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Original Research Article

PLGA/PEG particles created by Nano-precipitation as drug delivery carriers for dipyridamole

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Abstract: Biodegradable polymeric particles that can gradually degrade inside the human body have been investigated as drug delivery carriers for different pharmaceutical agents. Particles that entrap pharmaceuticals can be applied in combination with stent technology, by releasing one or more anti-coagulant agents in a controllable manner, in order to inhibit thrombosis and minimize inflammation caused by the immune system of the patient. In this research work, the anti-platelet drug dipyridamole (DIP) was incorporated in poly (lactic-co-glycolic acid) (PLGA) and polyethylene glycol (PEG) sub-micron particles, which were created using the Nano-precipitation method. The fabricated particles were loaded with different amounts of DIP in order to evaluate the effect of the drug's concentration on their physical properties. The encapsulation efficiency of the particles and the drug's release kinetics profile were investigated. The round-shaped sub-micron particles' average diameter was around 150 nm. The ratio between the two co-polymers of PLGA did not critically affect the particles' properties. The results highlight the fact that z-potential values of the particles in the dispersion decreased as the drug content increased while PEG created a surface coating that influenced release kinetics, which were primarily governed by Fickian diffusion. Taken together, the preliminary findings of this work demonstrate the versatility of the PLGA/PEG particles and therefore, their potential as promising drug delivery candidates.
Keywords: Biomaterials, dipyridamole, drug delivery systems, particles, polymeric composites.

INTRODUCTION

Biodegradable particles of different shapes and size have been thoroughly investigated over the past few decades as drug delivery systems (DDSs) mainly due to their biodegradability[1]. Additionally, biodegradable particles have been frequently used to improve the therapeutic index of drugs, improving their bioavailability, solubility and retention time [2].

Various biodegradable polymers have been investigated for the engineering of particles and other formulations, such as polycaprolactone (PCL), polyethylene glycol (PEG), poly (lactic acid) (PLA), poly (glycolic acid) (PGA), and their copolymer poly (lactic-co-glycolic acid)(PLGA) [3-5]. Apart from their biodegradability and biocompatibility, PLGA formulations have several advantages, such as process ability and the possibility of a sustained local release of the entrapped agent [6, 7]. Furthermore, the use of PLGA is associated with minimal systemic toxicity [8]. Thus, PLGA has received considerable attention due to its attractive properties and has been intensively used in many biomedical applications [8 - 12].

Dipyridamole (DIP) is an anti-thrombotic and anti-proliferative agent that has been used in patients suffering from cardiovascular diseases [13 – 15]. Based on previous literature findings, DIP was successfully entrapped into PLGA sub-micron particles for sustained release [7, 8, 16]. However, only few studies have shed light on the effect of fabrication and process parameters on the physicochemical properties of the PLGA formulations, as well as on their encapsulation efficiency and the release kinetics profile of the incorporated pharmaceutical agent.

Taken together, the aim of this study was to fabricate DIP-loaded PLGA/PEG particles using the Nano-precipitation method [17], study how the values of size and z-potential of the particles are affected by the drug's concentration and calculate the particles' encapsulation efficiency and the cumulative release of DIP.

CASE REPORT

Poly (lactic-co-glycolic acid) (Mw: 40000-75000), lactic/ glycolic acid ratio 65:35 (PLGA 6535), poly (lactic-co-glycolic acid) (Mw: 66000-107000), lactic/ glycolic acid ratio 75:25 (PLGA 7525), polyethylene glycol (PEG), (Mw: 2000), dipyridamole (DIP) powder, acetone 99.9%, and chloroform ≥99% were all purchased from Sigma-Aldrich. All other reagents and solvents were of analytical grade.

DIP-loaded PLGA/ PEG particles were fabricated by the method of nanoprecipitation [17]. Acetone was chosen as the organic medium, while bi-distilled water was used as the aqueous medium. PLGA concentration was 10 mg/mL in the organic phase [18]. DIP concentration in the organic phase varied from 0.25 mg/mL to 1.0 mg/mL and the ratio between organic and aqueous phase was determined at 1:3. PEG was dissolved in the aqueous phase at a concentration of 10 mg/mL. The particle dispersion was carefully placed in a rotary evaporator to allow the adequate evaporation of the organic solvent.

