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Research Article

KINETICS AND DRUG RELEASE STUDIES OF ISONIAZID ENCAPSULATED WITH PLA-CO-PEG/GOLD NANOPARTICLES

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ABSTRACT

PolyLactic Acid-PolyEthylene Glycol(PLA-co-PEG) copolyester was synthesized from oligomer of L-lactic acid and PolyEthylene Glycol (PEG) using stannous octoate as catalyst. Isoniazid containing PLA-co-PEG nanocapsules were prepared in the presence of gold nanoparticles by solvent evaporation method. Drug-polymer interaction studies were carried out using Fourier transform-infrared analysis. Scanning electron microscopic analysis was used to study the surface morphology of the nanoparticles. The particles were nearly spherical in shape with an average size of 200-250 nm. The diameter of gold nanoparticles was determined using TEM analysis and was found to be in order of 5-20 nm. Encapsulation efficiency (%) of isoniazid in PLA-co-PEG/gold nanoparticles showed considerable increase over nanocapsules containing no gold nanoparticles. Drug release profiles for nanocapsules were carried out in 0.1M phosphate buffer (pH 7.0) and 0.1M HCl solution. The drug release was found to be sustained and controlled for nanocapsules prepared with gold nanoparticles. Well controlled and sustained release was found to be in 0.1M HCl solution. The invitro drug release date was applied to various kinetic equations. The values of regression coefficient indicated that Higuchi matrix model was best fitted with the kinetic data of isoniazid.

Keywords: Encapsulation, Controlled, Nanocapsules, Buffer, Versatile

INTRODUCTION

Tuberculosis (TB), a chronic bacterial infection, causes more deaths worldwide than any other infectious disease[1]. TB is spread through the air and usually infects the lungs, although other organs are sometimes involved. Around 2 billion people, one-third of the world's population-are infected with the TB organism, Mycobacterium tuberculosis[2,3]. Although several treatment protocols for active TB are in wide use by specialists, and protocols sometimes change due to advancement in our understanding of optimal therapy, they generally share three principles the regimen must include several drugs to which the organisms are susceptible, the patient must take the medication on a regular basis, therapy must continue for a sufficient time.

Nanoparticle-based drug delivery systems have considerable potential for treatment of tuberculosis (TB). Nanostructured biomaterials, nanoparticles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity, and functionalizable structure[4]. The important technological advantages of nanoparticles used as drug carriers are high stability, high carrier capacity, feasibility of incorporation of both hydrophilic and hydrophobic substances, and feasibility of variable routes of administration, including oral application and inhalation[5]. Nanoparticles can also be designed to allow controlled (sustained) drug release from the matrix. These properties of nanoparticles enable improvement of drug bioavailability and reduction of the dosing frequency, and may resolve the problem of nonadherence to prescribed therapy, which is one of the major obstacles in the control of TB epidemics.

Gold nanoparticles have demonstrated remarkable properties, displaying non-toxicity to human cells and biocompatibility[6,7]. 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. Gold nanoparticle probes[8] were used as assays for Mycobacterium tuberculosis and mycobacterium tuberculosis complex from clinical specimens.

Controlled drug delivery occurs when a natural or synthetic polymer like PLG[9] is combined with a drug or other active agent in such a way that the active agent is released from the polymer in a predesigned manner. PolyLactic Acid (PLA) is a synthetic biodegradable polymer that finds application in tissue engineering, medical materials, drug carriers because of their biodegradability, good security, low immunity, and good mechanical strength[10]. However, PLA applications are limited due to its weak hydrophilicity, long degradation time, and low drug loading of polar drugs. Hence to improve the crystallinity and tensile strength, Poly (ethylene glycol) is used in block or graft copolymer synthesis with PLA. PLA-co-PEG also finds application in medicinal field as carriers for drug targeting.

Isoniazid is an antibiotic. Isoniazid drug is used to treat tuberculosis and other mycobacterial infections[11-14]. Isoniazid is the most effective antituberculosis drug currently available. The drug is characterized by short half life ranging from 1hr to 5 hr depending on the rate of metabolism. Isoniazid is metabolized in the liver by acetylation and dehydrazination. The rate of acetylation is genetically determined and subject to individual variation. INH is less permeated through the stomach and is mainly absorbed through the intestine because it occurs in the protonated form at acidic pH. Hence a drug formulation with controlled release of INH is required especially in the small intestine. Micro emulsions[15,16] containing isoniazid were formulated for drug delivery systems.

Hence an attempt has been made to synthesize PLA-co-PEG copolymer encapsulated with isoniazid containing gold nanoparticles, which may provide prolonged drug delivery. Finally the release kinetics of the nanoparticles was also carried out.

MATERIALS AND METHODS

Isoniazid, lactic acid, Poly ethylene glycol was purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4.3H_2O$, 98%), tri sodium citrate (99%), methanol and all other solvents used in these studies were purchased from SRL India. All chemical reagents were prepared with double distilled water.

