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Encapsulation of Isoniazid in Chitosan-Gum Arabic and Poly (Lactic-Co-Glycolic Acid) PVA Particles to Provide a Sustained Release Formulation

Keywords: Nanotechnology; Nanomedicine; Nanoparticles; Tuberculosis; Isoniazid; PLGA; Chitosan gum Arabic

Abstract

Background: Tuberculosis remains one of the greatest health challenges worldwide, hence the need for development of better diagnostic tools and effective treatments. Nanotechnology and particulate drug delivery systems are examples of systems which can enhance drug delivery. Isoniazid is a key pharmaceutical agent in fixed dose combination therapy for the treatment of tuberculosis. Encapsulation of isoniazid could enhance its release properties leading to reduction in frequency of dosing which has a positive impact on patient adherence to treatment.

Methods: Isoniazid was encapsulated in poly (lactic-co-glycolic acid) (PLGA) and polyvinyl alcohol (PVA) by a single emulsion evaporation technique and in chitosan gum Arabic particles by an ionic gelation method. The particles were characterized by particle size and distribution, zeta potential, encapsulation efficiency and in vitro drug release.

Results: PLGA-PVA particles had a mean particle size of $1.08 \pm 0.69 \mu\text{m}$ and chitosan gum Arabic particles had a mean particle size of $314 \pm 559 \mu\text{m}$. The mean zeta potential of the particles was $-10.6 \pm 1.3 \text{ mV}$ and $-12.6 \pm 2.9 \text{ mV}$ for Cs-GAR and PLGA-PVA particles respectively. PLGA-PVA particles had an encapsulation efficiency of 87 % and Cs-GAR particles had an encapsulation efficiency of 69 %. The results for the in vitro drug release indicated a prolonged drug release pattern, in pH 1.2, 88% of isoniazid encapsulated was released from CS-GAR and 40.4% from PLGA-PVA. In pH 6.8, 45% of isoniazid was released from the PLGA-PVA particles and 8% from Cs-GAR particles. In pH 7.4, 50% was released from PLGA-PVA particles and 7% from Cs-GAR particles over the 24 hours.

Conclusion: Isoniazid was successfully encapsulated in PLGA-PVA and CS-GAR polymers. The particles demonstrated a prolonged drug release pattern and PLGA-PVA formulation provided particles in the nanometer range which can be used in further investigations to assess their impact on bioavailability and therapeutic activity.

Introduction

Mycobacterium tuberculosis (Mtb) is one of the major human pathogens that infects one third of the World's population causing the disease, tuberculosis (TB) in approximately 9.4 million people each year [1]. The increased vulnerability to TB in HIV-infected populations has resulted in the increase in the incidence of TB. Multi drug resistant TB (MDR-TB) has also been on the rise in both developed and developing nations in the past decade [2]. In 2012 World Health Organization (WHO) estimated 1.3 million deaths from tuberculosis including 320 000 deaths among the HIV-infected people [3].



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The currently available treatment of tuberculosis requires multi-drug therapy, comprising of an initial intensive phase of a fixed dose combination with isoniazid (INH), rifampicin (RIF), pyrazinamide (PYZ) and ethambutol (ETB) daily for two months and a continuous phase of RIF and INH for a further four months [4]. The regimen is more than 50 years and isoniazid (INH) has been shown to be one of the most effective [1,5]. Due to the high percentage of side effects associated with the treatment and the long duration of therapy, there is low patient adherence to medication leading to the emergence of multi drug resistant TB (MDR) [5]. Therefore, there is need for the development of new approaches such as development of novel drugs, modification of old drugs and generation of new delivery systems [1].

Nanotechnology is one of the formulation approaches to overcome the shortcomings of the conventional drugs. The technology allows us to deliver the old drugs more efficaciously thereby reducing the drug dose [6], frequency and duration of therapy and increasing the bioavailability of the drug [7]. This formulation approach can improve patient adherence, reduce the pill burden and duration of treatment therefore leading to reduction in drug resistance [6].

