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Encapsulation of Zn-DTPA into poly lactic-*co*-glycolic acid nanoparticles via a modified double emulsion method for extended release into lung fluid

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Abstract

Diethylenetriaminepentaacetate (DTPA) is an approved chemical agent for decorporation of internalized actinides. During a radiological or nuclear incident, inhalation of radioactive material into the lungs is one of the main paths of hazardous exposure. High affinity of DTPA toward actinides makes it a suitable actinide decorporation agent that accelerates their excretion from the human body. Polymeric nanoparticles are ideal for use in prolonging drug release, as they are biocompatible, non-toxic, and have been approved for therapeutic use by the Federal Drug Administration. Poly lactic-co-glycolic acid (PLGA) was chosen for this research as previous literature has shown that PLGA NPs would delay the drug release of other therapeutic agents. PLGA NPs encapsulating Zn-DTPA were synthesized for the first time to extend the DTPA release in human lungs. Since the physicochemical properties of a particle such as its size and functional groups can influence its uptake by lung cells and tissues *in vivo*, the synthesized PLGA NPs encapsulating Zn-DTPA were characterized by transmission electron microscopy, Fourier-transform infrared spectroscopy, and dynamic light scattering. The loading capacity of Zn-DTPA in the PLGA NPs achieved in this formulation was determined to be 10(±1)% (w/w) through use of Liquid Chromatography-Mass Spectrometry (LC-MS). Extended release of Zn-DTPA from the PLGA NPs into simulated lung fluid was confirmed via dialysis experiments using continuous Ultraviolet (UV) absorbance monitoring. LC-MS was also used to demonstrate the extended release of DTPA from these PLGA NPs; a significantly longer half-time was achieved compared to that obtained for Zn-DTPA itself. As the dialysis lung fluid volume increased, longer half-times were observed for both Zn-DTPA and Zn-DTPA encapsulated by PLGA NPs. Furthermore, as expected, decreasing the concentration of Zn-DTPA in dialysis experiments increased the half-time. The purpose of this study was to extend the drug release through an optimized formulation of DTPA encapsulated PLGA NPs as compared to the free DTPA treatment *in vitro* study.

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Introduction

Over the past two decades, pharmaceutical research has focused on developing new drug delivery systems that offer high therapeutic potential by delivering the drug effectively at the target site to enhance both treatment efficacy and drug tolerability [1,2]. Drug delivery with nanoparticles enables the medicine to be released over a long period of time [3,4]. Thanks to their controllable physical properties, synthetic polymeric nanoparticles can improve drug delivery by releasing therapeutic agents over long time periods without causing toxic effects [5,6]. These physical properties, such as nanoparticle size and stability, can be controlled through optimization of various synthesis parameters such as drug to polymer mass ratios, organic solvents, and surfactants [7]. The U.S. Food and Drug Administration and the European Medicines Agency have approved the use of polymeric nanoparticles for therapeutic drug delivery [8,9]. Among various synthetic polymers, Poly lactic-co-glycolic acid (PLGA) is widely used in the preparation of nanoparticles (NPs). PLGA exhibits attractive properties including low cytotoxicity, high biocompatibility and sustain drug release for extended period [10]. Different forms of PLGA NPs can be prepared by varying the ratio of lactic acid and glycolic acid, the two monomers that form PLGA [11]. PLGA NPs have been applied successfully for the extended drug release of anticancer drugs [12]. Although PLGA NPs have been used successfully in various medical applications, formation of reproducible PLGA NPs remains a challenge [13]. In this work we report an optimized condition for the preparation of Zn-DTPA encapsulated PLGA NPs.

Radioactive actinides such as thorium 232Th, Uranium 238U, Plutonium 239Pu and Americium 241Am can enter the human body during nuclear accidents. The internalized actinides would continue to decay, emitting ionizing radiation that may cause cancer or other severe damages to the biological system. Removal of these contaminants requires decorporation therapy [14,15]. An effective decorporation agent should exhibit several properties under physiological conditions, including (i) functional groups that can chelate the actinides with high selectivity, (ii) low affinity to elements essential to the human body, (iii) good biocompatibility, (vi) availability to the contaminated individuals shortly after a nuclear incident [16-18].