Apparatus

Polymer samples were characterized by ^1H- NMR spectra recorded at 300 MHz using Bruker spectrometer and $CDCl_3$ as solvent. Sample was characterized by UV-vis spectrophotometry (Perkin-Elmer Lambda 25). The path length was 1cm and matched 1cm x 1cm cuvuttes were used. HR-TEM was undertaken employing a JEOL instrument with an accelerating voltage of 120 KV. FTIR spectroscopy was performed using a PE IR SPECTRUM ASCII PEDS 1.60 spectrometer and samples were presented as KBr pellets.

Spectra were acquired at room temperature at resolution of 4 cm^{-1}. 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 PLA-co-PEG copolymer

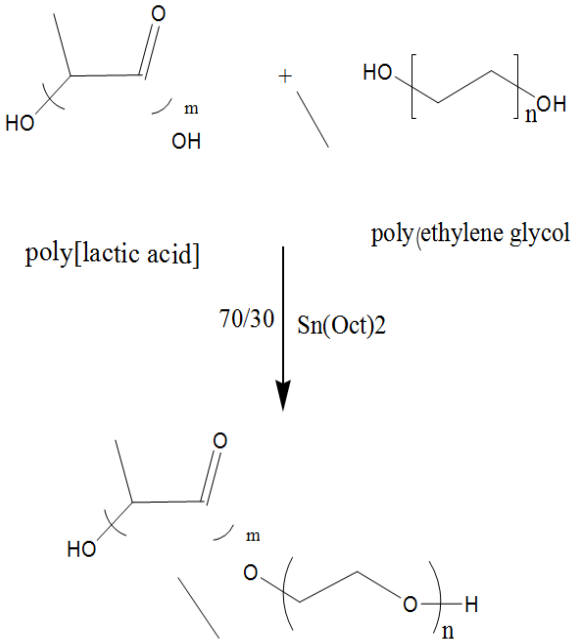
PolyLactic Acid- PolyEthylene Glycol (PLA-co-PEG) copolyester was synthesized (Scheme 1) from oligomer of L-lactic acid and PolyEthylene Glycol (PEG) in the weight ratio 70/30 using stannous octoate as catalyst. The reaction was carried out at 160°C using silicone oil bath for 24 hrs under nitrogen atmosphere. The resulting copolyester was dissolved in chloroform, and precipitated from methanol.

Preparation of Citrate -capped Gold Nanoparticles

5.0X 10^{-6} mol of $HauCl_4$ was taken, dissolved in 20 ml of deionised water. The faint yellowish solution was heated until boiling, while 1ml of 0.5% sodium citrate solution was added under vigorous stirring. It was stirred for the next 30 mins. The colour of the solution gradually changed from faint yellowish to wine red. Water was added to the solution to bring the volume back up to 20ml. It gives a characteristic absorbance at 518nm in the UV-vis spectrum.

Preparation of Nanocapsules

Nanocapsules were prepared by the solvent evaporation method. For PLA-PEG/INH-Au experiment, isoniazid (30mg) was added in 5ml nanogold aqueous suspension (ultrasonication was carried out for 30min to help the adsorption of isoniazid on gold nanoparticles) and the solution was preemulsified with an organic polymer solution (400mg PLA-co-PEG + 5ml Dichloromethane) for 5min through the stirring process. The resulting solution was emulsified with 300ml of water containing 1% PVA under stirring conditions (REMI RQ 126/D and 2000rpm). This was continued until the organic solvent was completely evaporated. The suspension became clear after all the nanocapsules precipitated out of the solution. These nanocapsules were collected and washed with deionized water to remove any undesirable residuals. Finally, the clean nanocapsules were dried in a vacuum oven at 40°C for 24hrs to ensure the complete removal of the organic solvent and deionized water. All the nanocapsules were stored in a desiccator at 25°C. PLA-co-PEG nanocapsules containing isoniazid were also prepared under similar conditions.



Scheme 1: Synthesis of PLA-co-PEG copolymer

Drug loading and Encapsulation efficiency

The drug loading of the nanocapsules was determined by first dissolving a precise amount of nanocapsules (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 nanoparticle via filtration, the absorbance of the loaded drug was measured with a UV-vis spectrophotometer. 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 was calculated as follows:

$$\text{Actual drug loading (\%)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of nanoparticles}} \times 100$$

$$\text{Encapsulation efficiency (\%)} = \frac{\text{Mass of drug in nanoparticles}}{\text{Mass of initial drug used}} \times 100$$

RESULTS AND DISCUSSION

NMR analysis of PLA-co-PEG copolyester

Figure 1 shows the ^1H NMR spectrum of PLA-co-PEG copolymer. As shown in the Figure 1, 3.64 ppm can be attributed to CH, CH_3 at PLA blocks and CH_2 at PEG blocks, respectively. The existence of a covalent bond between LA and PEG block is evident from a low-intensity multiplet at 4.35ppm representing methylene protons of the acylated end unit of PEG chain (–CH_2–O–CO–), this confirms the occurrence of copolymerization of LA and PEG. The singlet at 5.25ppm is assigned to methine protons.

