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Original Article

Preparation and evaluation of ampicillin solid lipid nanoparticles

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ABSTRACT

Solid lipid nanoparticles (SLNs) have been studied as a drug-delivery system for the controlling of drug release. These systems have many important advantages, such as biocompatibility, good tolerability, and ease of scale-up. Ampicillin as a β-lactam antibiotic was studied to load on SLNs for control of drug release to increase administration intervals and decrease dose of drug to increase patient compliance and decrease antibiotic resistance. The size of ampicillin loaded nanoparticles, drug loading, drug release profile, morphology and antibacterial effect were studied. The conventional broth macrodilution tube method was used to determine the minimum inhibitory concentration (MIC) and minimum bacteriostatic concentration (MBC) of ampicillin SLNs with respect to P.aeruginosa, E. coli and S. aureus in vitro. Prepared particles show 150 nm of size. Drug loading efficiency was 77±3%, all prepared particles had spherical shape. After 24 hours more than 95% of loaded drug was detected in release samples. MIC and MBC of ampicillin loaded nanoparticles decreased in comparison with free ampicillin against P.aeruginosa, E. coli and S. aureus.

Key words: Solid lipid nanoparticles, ampicillin, drug release, antibacterial efficacy, particle size.

INTRODUCTION

An antimicrobial refers to a substance that kills or inhibits the growth of microorganisms. Since the discovery of antimicrobial drugs in the 1960s, many infectious diseases have been overcome. β-lactams such as penicillins and cephalosporins inhibit bacteria cell wall synthesis. Despite the great progress in antimicrobial development, many infectious diseases, especially intracellular infections, remain difficult to treat. One major reason is that many antimicrobials are difficult to transport through cell membranes and have low activity inside the cells, thereby imposing negligible inhibitory or bactericidal effects on the intracellular bacteria. Over the last few decades, the applications of nanotechnology in medicine have been extensively explored in many medical areas, especially in drug delivery. Nanotechnology concerns the understanding and control of matters in the 1-100 nm range, at which scale materials have unique physicochemical properties including ultra small size, large surface to mass ratio, high reactivity and unique interactions with biological systems. By loading drugs into nanoparticles through physical encapsulation, adsorption, or chemical conjugation, the pharmacokinetics and therapeutic index of the drugs can be significantly improved in contrast to the free drug counterparts.[1] Fattal *et al.*, tested the effectiveness of ampicillin bound to nanoparticles of polyisohexylcyanoacrylate (PIHCA) in treating C57BL/6 mice experimentally infected with Salmonella typhimurium C5. Their study showed that Lower doses delayed but did not reduce mortality. The sharp increase in the therapeutic index of ampicillin after linkage to PIHCA nanoparticles was explained by studies of the distribution of ampicillin, which showed that when bound to nanoparticles, the ampicillin was concentrated mainly in the liver and spleen. These findings warrant further development of

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intracellular targeting of antibiotics on biodegradable polymeric carriers such as PIHCA.[2] Saha *et al*, developed ampicillin trihydrate-loaded chitosan nanoparticles, The nanoparticles demonstrated superior antimicrobial activity to plain nanoparticles and the reference, due probably to the synergistic effect of chitosan and ampicillin trihydrate.[3] Some studies were done to demonstrate effect of antibiotic loaded SLNs to decrease MIC and MBC of antibiotics on behalf of the decreasing dose of administration and patient compliance by Ghaffari et al. [4] In previous studies it has been reported that liposomal encapsulated tobramycin showed considerable antimicrobial effect at concentrations below the MIC of the free antibiotic in vitro. [5,6] Some studies were carried out that presence of phospholipids could decrease MIC and MBC of β-lactam antibiotics. According these studies P.aeruginosa is resistant to many antibiotics, part of this resistance can be because of low permeability of P. aeruginosa outer membrane to many antibiotics or inactivation of antibiotics by P. aeruginosa. Many studies were done to make Gram- negative bacteria outer membrane permeable to antibiotics.[7-10]

MATERIALS AND METHODS

Materials: Cholesterol (Merck, Germany) was used as lipid materials of SLNs. Tween 80 (Merck) was used as surfactant. Ampicillin (Kosar Pharmaceutical C., Tehran, Iran) was used as the active pharmaceutical ingredient. Ethanol and acetone (Merck Chemical Company, Germany) were organic solvents.

