

Research Article

Comparative Drug Release Studies of 5-Fluorouracil and 6-Thioguanine Entrapped in Peg-Co-Pla/Gold Nanocapsules

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Abstract

Poly lactic acid-polyethylene glycol (PLA-co-PEG) copolyester was synthesized from oligomer of L-lactic acid and poly ethylene glycol (PEG) using stannous octoate as catalyst. 5-Fluorouracil and 6-Thioguanine containing Poly lactic acid-polyethylene glycol (PLA-co-PEG) nanocapsules were prepared in the presence and absence of gold nanoparticles via the W/O/W emulsification solvent-evaporation method. The morphologies of prepared nanocapsules were observed by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The structure of copolymer was confirmed using Nuclear magnetic resonance spectroscopy. From SEM and TEM measurements, the average size of the polymer nanocapsules and gold nanoparticles were found to be in range of 230-260 nm and 18-20 nm respectively. In general the drug release was quicker in Phosphate buffer saline (pH 7.4) compared to 0.1M hydrochloric

acid and this may be due to higher solubility, higher swelling and penetration properties of PLA-co-PEG in PBS compared to HCl. Drug release was found to be slower in 6-Thioguanine due to the hydrophobic nature of the drug. The drug release was found to be 98% for Fluorouracil and 90% for Thioguanine in 0.1M Phosphate buffer medium. Polymer nanocapsules with gold show a prolonged controlled release with higher encapsulation efficiency compared to that of polymer nanocapsules in the absence of gold nanoparticles. Well controlled and sustained release was obtained in 0.1M HCl and the order of release for anticancer drugs was found to be 33% for Thioguanine, 60% for Fluorouracil. It may be due to the more entrapping efficiency of gold and less diffusivity of drugs from the nanocapsules.

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Introduction

Introducing polyethylene glycol (PEG) into poly(l-lactide) homopolymers can increase the degradation rate, decrease the acidity of the degraded products, and increase the hydrophilicity of the polymer carriers. Hydrophilic PEG segments in PLA copolymers may also enhance the diffusivity of water or drugs in polymer carriers. The non-toxic nature of PEG is certainly a great advantage in utilizing it as a component of PLA for medical purposes. Diverse drugs with a hydrophobic and hydrophilic (Govander et al 2000, Jie Ren et al 2007) in nature, antigens (Deng et al 1999) can be loaded with high efficacy in to the core, allowing them to be soluble in aqueous media. Block copolymers of enantiomeric PLA-PEG and PDLA-PEG (Chen et al 2007) were also used for drug release applications.

However, in recent years additional polymers designed primarily for medical applications have entered the area of controlled release. Many of these materials are designed to degrade within the body, among them are polylactides (PLA) (Ciftci et al 1993, Proikakis et al 2006), polyglycolides (PGA), poly(lactide-co-glycolides) (PLGA) (Qurrat ul-Ain et al 2003), Methoxypoly(ethyleneglycol)-Poly(lactide) (MPEG-PLA) (Kim et al 1998, Dong and Feng 2007), polyanhydrides, polyorthoesters. Novel functionalized polymers were also been used for drug delivery application for their low toxicity, high efficiency and drug loading (Kallinteri et al 2005).

In the past 30 years, the explosive growth of nanotechnology has burst into challenging innovations in pharmacology, which is in the process of revolutionizing the delivery of biologically active compounds. Research into delivery and targeting of therapeutic and diagnostic agents with nanoparticles is at the forefront of nanomedicine for several reasons. First, traditional oral or injectable drugs are not necessarily the most efficient formulations for a given product. This is particularly true for new biologics such as proteins and nucleic acids that require novel delivery technologies to optimize efficacy, minimize side effects and lead to better patient compliance. Since the efficiency of drug delivery is directly related to particle size nanoparticle formulations can enhance

bioavailability, improve timed/controlled release of drugs and enable more precise targeting to the level of direct intracellular delivery.