UV-visible spectroscopy

Figure 2(a) shows the UV visible spectrum of citrate stabilized gold nanoparticles. The peak at 518 nm confirms the presence of gold nanoparticles. Pure drug shows a maximum at 263nm. From Figure 2(b), with addition of isoniazid to gold nanosolution, band of pure drug and colloidal gold decreases steadily with time at an emergence of new peak at 612nm. Appearance of new peak is due to the aggregation of gold nanoparticles and the replacement of citrate by isoniazid leading to the formation of gold drug complex.

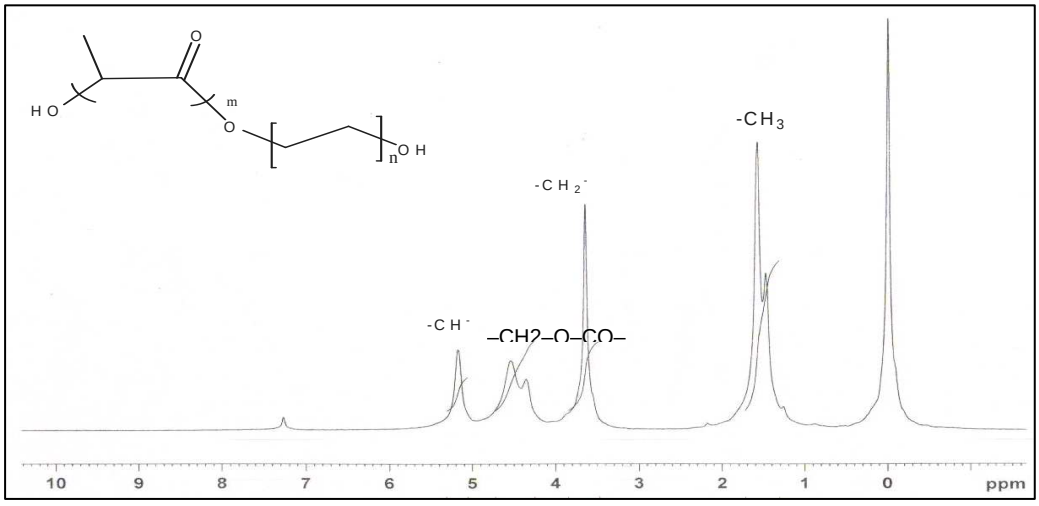


Fig. 1: ^{1}H-NMR spectrum of PLA-co-PEG copolyester

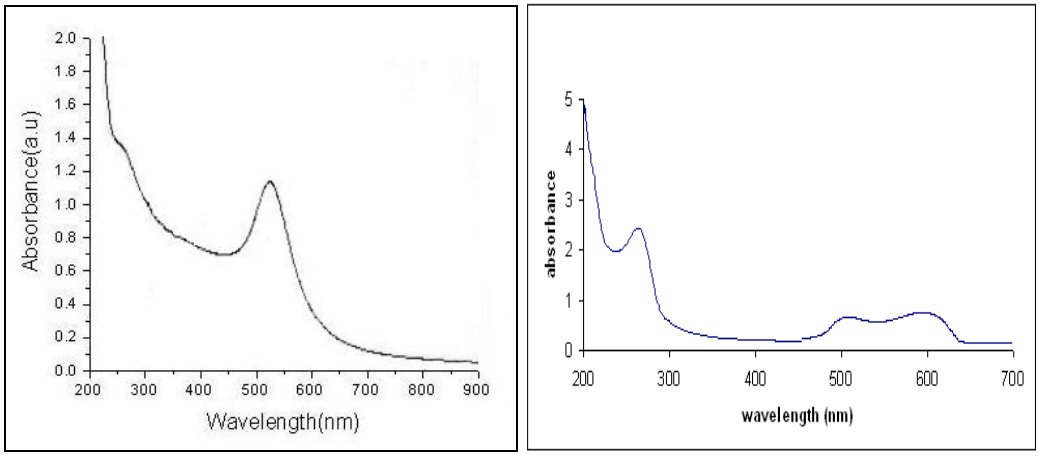


Fig. 2: UV-Visible spectrum of (a) Gold nanoparticles (b) Gold-Isoniazid nanoparticles

