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Enhanced antibacterial effect by antibiotic loaded starch nanoparticle

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Abstract The soluble form of starch nanoparticle (SNP) is used for its advantage to encapsulate the antibiotics. In this study, SNP encapsulated antibiotics were introduced to the bacterium, *Streptococcus pyogenes* and the inhibitory effect was studied. The preparation of SNP loaded antibiotics was carried out using microemulsion nanoprecipitation method which does not require sophisticated equipment, hazardous reagents and extreme conditions. The starch was dissolved in urea alkaline solution and precipitated in ethanolic emulsion system which yields smaller sized nanoparticles with larger surface area thus increasing the efficiency of low water soluble drug molecules loading. The SNP obtained are not vulnerable to clumping. The antimicrobial study was done using the disk diffusion test with different amounts of starch loaded with antibiotics. The encapsulation of single/multiple antibiotics and their inhibition is an indicator of the efficacy of SNPs. This study improved the effectiveness of drugs capability that are produced from a cheap source that brought about a large impact.
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1. Introduction

Nanoparticles have been extensively used in a wide range of downstream applications both in soluble and insoluble forms. Among different soluble nanoparticles, starch was profoundly one of the potentials. Starch is a natural polymer, renewable and biodegradable. It is the second most abundant biomass material in nature and found in many plant sources. The starch industry produced starches by the methods wet grinding, sieving and drying (Le Corre et al., 2010). Starch models are described as concentric semi-crystalline multistate structures that are involved in the production of new nano-elements. The disruption of amorphous domains of semi-crystalline granular by acid hydrolysis will produce starch nanocrystal, while gelatinized starch will form starch in the form of nanoparticles. Several methods have been known to produce starch nanoparticle (SNP), such as high-pressure mini-emulsion cross-linking, homogenization, emulsion, microemulsion and nanoprecipitation (Chin et al., 2014b, 2011) In this study, microemulsion method will be considered in producing starch particle having less than 100 nm. In the past, controllable particle sizes of SNP were successfully synthesized by

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precipitation in microemulsion system under controlled conditions. All SNPs obtained are supposed to be spherical in shape and have uniform particle size distribution.

Antibiotics are known to have inhibitory ability against microorganisms. Different bacteria will have different antibiotic sensitivities and some are still resisting. It was stated that liposome and nanoparticles loaded with antibiotics, have increased drug concentration (higher inhibition) at infected sites with minimized drug toxicity (Pinto-Alphandary et al., 2000). In this study, we take an advantage of soluble forms of SNP, which could encapsulate antibiotics, to be used to control the bacterial growth, with a view to apply in anti-bacterial applications. We have aimed to create encapsulated antibiotics to be used as a broad spectrum against harmful pathogenic organisms.

Metal nanoparticles such as silver, selenium and gold are not soluble thus making the encapsulation procedure difficult. Furthermore, these metals are not easy to break and digest by the living system. However, in controlling microbial growth, metal nanoparticles have known to exhibit inhibitory effect with the aid of capturing antibiotics on them (Dizaj et al., 2014). The soluble form of SNP is availed to encapsulate antibiotics to be used in controlling microbial growth in this study. Starch in this particle can easily be digested, is cheaper, available with continuous supply from various sources, and most favorable as it does not create health issues. SNPs have also been reported in many studies to exhibit and help in medicinal and other anti-bacterial applications (Meyabadi et al., 2014; Moon et al., 2011; Sharma and Varma, 2014). Multiple antibiotics can also be encapsulated to function as an effective approach in controlling microbes.