The surface morphology of the particles with the highest initial DIP concentration was assessed by atomic force microscopy (AFM) (NT-MDT Solver P47H Pro). Silicon wafers of 10×10 mm^2 were dip-coated inside the particle dispersions and left to dry overnight at room temperature. Phase and topography pictures were obtained at different resolutions (1×1, 5×5 and 10×10 μm^2).

The size of the fabricated particles was calculated by photon correlation spectroscopy and the z-potential of the particle dispersions were calculated via aqueous electrophoresis measurements (Zetasizer 3600, Malvern). The experiments were performed in triplicates.

In order to determine the encapsulation efficiency of the particles, the latter were dissolved in 1 mL of chloroform. Their absorbance was measured at a fixed wavelength of 285 nm using a UV-vis spectrometer (LIBRA S22, Biochrom). A standard curve of absorbance versus concentration was previously prepared using different concentrations of DIP in chloroform (2-20 μg/mL). The encapsulation efficiency was calculated as the % percentage of the total mass of the drug encapsulated in the particles divided by the total mass of the drug initially used.

The in vitro release studies were performed by incubating the DIP- loaded particles (DIP conc. 1mg/mL) in phosphate buffer saline (PBS) (pH 7.4, T = 37^oC) under constant stirring (90 rpm). At predetermined time points, 1 mL of the particle dispersion was collected inside a 2 mL Eppendorf tube. After a set of centrifuging steps (3×, 20000 rpm, 15min) to collect only the amount of the drug released from the particles, the latter was re-dispersed in PBS and the absorbance of DIP was measured at 291 nm. The cumulative release of DIP was calculated as the % percentage of the total mass of DIP released from the particles divided by the total mass of DIP initially encapsulated inside the particles. The experiments were performed in triplicates.

In order to determine the release kinetics mechanism, the experimental data were mathematically fitted using the "Peppas equation" [19, 20].

$Q = kt^{\mathbf{n}}$

Where Q is the drug release percentage, t is the release time, k is a constant depended on the characteristics of the particles and **n** is the release exponent which indicates the mechanism [21].

DISCUSSION

The results presented in Table 1 indicate that the average particle diameter ranged from 147 ±1.79 to 155.94± 7.37 nm. From the obtained data, it can be concluded that it was not critically affected by the incorporation of the drug. The z-potential values were negative for all particle dispersions ranging from -14.03 ± 0.78 to --17.09 ± 0.39 mV. The incorporation of the drug slightly reduced the overall negative surface charge of the particles, resulting in decreased z-potential values, concentration dependent.

From the obtained AFM pictures in Figure 1, it can be concluded that the particles had a discrete spherical outline while a phase separation between the inner core and the surface was evident. More specifically, a surface coating of 20-30 nm was observed (Figure 1B), which can be attributed to the accumulation of PEG in the surface of the particles and the phase separation between PLGA and PEG [22].

PLGA 6535/ PEG particles exhibited significantly higher encapsulation efficiency (16.61 ±0.36%) compared to PLGA 7525/ PEG (11.16 ±0.78%), respectively (student's t-test, $p<0.05$). This observation can be attributed to the formulation process, and the dissolution of the polymer into the organic phase [17, 18]. More specifically, PLGA 6535 was dissolved faster compared to the other type while DIP was already dissolved in acetone before the addition of the polymer.

Regarding the drug release experiments, a burst release of DIP was observed in both types of PLGA/ PEG particles, which was followed by a slower release stage (Figure 2). The initial burst phenomenon can be explained by the DIP molecules close to or at the surface of the particles as well as to possible presence of pores through the polymeric matrix due to PEG erosion inside PBS [2, 5, 8, 16, 18, 21]. The possible loss of mass due to the breakdown of the esteric bonds between PLGA and PEG, leads to increased levels of hydrophilicity of the polymeric matrix through time

[22]. Since the size of the particles remained relatively stable, the porosity of the polymeric matrix increased, resulting in an increased diffusion of DIP. After fitting the experimental data using the Peppas equation the release exponents n were obtained (PLGA 6535/ PEG: n=0.37, R^2=0.993; PLGA 7525/ PEG: n=0.31, R^2=0.986). A release exponent n ≤ 0.43 for a spherical formulation corresponds to Fickian diffusion [19 - 21]. Therefore, the release of DIP was primarily governed by the diffusion through the polymeric matrix and secondarily by polymer (PEG) erosion through a biphasic kinetics profile.