Methods:

Preparation of SLNs: Different amounts of lipids were added to 6 mL of acetone and 18 mL of ethanol, and the mixture was heated in a water bath at 70°C. Ampicillin was added to different volumes of deionized water containing 1% (w/w) tween 80 as surfactant.[11] Then, the hot oily phase was added to water at room temperature under homogenizing at 11,000 rpm, using IKA® (Staufen, Germany) T-18 basic, Ultra-Turrax® (Germany) for 20 minutes, and then the mixture was sonicated at 45–50°C for 10 minutes, using a bath-sonicator system (Tecna 6; Tecno-Gaz, Sala Baganza, Italy). SLNs were made when the mixture temperature was decreased to 25°C. The particle size of the nanoparticles was measured by a Zetasizer (ZEN3600; Malvern Instruments, Malvern, Britain). For the determination of drug-loading efficiency, the samples were centrifuged at 26,000 rpm (round per minute) for 35 minutes, at 4°C by a Sigma Laboratories centrifuge (Osterode am Harz, Germany). The drug concentration in the supernatant was analyzed, and the drug-loading efficiency was calculated by using the reverse method applying Equation 1:[12,13]

%Drug loading efficiency:

$$\frac{Drug_{total} - Drug_{supernatant}}{Drug_{total}} \times 100$$

Drug-release study: A release study was performed by using the dialysis sack method by DO405 Dialysis tubing 23 × 15 mm (Sigma, Germany). First, 5 mL of prepared formulation was placed in a dialyzing membrane (10–12 KD) immersed in 50 mL of phosphate buffer (pH 7.4). Next, 2-mL samples were withdrawn in a predetermined time interval, and drug concentration was analyzed by using UV spectrophotometry method.

Morphology study: Morphology of the nanoparticles was characterized by scanning electron microscopy (SEM). The nanoparticles were mounted on aluminum stubs, sputter-coated with a thin layer of Au/Pd, and examined by using an SEM (Philips XL30, Almelo, Netherlands) instrument.

Antimicrobial effect studies: To determine if there is any relationship between the activity of SLNs of ampicillin and drug release profile from colloidal vehicle, and also to compare between the activity of nanoparticles of ampicillin (directly after preparing particles in original medium) with that of free drug, the "well diffusion test" was carried out using P. aeruginosa (ATCC 27853) and E. coli (ATCC 25922) as the Gram-negative pathogenic strains and S. aureus (ATCC 25923) as Gram-positive strain. The bacterial suspensions with a cell density equivalent to 0.5 McFarland (1.5 × 108 CFU/mL) were transferred individually onto the surface of Muller–Hinton agar plates using sterile swabs. Wells with 8 mm diameters were prepared by punching a sterile cork borer onto agar plates and removing the agar to form a well. Aliquots of 100 µl of each of two control solutions, free-drug and blank-SLNs, were delivered into the wells, and third well was prepared for SLNs of ampicillin as the test sample. After incubation time for about 24–48 h, at 35°C–37°C, the zones of inhibition around the wells were measured in mm using a caliper.

RESULTS AND DISCUSSION

Particle size analysis: The evaluation of particles using Malvern zeta sizer (ZEN3600) showed that a normal distribution of particle size in which more

than 70% of particles were smaller than 95 nm in size also 85% and 96% of prepared particles were smaller than 150 and 285 nm respectively. Figure 1 shows the particle size distribution profile.

Drug loading efficiency: Using equation 1, results showed that the loading efficiency of ampicillin was 77±3 %. UV spectroscopy method was used to detection of ampicillin at 207 nm wave length using Shimadzu spectrophotometer (UV 1650PC).