Gold nanoparticles have demonstrated remarkable properties, displaying non-toxicity to human cells and biocompatibility (Hainfeld et al 2004, Rena and Chow 2003, Gu et al 2003). Coating nanometer sized colloidal gold particles completely alters the biodistribution of these particles, allowing them to find solid tumors and deliver therapeutic payloads while bypassing normal cells. This versatile drug delivery platform is able to bring potent anticancer drugs directly to the tumor. Hence Polymer nanocapsules were prepared using gold nanoparticles and their effect on morphology and drug release profiles were studied.

Materials and Methods

The precursor materials required for the synthesis of various nanomaterials like chloroauric acid ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, 98%), polyvinyl alcohol, stannous octoate were obtained from Aldrich. L-lactic acid, polyethylene glycol, tri sodium citrate (99%), methanol were obtained from SRL India. Hydrochloric acid, chloroform, dichloromethane, acetone etc., were obtained from SRL India. All chemical reagents were prepared with double distilled water. All the solvents used were laboratory grade and distilled prior to use. UV-Vis spectra of the nanoparticles samples were measured on a Perkin Elmer lambda Spectrophotometer. For size analysis, a drop of nanoparticles was suspended onto a copper coated grid. The grids were dried in a desiccator overnight and examined using a HR-TEM (JEOL) with an accelerating potential of 120 kV. A JEOL JSM-6360 field emission scanning electron microscope was used and the samples were prepared by coating gold on the surface of the sample for SEM measurements.

Synthesis of poly lactic acid-co-poly ethylene glycol copolyester

Poly lactic acid-polyethylene glycol (PLA-co-PEG) copolyester was synthesized from oligomer of L-lactic acid and poly ethylene glycol (PEG) in the weight ratio 70/30 using stannous octoate as catalyst. The reaction was carried out at 160 °C using silicone oil bath for 24 hrs under nitrogen atmosphere. The resulting copolyester was dissolved in chloroform, and precipitated from methanol.

Preparation of citrate-capped gold nanoparticles

Trisodium citrate (38.8mM, 50 cm³) was added to a boiling HAuCl_4 solution (1 mM, 500 cm³). As a result, the previously yellow solution of gold chloride turned to wine red color and gave a characteristic absorbance at 518 nm in the UV-vis spectrum.

Preparation of polymer/drug@Au polymer nanocapsules

Nanocapsules were prepared by the solvent evaporation method similar to that previously reported (Beck et al 1979). For polymer/drug with gold nanoparticles experiment, drug (30 mg) was added in 5mL nanogold aqueous suspension (with a 30-minute shaking under ultrasound to help the adsorption of drug on gold nanoparticles) and was added to an organic polymer solution (400 mg of copolymer + 8mL CH_2Cl_2) under stirring condition. This was continued until the solvent was completely evaporated. The suspension became clear after all the nanocapsules precipitated out of the solution. These nanocapsules were collected by filtration and washed with deionized water to remove any undesirable residuals. Finally, the clean nanocapsules were dried in a vacuum oven at 40 °C for 24 hours to ensure a complete removal of the solvent and deionized water. All the nanocapsules were stored in a desiccator at 25 °C. Polymer/drug nanocapsules were also prepared under similar conditions.

Drug loading and Encapsulation efficiency

The drug loading of the nanocapsules was determined by first dissolving a precise amount of microcapsules (10 mg) in methylene chloride and then diluting the mixture with 0.1N hydrochloric acid. After the evaporation of methylene chloride and subsequent removal of copolymer, gold and iron oxide nanoparticle via filtration, the absorbance of the loaded drug was measured with a UV-vis spectrophotometer (Pignatello et al 2001). The measured absorbance was then converted to the weight of the drug based on the standard calibration curve, which was constructed with UV-vis spectrophotometer on 0.1N hydrochloric acid each containing a known amount of drug. Finally the percentage encapsulation efficiency of nanocapsules were calculated

Results and discussion

Transmission electron microscopy

From the TEM measurements, the average diameters of the gold nanoparticles were found to be in the range of 18–20 nm. Figure 2(a) and (b) shows TEM pictures of drugs (anticancer and antituberculosis drugs) capped gold nanoparticles. From the TEM

pictures, the aggregations of gold nanoparticles were observed which confirms the complex formation between drugs and gold nanoparticles. This can also be visualized by the colour changes from wine red to blue color.