An effective decorporation agent of actinides is Diethylenetriaminepentaacetate (DTPA) in its sodium, calcium, or zinc forms [19]. It is usually administered through injection, but due to low retention of the agent in the human body, frequent injections of DTPA are needed which can cause severe side effect through depletion of essential metals in the body [20,21]. DTPA binds with actinides forming stable complex structures which can be excreted rapidly from the human body via urine [22]. A previous study showed that an early treatment with DTPA resulted in a marked decrease of inhaled soluble Pu and Am in the livers and bones of rats [23,24].

Inhalation drug delivery can be considered as the best way of treating lung contamination, which enhances the drug bioavailability in the lungs, compared to other systemic methods [25,26]. Particle size plays a significant role in whether the particles can access the deposition site in the lower respiratory system (including bronchioles and alveoli) for the removal of the contaminants [27]. In order to reach the lower respiratory system, the aerodynamic diameter of inhalable nanoparticles must be within an optimal size range of 1-5 µm. Particles with sizes larger than 5 µm tend to deposit in the mouth, hence decreasing the therapeutic dose that reaches the lungs. On the other hand, particles smaller than 1 µm may escape from the lower respiratory tract during the exhalation cycle [28,29].

Mucus, consisting mostly of water and protein, creates a tangled network of gel-like substance within the lungs. [30]. When drug molecules are administered by inhalation through the mouth or nose, they must pass through the mucosal surface in order to reach the underlying cells; however most drugs interact with mucus which prevents their delivery into the target site, resulting in less therapeutic effects. As such, developing a new therapeutic delivery system that can pass through mucus without any interaction is demanded for better therapeutic efficacy [31]. PLGA NPs coated with PEG showed a rapid penetration through mucus *in vitro* studies [32].

Different methods including sample and separate, continuous flow and dialysis membrane have been used to determine the release rate of drug from nanoparticles [33]. Dynamic dialysis is the most common *in vitro* method to evaluate drug release from nanoparticle delivery systems [34]. The donor compartment contains nanoparticles in the donor fluid, which pass through the dialysis membrane into the receptor compartment. The increase of drug concentration in the acceptor lung fluid could be used to estimate the drug release kinetics [35,36]. However, dialysis can also lead to inaccurate data interpretation about nanoparticle release half-time, simply due to the dual barriers within the dialysis setup [37]. Drug release kinetics is important for drug carrier design and drug development since it advances our understanding of dosage form behavior, and defines a parameter to evaluate the safety and efficiency of drug administration. *In vitro* release kinetics is being vastly used to evaluate *in vivo* behavior and to test drug carrier performance [38].

To address the issue with the depletion of essential metal-requiring biomolecules that can be resulted from the long-term use of a chelation agent such as DTPA, Zn- DTPA encapsulated PLGA NPs (for sustainable drug release) was prepared via double emulsion method in this study. The prepared Zn-DTPA encapsulated PLGA NPs were characterized by various instrumental analysis techniques. Dialysis and individual sample methods were used to study the drug release from the PLGA NPs in vitro. The PLGA NPs exhibited a sustained release of Zn-DTPA. Binding capacity of PLGA NPs was determined by LC-MS analysis.

Materials

Poly lactic-co-glycolic acid (PLGA) (lactide:glycolide ratio of 50:50, MW: 24,000-38,000), acetone (ACE), Diethylenetriaminepentaacetic Acid (DTPA-H_5), Dichloromethane (DCM), Tween 80, and polyvinyl alcohol , magnesium chloride, potassium chloride, sodium chloride, sodium citrate dehydrate, sodium hydrogen carbonate, sodium hydroxide, sodium sulfate, dihydrate sodium acetate, disodium hydrogen phosphate, and zinc acetate were purchased from Sigma-Aldrich, and were ACS grade or greater (Millwaukee, Wisconsin, USA). Pentetate zinc trisodium (Zn-DTPA) (200 mg/mL) was purchased from Heyl Chemisch-pharmazeutische Fabrik, Berlin. Regenerated cellulose dialysis membrane (MWCO: 6-8 kDa) was purchased from Thermo Fisher Scientific (Waltham, Massachusetts, USA). Distilled deionized water was used throughout this work.

