Preparation and characterization of polyvinyl alcohol-chitosan-tripolyphosphate hydrogel for extended release of anti-tuberculosis drugs

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Abstract. Polyvinyl alcohol (PVA)-chitosan-tripolyphosphate hydrogels were prepared using a solvent casting-evaporation method to obtain anti-TB drugs extended release formulations. X-ray diffraction and scanning electron microscope results indicated that chitosan reduced the crystallinity of PVA hydrogels and the polymers formed homogeneous mixtures. The added chitosan reduced the encapsulation efficiency, and, the rate of drug release. The profile release of the four drugs indicated that the formulation of 80% PVA-20% chitosan hydrogel matrix had the highest drug release rate in a short period of time in phosphate buffer solution of pH 7.4. The maximum drug loading efficiency was obtained in a matrix formulation contain only PVA, while the addition of sodium tripolyphosphate increased the drug loading efficiency and also reduced the amount of drug released. The hydrogel matrix formulation of PVA-chitosan-tripolyphosphate showed its potential to be used as an extended delivery system for anti-TB drugs.

1. Introduction

Tuberculosis (TB) is an infectious disease caused by Mycobacterium tuberculosis that could affect various organs, such as lung, pericardium, meninges, and also backbone [1,2]. One of the most common types of TBC is osteoarticular tuberculosis, an extrapulmonary tuberculosis affecting bones, including backbones. The current treatment method is by surgery to remove the infected part, and continued by oral drug treatment of isoniazid (INH), pyrazinamide (PZA), ethambutol (ETH), and rifampicin (RFP) for two months, followed by isoniazid and rifampicin treatment for four more months to kill remaining active and dormant bacteria [3]. Unfortunately, this treatment could potentially produce discomfort and damage to the liver of the patients, as well as, drug resistant bacteria. These problems call for the development of an extended drug release formulation, in which drugs are accumulated in a depot to be later released slowly in the infected site. TB treatment using an extended drug release system has several advantages, such as decreased drug dosage, reduced side effect to the patients’ liver, and drastically reduced drug consumption frequency. Xue et al. [4] showed that TB drugs encapsulated in poly-lactide co-glycolic acid (PLGA) could be released in 25 days. A study on alginate-PLGA core-shell for slow release of TB drugs indicate that only 0.4% of the drugs were released in 25 days [5]. Polyvinyl alcohol (PVA) is a synthetic polymer vastly used for biomedical application having biodegradable, hydrophilic, non-toxic, and biocompatible characteristics [6], as well as, a relatively high drug encapsulation efficiency of hydrophilic drugs [7].
However, PVA hydrogels in aqueous media swollen easily facilitating the release of drugs. Rogojanu et al. [8] showed that 50% of salicylic acid was released in only 10 h, while Takamura et al [9] demonstrated that 45% of flurbiprofen was released in 3 h. The addition of chitosan (CS) to the PVA matrix slowed the bovine serum albumin release 60% of the total amount in 25 h [10]. It is also known that chitosan could form crosslinking in the presence of sodium tripolyphosphate (STPP) [11]. In this research, the previously prepared PVA-chitosan matrix was immersed in the tripolyphosphate solution in order to form crosslinking the chitosan in the matrix. The aim was to produce PVA hydrogel matrices with a high encapsulation efficiency and a slow release of anti-TB drugs to be used as an implant material for spinal tuberculosis treatment.

2. Methods

2.1. Material
Polyvinyl alcohol (degree of hydrolysis of 99%), chitosan (15-75 kDa, degree of deacetylation of 84%), acetic acid, and sodium tripolyphosphate were purchased from Sigma-Aldrich. Anti-TB drugs isoniazide (INH), pyrazinamide (PZA), ethambutol (ETH), and rifampicin (RFP) were supplied by Phapros.

