Preparation, Characterization, and *in - vivo* Evaluation of Daptomycin Poly(D,L-lactide-co-glycolide) Microspheres

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Abstract

Polymeric microsphere devices occupy a wide range in the field of controlled drug delivery. Subcutaneous injectable preparations of Poly(Lactide-co-Glycolide) (PLGA) microsphere of Daptomycine were prepared by solvent extraction/evaporation technique using different copolymers ratio and molecular weights. Four formulations were prepared (F1-F4) and characterized in term of particle size, surface morphology, bulk density and porosity in addition to the drug content. The effects of the above parameters on the *in-vitro* release study were evaluated. These formulas were evaluated also for their *in-vivo* release profile using rat (as an animal model) and their serum daptomycin concentration were evaluated accordingly. A trapezoidal method was used to estimate the cumulative AUC parameter and the effect of copolymers ratio and molecular weights on the shape of AUC for four formulas were evaluated. Simulation study was performed (for 7 or 10 days dosing of F1 and F2 and 15 day dosing for F3 and F4) as a predictor of the steady state concentration and other pharmacokinetics parameters. It was inferred from this study that by using tailored formulation approach, long and short acting preparations of injectable daptomycin microsphere could be developed helping to provide the clinicians more flexibility to select the suitable preparation according to the patient need.

Key words: Daptomycin, trapezoidal,microsphers ,simulation .

تحضير وتوصيف وتقييم داخل الجسم لدواء دابتومايسين باستخدام Poly(D,L-lactide-co-glycolide) ككرات مجهرية

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الخلاصة

الأجهزة المكروية البوليمرية تحتل حيز كبير في مجال الأدوية ذات التحرر المسيطر عليه. تم تحضير حقن تحت الجلد لدواء الدابتومايسين بشكل مكورات مجهرية مع (PLGA) بواسطة تقنية استخراج / تبخير المذيب باستخدام نسبة بوليمرات مختلفة و الأوزان الجزيئية . وتم إعداد أربعة تركيبات (F1- F4) و تم تقييمها من ناحية حجم الجسيمات ، مورفولوجيا السطح، الكثافة الظاهرية والمسامية بالإضافة إلى محتوى الدواء. تم تقييم تأثير العوامل أعلاه على دراسة تحرر الدواء مختبريا . كذلك تم تقييم هذه الصيغ في الجسم الحي باستخدام الفئران (كنموذج حيواني) و جرى تعيين تركيزالدواء في الدم. وقد استخدمت طريقة شبه منحرف لتقدير المساحة تحت المنحني التراكمي وتأثير نسبة البوليمرات و اوزانها الجزيئية على شكل المساحة تحت المنحني للاربعة صيغ. أجريت دراسة المحاكاة (لمدة ٧ أو ١٠ ايام للصيغ F1و F2 و ١٥ يوم الجرعات ل F3 و F4 باعتباره مؤشرا لتركيز الحالة المستقرة والمعلمات الدوائية الأخرى. تم الاستدلال من هذه الدراسة باستخدام نهج صياغة مصممة ،ان حقن دابتومايسين مكروية قصيرة وطويلة الامد امكن تطويره مما يساعد على توفير المزيد من المرونة للأطباء لاختيارالمستحضر المناسب وفقا لحاجة المريض

الكلمات المفتاحية:دابتومايسين ، شبه مفرق ، كرات مجهرية .

Introduction

Controlled release delivery systems were being developed to address many difficulties associated with traditional methods of administration. One of the most important advantages of controlled drug delivery is reduced frequency of administration, a thing that significantly improves patient compliance and convenience with a consequent improvement in the efficacy of the treatment [(1)]. Due to their attractive properties, microspheres occupy a unique position in controlled delivery technology, and have shown to control release profiles of drugs having a wide range of molecular weights, applying different types of polymers. Biodegradable polymers are the most interesting type since it hold several advantages including their biocompatibility and biodegradability where it would degrade into nontoxic, absorbable subunits which

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would be subsequently metabolized, in addition to that these polymers exhibit a predictable erosion so it do not exhibit dose dumping and would retain its characteristics even after depletion of the drug. The release of the active agent occurs by either gradual bioerosion of the drug containing polymer matrix, or by cleavage of unstable bonds by which the drug is coupled to the polymer matrix so the release rate is governed by the biodegradation process[2]. Biodegradable polymer includes synthetic and natural type. The linear polyesters including poly lactic acid-co-glycolic acid (PLGA) are the most widely investigated type [3,4].

The exact kinetics of drug release could be determined and fine-tuned by careful design of the microsphere composition including the type of Polymer used, the polymer molecular weight, the copolymer composition, the nature of any excipients added to the microsphere formulation and the microsphere size, all of these factors have a strong impact on the delivery rates[5].

