range of 7. Initial drug loading was 10% w/w of the polymer weight.
molecular weight placebo PLGA nanoparticles and monensin PLGA nanoparticles (Mw. 19,000 and 110,000 Da).

The DSC trace of monensin (Fig. 2(a)) showed a sharp endothermic peak at 269.23 °C, (its melting point). Figures 2(b) and (c) showing the thermograms of both placebo nanoparticles. In Figures 2(d) and (e) thermograms of different physical mixture samples of pure monensin and various molecular weight placebo PLGA nanoparticles (19,000 and 110,000 Da respectively) showed nearly the same thermal behavior as the individual components, indicating that there was no interaction between the drug and the polymer in the solid state. In case of different molecular weight monensin loaded PLGA nanoparticle (Figs. 2(f) to (g) respectively) showed the absence of the endothermic peak of the monensin at 269.23 °C in the DSC.

It suggests that the monensin existed in an amorphous or disordered-crystalline phase as a molecular dispersion or a solid solution state in polymeric matrix. The reduction in the peak size of the physical mixture of monensin–PLGA is due to the presence of smaller amount of monensin in the respective physical mixture in comparison with pure monensin.

3.4. FT-IR of Monensin-PLGA Nanoparticle Formulations

FTIR analysis measures the selective absorption of light by the vibration modes of specific chemical bonds in the sample. The observation of vibration spectrum of encapsulated drug allows evaluating the kind of interaction occurring between the drug and polymer, because the vibrations of
the atoms involved in this interaction can suffer alterations in frequency and intensity.32

Figures 3(A) and (B) exhibited FT-IR spectra of monensin powder, the physical mixture of different molecular weight placebo PLGA-nanoparticles with monensin (1:1), various placebo PLGA nanoparticles samples and monensin-PLGA nanoparticles samples of different molecular weight 19 kDa and 110 kDa. The pure monensin sample showed the main peaks contributed by an intense and broad band in the region 3200–2100 cm\(^{-1}\), corresponding to the proton vibrations in a medium short hydrogen bond formed between O(11)H group and the O(1) oxygen atom of the carboxylate group and one sharp peak at 1558 cm\(^{-1}\), corresponding to aromatic C–C bending. The spectra for PLGA of different molecular weight show peaks at 1757 cm\(^{-1}\) which is its characteristic peak due to carbonyl group stretch, C–O stretching at 1050–1250 cm\(^{-1}\).

The physical mixture for 19 kDa and 110 kDa formulations showed peaks resulting from simple superposition of their separated components in the infrared spectra. In the case of monensin loaded nanoparticles, the peaks of monensin very less intense due to low drug concentration in the nanoparticles. It is also observed that there are no major shifting as well as no loss of functional peaks between the spectra of drug, polymer and drug loaded nanoparticles.

The spectral analysis indicated that the specific functional groups of polymeric material in the nanoparticles surface have almost the same chemical characteristics of the pure polymer and the drug entrapped shows their main characteristics peaks. The study suggests there is no occurrence of molecular interactions that could alter the chemical structure of the drug in the time of study.

### 3.5. Encapsulation Efficiency

The spectrophotometric method was used for determination of the encapsulation efficiency of monensin PLGA nanoparticle. Encapsulation efficiency increased with increase in molecular weight of polymer in nanoparticle preparation. It was observed that 18.1% of monensin could be entrapped in monensin-PLGA nanoparticle (19,000 Da) whereas for high molecular weight nanoparticle of Mw. 110,000 Da, it was found to be 28%, under the conditions employed in the study (Table I).

The spectrophotometric method cannot be used for analysis of trace amounts of monensin. Therefore, the radioactive method is used for in vitro release studies. With radioactive method the entrapment efficiency was found to be same with no significant difference for both the preparations.