Research and development in nanotechnology based drug delivery systems has shown potential in the improvement of the chemotherapy of tuberculosis. Strategies have been employed in the development of targeted nano drug delivery systems that reach the mycobacteria within granulomas reducing non-specific side effects associated with the treatment [1]. A number of carriers have been developed for the anti-mycobacterial antibiotics and poly lactide-co-glycolic acid (PLGA) is one of the most studied polymers [1].

Of the drugs currently used in the treatment of tuberculosis, several factors including drug toxicity and the rate of primary and acquired resistance is higher for isoniazid than for other anti TB drugs [8]. Isoniazid also presents with poor pharmacokinetic and physicochemical properties. It has a low permeability and is also characterized by a short half-life ranging from one hour to four hours and less than one hour in rapid acetylators [8].

The present study was aimed at developing INH nanoparticles that can show sustained drug release pattern. The oral route being

the most preferred route of administration, the intended dosage form is isoniazid sustained release tablets. In this study isoniazid was encapsulated in biodegradable and biocompatible polymers, chitosan-gum arabic (CS-GAR) and PLGA particles the effect of the particles on drug release was investigated. The study provides important preliminary data on the impact of the particles on improving the release properties of isoniazid. Considering that some of the materials used in this formulation are of low costs and some are locally available e.g. gum arabic, this research provides important information on the possibility of fabrication of nanoparticles in resource limited settings. The work done in this research can also be considered as the stepping stone for further investigations on the impact of the particles on bioavailability and therapeutic activity of isoniazid.

Materials and Methods

Chitosan (LMW), poly (lactide-co-glycolide acid) (PLGA), acetonitrile HPLC grade and polyvinyl alcohol (PVA) were purchased from SIGMA ALDRICH, USA. Isoniazid and gum arabic, disodium hydrogen orthophosphate (Na_2HPO_4) and sodium hydroxide (NaOH) were purchased from ASSOCIATED CHEMICAL ENTERPRISE, SOUTH AFRICA. The equipment used include magnetic stirrer from SIGMA ALDRICH COMPANY, GERMANY, sonicator 90s (WESTWOOD ULTRASONICS, UK), vortex (HEIDOLPH REAX 2000, Germany), ultracentrifuge (HERMLE Z160M, GERMANY), water bath (BUCHI, SWITZERLAND), UV-VIS Spectrophotometer (SHIMADZU, JAPAN) and a pH Meter (JENWAY, UK). Distilled water was used in all the experiments.

Fabrication of isoniazid-loaded PLGA particles using the solvent evaporation method

An accurately weighed 200 mg of PLGA was added to 10ml of ethyl acetate and stirred overnight to dissolve using a magnetic stirrer (Sigma Aldrich Company, Germany) at 25°C. Isoniazid was added to the nanoparticle formulation in a drug to polymer ratio of 1:1 w/w. 200 mg of isoniazid and 100 mg of PVA were dissolved in 10 ml water using mild heat. PLGA solution was then added drop wise to PVA solution under stirring at 1000 rpm. The emulsion was stirred at room temperature for 1 hour in a closed beaker to prevent evaporation of solvent. After stirring, the ethyl acetate was evaporated under stirring at 500 rpm overnight in a fume cupboard with beaker open to allow evaporation of solvent. The resultant suspension was placed in 1 ml eppendorf tubes and centrifuged at room temperature using an ultracentrifuge (Hermle Z160M, Germany) at 14000 rpm for 15 minutes to collect the particles.

After centrifuging the supernatant was discarded and the particle pellets were washed thrice using de-ionised water.

Fabrication of isoniazid-loaded chitosan gum arabic particles using the ionic gelation method

The method for the preparation of isoniazid loaded chitosan, gum arabic and was adapted from a method previously described by Avadi et al. [9]. One gram of chitosan was dissolved in 100 mL of 0.175 % v/v acetic acid to obtain a concentration of 10 mg/mL (1% w/v); this was done under stirring using a magnetic stirrer (Sigma Aldrich Company, Germany), at 200-300 rpm at room temperature overnight. In the

second step, one gram of isoniazid and 500 mg of gum arabic were dissolved in 100 mL of water to obtain a concentration of 10 mg/mL (1%) and 5 mg/mL (0.5%) respectively, this was done under stirring at room temperature for 30 minutes. To the resultant solution, 6.7 mL of gum arabic solution containing isoniazid was added drop wise to 13.3 mL of chitosan solution under magnetic stirring at 1000 rpm at room temperature. The suspension was stirred for an hour and then placed in 1ml eppendorf tubes for centrifuging. The particles were recovered by centrifuging the colloidal suspension at 25°C and 14000 rpm for 15 minutes using an ultra-centrifuge (Hermle Z 160m, Germany). The supernatant was discarded and the particle pellets were washed thrice using de-ionized water.