Daptomycin is novel cyclic lipopeptide antibiotic with molecular weight 1620.67 g/mol. It's a tridecapeptide comprising several non-proteinogenic amino acids with an N-terminal decanoyl fatty acid side chain and a decapeptide lactone core that resulting from the cyclization of the Thr-4 hydroxyl group onto the C-terminal carboxylate. Daptomycin is produced from the fermentation culture of *Streptomyces roseosporus* and it shares a similar structure, and possibly a related mode of action, to other acidic lipopeptide antibiotics, these include the calcium-dependent antibiotics [6-8]. Because of its unique structure which consists of a 13-member amino acid, a noval mechanism of bactericidal action has been achieved which involves insertion of the lipophilic daptomycin tail into the bacterial cell membrane, causing rapid membrane depolarization and a potassium ion efflux. This is followed by arrest of DNA, RNA and protein synthesis [9-11].

Daptomycin is highly water soluble, this is due to its predominantly acidic nature and the negative charge at neutral pH while the presence of lipid tails and some hydrophobic amino acids offer amphipathic properties to its structure. The pKa values for individual daptomycin residues at neutral media are 2.9, 3.5, 4.3, 4.7, and 10.5 while its melting point is 215°C [12,13].

On 2003, the Food and Drug Administration (FDA) approved daptomycin for the treatment of complicated skin and skin structure infections in adults. In 2006 an indication was added for *Staphylococcus aureus* right-sided infective endocarditis. Daptomycin provides a useful alternative to standard therapies for both methicillin susceptible (MSSA) and methicillin-resistant (MRSA) *Staphylococcus aureus*, and in some cases, vancomycin-resistant enterococcus (VRE) [14]. Adult dosing 4-6mg/kg/day and it available in 500mg of lyophilized powder per10 ml vial for IV injection over a two minute period or by infusion over a thirty minute period [13].

In accordance to above, the delivery of daptomycin using polymeric carriers, dosed subcutaneously or intramuscularly, is an effective strategy in mitigating patient compliance concerns and related issues as it ensures adherence to therapy leading to improved patient outcomes. This fact is further corroborated by several publications that have emphasized the development and clinical use of long acting dosage forms used in the treatment of systemic and life-threatening infections caused by Gram-positive organisms [15]. Thus, the PLGA polymer is an ideal delivery matrix for daptomycin that could provide initial and sustained levels based on the choice of the polymer used.

Therefore, the goal of this study was to develop and subsequently investigate the suitability of using PLGA polymers having varying properties like copolymer composition and molecular weight to provide tailored *In-vivo* release of such novel antibiotic via the subcutaneous route.

Materials and Methods

Materials

Daptomycin (molecular weight 1619.7086, soluble in water; sparingly soluble in acetonitrile, and soluble in dichloromethane) was purchased from Cipla Ltd., Bombay, India. PLGA having varying molecular weights (15 and 131kDa of 75:25 PLGA, 30 kDa of 50 : 50 PLGA, 82 kDa of 65 : 35 PLGA) was purchased from Boehringer Ingelheim (Ingelheim, Germany) and Alkermes (Cambridge, MA, USA). All other chemicals were obtained commercially as analytical grade reagents.

Method

Preparation of microspheres

The four PLGA copolymer ratios and molecular weights evaluated were:(a)15 kDa PLGA, 75 : 25 lactide : glycolide (Formula F1),(b)30 kDa PLGA, 50 : 50 lactide : glycolide (Formula F2) , (c)82 kDa PLGA, 65 : 35 lactide : glycolide (Formula F3),(d)131 kDa PLGA, 75 : 25 lactide : glycolide (Formula F4).

The microspheres were prepared by a solvent extraction/evaporation method and recovered by filtration [16]. Briefly, a solution of drug and

polymer (10–20% polymer concentration) in dichloromethane was injected into an aqueous continuous phase at a ratio between 250 and 350 parts of polymer phase: aqueous phase, under stirring with a Silverson L4R mixer (Silverson machines, MA, USA) at 5000 rpm. Subsequently, the solvents were removed by stirring after which the microspheres were recovered by filtration, suspended in a suitable vehicle, filled into vials, and freeze dried.

Characterization

The microspheres were characterized for mean particle size, surface morphology, bulk density, drug content, and *in-vivo* performance.

Particle Size

Particle size distribution of the microspheres prior to vialing was determined using a laser diffraction technique (Malvern 2600c Particle Sizer, Malvern, UK). The particles were suspended in 0.05% Tween 80 and counted using a laser sensor. The average particle size was expressed as volume mean diameter in microns (μm) [17].

Surface morphology

The surface morphology was examined by scanning electron microscopy (SEM) (Hitachi S800, Japan) at an appropriate magnification, after palladium/gold coating of the microsphere sample on an aluminum stub[18].

Bulk density

Bulk density was determined by transferring known quantity of Micro spheres to graduated cylinder and tapping 100 times from a vertical distance of approximately 0.5 inches at 2 sec interval. The tapping process was repeated until the volume occupied by particles remained unchanged. The final volume was recorded as tapped volume, V_b, and the tapped bulk density (g/cc) was calculated as M/V_b, where "M" was the weight of microspheres employed [19].