These results show the polymer molecular weight influences the encapsulation efficiency of monensin in nanoparticles. One possible reason for this influence may be due to increase in molecular weight causing the increase viscosity of polymer solution. Thus the escape of drug molecules from denser polymer matrix becomes difficult; thereby the encapsulation efficiency increases in higher molecular weight polymer formulations.29,30 In order to investigate the relationship of encapsulation efficiency (EE) and the particle size, several experiments were carried out, where the PLGA (19,000 Da) mass in the organic solvent was altered from 50, 100 to 200 mg (a constant monensin amount 5 mg) in other same prepared condition. The results were presented in Table II. As shown in the table, when PLGA mass were increased from 50 to 200 mg, the

![Fig. 3.](#) (A) FT-IR spectra of monensin loaded PLGA nanoparticles for molecular weight 19 kDa, and (B) FT-IR spectra of monensin loaded PLGA nanoparticles for molecular weight 110 kDa. Here, MON denotes monensin powder, PLGA for respective molecular weight placebo nanoparticles, PHY. MIX for physical mixture of monensin and placebo PLGA nanoparticles in ratio (1:1) and MON-PLGA NP denotes monensin loaded PLGA nanoparticles of respective molecular weight.

mean size of nanoparticles were increased from 142 to 228 nm. Moreover, the EE was also increased from 18% to 54% with the increase in the particle size. Three reasons for drug encapsulation efficiency with increase in polymer concentration are:

(i) the increase in PLGA concentration cause increase in the viscosity of organic phase. Viscous forces oppose diffusion of drug molecules to aqueous phase from organic phase,21

(ii) Increase in PLGA concentration leads to increase in size of nanoparticle; hence the drug content encapsulation area also increases.33

3.6. In Vitro Release of Monensin from Monensin PLGA Nanoparticle

Monensin loaded PLGA nanoparticles formulations prepared with different molecular weight PLGA showed biphasic release profile (Fig. 4). The release profile of monensin from all formulations of PLGA nanoparticle exhibited exponential rapid release phase followed by slower release phase. However, the release rate significantly dependent on molecular weight of PLGA used in respective formulations. Amount of drug released within first 8 h of incubation was considered as burst release. The maximum burst release of monensin observed when PLGA nanoparticle prepared with polymer molecular weight 19 kDa. The release of monensin reduced for high molecular weight of PLGA (110,000 Da) in formulation. The burst release of monensin from 110 kDa was found to be 30%. The results are in agreement with earlier reports by Graves et al., who have demonstrated that increase in molecular weight of PLGA, produce denser polymer wall and reduced drug dissolution from the formulation.34

The release profile was followed by a constant slow release until to 45% of accumulative amount of monensin was released from monensin loaded PLGA nanoparticle formulations preparing with 110 kDa from until 96 hr (4 days).

This type of exponential release pattern for PLGA nanoparticles are also have been reported by several authors.35–37 The results showed release of monensin is faster from nanoparticles prepared with the low molecular weight PLGA as compared to the nanoparticles prepared from higher molecular weight PLGA. The rapid release of monensin from PLGA nanoparticles prepared with polymer Mw. 19000 Da was probably due to the fact that the high molecular weight polymer (110,000 Da) have longer polymer chain than low molecular weight polymer (19,000 Da). Increase in polymer chains provides more hydrophobicity to the nanoparticles. Due to less hydrophobicity, the hydration rate of the lower molecular weight nanoparticle is higher than the high molecular weight nanoparticles. The short chain molecules have greater solubility, forming channels and pores in matrix leads to fast drug release. This phenomenon is much reduced for the high molecular weight polymers, because the chain length increases, hydrophobicity increases.38 At second stage of release, the release of monensin from nanoparticles was a typical sustaining release and would mainly depend on the drug diffusion and the matrix erosion which is a slower process.39

The other reason for the difference in the release profiles of monensin from nanoparticles prepared with polymer having different molecular weight probably due to the factor of different glass transition temperatures ($T_g$). It has been reported by various research groups that the glass transition temperatures ($T_g$) of the polymers plays a key role in the release rate from different molecular weight polymers. The $T_g$ increases with increase in polymer molecular weight.25 Probably, in low molecular weight polymer formulation (19 kDa) as $T_g$ goes lower the polymer chain motion would be increased at a given temperature, resulting in increased diffusivity of small drug molecules. Hence the release of monensin is faster in nanoparticles prepared with low molecular weight polymers. These results agree with earlier report by Jalil and Nixon.38

![Fig. 4](image)

In vitro release profiles of monensin loaded PLGA (50:50) nanoparticles of different molecular weights (19 kDa and 110 kDa) in pH 7.4 phosphate buffer. Data points shown are mean ± standard deviation ($n = 3$).