FT-IR Spectroscopy

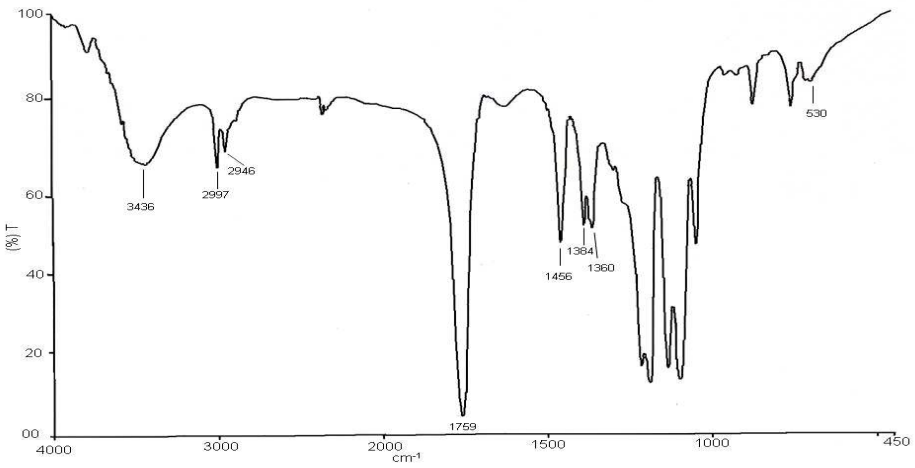


Fig. 3(a): FT-IR spectrum of PLA-co-PEG copolymer

Fig. 3(a): shows the FT-IR spectrum of PLA-co-PEG copolymer. The peaks appeared at 2967 cm^{-1} was due to the presence of -C-H stretching in PLA-co-PEG. C-O stretching frequency appeared at 1428 cm^{-1} and 1384 cm^{-1} indicates -C(O)-O-C-(ester C-O) group formation in PLA-co-PEG. The band appeared at 1759 cm^{-1} indicates the presence of ester carbonyl (CO) group in PLA-co-PEG. From the above stretching frequency, it confirms the formation of PLA-co-PEG copolymer. Isoniazid shows bands at 3098cm^{-1}, 3050cm^{-1} and 3014cm^{-1} for C-H asymmetric and symmetric vibrations respectively. Figures 3(b) show the FT-IR spectrum of PLA-co-PEG/INH-Au nanocapsules. This spectra contains the peaks corresponding to stretching frequency of PLA-co-PEG along with the peaks of INH stretching frequencies, which confirms the presence of INH in the nanocapsules of PLA-co-PEG/INH-Au.

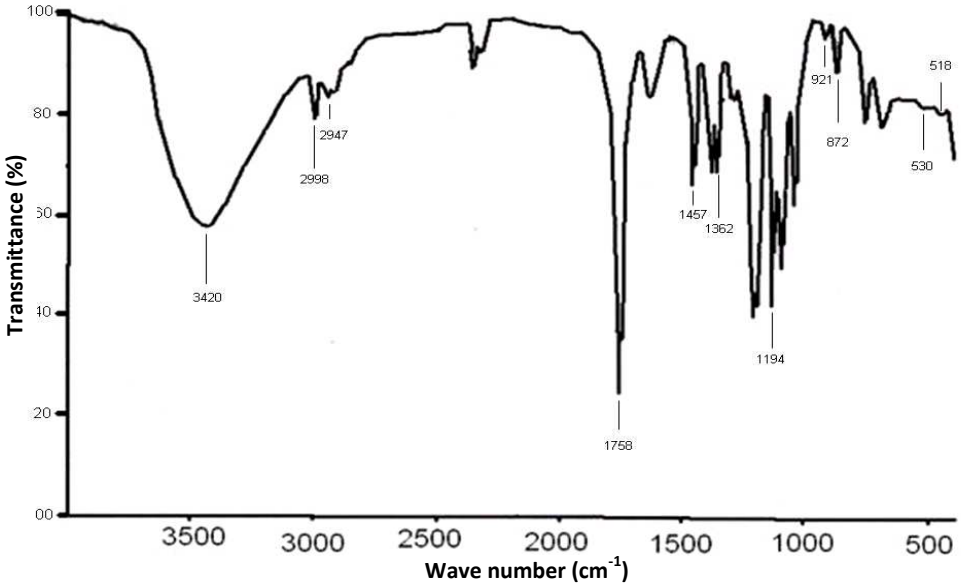


Fig. 3(b): FT-IR spectrum of PLA-co-PEG/INH-Au nanocapsules

Transmission Electron Microscopy (TEM)

Figure 4(a) show the TEM pictures of drug capped gold nanoparticles. From the figure the diameter of gold nanoparticles was found to be in the range of 5-20nm From the TEM pictures the aggregations of gold nanoparticles were observed which confirms the complex formation between isoniazid and gold nanoparticles. Figure 4(b) show the transmission electron micrography of gold/drug encapsulated polymer nanocapsules. The PLA-co-PEG molecules adsorbed on the surface of the nanocapsules prevented the coalescence of nanocapsules. Therefore, it appears that PEG coating can ensure better stabilization.