2. Experimental

2.1. Materials

For this study, corn starch powder was obtained from the local departmental store. Sodium hydroxide (NaOH) pellet and 46% Nitrogen Urea were used to dilute the starch. The microemulsion components, 250 mg penicillin and 250 mg streptomycin were from the local Pharmacy. Sunflower oil from the local departmental store acts as an oil phase; incorporation with absolute ethanol as the solvent phase is done. Tween-20 was obtained from Sigma-Aldrich, USA. The common human bacterium, *Streptococcus pyogenes* was supplied by University of Malaya, Malaysia. *Escherichia coli* was from School of Bioprocess Engineering, Universiti Malaysia Perlis. For culturing the bacteria, Luria Broth (LB) was from EMO Millipore Corporation (USA), and the agar plate was prepared by using nutrient agar.

2.2. Preparation of starch solution

The preparation of starch solution and SNPs was carried out in 250 ml beakers. Micropipette (10–100 µl) from Germany was used to measure the amount of the required reagents and as a tool to drop the starch solution into the microemulsion system. Micro-centrifuge was used to separate the nanoparticles and the surfactant. Magnetic stirrer and hot plate were used to ensure the solution is mixed well and prevent the agglomeration among the nanoparticles themselves.

2.3. Preparation of starch nanoparticles (SNPs)

For aqueous alkaline urea solution, the mixture contained 8:10 of NaOH and 46% urea solution, respectively. The mixture was then added with 0.05, 0.1, 0.2, 0.5, 1.0 and 2.0 g of corn starch powder to vary the concentrations. The mixture was heated at 80 °C and stirred continuously at 700 rpm for 30 min or until the starch is well dissolved. The starch was considered to be diluted when the solution is homogeneous. Microemulsion nanoprecipitation method was used in this study to prepare the smaller and uniform sized SNPs. 10 ml of 95% ethanol, 10 µl of Tween-20 (surfactant), and 120 µl of sunflower oil (oil phase) were added and mixed by stirring continuously for 1 hour at 900 rpm.

Starch solution was added dropwise into the microemulsion solution. Between each drop, waited for a while to ensure the nanoparticles are well formed and do not clump. The nanoparticle was then centrifuged at 4500 rpm for 20 minutes to separate the SNPs and the remaining solution. Nanoparticles were washed twice with ethanol to remove the unincorporated components by centrifuging at 10000 rpm for 5 min.

For nanoparticles with antibiotic loading, 120 µl of sunflower oil, 10 µl of surfactant, 10 µl antibiotic solution and 10 ml of ethanol were stirred continuously at 900 rpm. The solution was varied with different amounts of antibiotic which are penicillin and/or streptomycin. About 0.5 ml of starch solution was added into the antibiotic solution. The mixture of SNPs was then washed with ethanol and dried as stated above. Likewise for multiple antibiotics-loaded SNPs, antibiotics (streptomycin and penicillin) were added to the microemulsion solution. Atomic Force Microscope (Digital Instruments Nanoscope) and Scanning Electron Microscope measurements (SEM: JSM-6460) have been done with 1 µm scan size.

2.4. Disk diffusion test

Active cultures were prepared by transferring a loop-full of culture to 5 ml of LB broth and incubated (at 37 °C) for 24 h. To measure the minimum inhibitory, different concentrations of antibiotic were wetted on the paper disk of 5 mm in diameter and placed on the agar plate containing bacteria, then incubated overnight. Penicillin and streptomycin were tested on *S. pyogenes*. On each plate 0.5, 1.0, 2.0, 5.0, and 10 mg/ml of antibiotic was placed with fixed volume of 10 µl on the agar plate containing inoculated bacterial species. Then the inhibition area formed was measured. To compare the effectiveness of SNPs, 10 µl of both single and multiple loaded SNPs was placed on paper disks and placed on bacterial agar plate. Paper disk with only antibiotic solution with the same concentration as in SNPs was also placed and considered as a control.

3. Results and discussion

Pathogenic bacterial infection causes disease that lead to severe illness worldwide. Different lines of study have been made to understand the bacterial mechanism (Yean et al., 2016; Kumarevel et al., 2004; Perumal et al., 2015). The enhancement or development of new antimicrobial agents to treat bacterial infections is of great interest. The objective of this

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