Preparation of Zn-DTPA encapsulated PLGA nanoparticles

Double-emulsion (water-in-oil-in-water (W/O/W)) solvent evaporation/diffusion technique was used to prepare the Zn-DTPA encapsulated polymeric nanoparticles [39]. Two hundred

mg of PLGA was dissolved in 10 mL of an organic mixture of acetone and dichloromethane with 1:1 (v/v) ratio containing Tween 80 (5% by volume) as an emulsifier. Zn-DTPA (500 mg) was dissolved in 2 mL of distilled deionized water, and homogenized with the polymer solution by sonication for 5 minutes. The obtained primary W/O emulsion was further added to 40 mL of water with homogenization (for 5 minutes) to achieve the stable double emulsion (W/O/W). The resulting emulsion was added gradually into 200 mL of water containing polyvinyl alcohol 0.1% (w/v) as a surfactant under stirring. Stirring was continued for 4 hours to solidify the nanoparticles and evaporate the remaining organic solvents [40,41]. The nanoparticles were collected along with 10 mL of residual supernatant after centrifugation at 600 rpm for 15 minutes at 18 °C. The products were dried by lyophilization in a Labconco model 7753020 freeze dryer (Kansas City, MO, USA) operating at a temperature of -53 °C. PLGA NPs as a control were prepared following the same above procedure in the absence of Zn-DTPA.

Characterization of nanoparticles by transmission electron microscopy

Transmission Electron Microscopy (TEM) was used to characterize the PLGA nanoparticles and Zn-DTPA encapsulated PLGA nanoparticles on an FEI Tecnai G2 F20 microscope (Hillsboro, OR, USA) operating at 200 kV.

Characterization of nanoparticles by Fourier-transforms infrared spectroscopy

The chemical structures of PLGA NPs and Zn-DTPA encapsulated PLGA NPs were characterized using an ABB (Bomem MB series, Quebec, Canada) Fourier-transform infrared (FTIR) spectrometer. Disc samples were prepared by grinding 2 mg of nanoparticles with 200 mg of spectrophotometric-grade KBr. All FTIR spectra were obtained in the spectral region of 600-4000 cm^{-1}.

Characterization of nanoparticles by dynamic light scattering

Dynamic Light Scattering (DLS) was used to measure the hydrodynamic size distribution of PLGA NPs and Zn-DTPA encapsulated PLGA NPs suspended in 10 mM potassium nitrate buffer. DLS analysis of each suspension was performed using a Brookhaven Instruments nano DLS particle size analyzer (Holtsville, NY, USA). Each suspension was measured in ten replicates of 10 s each for high accuracy.

Determination of drug loading of Zn-DTPA

The content of Zn-DTPA in PLGA NPs prepared using Zn-DTPA was determined by LC-MS. After centrifugation, Zn-DTPA encapsulated PLGA NPs were collected, dried, and re-suspended in 0.1% formic acid to determine the amount of the drug in PLGA NPs. The concentration of Zn-DTPA in the PLGA NPs was calculated from a standard curve, prepared by known concentrations of Zn-DTPA.

Determination of Zn-DTPA concentration by drug release automatic monitoring system

The effect of different volumes of lung fluid inside the dialysis bags on the drug release rate was investigated on Zn-DTPA itself and Zn-DTPA encapsulated PLGA NPs. A drug release automatic monitoring system (DREAMS) was adapted from the design by Xie et al. to study the continuous release of Zn-DTPA from the PLGA NPs by measuring the increasing UV absorbance during dialysis [37]. In this system, a beaker (the acceptor compartment) contained a constant volume of lung fluid (120 mL) kept under magnetic stirring at 37±1°C. A dialysis bag (the donor compartment) containing either Zn-DTPA or Zn-DTPA encapsulated PLGA NPs dispersed in various volumes of lung fluid (0.5, 5, 7, 10 and 15 mL) was immersed in the acceptor compartment. A mini pump transported the lung fluid from the acceptor compartment to the UV detector. As the Zn-DTPA concentration increased in the acceptor compartment due to the dialysis from the donor compartment, the UV absorbance increased proportionally.

The effect of different concentrations of Zn-DTPA on the release half-time

Several quantities of Zn-DTPA (3, 10, and 20 mg) were dispersed in 6 mL lung fluid at a concentration of (0.5, 1.7, and 3.3 mg/mL) respectively inside dialysis bag that were then immersed in 120 mL of lung fluid in a beaker to study the effect of different concentrations of the plain drug on the release half-time. A DREAMS was used for this experiment. The release half-times of different concentrations of Zn-DTPA were compared with each other based on their UV detector signals at 10 hours since their signals at 10 hours were assumed to be at equilibrium (no changes observed afterward).