Figure 4(b) clearly show the gold nanoparticles are covered by polymer shells. It also shows the aggregation of metal nanoparticles particularly with gold which confirms the presence of drug in the polymer nanocapsules.

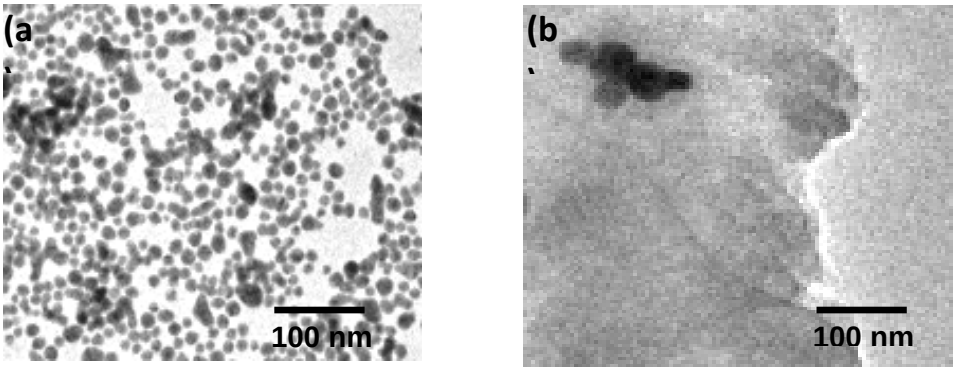


Fig. 4: TEM images of (a) gold-INH nanoparticles (b) PLA-co-PEG/ INH-Au nanoparticles

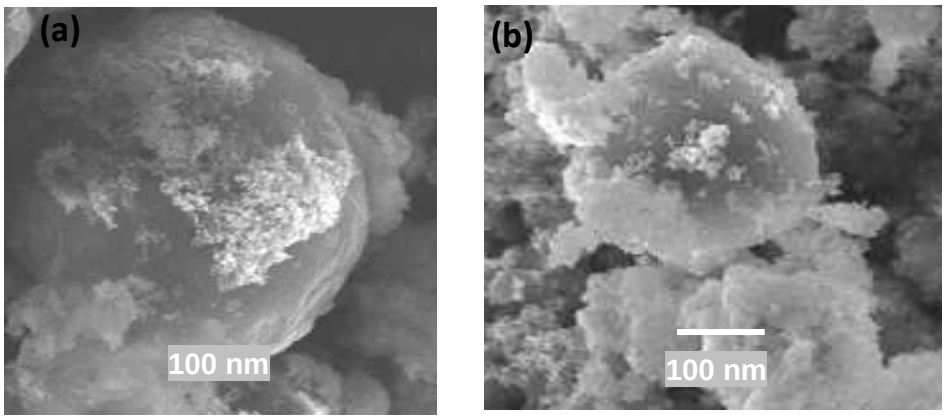


Fig. 5: SEM images of (a) PLA-co-PEG/INH (b) PLA-co-PEG/ INH-Au nanoparticles.

Scanning Electron Microscopy (SEM)

From the SEM morphology the average size of the nanocapsules was found to be in the range of 200-250 nm. From the Figures 5(a)-(b) nanocapsules made from PLA-co-PEG have straight open channels. The surface of nanocapsules appeared to be smooth, spherical in shape. Nanocapsules prepared using gold nanoparticles was smooth, whereas nanocapsules prepared in absence of gold nanoparticles was bumpy and contains bigger pores. These observations reveal that the adsorption of drugs on gold nanoparticles slowed down its diffusion process.

Drug Release study

The release profiles of Isoniazid from PLA-co-PEG nanocapsules in hydrochloric acid (0.1M) and phosphate buffer saline (pH 7.4) were shown in Figure 6. 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 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. Interestingly, the release profile is indicative of diffusion-controlled release only over this time period. The drug release is diffusion controlled as the drug can travel through the pores formed during the hardening phase.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.