Scanning Electron Microscopy

Figures 3 (a)-(d) show the SEM images of PLA-co-PEG/drug@Au, PLA-co-PEG/drug@Fe₃O₄ nanocapsules. The nanocapsules made from PLA-co-PEG have straight open channels. Scanning electron microscopy observations revealed that the shape of nanocapsules was spherical and few pores were observed on the surface of nanocapsules. From the SEM measurements, the size of nanocapsules was found to be in the range of 230-260 nm. The surface of nanocapsules appeared to be smooth and there was no evidence of collapsing. The surface of PLA-co-PEG nanocapsules was observed in the sinking area, due to the swelling characteristics of polyethylene glycol. The drug molecules adsorbed on the surface of the nanospheres prevented the coalescence of nanospheres. Therefore, it appears that PLA-co-PEG coating can ensure better stabilization. From the Figures 3 (a)-(d) it was confirmed that particles of nonporous spherical shape with smooth surface had been prepared. Nanocapsules prepared using gold nanoparticles was smooth, whereas nanocapsules prepared in absence of gold nanoparticles was bumpier and contains bigger pores. These observations reveal that the adsorption of drugs on gold nanoparticles slowed down its diffusion process

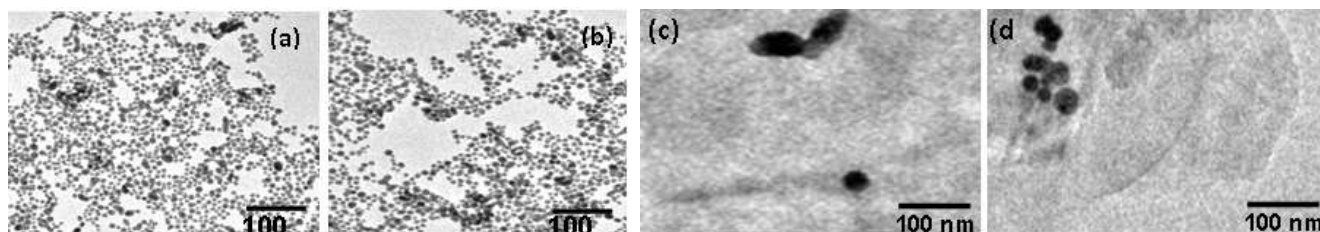


Figure 2 TEM pictures of drugs capped gold nanoparticles (a) TG@Au, (b) FU@Au, (c) TEM images of PLA-co-PEG/FU@Au and (d) TEM images of PLA-co-PEG/TG@Au nanoparticles

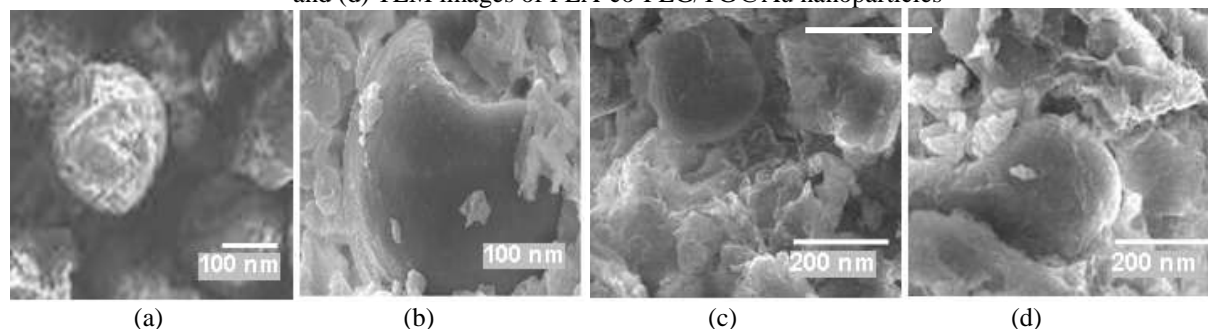


Figure 3 (a) SEM images of PLA-co-PEG/TG (b) PLA-co-PEG/TG@Au, (c) SEM images of PLA-co-PEG/FU (d) PLA-co-PEG/FU@Au