***In vitro* release of Zn-DTPA encapsulated PLGA nanoparticles via dialysis**

Gamble's solution was prepared with pH 7.4 for use as Simulated Lung Fluid (SLF) for in vitro release tests [42]. Zn-DTPA encapsulated PLGA NPs were dispersed in 6mL of SLF, and the dispersion was transferred into a dialysis membrane tube. Subsequently, the dialysis membrane tube was immersed in a beaker containing 120 mL of SLF. The dialysis process was performed at 37±1°C under magnetic stirring. A DREAM has been used during this release test experiment.

***In vitro* release of Zn-DTPA encapsulated PLGA nanoparticles individual sample method**

Zn-DTPA encapsulated PLGA NPs (266mg) were dispersed in 32 mL lung fluid (Gamble's solution), and kept under stirring for 5 minutes. Then, 0.4 µL of the dispersed nanoparticles was transferred into 1.5 mL centrifugation tubes. At appropriate time intervals, the dispersed NPs were centrifuged, and 20 µL of the supernatant was transfer into a vial, and diluted with 100 µL of iron (III) chloride solution at 5 mg/mL and 880 µL of 0.1% formic acid to obtain 1 mL as a total volume [43]. Samples were complexed to $Fe\text{-}DTPA^{2-}$ due to the highest affinity of $DTPA^{5-}$ towards the Fe3+ metal ion compared to various metal ions such as Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} that are present in the lung fluid [44]. The *in vitro* release test was conducted for 16 hours. Liquid Chromatography- Mass Spectrometry (LC-MS) (single ion monitoring) was used to determine the concentration of Zn-DTPA released from PLGA NPs and Zn-DTPA standard solutions upon the formation of $Fe\text{-}H_2DTPA$, at m/z 445. A C18 column was used in LC (50 mm x 2.1 mm, 1.8 micron) maintained at room temperature. The mobile phase was prepared by mixing 98% of the solvent A (0.1% formic acid in ultrapure water) and 2% of the solvent B (100 % acetonitrile) at a flow rate of 0.4 mL/min. Mass spectrometric analysis was performed using an Agilent Technologies model 6460 triple quad MS/MS system equipped with an Electrospray Ionization (ESI) source operating in the negative mode. The operating parameters were: nitrogen gas flow rate = 9.8 L/min, gas temperature = 300°C, nebulizer

pressure = 15 psi, capillary voltage = 4000 V, fragmentor voltage = 135 V, and cell accelerator voltage = 7 V.

Results and Discussion

The resultant Zn-DTPA encapsulated PLGA NPs were characterized and examined for extended drug release in the *in vitro* study. They were characterized to determine if there was any difference between those with encapsulated Zn-DTPA and those without (as a control), in terms of their TEM morphologies and DLS size distributions. The PLGA NPs exhibit a particle size of 88±5 nm as shown in the TEM images (Figure 1). The Zn-DTPA encapsulated PLGA NPs showed a significant increase in the particle size (105±7 nm). This increase could be attributed to the encapsulation of Zn-DTPA in the PLGA NPs. As can be seen, PLGA NPs and Zn-DTPA encapsulated PLGA NPs exhibited a spherical shape.

The size of polymer droplet depends on the sonication power. High sonication power would lead to the formation of smaller particle sizes, as the organic and aqueous phases are totally homogenized [11]. The PLGA NPs and Zn-DTPA encapsulated PLGA NPs exhibited large hydrodynamic diameters of 472.4 and 643.4 nm, respectively, at a sonication power of 100 W (100% of power). As the sonication power was increased (300 W at 50%) using an ultrasonic homogenizer with a probe, the PLGA NPs exhibited a smaller hydrodynamic size of 331 nm. The Zn-DTPA encapsulated PLGA NPs showed significant increases in their hydrodynamic diameters compared to the PLGA NPs that were prepared at an identical sonication power, as listed in Table 1. It could be implied that the encapsulation of Zn-DTPA into the PLGA NPs would result in increasing the hydrodynamic size [11]. The size of Zn-DTPA encapsulated PLGA nanoparticles (30-500 nm in diameter) was optimal for trapping by, and penetration through, mucus in the lungs [45].