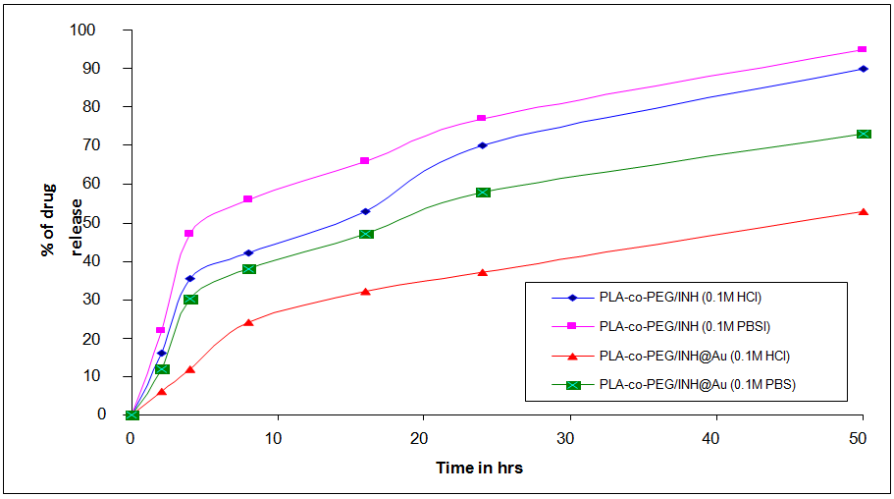


Fig. 6: Drug release profiles for PLA-co-PEG/INH-Au in 0.1M PBS and 0.1M HCl

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 smooth PEG-co-PLA/INH-Au nanocapsules with smaller pores on the surface. Well controlled and sustained release was obtained in 0.1M HCl and was found to be 53% for Isoniazid. The encapsulation efficiency and drug loading was found to be 75% and 5.62 respectively.

Kinetic Studies

Data of the isoniazid release from polymer nanocapsule were analysed for release kinetics. Drug release kinetics from nanocapsules is being analysed by Zero-order kinetics, First order kinetics and Higuchi model.

Zero order kinetics

The table 1 represents the calculated values of release constants and release exponent (n) determined by fitting the release data into the respective equations along with regression coefficients (R^2). Figure 7(a)-(b) shows zero order kinetics for HCl and PBS medium.

Zero order kinetics model follows the equation as $(Q/Q_0) = k_0 t$,

Where, k_0 is the zero order rate constant; Q denotes the amount of isoniazid released and Q_0 denotes the amount of isoniazid present initially. Due to the deviation of regression coefficient these curves precludes the possibility of Zero order kinetics.

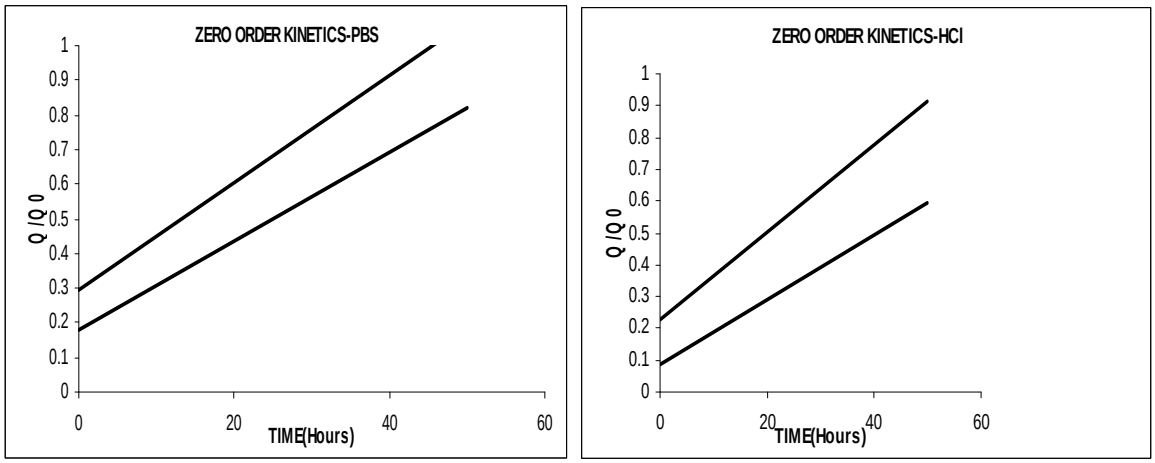


Fig. 7: Zero order kinetics (a) HCl (b) PBS

Table 1: Zero Order Kinetics

Nanocapsules	HCL		PBS	
	K	R^2	K	R^2
PLA-PEG/INH	0.2275	0.7902	0.2929	0.7459
PLA-PEG/INH-Au	0.0886	0.9048	0.1803	0.8036

Table 2: First Order Kinetics

Nanocapsules	HCL		PBS	
	K	R^2	K	R^2
PLA-PEG/INH	1.7815	0.5041	1.5772	0.2684
PLA-PEG/INH-Au	2.63	0.5892	2.284	0.39