### Table II. Effect of PLGA concentration on the size, size distribution, zeta potential and encapsulation efficiency in monensin PLGA nanoparticles

<table>
<thead>
<tr>
<th>PLGA (mg)</th>
<th>Monensin (mg)</th>
<th>PS (nm)</th>
<th>PDI</th>
<th>EE (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>5</td>
<td>142±15</td>
<td>0.088±0.009</td>
<td>17.2±2.3</td>
</tr>
<tr>
<td>100</td>
<td>5</td>
<td>228±19</td>
<td>0.120±0.011</td>
<td>36.1±3.1</td>
</tr>
<tr>
<td>200</td>
<td>5</td>
<td>317±23</td>
<td>0.112±0.012</td>
<td>54.1±5.1</td>
</tr>
</tbody>
</table>

Notes: PS: particle size, PDI: polydispersity index, ZP: zeta potential, EE: entrapment efficiency. Values given are mean±standard deviation ($n = 3$).
3.7. Evaluation of Antimalarial Activity of Monensin Loaded PLGA Nanoparticle

The effects of the monensin, monensin loaded PLGA nanoparticles and PLGA nanoparticles (19,000 and 110,000 Da) on *P. falciparum* growth in vitro were measured in microtiter plates as described by Desardins et al.\(^2\) Figure 5 exhibits effects of monensin and monensin PLGA nanoparticles on the growth of *P. falciparum* cultured in human erythrocytes in vitro. Monensin and monensin-PLGA nanoparticles found to be very effective with IC\(_{50}\)s of nanomolar range against intraerythrocytic stage of *P. falciparum*. Free drug monensin has significant antimalarial activity in nanomolar range with the IC\(_{50}\) of 8.03 ± 0.21 ng/ml. The monensin PLGA nanoparticles have IC\(_{50}\) of 0.83 ± 0.03 ng/ml. The effect of PLGA nanoparticle (placebo) was also measured on the growth of *P. falciparum* in vitro. No inhibition of *P. falciparum* growth was observed by placebo formulation.

The enhancement of monensin activity mediated by its incorporation into nanoparticles can be explained by the fact that these systems can act as reservoir for monensin, protecting the drug from binding to the serum proteins. Gibot et al., clearly showed the decrease of antimalarial activity of monensin due to binding of serum proteins.\(^4\) Hence monensin nanoparticles shows greater antimalarial efficacy than free monensin. It is observed that the monensin nanoparticle formulation with high molecular weight PLGA (110 kDa) was most effective as compared to its low molecular weight PLGA nanoparticle counterparts. In vitro release studies showed high burst release from low molecular weight nanoparticles. High burst release causes therapeutically wasteful of drug as well as rapid exhaustion of the delivery system.\(^4\) For complete inhibition of parasitemia at least 30 hr treatment of with monensin is required.\(^1\) In low molecular weight nanoparticles, approx. 70% drug is released (an initial large bolus of monensin released in medium is available as free drug for binding the serum proteins) in 12 hr and subsequently 5% of drug is released in next 12 to 42 hr available to kill the parasite. However High Molecular weight nanoparticles showed slow release of drug with small burst release (approx. 30%), But subsequently releases 12% monensin in 12 hr to 42 hr period of time. Hence more amount of monensin is available for the subsequent hours for better inhibition of parasite growth as compared to low mol wt polymer.