Drug content

Daptomycin content in the microspheres was analyzed by a reverse phase HPLC method using the stationary phase, Hypersil 5 ODS in a stainless steel column, 100 x 4.6 mm (Thermo-Sep Electron Corp., Waltham, MA, USA) with a guard column of the same material. The mobile phase was 0.2 M phosphate buffer (pH 5.5) and acetonitrile (70:30). The pump flow rate was 1.5 ml/min. Detection was by UV absorbance at 223 nm (model UVD170U, Dionex Corp., Sunnyvale, CA, USA). Serum (100 μL) was mixed with acetonitrile (100 μL), allowed to stand for 5 min and centrifuged at 13000g for 5 min. An aliquot of 10 μL of the supernatant was injected [20].Measurements were performed in triplicate. Drug content (%) was expressed as the weight of drug in microspheres/weight of microspheres × 100. Encapsulation efficiency (%) was also calculated for the four formulations.

In-vivo study

Four groups of male Sprague-Dawley rats (per group) weighing approximately 300 g were used to evaluate *in-vivo* performance of Daptpmycin microspheres. The microspheres were injected subcutaneously at the back of the neck (15–30 mg/kg Daptomycin/rat) after reconstitution with 0.9% of sodium chloride for injection, USP. Blood samples were collected from the tail vein. The samples were centrifuged in Microtainer tubes (Becton Dickinson, Franklin Lakes, NJ) and serum was collected. Serum samples were frozen and stored at −20°C until analysis. Serum samples were analyzed using a validated method [21].

Simulation studies

Multiple dosing pharmacokinetic (MDP) studies are commonly used to help the selection of an appropriate dosing regimen for a given formulation [22].

Since Daptomycin follows linear pharmacokinetics after multiple intravenous dosing, the plasma concentrations observed after multiple dosing of Daptomycin can be linearly related to the dose and can be predicted from the AUC after administration of a single dose [23]. Therefore, this linearity allows simulations of multiple dose pharmacokinetics after continual dosing to be performed using the superposition principle.

In the current study, simulations of serum *in-vivo* levels were obtained after subcutaneous administration of formulations F1 and F2 (single dose at 15 mg/kg), and formulations F3 and F4 (single dose at 30 mg/kg) were performed using the superposition principle. A 7- and 10-day dosing regimen was used with formulations F1 and F2 while a 15-day dosing was used with formulations F3 and F4. A total of 4 doses were selected for the simulation study as this would be an interpreter of steady state concentrations for this molecule.

Results and Discussions

Characterization of daptomycin microspheres

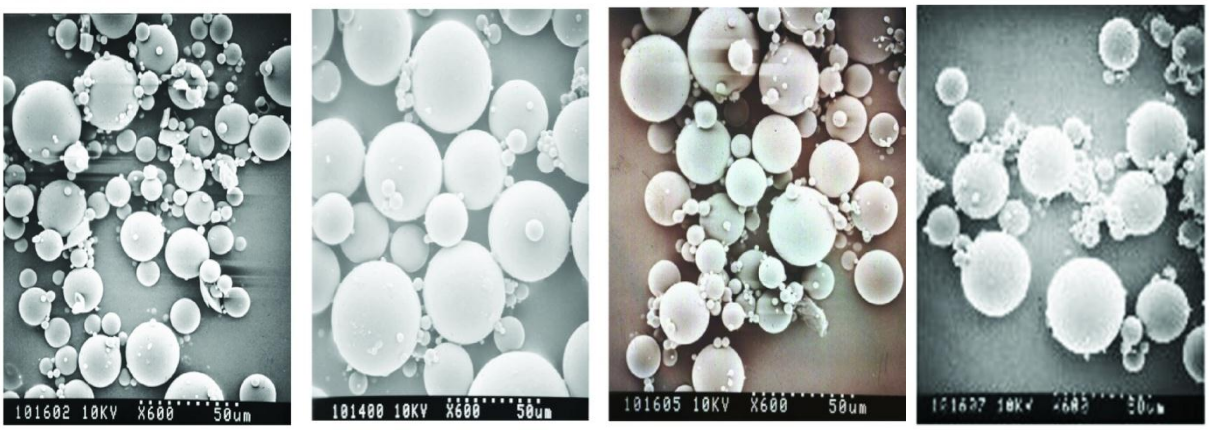
Particle shape, size, and morphology

The scanning electron microscope (SEM) images of formulations F1-F4 are provided in figure 1. The scanning showed that the microspheres having a spherical shape with a smooth nonporous surface and homogeneous particle size distribution. Particle size analysis revealed that formulas F1,F2,F3 and F4 had a mean volume diameter of 20.0, 19.8, 25.3, and 23.6 μm, respectively (Table1). The mean volume diameter was found to be similar in

formulas F1and F2, both prepared from lower molecular weight PLGA, while the same was true for formulas F3 and F4, manufactured using higher molecular weight PLGA.