Drug release profile: The release profile of formulation was studied and as predicted for SLNs, a sustained release profile was observed, and ampicillin was released for 24 hours, and after that time, 96% of loaded drug was detected in samples. Figure 2 shows the formulation of the ampicillin SLN release profile. At the first 30 minutes a burst effect was observed and about 30% of loaded drug was detected in samples. As predicted for SLNs, a sustained release profile was obtained, and after 24 hours more than 98% of loaded drug was detected in samples. The first burst effect could be as loading dose of treatment line in clinics and the part of drug which was detected during 24 hours can be used as maintenance dose in patient treatment program.

Morphology study: Morphologic study for formulation was done by taking SEM pictures of prepared SLNs. Figure 3a,b shows the spherical shape of prepared SLNs. Studies showed that the predicted particle size and measured size with a nanosizer is comparable with the size of particles which were detected by SEM.

Antibacterial effect study: The antimicrobial activity of the ampicillin SLNs after preparation in first dispersion is shown in figures 4a,b,c. Results show that drug free SLNs don't show antibacterial efficacy but ampicillin loaded nanoparticles increase antibacterial efficacy of ampicillin in comparison with free drug. Table 1. shows the MIC (Minimum Inhibitory Concentration) and MBC (Minimum Bacteriocidal Concentration) of ampicillin loaded nanoparticles against three strains of bacteria. All figures confirm that SLNs which were free of drug, didn't show any antibacterial effect. Figure 4a shows that free drug could not affect P.aeruginosa significantly to inhibit growth of bacteria but ampicillin loaded nanoparticles show significant antibacterial effects. Figure 4b conform that loading of ampicillin on nanoparticles could increase inhibitory zone of ampicillin against E.coli. Because ampicillin was strongly effective on Gram-positive bacteria, in figure 4c the significant inhibitory margin was not detectable between ampicillin and ampicillin loaded nanoparticles. In some studies the MIC and MBC of ampicillin for P. aeruginosa reported equal to 2 and 4 g/L respectively.[7] In other study Pseudomonas spp called resistance to ampicillin in mg/L ranges. [15] In this study SLNs as lipophilic carrier caused to decrease the MIC and MBC of ampicillin for P.aeruginosa significantly. Based on figure 4a the free ampicillin could not affect the P.aeruginosa which was studied here but SLN loaded ampicillin shows effectiveness. Some studies informed that MIC of ampicillin for Entrobacteriacea is between 0.25-128 mg/L.[14] In the present study MIC and MBC of SLN loaded antibiotic detected equal to 0.5 and 1 mg/L respectively against E.coli, also figure 4b shows that the inhibitory zone of ampicillin grows significantly in SLN loaded form comparison with free drug. Ampicillin is an effective antibiotic agains Gram-positive bacteria and at the least concentration could effect on S.aureus (table 1). Some studies were done to show the synergic effect of ampicillin and alkaloids against ampicillin and/or methicillin resistants bacterial strains.[15] Ghaffari et al. study showed loading of amikacin on SLNs could decrease MIC and MBC of the antibiotic against P.aeruginosa. [4]

CONCLUSION

The aim of this study was to evaluate the properties of prepared ampicillin loaded SLNs. The loaded drug show less MIC and MBC than free ampicillin. Some reasons could be the lipophilic nature of SLNs that enhanced cellular entrance of drug into bacterial membrane and the small size of particles. Consequently it could be concluded that ampicillin might be administered in lower doses or longer intervals by delivering as solid lipid nanoparticles to reduce hospitalization cost and risk of antibiotic resistance also increase patient compliance.

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Table 1. MIC and MBC of ampicillin loaded SLNs against three strains of bacteria

MBC (micro g/ml)	**MIC (micro g/ml)**	**Bacteria strain**
0.5	0.25	**S.aureus**
1	0.5	**E.coli**
8	4	**P.aeruginosa**