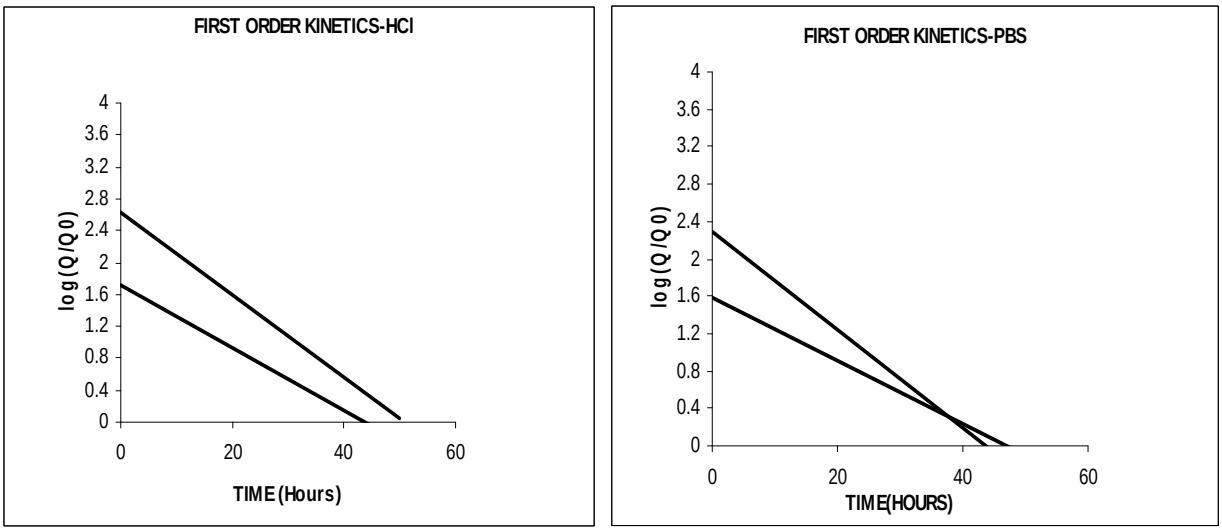


Fig. 8: First order kinetics (a) HCl (b) PBS

First order kinetics

The table 2 represents the calculated values of release constants and release exponent (n) determined by fitting the release data into the respective equations along with regression coefficients (R^2). Figure 8(a)-(b) shows First order profiles in HCl and PBS. First order kinetics model follows the equation as $\log(Q/Q_0) = -k_1 t / 2.303$, where k_1 is the first order rate constant. Due to the lack of linearity these curves precludes the possibility of first order kinetics.

Higuchi Model

The table 3 represents the calculated values of release constants and release exponent (n) determined by fitting the release data into the respective equations along with regression coefficients (R^2). Figure 9(a)-(b) shows half order kinetics for HCl and PBS solution. Tabular values of R^2 indicated that the Higuchi model was best fitted with the release kinetic data of isoniazid for both (HCl and PBS) the medium.

Higuchi equation : $Q/Q_0 = k_h t^{1/2}$

Q denotes amount of thioguanine drug released

Q_0 denotes the amount of thioguanine drug in nanocapsule initially

k_h is the Higuchi matrix release kinetics

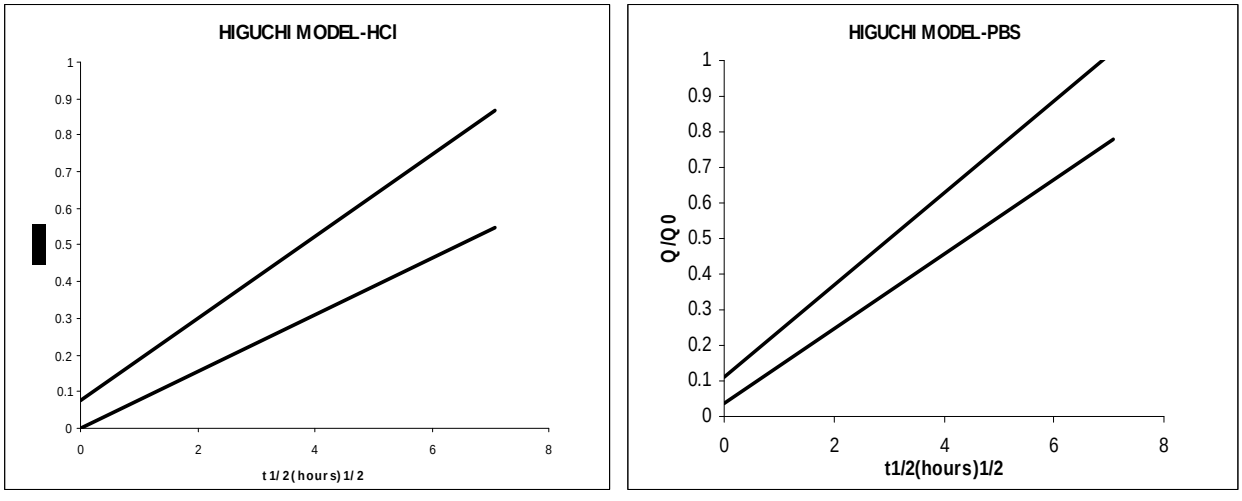


Fig. 9: Higuchi model kinetics (a) HCl (b) PBS

Table 3: Higuchi model Kinetics

	HCL		PBS	
NANOCAPSULES	**K**	**R^2**	**K**	**R^2**
PLA-PEG/INH	0.0744	0.9542	0.1109	0.9395
PLA-PEG/INH-Au	0.0011	0.9884	0.0383	0.9643