Table (1) Properties of daptomycin PLGA microspheres

Formulation	F1	F2	F3	F4
PLGA type	75 : 25	50 : 50	65 : 35	75 : 25
Polymer MW	15 kDa	30 kDa	82 kDa	131 kDa
Drug content, %	30	34	44	44
Bulk density, g/ml	0.99	1.1	1.0	1.36
Mean particle size (μm)	20.0	19.8	25.3	23.6
Dose of Daptomycin	15 mg/kg	15 mg/kg	30 mg/kg	30 mg/kg



F1 **F2** **F3** **F4**

Figure 1: Scanning electron micrographs of Daptomycin PLGA microspheres of different formulations

According to the published literatures, the particle size was found to have an important effect on the drug release in the field of drug delivery. For instance, a reduction in particle size is a common strategy to enhance dissolution rate of soluble drugs [(24)]. Particle size remains one of the key parameters that affect the degradation rate of the PLGA polymer matrix and thereby drug release rates [(25)]. A reduction in particle size generally depicts an increase in surface area to volume ratio, resulting in a large surface area available for the dissolution media penetration into the particles and also for a rapid escape of any entrapped in the easily accessible porous network. For smaller sized particles, the amount of surface associated drug is expected to be large, and hence, initial burst is not unexpected [(27)].

Based on the small particle size of the Daptomycin microspheres, it was inferred that an initial burst would be exhibited by all the formulations evaluated. However, a shorter duration of release was expected for Formulations A and B, due to lower molecular weight. This suggests that the in vivo behavior of Daptomycin from PLGA polymeric degradation products. Additionally, with PLGA microsphere dosage forms, the initial burst release phenomenon depends on particle size. Yagnesh Bhatt et al, reported that the Processing conditions employed during preparation of microparticles determine the properties of the microparticles, such as particle size and the reduction in particle size was found to have dramatic effect on the initial rapid release from Bovine serum albumin (BSA) loaded PLGA microparticles [(26)]. Thus, the initial burst effect depends on two parameters: (a) amount of drug loosely associated with the surface and (b) drug microspheres could be manipulated to provide varying duration of action.

Bulk density

The results of bulk density studies are summarized in Table 1. Bulk density values for the formulations were varied greatly and were determined to be 0.99, 1.1, 1.00, and 1.36 g/cc for formulations F1,F2,F3 and F4, respectively. These data suggest that all four formulations showed intermediate to high bulk density values. Between the formulations, a comparison of the data revealed the lowest values for formulas F1 and F3, while formula

F4 exhibited the highest bulk density value, with an intermediate bulk density value for formulation F2.

The evaluation of bulk density would provide an important information about the porous network in the drug loaded microspheres. Thus, any variation in the density or porosity influences the other parameter and hence, impacts drug release behavior. Low bulk density values are a qualitative indicator of the porous network inside the microspheres. Additionally, low bulk density values are also observed with irregular or nonspherical microspheres that display nonoptimal packing. Further, these values can also be correlated with specific surface area and onset of mass loss. Microspheres with high bulk density typically exhibit low values of specific surface area. Conversely, microspheres with a highly porous network will have a low bulk density and thus a faster drug release rate [28, 29].

It was concluded from the bulk density data, that the specific surface area was the lowest for F4, but the highest for formulations F1 and F3. Generally, low bulk density (high porosity) values in microspheres translate to faster drug release; same finding has been seen by Byrne R *et a*l who studied the influence of the porous microstructure on the drug release from aluminosilicate pellets [30]. Hence, certain predictions have been made with bulk density and particle size data: (a) particle size values for the four formulations were similar implying that the impact of this parameter on drug release would be comparable across formulations F1–F4, and (b) due to slightly lower bulk density values for formulations F1 and F3, they were expected to show a higher initial burst than formulations F2 and F4.

Drug content

Results of drug content for Daptomycin PLGA microspheres, as determined by HPLC, are presented in Table 1, F1 had the lowest drug content (30%) while F2 had 34% and formulations F3 and F4 had the highest drug loading (44%). A noteworthy observation was that the encapsulation efficiency was 100% for all the microsphere formulations. These results suggest that the solvent extraction/evaporation method is suitable method for the preparation of Daptomycin microspheres.

In-vivo studies

Serum levels of Daptomycin for Formulations F1-F4

Serum levels of Daptomycin after administration of formulas F1 and F2, of 15 mg/kg dose, and formulas F3, and F4 of 30 mg/kg dose, are shown in Figure 2. In general, formulations F1-F4 exhibited similar release profiles in that they show an initial burst release followed by a brief decline leading to a secondary peak and a final slow decay phase for the four formulations.