Figure 2 shows the FT-IR spectra of Zn-DTPA, PLGA, and Zn-DTPA encapsulated PLGA. The broad strong characteristic peak at 3425 cm^{-1} corresponds to N–H stretching for the pure Zn-DTPA (2a) and a peak at 2940.4 cm^{-1} is originated from C-H stretching vibration. The strong characteristic peak at 1757 cm^{-1} corresponds to C= O stretching for the PLGA (2b). The FT-IR spectra of the Zn-DTPA-encapsulated polymeric nanoparticles (2c) shows a peak at 3497 cm^{-1} which could be assigned to N–H stretching vibration. The band in the PLGA at 2953 cm^{-1} corresponding to the C-H stretching vibrations shifted to 2921 cm^{-1} in Zn-DTPA encapsulated PLGA. The band at 1442.8 cm^{-1} in the PLGA corresponding to C-H bending shifted into 1458.9 cm^{-1} in Zn-DTPA encapsulated PLGA. The Zn-O band in the Zn-DTPA appeared at 777.2 cm^{-1} and shifted to 729 cm^{-1} in the Zn-DTPA encapsulated PLGA. No significant shifts were observed for other bands, which suggests the encapsulation of Zn-DTPA into the nanoparticles via physical interaction.

The encapsulation of hydrophilic drugs into the polymeric nanoparticles via the double emulsion method exhibit several limitations such as low drug loading and low encapsulation efficiency. The hydrophilic drugs penetrate rapidly from the inner aqueous phase to the external aqueous phase resulting in low encapsulation. To overcome these limitations, a modified double-emulsion solvent diffusion/ evaporation technique was used in which acetone was a co-solvent. Due to the miscibility of acetone in water, it can diffuse from the inner to the external aqueous phase inducing nanoparticle solidification. This prevents the hydrophilic drug to penetrate to the external phase leading to higher drug loading [38].

The prepared PLGA NPs exhibited a drug loading capacity of (10.6 ± 0.7)% based on LC-MS analysis, and the capacity was calculated from the following equation:

Drug loading (%) = (mass of trapped drug in the nanoparticles/ mass of polymeric nanoparticles) × 100 (Equation 1)

The drug released in lung fluid was monitored at a wavelength of 210 nm as Zn-DTPA showed the highest absorbance at this wavelength. Figure 3 shows that changing the volumes inside the dialysis bags influenced the drug release rates in both plain and Zn-DTPA released from PLGA NPs. As the inside volume increased, longer half-times were observed. This could be attributed to the longer diffusion distances for DTPA molecules inside larger lung fluid volumes. Therefore, the optimum volume of lung fluid inside the dialysis bag, which had less effect on the drug release rate, was obtained in order to find an accurate half-time. Increasing the inside volume from 0.5 to 15 mL increased the half-time of the plain drug from 7 to 25 minutes while the half-time of the Zn-DTPA released from the PLGA NPs increased from 21 to 45 minutes at the same volumes. This proved a delayed and extended drug release from the PLGA NPs.

Figure 4 shows the drug release of plain Zn-DTPA at concentrations of 0.5, 1.7, and 3.3 mg/mL. As the concentrations of the drug inside the dialysis bag increased from 0.5 to 3.3 mg/mL, the half-time corresponding UV signal (when half of the Zn-DTPA was released) decreased from 2.3 to 1.6 hour resulting in an average release half time of 2 ± 0.4 hours. The diffusion rate of the drug was reduced when the concentrations of Zn-DTPA decreased leading to longer release half time.

In vitro drug release

There are mainly three possible mechanisms for the drug molecules to be released from PLGA NPs used in the drug delivery system including diffusion through water-filled pores, diffusion through polymer matrix, and dissolution of the encapsulating polymer [46]. In order to control the drug release, it is very important to determine factors that affect the drug release rate. Several factors influence the drug release rate from polymeric nanoparticle, namely environmental conditions, physico-chemical processes within PLGA matrices, polymer-drug interaction, polymer-polymer interaction, or a combination of these factors [42].

Encapsulation of ZnDTPA into PLGA NPs did not alter the drug structure since the released drug formed FeH_2DTPA that was detected by LC-MS (single ion monitoring) at the same molecular ion (m/z = 445). Therefore, the chemical structure and function of DTPA remained unchanged, and the encapsulation should not reduce its therapeutic efficacy in decorporating actinides. For instance, Ca-DTPA had previously been encapsulated into liposome (or lipid nanoparticles) and showed even higher efficacy for the removal of actinides in animal experiments compared to that of the free DTPA [47]. The liposomes improved the therapeutic efficacy through an extended drug release resulting in a gradual decorporation and removal of actinide residues from patient organs. [48]. Administration of therapeutic agents via inhalation can result in higher distribution of the drugs in the lungs compared to other routes. However, inhalable therapeutic agents may be eliminated rapidly by alveolar macrophages upon deposition into the lungs [45]. Also, encapsulation of the anti-inflammatory drug PS341 into PLGA NPs increased its bioavailability and half-life upon accumulation in the lungs compared to the plain PS341 [49].