Characterization of isoniazid loaded chitosan-arabic gum and PLGA particles

Particle size, size distribution and zeta potential: The particle size, size distribution, polydispersity index, zeta potential of isoniazid loaded PLGA and chitosan-gum arabic particles were analyzed by photon correlation spectroscopy (PCS) using a zetasizer 2000 (Malvern Instruments, UK) at 25°C at a measuring angle of 173°. The particle pellets prepared as above were dissolved in de-ionised water to form colloidal suspensions which were measured in triplicate as prepared without dilution.

Encapsulation Efficiency: Isoniazid loaded particle pellet in one 1 mL eppendorf tube were destroyed using acetonitrile. The resultant solution was passed through a membrane filter (0.22µm pore size). The amount of isoniazid contained in the destroyed nanoparticles was determined using a UV/Vis spectrophotometer (Shimadzu UV-1700, Japan) set at 263 nm. Encapsulation efficiency was then calculated using the formula below;

$$\text{Encapsulation Efficiency (EE)} = \frac{\text{total amount of isoniazid in particles}}{\text{total amount of initial isoniazid added to nanoparticle preparation}} \times 100\%$$

In vitro drug release study design: The method for drug release was adapted from Pandey et al. [10]. The particles were prepared and washed as described above and in vitro drug release was then carried out at 37°C in a buffer solution of pH 1.2, pH 6.8) and pH 7.4 to determine the release properties of the formulations. The buffer solutions were prepared according to the United States Pharmacopoeia (USP 2007). The pH experiments were done in triplicate.

An amount of 1 mL phosphate buffer solution of pH 1.2, pH 6.8 or pH 7.4 was placed in eppendorf tubes sufficient for all the time points required and these contained the washed samples of nanoparticles. The eppendorf tubes were placed in the water bath (Buchi, Switzerland). The samples were withdrawn for analysis at pre-determined time intervals at 30 minutes and 1, 1.5, 2, 2.5, 3, 6, 24 hours. The absorbance of the solutions which represents the drug content of the samples was analyzed using the UV-VIS spectrometer (Shimadzu UV-1700, Japan) at 263 nm. The absorbance measurements were carried out in triplicates.

Results and Discussion

Preparation of nanoparticles

The isoniazid loaded chitosan gum arabic particles were prepared by ionic gelation, a mild and simple method. This method is based on the electrostatic interactions between amine group of chitosan and the negatively charged group of pollinations of gum arabic forming intramolecular and intermolecular cross-linking mediated by these polyanions [9,11]. Chitosan is poorly soluble in water therefore addition of acid was done to improve its solubility, which occurs as a result of protonation of amino groups [12].

Isoniazid loaded PLGA-PVA particles were prepared by the solvent evaporation technique. In this method, there was emulsification of the polymer solution (PLGA) in an aqueous solution to form an oil/water emulsion, ethylacetate as the organic solvent was evaporated by continuous stirring which induces the precipitation of polymer as nanoparticles [11].

Particle size distribution

Particle size distribution is one of the most important aspects of formulation of particulate systems. The PLGA-PVA particles had a mean particle size of $1.08 \pm 0.69 \mu\text{m}$ and CS-GAR particles had a mean particle size of $314 \pm 559 \mu\text{m}$. The mean zeta potential of the particles was $-10.6 \pm 1.3 \text{ mV}$ and $-12.6 \pm 2.9 \text{ mV}$ for Cs-GAR and PLGA-PVA particles respectively. Likely due to low zeta potential, the particles aggregated to form microparticles due to van der Waal's interparticle attractions. Freeze drying of the samples could have been employed to improve the stability of the particles [13].