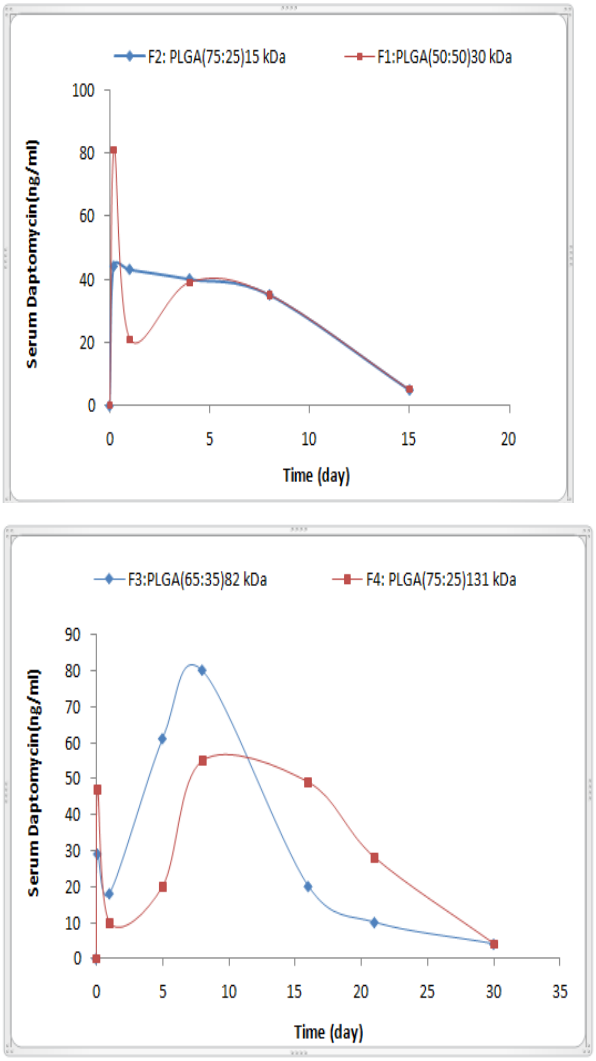


Figure2: *In-vivo* release of Daptomycin PLGA microspheres (formulations F1 and F2 (15 mg/ kg dose) ,and formulations F3 and F4 (30 mg/kg dose).

As expected, F1 exhibited the highest initial burst (81 ng/ml) followed by a sharp drop that characterized the trough (21 ng/ml, day 1) leading to a second peak of around 39 ng/ml at day 4, after which levels exhibited a slow decline to day 15 (Figure 2). In comparison, F2 exhibited an intermediate initial burst (45 ng/ml) followed by very slight dip in levels (43 ng/ml, day 1) and a secondary peak where values were comparable to the initial burst and trough (39 ng/ml, day 4), with a slow drop in levels till the last time point (day 15). With formulations F1 and F2 administered of 15 mg/kg dose, the short duration of action (15 days) was expected and attributed to a

combination of the properties of PLGA polymer (copolymer ratio and molecular weight) and microspheres (bulk density and drug content). The high initial burst for formulation F1 was attributed to a combination of small particle size and low bulk density that allowed for easily accessible drug residing on the surface or in the pores of the microspheres to be released rapidly *in-vivo*, while the intermediate burst for formulation F2 was recognized for its high bulk density (low porosity).

For formulations F3 and F4, administration of 30 mg/kg dose, the duration of action was significantly longer than formulations F1 and F2 (Figure 2). With formulation F3, initial levels were low (29 ng/ml, 6 hours), dropping even lower to reach a trough value of 18 ng/ml at day 1, then serum Daptomycin values rose sharply to reach 61 ng/ml by day 4. The true secondary peak level for formulation F3 was achieved by day 8 (78 ng/ml) after which levels dropped equally sharply to reach about 3 ng/ml by day 30. Unlike formulation F3 where initial burst was lowest, intermediate burst levels were observed with formulation F4 (48 ng/ml) that dropped to a stark trough value of 5 ng/ml (day 1). After the trough, serum Daptomycin values began a steady ascent to reach 51 ng/ml (day 8) after which levels once again dropped to reach a final minimum of 3 ng/ml by day 30 (Figure 2).

Serum Daptomycin profiles obtained for formulations F3 and F4 can be explained on the basis of the *in-vitro* characterization results. As stated in Section 3.1.3, a low to intermediate burst was expected for formulations F3 and F4. Since the bulk density and drug content values were high, a low to intermediate burst implied that the drug remaining in the microspheres would be released in a more sustained fashion. Considering the factor of in the higher lactide content and high polymer molecular weight, the extended duration *in-vivo* release was expected for these two formulations. Between formulations F3 and F4, the former was manufactured from a 65: 35 PLGA polymer and hence, faster release of drug to reach a secondary peak was predicted; the *in-vivo* results are in agreement with predicted behavior of these polymeric formulations.

Cumulative AUC for Formulations F1,F2,F3 and F4

The cumulative area under the curve (AUC), a key pharmacokinetic parameter, for the four formulations, as calculated by the commonly used trapezoidal method (equation 1), is shown in Table 2.

$$AUC_{(t_1-t_2)} = \left[\frac{(C_1 + C_2)}{2}\right] \times (t_2 - t_1)$$

Table (2) Cumulative AUC for Daptomycin PLGA microspheres.