Figure 5 exhibits the drug release profile Zn-DTPA encapsulated PLGA NPs for a drug amount of 30 mg at a ratio of 2.5:1 (w/w). The *in vitro* drug release experiment in the simulated lung fluid was performed for 16 hours via dialysis at pH 7.4 and 37°C in order to test the extended drug release of PLGA NPs. The drug release profiles exhibit a fast release within the first hour, followed by a sustained release over the next 15 hours. However, the extended drug release could also be due to the use of the dialysis membrane and not only due to the PLGA NPs. As such, to confirm the extended drug release of PLGA NPs, individual sample method was used as an alternative method. In this method, the Zn-DTPA encapsulated PLGA was dispersed into lung fluid without any barrier (like a dialysis membrane), and the sample was centrifuged at different time, and analyzed to assess the release of Zn-DTPA from PLGA NPs. Figure 6 shows the drug release profile of Zn-DTPA encapsulated PLGA NPs from an individual sample method. The free Zn-DTPA, which left in the supernatant before freeze-drying process can account for the initial rapid rise in DTPA concentration. This free Zn-DTPA could serve as an urgent dose for victims after a nuclear accident, followed by a slow and sustained release of encapsulated Zn-DTPA from the PLGA NPs over 10 hours. After that, the Zn-DTPA concentration did not change significantly in the next 16 hours. The half time of Zn-DTPA released from PLGA NPs was 3.5 hours, which confirmed extended drug release from the PLGA NPs.

PLGA was not used as a control during the drug release because LC-MS analysis was performed in the single ion-monitoring mode to detect only Zn-DTPA as FeH_2DTPA (at m/z 445) after PLGA nanoparticles were sedimented by microcentrifugation. Alternatively PLGA NPs with encapsulated Zn-DTPA were placed in a dialysis bag and only Zn-DTPA could diffuse through the membrane to reach the acceptor compartment for UV detection. Thus PLGA NPs could not interfere with the UV detection of any released Zn-DTPA. PLGA is safe and nontoxic after hydrolysis in vivo; the US Food and Drug Administration have approved it [50]. Unloaded PLGA NPs exhibited negligible cytotoxicity in *in vitro* studies wherein mouse embryonic fibroblasts NIH3T3, a highly sensitive cell line, were treated with PLGA nanoparticles [51]. Their health was monitored by a resazurin-based assay [52]. PLGA NPs of various sizes remain in the mice lungs for seven days without any clearance but they did not trigger the immune system.

Conclusion

To the best of our knowledge, PLGA NPs were employed for the first time for the encapsulation of Zn-DTPA, a hydrophilic drug. The double-emulsion solvent diffusion/evaporation technique was used successfully for the encapsulation of Zn-DTPA into PLGA NPs. The Zn- DTPA encapsulated PLGA NPs exhibited a spherical shape with a mean diameter of 105±7 nm and a hydrodynamic size of 372±5 nm. Several characteristic peaks of Zn-DTPA in the FTIR spectra of the Zn-DTPA encapsulated PLGA NPs revealed the encapsulation of Zn-DTPA into the PLGA NPs. The concentration level of the drug inside the dialysis bag would affect the release half-time. These polymeric NPs were synthesized and applied for the first time for the extended drug release of hydrophilic drug *in vitro* and exhibited a drug loading capacity of 10.6% by weight. DTPA release from nanoparticles was found to be significantly slower compared to the plain drug. At this stage, our research mainly focused on the preparation of different types of nano-carriers for the extended release of Zn-DTPA. However, more research is needed to investigate the performance of Zn-DTPA encapsulated PLGA NPs in *in vivo* experiments and also to examine the effects of modification of the PLGA NPs on loading capacity and release kinetics of the drug. The carrier that exhibits an optimally extended drug release profile will be examined further through release experiments in cell lines and animal pharmacokinetic models with comparison to the plain drug.