CONCLUSION

PLA-co-PEG nanocapsules encapsulated with isoniazid containing gold nanoparticles were prepared by solvent evaporation method. The synthesized copolymer was characterized by NMR spectroscopy. From TEM analysis, the diameter of the gold nanoparticles was found to be in the range 5-20 nm. SEM shows the nanocapsules were spherical in shape with average size 200-250 nm. FTIR studies indicated that there were no drug interactions with the polymer nanocapsules. Invitro drug release for nanocapsules was carried out in two media namely 0.1M PBS and 0.1M HCl. Nanocapsules with gold nanoparticles showed a well controlled and sustained release profile in both the solutions. On applying the drug release data to various kinetic models, Isoniazid was best fillted with Higuchi model indicating uniform distribution of drug over the nanocapsules.

REFERENCE

1. Lisa Clarie du toit, Viness pillay and Michael paul Dankwerts. Tuberculosis Chemotherapy: current drug delivery approaches'. Respiratory Research 2006; 7:118 doi:10.1186/1465-9921-7-118.
2. Arentz M, Hawn TR. Tuberculosis infection: insight from immunogenomics. Drug Discov Today Dis Mech. 2000; 4: 23-236.
3. M.Sarat chandra prasad, B.Nagendra babu, M.Ajay, S.Brito raj, N.Audinarayana, K.Bhaskar reddy. Design and characterization of modified release isoniazid and salbutamol sulphate inlay tablet. International journal of Pharmacy and Pharmaceutical sciences 2011; 3:153-159
4. L. Zhang, D. Pornpattananangkul, C.-M.J. Hu and C.-M. Huang. Development of nanoparticles for antimicrobial drug delivery. Current Medicinal Chemistry 2010; 17: 585-594.
5. Gelperina S, Kisich K, Iseman MD, Heifets L. The potential advantages of nanoparticle drug delivery systems in chemotherapy of tuberculosis. American Journal of Respiratory and Critical Care Medicine 2005; 172:1487-1490.
6. Hainfeld J.F., Slatkin D.N. and Smilowitz H.M. The use of gold nanoparticles to enhance radiotherapy in mice. Phys. Med. Biol 2004; 49: 309-315.
7. Rena L. and Chow G.M. Synthesis of nir-sensitive Au–Au2S nanocolloids for drug delivery. Materials Science and Engineering C 2003; 23:113-116.
8. Po-Chi Soo, Yu-Tze Horng, Kai-Chih Chang, Jann-Yuan Wang, Po-Ren Hsueh,Chun-Yu Chuang. A simple gold nanoparticle probes assay for identification of Mycobacterium tuberculosis and Mycobacterium tuberculosis complex from clinical specimens. Molecular and cellular probes 2009; 23: 240-246.
9. Madan madhu, Lewis shaila and aig jamal anwar. Biodegradable injectable implant systems for sustained delivery using Poly(lactide-co-glycolide) copolymers. International journal of Pharmacy and Pharmaceutical sciences 2009; 1:103-107.
10. Ren Zhong Xiao, Zhao Wu Zeng, Guang Lin Zhou,Jun Jie Wang, Fan Zhu Li, and An Ming Wang.,Recent advance in PLA-co-PEG block copolymer nanoparticles. International journal of nanomedicine 2010; 5: 1057-1065.
11. Qurrat ul-Ain., Sharma S.and Khuller G.K. Chemotherapeutic Potential of Orally Administered PLGA microparticles containing Isoniazid, Rifampin, and Pyrazinamide against experimental Tuberculosis. Antimicrobial Agents and Chemotherapy 2003;47: pp. 3005-3007.
12. Khuller G.K. and Pandey R. Sustained Release Drug Delivery Systems in Management of Tuberculosis. Indian Journal of Chest Disease and Allied Sciences 2003; 45: 229 -230.
13. Nagaraja P., Sunitha K., Vasantha R. and Yathirajan H. Novel method for the spectrophotmetric determination of Isoniazid and Ritodrine Hydrochloride Turkish Journal of Chemistry 2002; 26:743-750.
14. Rastogi R., Sultana R.Y., Aqil M., Ali A., Kumar S., Chuttani K. and Mishra A.K. Alginate microspheres of isoniazid for oral sustained drug delivery. International Journal of Pharmaceutics 2007; 334: 71-77.
15. Mehta S.K., Kaur G. and Bhasin K.K. Incorporation of Antitubercular Drug Isoniazid in Pharmaceutically Accepted Microemulsion: Effect on Microstructure and Physical Parameters. Pharmaceutical Research 2007; 25: DOI: 10.1007/s11095-007-9355-8.
16. Aliasgar J. Kundawala, Vishnu A. Patel, Harsha V. Patel and Dhaglaram Choudhary. Isoniazid loaded chitosan microspheres for pulmonary delivery: Preparation and characterization. Pelagia Research Library 2011; 2: 88-97.