Formulation	F1	F2	F3	F4
Dose	15 mg/kg	15 mg/kg	30 mg/kg	30 mg/kg
Cumulative AUC (ng / ml.day)	480	549	1300	1232

Where (t) is time in hours and (C) represents serum concentration of Daptomycin in ng/ml. Results from AUC calculations indicate that formula F1 exhibited the lowest cumulative AUC through 15 days (480 ng/ml.day), with a slight increase in the value for formula F2 (549 ng/ml.day). The lower cumulative AUC values for formulations F1 and F2 were attributed to the low polymer molecular weights and low drug content for both formulations. A closer examination of the data revealed that despite the high burst with F1 that contributed about 3% to the cumulative AUC, the net contributions of the time points after the secondary peak were similar to that of F2. This was attributed to the lack of the characteristic peak and trough release profile observed with F2 (Figure 2) where burst release contributed a meager 1% to the total cumulative AUC. For this reason, the total cumulative AUC value for F2 was slightly higher than F1.

Formulations F3 and F4, administered at a higher dose (30 mg/kg), demonstrated cumulative AUC values of 1300 and 1232 ng/ml.day through 30 days, respectively, which were higher than those observed with Formulations F1 and F2 that were administered at 15 mg/kg dose (Table 2). These formulations exhibited low to intermediate initial burst; therefore, the percent of cumulative AUC contributed by this phenomenon was less than 0.4% for Formulations F3 and F4. A lower amount of initial burst also suggested that the extended duration of PLGA release was due to

Daptomycin entrapped in the polymer that was released slowly upon hydrolytic degradation of the 65:35 or 75:25 lactide : glycolide copolymer. In general, analysis of cumulative F1-F4 revealed the following significant points:(a) The contribution of initial burst towards the total AUC for all formulations was minor (equal to or less than 3%).(b) Daptomycin was well entrapped in the PLGA polymer matrix and was responsible for over 97% of the cumulative AUC *in- vivo*.(c)The cumulative AUC obtained with F3 and F4 was nearly 2 to 3 times greater than that observed with F1 and F2, suggesting that by selection of an appropriate polymer molecular weight and proportions, the release of Daptomycin from PLGA microsphere would be customized.

Simulation of multiple dosing

Figure 3 shows serum levels for formulations F1 and F2, after 4 doses, when administered weekly or once every 10 days. Once weekly and 10-day dosing regimen were selected for formulations F1 and F2, where the duration of action was short. Once weekly simulation for F1 revealed that pulsatile behavior was to be expected *in-vivo*, similar to what was observed with administration of a single dose. Simulations for doses 2-4 show that levels between 40 and 110 ng/ml are easily achieved with weekly dosing with a slightly lower range for the 10-day dosing. With Formulation B, weekly dosing provides serum levels ranging between 50 and 80 ng/ml while 10-day dosing affords slightly lower levels, in a manner similar to that observed with F1. The difference between the maximum and minimum serum levels for F2 was the smallest among all the formulations evaluated. Irrespective of the dosing regimen, Figure 3 indicates that steady state levels are attained between doses 2 and 4 for F1 and F2.

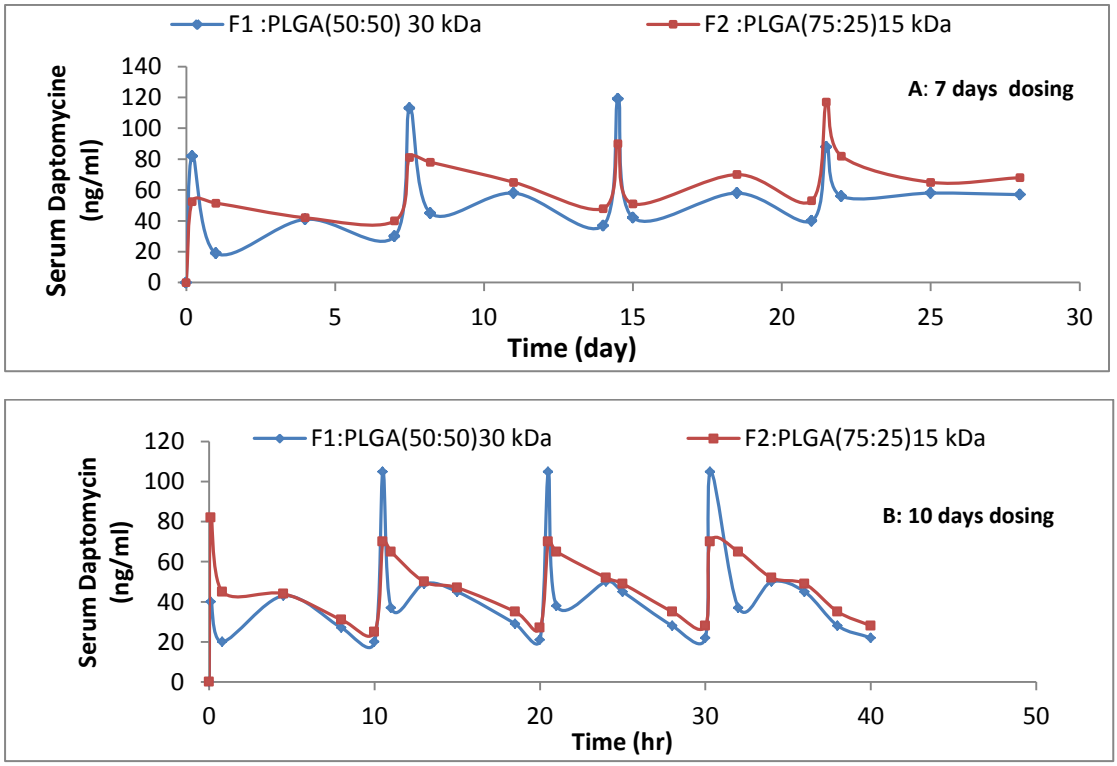


Figure 3: Simulation of multiple dosing regimens after 15 mg/kg dose of formulas F1and F2 ,A: every 7 days ,B:every 10 days.