Table 1: Average size and z-potential of the particle dispersions for different PLGA type and DIP concentrations

Sample	**DIP concentration** (mg/mL)	**Size** (nm)	**z-potential** (mV)
	mean ± SD	**mean ± SD**	**mean ± SD**
PLGA 6535 PEG	0	153.47 ± 10.08	-14.12 ± 0.83
PLGA 7525 PEG	0	155.94± 7.37	-14.03 ± 0.78
PLGA 6535 PEG DIP	0.25	147± 1.79	-15.52 ± 0.85
PLGA 7525 PEG DIP	0.25	149.5± 4.49	-15.67 ± 1.01
PLGA 6535 PEG DIP	0.5	155 ± 5.4	-15.77± 0.93
PLGA 7525 PEG DIP	0.5	154.67 ± 9.52	-16.05 ± 1.36
PLGA 6535 PEG DIP	1	150.44 ± 9.1	-16.83 ± 0.5
PLGA 7525 PEG DIP	1	149 ± 8.37	-17.09 ± 0.39

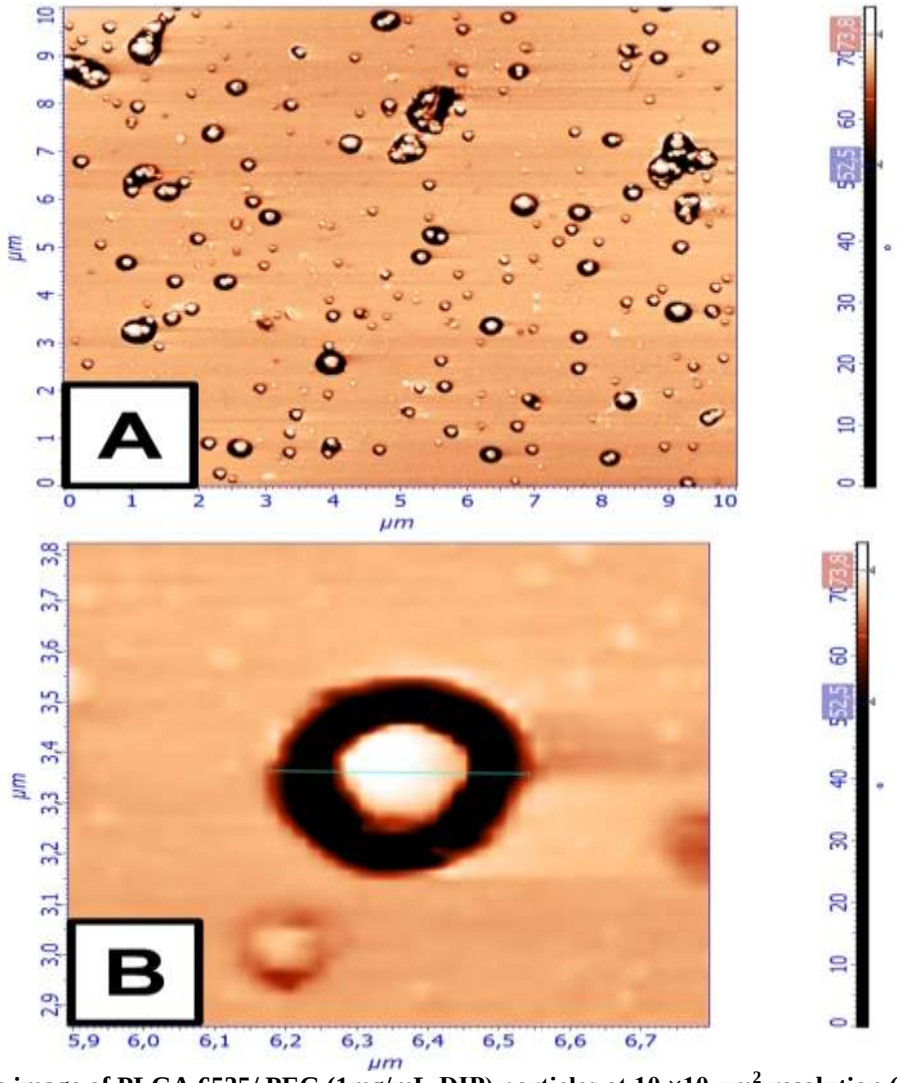

Fig-1: AFM phase image of PLGA 6535/ PEG (1mg/mL DIP) particles at 10 ×10 µm^2 resolution (A); AFM phase image of PLGA 7525/ PEG (1mg/mL DIP) particles at 1×1 µm^{2}resolution (B)