2.2. Preparation of PVA-CS-drug-STPP hydrogel matrices
A casting solvent evaporation method was used in the preparation of the matrices. Chitosan was dissolved in 2% acetic acid solution to obtain 4%-w/v chitosan solution. PVA was dissolved in distilled water by heating at 90 °C and stirred until PVA was completely dissolved to obtain a 4%-w/v PVA solution. The PVA solution was mixed thoroughly with the chitosan solution at room temperature resulting in mixtures with PVA to chitosan mass ratios of 100:0, 80:20, 60:40, and 40:60. Each of the TB drugs (2 mg) was added to the PVA-chitosan solution (50 ml), the mixture was agitated using a sonicator until they become homogenous, and allowed to stand at room temperature to remove the bubble from the agitated solution. The mixture was poured into 5 cm petri dishes (20 ml), and then evaporated in an incubator at 37 °C until a constant weight was obtained. The crosslinking process was carried out by immersing the hydrogels in 2 or 4% STPP solution for 30 min. The crosslinked hydrogels were dried in the incubator at 37 °C until the weight was constant.

2.3. Drug encapsulation efficiency
The drug encapsulation efficiency was determined based on mass of drugs encapsulated in the matrices and mass of drugs dissolved in the STTP solution during the crosslinking process. The STTP solution after the crosslinking process was collected for analyzing the non-encapsulated drug using high performance liquid chromatography (HPLC) analysis. The drug encapsulation efficiency was calculated using equation (1):

\[
\text{encapsulation efficiency} (\%) = \frac{\text{initial drug weight} - \text{drug loss to STPP}}{\text{initial drug weight}} \times 100\%
\]  

(1)

2.4. In vitro drug release test
In vitro drug release test was conducted in PBS of pH 7.4. The drug-matrix samples of 15 mg were placed in a dialysis membrane containing 20 ml of PBS. The dialysis membrane was immersed in test tube containing 40 ml of PBS. The test tube was placed in a water bath at 37 °C and stirred at 250 rpm. For assaying the drug released, the PBS solution samples (4 ml) were withdrawn several times up to 72 h. The same volume of the fresh PBS solution was added to keep a constant volume. The drug (isoniazid, pyrazinamide, ethambutol) contents in the sample solution were assayed using an HPLC-UV detector at a wavelength of 250 nm and mobile phase composed of 8% acetonitrile and 92% 20 mM NaH₂PO₄ solution of pH 6.8. Mobile phase composed of 50% acetonitrile and 50% 20 mM NaH₂PO₄ with a pH of 6.8 was used for rifampicin content determination at a wavelength of 333 nm.
2.5. Morphology crystallinity of hydrogels
The surface morphology of hydrogel matrices was determined using the scanning electron microscope (SEM) SEI JEOL-JSM 6510LA with the secondary electron image mode of 20kV acceleration voltage and 500-10,000x magnification. X-Ray Diffraction (XRD) pattern of hydrogel matrices was obtained using SHIMADZU XRD 7000 MAXIMA-X with Cu tube. All samples were analyzed between 20 angles of 10° and 80° using the voltage of 40 kV, current of 30 mA, and time per step of 1 second.

3. Results and discussion

3.1. Drug encapsulation efficiency
Table 1 gives the formulation code, PVA to chitosan mass ratio in the hydrogel matrices, the concentration of the STTP solution used, and, the drug encapsulation efficiency for each anti-TB drug as determined using equation (1).

| Formula | PVA:chitosan mass ratio | [STPP] (%-w/v) | Encapsulation efficiency (%) |
|----------|-------------------------|----------------|-----------------------------|
| 100A     | 100:0                   | 2              | 98.6 57.7 62.9 99.6         |
| 100B     | 100:0                   | 4              | 98.4 40.8 78.8 99.3         |
| 80A      | 80:20                   | 2              | 99.7 28.1 45.0 99.9         |
| 80B      | 80:20                   | 4              | 99.3 24.5 37.9 99.9         |
| 60A      | 60:40                   | 2              | 98.8 29.7 55.2 99.9         |
| 60B      | 60:40                   | 4              | 98.9 35.1 53.9 99.9         |
| 40A      | 40:60                   | 2              | 97.8 27.2 49.1 99.8         |
| 40B      | 40:60                   | 4              | 97.4 18.1 54.0 99.8         |

The encapsulation efficiencies of the anti-TB drugs follow the order of: RFP~INH>ETH>PZA. This trend could be correlated to water solubilities of these drugs in the order of: RFP<INH<PZA<ETH or 0.0003<42<363<649 mg/mL, respectively. Although PZA is slightly less soluble in water than ETH, the encapsulation efficiency values of this drug were the lowest among the four drugs, indicating that PZA is leached out of the chitosan-PVA hydrogels more extensively. This might be due to the interaction between the more positively charged amide functional group of PZA, stabilized by the amide resonance structures, and the more negatively charged tripolyphosphate anion.