A 15-day dosing regimen was performed on formulations F3 and F4, where the duration of action was considerably longer (Figure 4). The 15-day simulation for formulations F3 and F4, shows that drug release from the latter formulation was pulsatile. However, serum levels ranged between 30 and 100 ng/ml for both batches through 4 doses. This infers that formulations F3 and F4, tailored to release drug for an extended duration, and it would be excellent candidates for 15-day administration. Such type of therapy has the added benefit of reducing the number of injections required to initiate and maintain adherence to therapy. Overall, simulations for the four formulations suggest that the Daptomycin PLGA microspheres provide a suitable initial burst and maintain release over a period of time *in-vivo*.

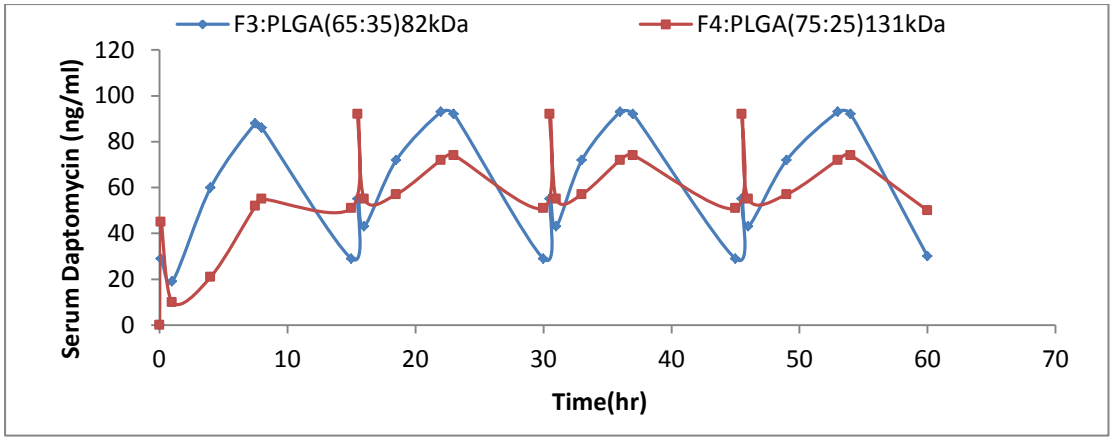


Figure 4: Simulation of multiple dosing regimens(30mg/kg every 15 days, total=4 doses) for daptomycin PLGA microsphere (F3 and F4)

Steady State Levels

A comparison of the average steady state concentration for formulations F1-F4 is shown in Figures 5 and 6. The average steady state concentrations were computed for the four formulations and was found in range of 54 and 64 ng/ml for weekly dosing of formulations F1 and F2, with slightly lower levels (39 and 46 ng/ml, respectively) for 10-day dosing(figure 5). A similar calculation for formulations F3 and F4 (15-day dosing) revealed steady state levels of 67 and 63 ng/ml, respectively (figure 6).