From Figures 2(c) & (d), it was concluded that the penetrations of drug metal nanoparticles were observed during the process of encapsulation drugs, which evidence the presence of drugs in the nanocapsules.

DRUG RELEASE STUDY

The release profiles of anticancer drugs like 5-Fluorouracil and 6-Thioguanine from PLA-co-PEG nanocapsules in hydrochloric acid (0.1M) and phosphate buffer saline (pH 7.4) were shown in Figures 4 and 5. In general the drug release was quicker in PBS compared to 0.1M hydrochloric acid and this may be due to higher solubility of PLA-co-PEG in phosphate buffer medium and higher swelling and penetration properties of PLA-co-PEG in PBS compared to HCl. The hydrophilic nature of PLA-co-PEG copolymer may be due to higher hydration rate and swelling characteristics of the ethylene glycol. So, water easily interacts with the hydrophilic segments in the PLA-co-PEG copolymers and hydrates the spheres. As the copolymer takes more water it becomes porous which increases the diffusion rate of drug. Hydrophilic PEG segments in PLA-co-PEG copolymers may also enhance the diffusivity of water or drugs in polymer carriers. The nanocapsules of PLA-co-PEG take up more water as a result, the copolymer swells and becomes more porous. A more porous membrane increased the release rate of the drug. The release profile is indicative of diffusion-controlled release only over this time period.

With respect to anticancer drugs, the drug release was found to be 98% for Fluorouracil and 90% for Thioguanine in 0.1M Phosphate buffer medium. This is a relative release of drugs with respect to complete release of drugs in same polymer. The drug released in initial period is due to diffusion and burst process; there is perhaps a later degradation controlled release for a small fraction of the drug. This profile is due to the hydrophilic nature of the drug which does not permit encapsulation in the core. When gold nanoparticles were incorporated in drug encapsulated polymers, much better control of release was obtained in both the media. This may be due to the formation of smoother with smaller pores on the surface PEG-co-PLA/drug@Au nanocapsules. Well controlled and sustained release was obtained in 0.1M HCl and the order of release for anticancer drugs was found to be 33% and 60% for Thioguanine and Fluorouracil respectively (for 50 hrs).The encapsulation efficiency and drug loading were calculated for PLA-PEG nanocapsules and were found to be in the range 40% to 75% and 3.00 to 5.62% respectively and the various values are presented in Table 1.