3.2. The SEM pictures and XRD patterns of the hydrogel matrices
Figure 1 shows SEM pictures (500x magnification) that show surface morphology of three PVA-CS matrices: (left) PVA:CS=100:0, 2%-STPP, pre-immersion; (center) PVA:CS=40:60, 4%-STPP, pre-immersion; (right) PVA:CS=40:60, 4%-STPP, post-immersion of 72 h. Sample 100A (PVA only) showed a smooth and even surface of the matrix. PVA thin film has a good structural integrity, smooth, even, and no crack [12]. Sample 40B-new (40% PVA, 60% chitosan in 4% STPP) also shows a homogenous, although a less smooth surface compared to sample 100A. This shows that PVA and chitosan were compatible polymers.
Sample 40B-72 (60% chitosan in 4% STPP after 72 hours release test) shows an uneven and rough surface, due to degradation and erosion of the polymer in the release media. Figure 2 shows an XRD pattern with a high intensity peak at 2θ of 20° due to the crystalline of the PVA-only matrix. The intensity of this peak was diminished after chitosan was incorporated into the hydrogel matrices due to the reduced crystallinity of the PVA matrices. An XRD pattern could be related to crystal size where a wider peak is a result of imperfect crystals [13]. The SEM and XRD data indicate that the amine or hydroxyl functional groups in chitosan were able to form bonds with the hydroxyl groups in PVA.

It is reported earlier that combining PVA with chitosan for preparing scaffold was occurring due to the formation of hydrogen bonding between hydroxyl groups of PVA and chitosan [14]. By immersing the matrix of PVA-CS-drug into the solution of STTP, the matrix seemed hardened because of the formation of networking between the positively charged amine group in chitosan and the negatively charged phosphate group of STPP [15]. The schematic of hydrogel matrix formation between PVA, chitosan and STPP, loaded with anti-TB drugs is shown in figure 3.
3.3. Drug release profile

The samples taken at a certain period of time were assayed for the drug content and the cumulative release plots are shown in figure 4 for anti-TB drugs INH, PZA, ETH, RFP. Although these drugs showed different release profiles in buffer solution of pH 7.4, the release profiles of INH and PZA followed a similar pattern. Less than 20% of INH were released from the PVA-only matrices (100A & 100B) due to the high crystallinity of the matrices (see figure 2). On the other hand, it took only about 4 h to achieve a 65% cumulative release of INH from the matrix with 20% chitosan (80A). A similar burst release of PZA from a matrix with similar composition (80A), reached 100% cumulative release in about 60 min. These drugs were readily released after the matrix was swollen with the buffer solution in a short period of time, probably due to the disrupted PVA network in the presence of chitosan. Matrices with increasing chitosan to PVA mass ratios (60A and 40A) showed even lower cumulative release of INH and PZA because of the more extensive crosslinked between chitosan and triplyphosphate, and also, due to the formation of hydrogen bonds between the carbonyl functional group of INH and PZA and the amine functional group of chitosan [16]. Ethambutol showed slightly longer release time, probably because the secondary amine functional groups do not interact as strongly as the primary amine functional group of INH and PZA.

![Graphs showing drug release profile](image)

**Figure 4.** Release profiles of anti-TB drugs from PVA-CS-STTP matrices.

The release profile of the RFP in buffer solution was quite different from the other three drugs. It was only about 45% released at 4 h when matrices contained chitosan of 20% and 40%. More importantly, the release of RFP in a matrix of 100% PVA was almost linear for 3 days, with the cumulative release of 5 and 45% from matrices prepared with 4 and 2%-w/v STTP, respectively. Based on its size, it is likely that RFP molecules would be entrapped in the PVA matrix, allowing for the desired zero-order release.
4. Conclusion
The encapsulation efficiencies of the anti-TB drugs follow the order RFP-INH>ETH>PZA that could be related to their water solubility values. The release profiles of the four drugs showed significant burst release up to 500 min (INH and PZA) and to 500 min (ETH and RFP). In general, the effect of the added chitosan was more drugs were released from hydrogels with PVA to chitosan ratio of 80:20, while hydrogels with higher content of chitosan hindered the release of the drugs. The hydrogels with the lower concentration of tripolyphosphate produced higher cumulative release of all drugs. In contrast to the burst release of the hydrophilic drugs, approximately linear release profiles of the RFP from PVA-only matrices were observed for 3 days. As a large molecule, it is likely that RFP would be entrapped in the PVA matrix, allowing for the desired extended zero-order release. The formulation of the PVA hydrogel matrix containing tripolyphosphate should be evaluated further in order to obtain longer release time of the drug.