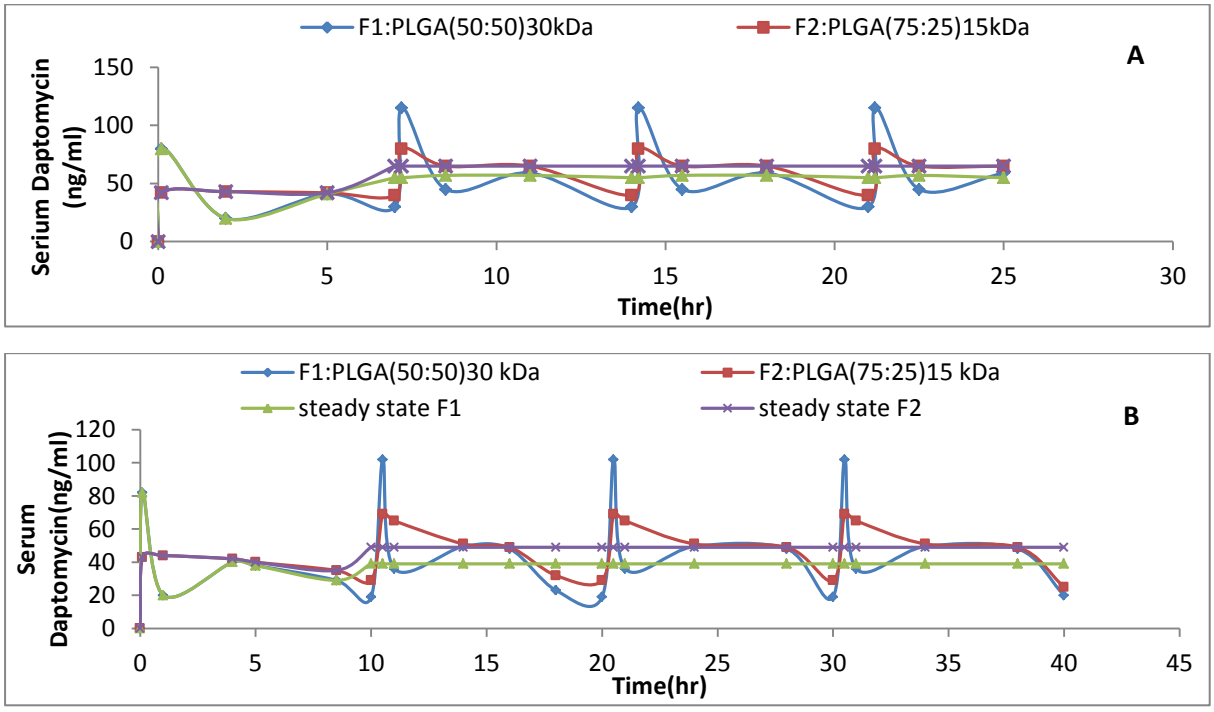


Figure 5: Average steady state concentration for formulation F1 and F2 , A :7day dosing B:10 day dosing

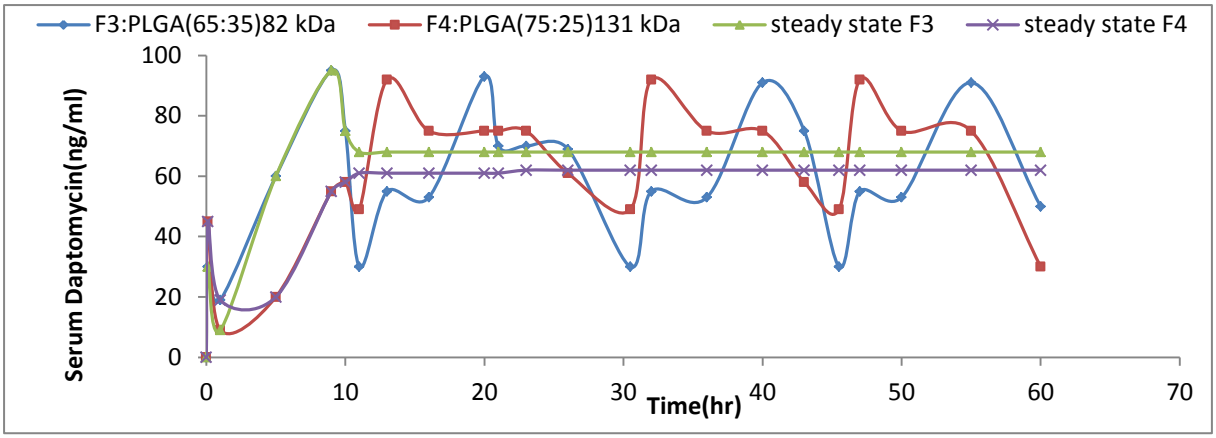


Figure 6: Average steady state concentration of formulation F3 and F4

Steady state values from the simulation studies provide information on the *In-vivo* behavior of the four formulations. For F1, dosed weekly, a high burst is expected after which levels drop nearly 35 ng/ml to reach 60 ng/ml and release drug in a sustained fashion through the 4-week dosing interval. Slightly higher and constant steady state levels are expected when F2 is dosed weekly. As expected, steady state levels for a 10-day dosing regimen are lower for F1 and F2 (Figure 5).

For the higher molecular weight longer acting PLGA formulations, higher levels could be achieved with 15-day dosing. In fact, the steady state levels achieved are higher than the initial burst and can be attributed to drug entrapped in the polymeric matrix.

These results bear strong clinical significance in that drug levels in vivo can be tailored to suit patient needs using a systematic scientific approach. Indeed, steady state levels for weekly, 10-day, or 15-day dosing range between 45 and 65 ng/ml, allowing the clinician to utilize a variety of dosage forms for a shorter or longer duration of therapy that is patient specific. Such an approach is highly effective in the treatment of patient populations with skin , lungs , and blood infections .

Conclusion

Preparation of injectable depot of Daptomycin antibiotic encapsulated within PLGA microspheres is an excellent delivery mechanism that offers the possibility of sustained drug release over a long duration of time. In this study, 4 long-acting formulations of varying molecular weight and copolymer compositions were developed with the purpose of illustrating that such formulation modification can provide medical professionals suitable choices in designing therapeutic strategies to treat patients with varying clinical needs. *In-vivo* experiments done in rats, revealed that Daptomycin formulations would be suitable for weekly, 10-day or, 15-day dosing and would achieve steady state levels by the second dose. The results showed the value of the tailored formulation approach in developing long-acting Daptomycin injectable depot preparations. Thus, proper selection of polymer composition and molecular weight will enable customizing drug release from PLGA formulations and reduction in the frequency of dosing.

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