The similar results obtained by Gasper et al., in their study in which they showed the activity of L-asperginase enzyme entrapped in high molecular weight PLGA nanoparticles remains sustained for more long-period of time as compared to the low molecular counterparts. In addition, they also reported that the low molecular weight preparations showed fast release of enzyme, while high molecular weight nanoparticle formulation showed slower release of the enzyme.\(^2\)

In addition, the mechanism of enhanced antimalarial efficacy of monensin nanoparticles may be due to bioadhesion of the nanoparticles to the erythrocyte membrane, facilitating increased drug penetration or increased internalization of the nanostructures, or both.\(^4\)

3.8. Hemolysis Assay

As discussed above the monensin in PLGA nanoparticles showed better antimalarial activity in comparison to free monensin. There are reports which show that monensin leads to Na\(^+\) entry and subsequent cell swelling in nucleated cells.\(^4\) Kim et al.,\(^4\) also reported that PLGA nanoparticles can damage erythrocytes which lead to hemolysis.\(^4\) In this investigation hemolysis assay was performed to explore whether the antimalarial activity of these formulations are due to above mentioned facts. The results showed no hemolytic activity of free monensin till 50 ng/ml concentration. These results tally well with the recent report by Bhavsar et al.\(^4\) They have reported that monensin does not exhibit any hemolytic activity upto 10 \(\mu\)M concentration. The placebo PLGA nanoparticles and monensin loaded PLGA nanoparticles did not show any hemolytic activity. The contradictory result from Kim et al., may be due to the usage of very less concentration of PLGA nanoparticles. Less concentration of PLGA nanoparticles were found to be sufficient for delivering monensin in desired concentration to the malaria infected cells. Thus the results of aforementioned assay proved that monensin and monensin loaded PLGA nanoparticles do not cause hemolytic activity to the uninfected and infected erythrocytes.

![Fig. 5. Effect of free monensin and various monensin loaded PLGA nanoparticle formulations on the growth of *P. falciparum: P. falciparum*-infected erythrocytes (2% final hematocrit and 1% parasitemia) were incubated for 48 h at 37 °C in the presence of different dosages of drug. Cell viabilities were assessed by measuring \([\text{H}]\) hypoxanthine (P. falciparum) incorporation into nucleic acids for 18 hr.](image)
4. CONCLUSION

In the present investigation, for the first time monensin loaded PLGA nanoparticles have been shown to have antimalarial activity. There are several reports for the antimalarial activity of free monensin, but due to its hydrophobicity its application is limited. Here monensin-PLGA nanoparticles are produced by emulsification solvent evaporation method. The monensin loaded particles were evaluated with respect to their size, shape, zeta potential, encapsulation efficiency and physical characteristics. The effect of monensin loaded PLGA nanoparticle in the inhibition of the growth of *P. falciparum* in culture was examined. DLS, TEM and AFM studies have showed that the mean particles size was found to be 152 nm with low polydispersity index (less than 0.1). TEM and AFM exhibited a fine spherical shape with the smooth surfaces. The DSC analysis measurement has suggested that the drug in the nanoparticle was found in the amorphous form. FT-IR showed that there is no interaction between the drug and the polymer. The encapsulation efficiency evaluated by spectrophotometric method and radioactive method and there is no significant difference found in the results. The monensin PLGA nanoparticles have 18% encapsulation efficiency for monensin. The *in vitro* release amount of monensin from monensin PLGA nanoparticles was successfully determined by radiolabelled monensin PLGA nanoparticles. The *in vitro* release profile of monensin from monensin loaded PLGA nanoparticles exhibited a typical biphasic release phenomenon, namely initial burst release and subsequently sustained release. The *in vitro* release also indicated that the release property of monensin from nanoparticles not only depended on adsorption of the drug but also on diffusion through the PLGA matrix. Monensin PLGA nanoparticles and PLGA nanoparticles show no hemolytic effect in the range of 50 ng to 0.1 ng/ml concentration. Hence it is proved that the antimalarial activity of monensin PLGA nanoparticles is not due to any hemolytic activity. The antimalarial activity was evaluated by semiautomated microdilution method. Results show that monensin and monensin nanoparticles have antimalarial properties in nanomolar range while PLGA nanoparticles (placebo) do not have any antimalarial property. But the monensin-PLGA nanoparticle formulation (110 kDa) is ten times more effective as an anti-malarial than the free monensin with IC50 of 0.82 ng/ml. All these results lead us to conclude that monensin PLGA nanoparticles have strong potential in antimalarial chemotherapy.

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References and Notes

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