Acknowledgment
The authors are grateful for the financial support received from the Ministry of Research, Technology and Higher Education of the Republic of Indonesia through the PDUPT Research Grant contract 410/UN2.R3.1/ HKP05.00/2018.

References
1. Fogel N 2015 Tuberculosis: A disease without boundaries Tuberculosis 95 pp 527-531
2. Centers for Disease Control and Prevention Tuberculosis 2016 [cited 7th May.2017] Available from: https://www.cdc.gov/tb/topic/basics/default.htm
3. World Health Organization Guidelines for Treatment of Tuberculosis 2010 [Internet] [cited 21st Nov. 2017] Available from: http://www.who.int/tb/publications/2010/9789241547833/en/
4. Xue M, Hu H, Jiang Y, Liu J, HE H, and Ye X 2012 Biodegradable Polymer-Coated, Gelatin Hydrogel/Bioceramics Ternary Composites for Antitubercular Drug Delivery and Tissue Regeneration J. Nanomaterial 2012
5. Lio D, Yeo D and Xu C 2016 Control of Alginate Core Size in Alginate – Poly (Lactic-Co-Glycolic) Acid Microparticles Nanoscale Res Lett. 11 pp 9
6. Paradossi G, Cavalieri F, Chiessi E, Spagnoli C and Cowan M K 2003 Poly(vinyl alcohol) as versatile biomaterial forpotential biomedical applications J. Mater. Sci.: Mater. Med. 14 pp 687–691
7. Li J and Mooney D J Designing hydrogels for controlled drug delivery. Nat Rev Mater. 1(12)
8. Rogojanu A, Rusu E, Olaru N, Dobromir M and Dorohoi D O 2011 Development and characterization of poly(vinyl alcohol) matrix for drug release. Dig. J. Nanomaterials and Bionanomaterials 6 pp. 809 – 818
9. Takamura A, Ishii F and Hidaka H 1991 Drug release from poly(vinyl alcohol) gel prepared by freeze-thaw procedure J. Controlled Release 20 pp 21 – 28
10. Wang Q, Du Y, and Fan L 2004 Properties of chitosan/poly(vinyl alcohol) films for drug controlled release J. Appl. Polym. Sci. 96 pp. 808 – 813
11. Shu X Z and Zhu K J 2002 Controlled drug release properties of ionically cross-linkie chitosan beads: the influence of anion structure Int. J. Pharm. 233 pp 217-225
12. Liu Y, Wang S, Lan W and Qin W 2017 Fabrication and Testing of PVA/Chitosan Bilayer Films for Strawberry Packaging Coatings 7(8) 109
13. Cho A R, Chun Y G, Kim B K and Park D J 2014 Preparation of Chitosan–TPP Microspheres as Resveratrol Carriers J. Food Sci. 79 pp 4
14. Alhosseini S N, Mozarthazineh F, Mozaafari M, Asgari S, Dodel M, Samadikuchaksarei A, Kargozar S and Jalali N 2012 Synthesis and characterization of electrospun polyvinyl alcohol nanofibrous scaffolds modified by blending with chitosan for neural tissue engineering Int. J. Nanomedicine 7 pp 25-34
15. Liang S, Liu L, Huang Q and Yam K L 2009 Preparation of single or double-network chitosan/poly(vinyl alcohol) gel films through selectively cross-linking method Carbohydr. Polym. 77 pp 718-724

16. Takahashi H, Chen R, Okamoto H and Danjo K, Acetaminophen particle design using chitosan and a spray-drying technique Chem. Pharm. Bull. 53(1) pp 37-41