Figures

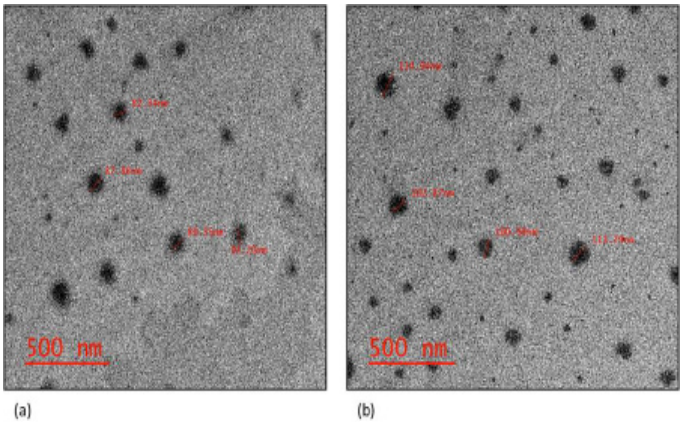

Figure 1: TEM images of (a) PLGA nanoparticles, and (b) Zn-DTPA encapsulated PLGA nanoparticles.

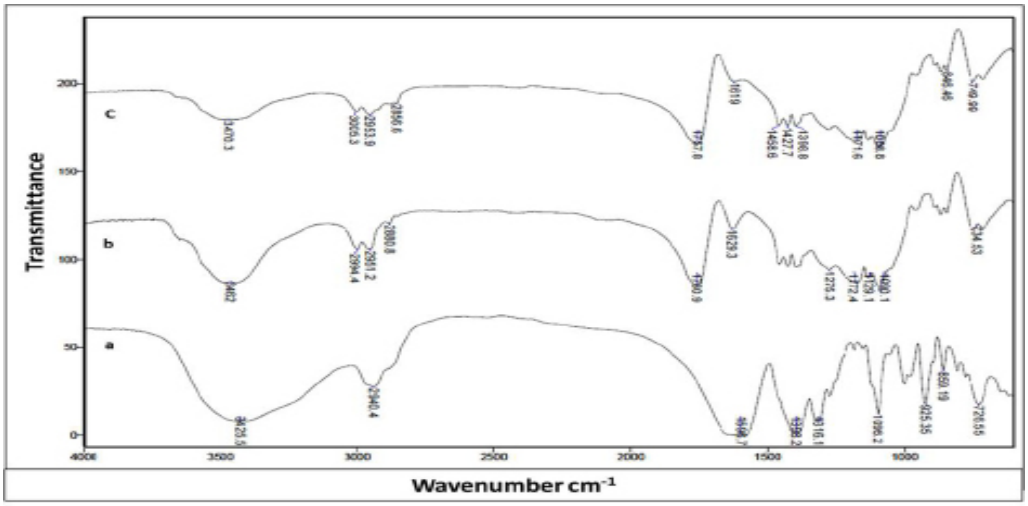

Figure 2: FTIR spectra of (a) Zn-DTPA, (b) PLGA, and (c) Zn-DTPA encapsulated PLGA nanoparticles.

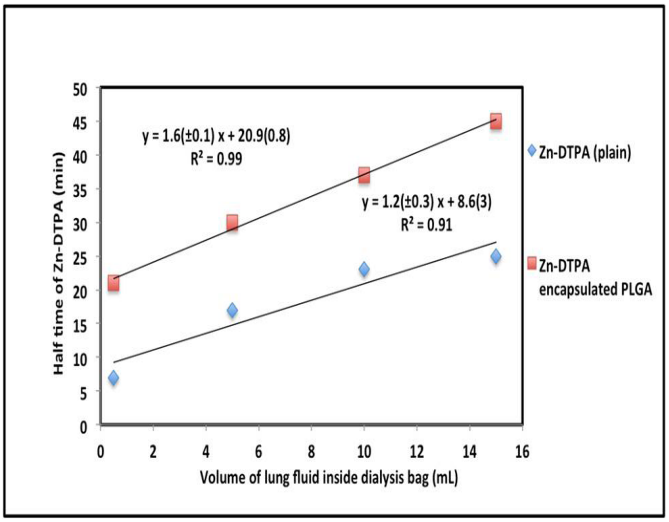

Figure 3: Effect of lung fluid volume inside the dialysis bag on half time of plain Zn-DTPA, and Zn-DTPA encapsulated PLGA Nanoparticles