Zeta potential is the charge at particles mobile surface and is used to determine the degree of flocculation or deflocculation in nanosystems. The more pronounced zeta potential values, being positive or negative tend to stabilize particle suspension [14]. The electrostatic repulsion between particles with the same electric charge prevents the aggregation of the particles. Particles with zeta potential values greater than $+25 \text{ mV}$ or less than -25 mV typically have high degrees of stability [15]. Dispersions with a low zeta potential value will eventually aggregate due to Van Der Waal inter-particle attractions.

In this study the chitosan-gum arabic nanoparticles were negatively charged this indicates that all the amino groups were neutralized during nanoparticle formation remaining with the negative charges from gum arabic. The PLGA-PVA particles were also negatively charged due to the COO⁻ end groups present on the PLGA polymer backbone.

Encapsulation efficiency

Encapsulation efficiency is an important parameter to evaluate drug loaded nanoparticles as it is more economical when high encapsulation efficiency can be obtained [12]. The encapsulation efficiency of isoniazid loaded PLGA nanoparticles was calculated to be 87%. The encapsulating efficiency of isoniazid loaded chitosan-acacia nanoparticles was calculated to be 69.9%.

Drug Loading

The drug loading capacity of chitosan-gum arabic nanoparticles

was 0.67 mg/mL. Chitosan did not completely dissolve in the acetic acid 0.175 % v/v, the solution was slightly viscous and as a consequence decreased the drug loading. Because of the high solubility of isoniazid, part of the drug which was entrapped to the surface of the nanoparticle could have been lost by dissolving in water during the washing steps.

Isoniazid loaded PLGA nanoparticles had relatively high drug loading of 0.89 mg/mL than the isoniazid loaded chitosan-gum arabic nanoparticles 0.69 mg/mL. The difference could have been due to smaller particle size ranges in PLGA nanoparticles hence larger surface area for drug entrapment than the chitosan nanoparticles which had a larger nanoparticle size range [16] and the differences in the characteristics of the polymers used.

Drug release profiles of isoniazid loaded PLGA, isoniazid loaded chitosan-gum arabic (CS-GAR) particles and free isoniazid

Drug release in buffer solution of pH 1.2 : The results indicated that the release into the saline buffer was faster from both particle formulations and the free drug due to faster hydrolysis of the drug-polymer bonds in the acid medium. The amount of isoniazid released from the CS-GAR particles was 88% of the total amount encapsulated and 53% from PLGA-PVA, this is because chitosan is more soluble in organic solutions of pH less than 6.5 [11]. It was also interesting to note that the amount of drug released from the isoniazid samples began to decrease; it is possible that some form of drug degradation was taking place. In the free drug sample, the decrease was noted within the first five hours, within the same period, the drug from chitosan-gum arabic particles was still being released into the medium. In PLGA-PVA nanoparticles the amount of drug only started to decrease after five hours, the amount of drug released did not reach 100% and this could have been because the rate of drug degradation had exceeded the rate of drug release from the polymer (Figure 1).

Drug release in isoniazid loaded PLGA and chitosan-gum arabic particles in pH 6.8 and pH 7.4: The diffusion of the drug and degradation of polymer are the main mechanisms of release [17]. Degradation of PLGA is slow; the release of drug would mainly depend on drug diffusion hence the slower release observed during the time of the experimental studies. PLGA undergoes degradation by hydrolysis or biodegradation through cleavage of its backbone ester linkages into oligomers and, finally monomers [18].

The release from chitosan-gum arabic nanoparticles was lower than that of PLGA. This could have been because chitosan has limited solubility at pH values above 6.5 therefore its degradation is low resulting in low drug release since drug release depends on the solubility of drug combined with the degree of polymer degradation [19]. Isoniazid also has a lower solubility in pH values greater than pH 6.8.

In pH 7.4, the total amount of free drug released initially was 89.7% and it was less than that observed in pH 6.8 and this could have been attributed to the decrease in solubility of isoniazid as the pH increases. The release from the polymers was similar to that observed in pH 6.8 (Figure 2 and Figure 3).