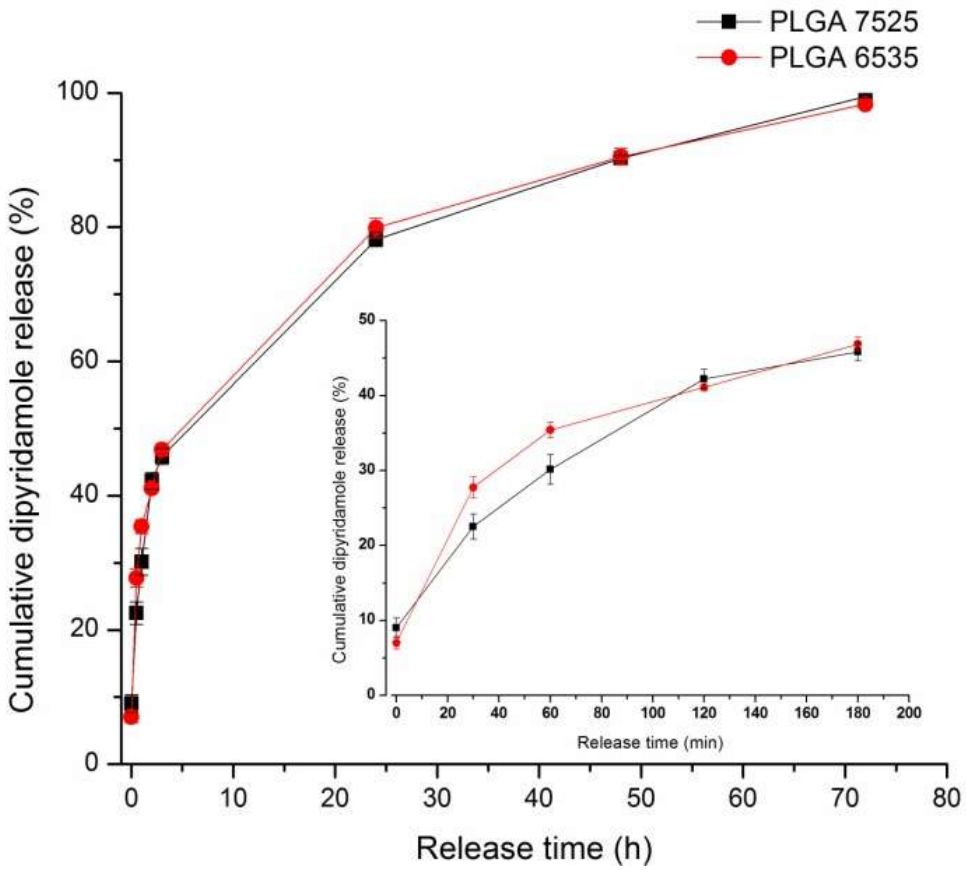

Fig-2: Cumulative in vitro drug release profiles of both PLGA 6535/PEG (red) and PLGA 7525/PEG (black) particles in PBS (pH 7.4, T = 37 ^{o}C), loaded with 1 mg/mL DIP

CONCLUSION

PLGA/ PEG round-shaped particles were successfully fabricated with a diameter below 170 nm that exhibited a surface coating of PEG, which affected the release kinetics of the encapsulated DIP. The release mechanism of DIP was primarily Fickian diffusion. In a nutshell, PLGA/PEG particles have to be further studied as potential DDS since they exhibited promising attributes.

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