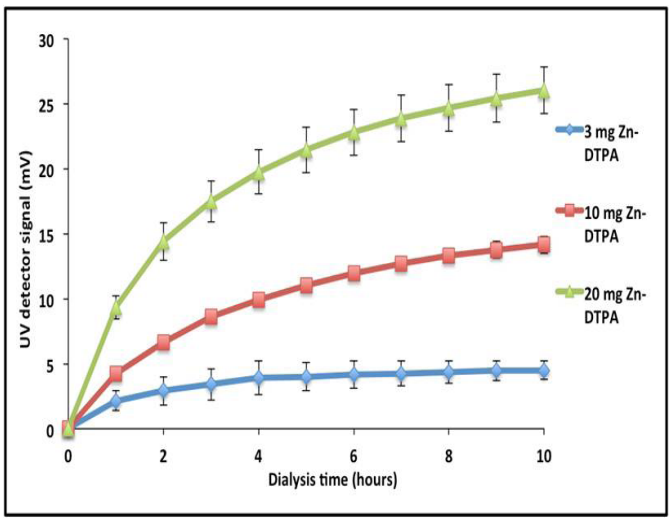

Figure 4: Effect of different quantities of Zn-DTPA on the release profiles.

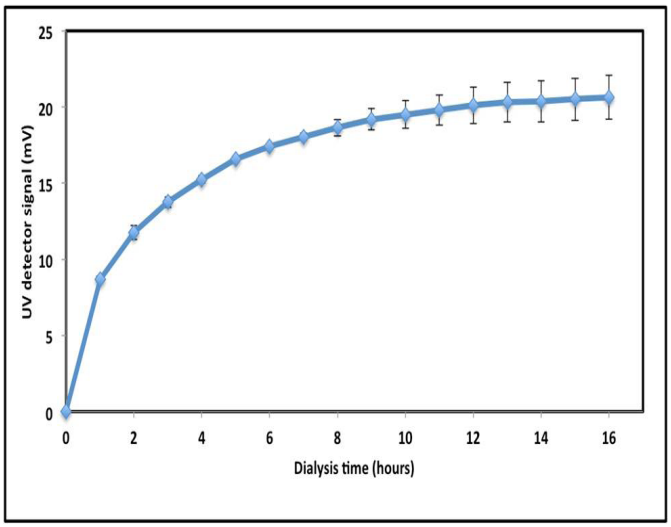

Figure 5: Drug release profile of Zn-DTPA encapsulated PLGA nanoparticles from the dialysis method with UV detection.

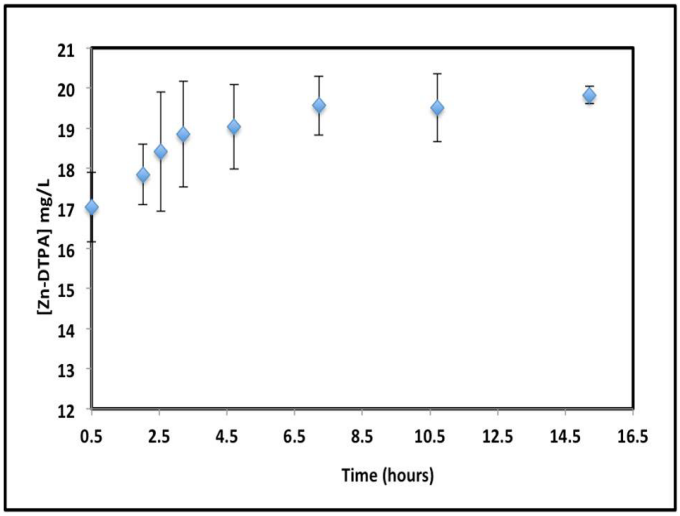

Figure 6: Drug release profile of Zn-DTPA encapsulated PLGA nanoparticles from the individual sample method with LC-MS detection.

Table

Table 1: Hydrodynamic diameters of PLGA nanoparticles and Zn-DTPA encapsulated PLGA nanoparticles. Data are given as mean ± SD (n=10)

Sample	Hydrodynamic diameters (nm)	Poly-dispersity
PLGA NPs (low intensity sonication)	427±32	0.334
Zn-DTPA encapsulated PLGA NPs (low intensity sonication)	643±54	0.312
PLGA NPs (low intensity sonication)	331±6	0.259
Zn-DTPAencapsulated PLGA NPs (low intensity sonication)	379±10	0.276

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