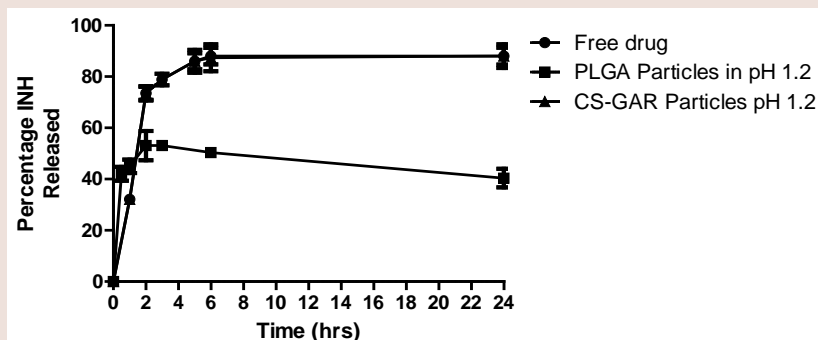


Figure 1: Drug release profile of isoniazid loaded PLGA-PVA, isoniazid loaded chitosan-gum arabic (CS-GAR) particles and free isoniazid in pH 1.2 saline buffer.

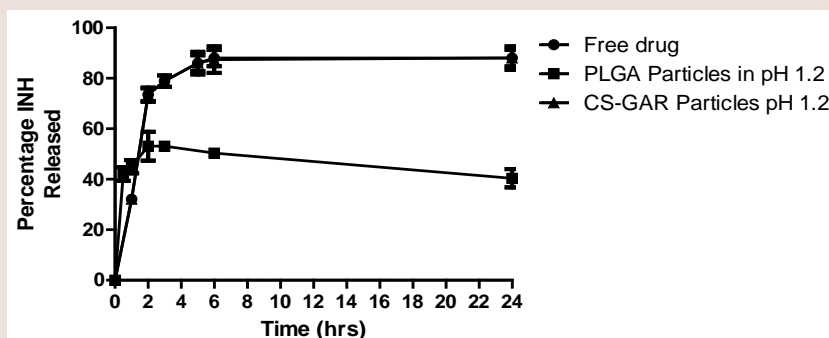


Figure 2: Drug release profile of PLGA-PVA and CS-GAR isoniazid particles compared to free drug in pH 6.8.

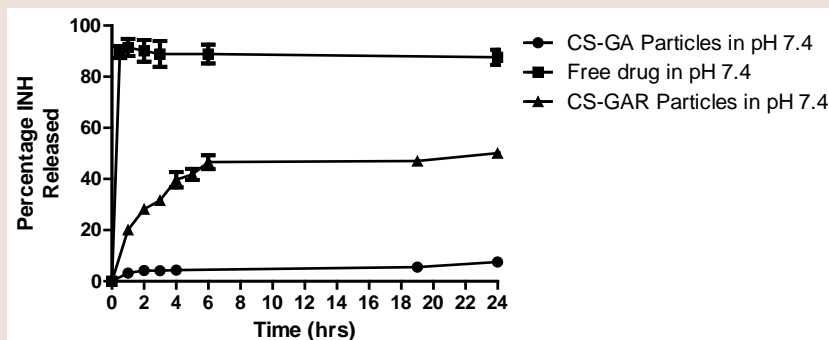


Figure 3: Drug release profile of PLGA-PVA, CS-GAR particles compared to the free drug in the pH 7.4.

Conclusion

From the present research work that was done in this study, it can be concluded that the formulation of isoniazid (INH) nanoparticles using PLGA-PVA and chitosan gum arabic polymers was done successfully. The solvent evaporation method and PLGA-PVA polymers were found to be more efficient in the fabrication of the particles in terms of size and encapsulating efficiency than chitosan-gum arabic. Both particle formulations showed a prolonged release pattern. The prolonged release aspect shown by the particles prepared in this study, indicate that the nanoparticle anti TB drug therapy can allow a reduction in dosing frequency improving the management of TB. Further investigations on the impact of the particles on the bioavailability, pharmacokinetics and pharmacodynamics of isoniazid in animals will be conducted.

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