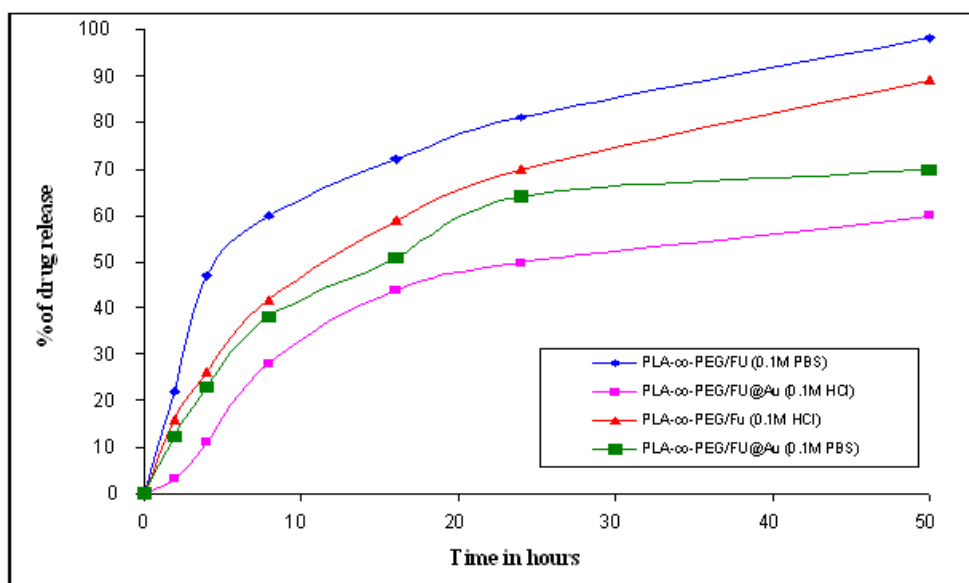


Figure 4 Drug release profiles for PLA-co-PEG/FU@Au in 0.1M PBS and 0.1M HCl

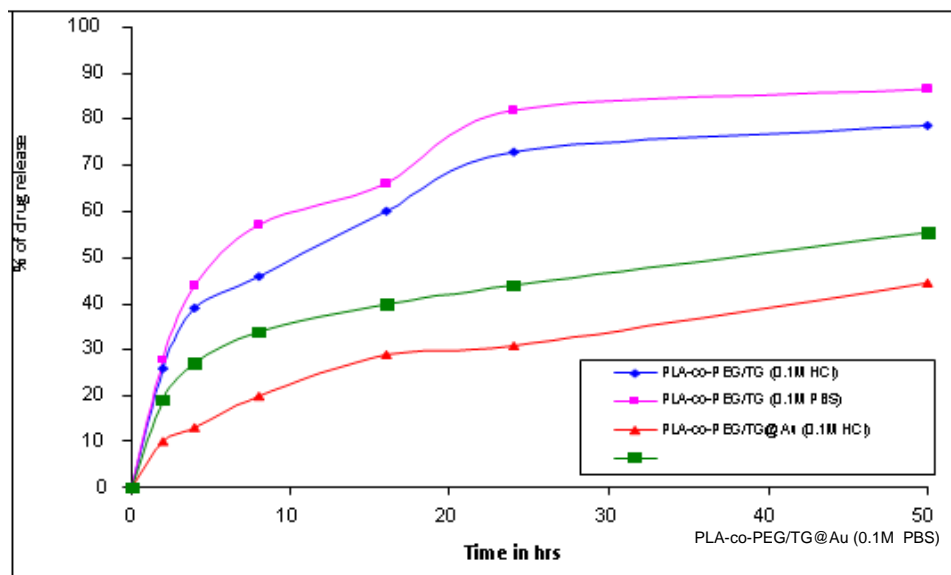


Figure 5 Drug release profiles for PLA-co-PEG/TG@Au in 0.1M PBS and 0.1M HCl

Table 1 Encapsulation efficiency and Drug loading of PLA-co-PEG nanocapsules

S.No	Nanocapsule	Drug (%)	loading	Encapsulation Efficiency (%)
1.	PLA-co-PEG/FU	3.00		40
2.	PLA-co-PEG/FU@Au	4.70		65
3.	PLA-co-PEG/TG	3.37		45
4.	PLA-co-PEG/TG@Au	5.62		75

Conclusion

In this work, PLA-co-PEG nanocapsules containing fluorouracil and thioguanine were prepared by the emulsion solvent evaporation method. The morphology of the nanocapsules was characterized by Transmission electron microscopy. From TEM and SEM measurements, the average diameter of gold nanoparticles was found to be in the range of 18-20 nm and the size of nanocapsules was found to be in the range of 230-260 nm. When gold nanoparticles were incorporated in drug encapsulated polymers, well controlled and sustained release was obtained in both the media, namely phosphate buffer saline and hydrochloric acid (0.1M). Thioguanine showed a controlled drug release profile when compared to fluorouracil because of its hydrophobic nature.

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