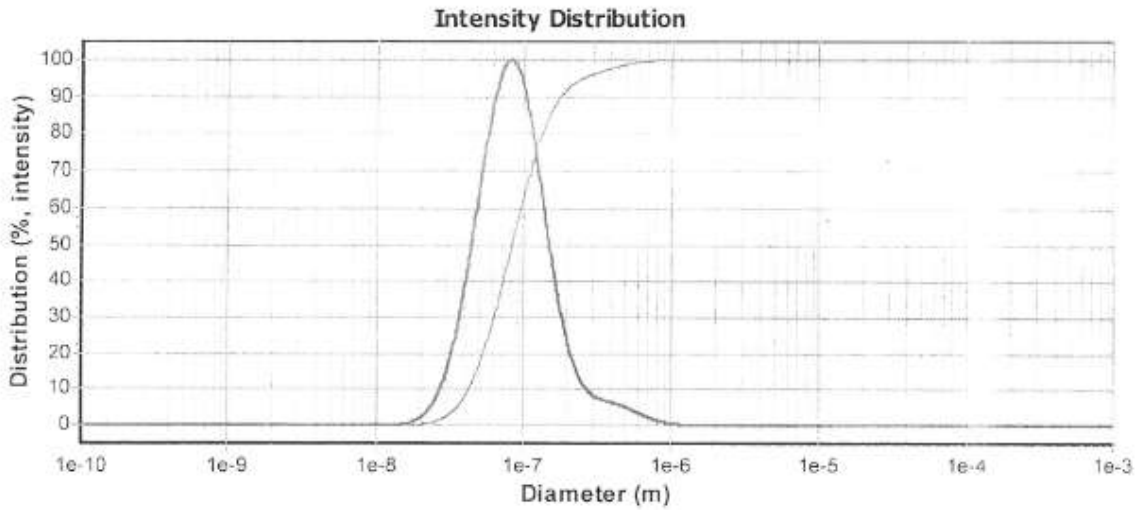

Figure 1. Particle size distribution of Ampicillin solid lipid nanoparticles.

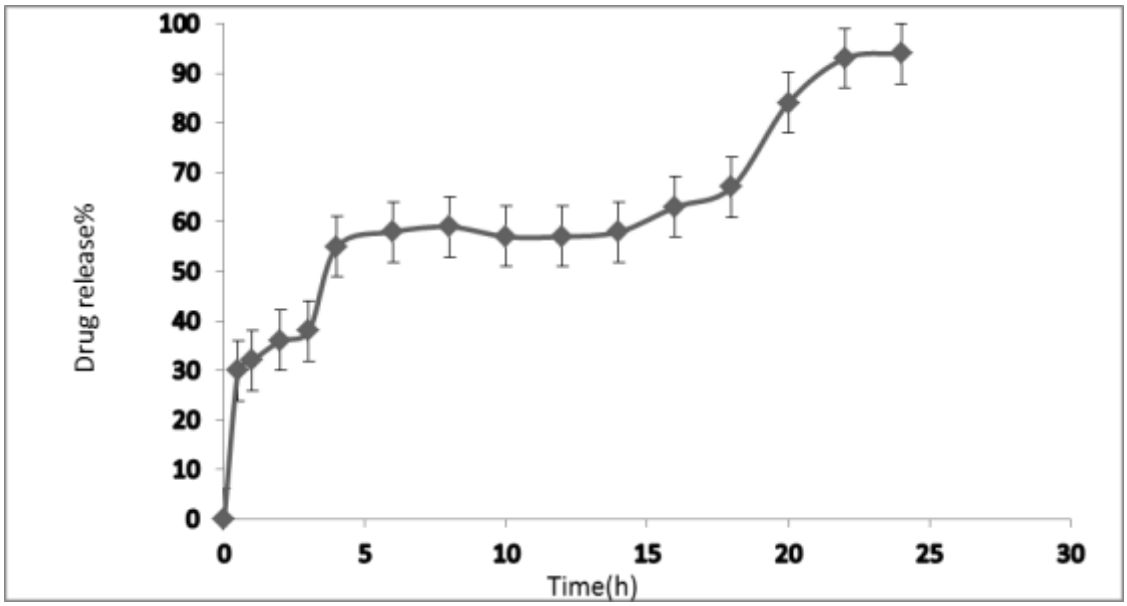

Figure 2. Drug release profile of Ampicillin solid lipid nanoparticles.

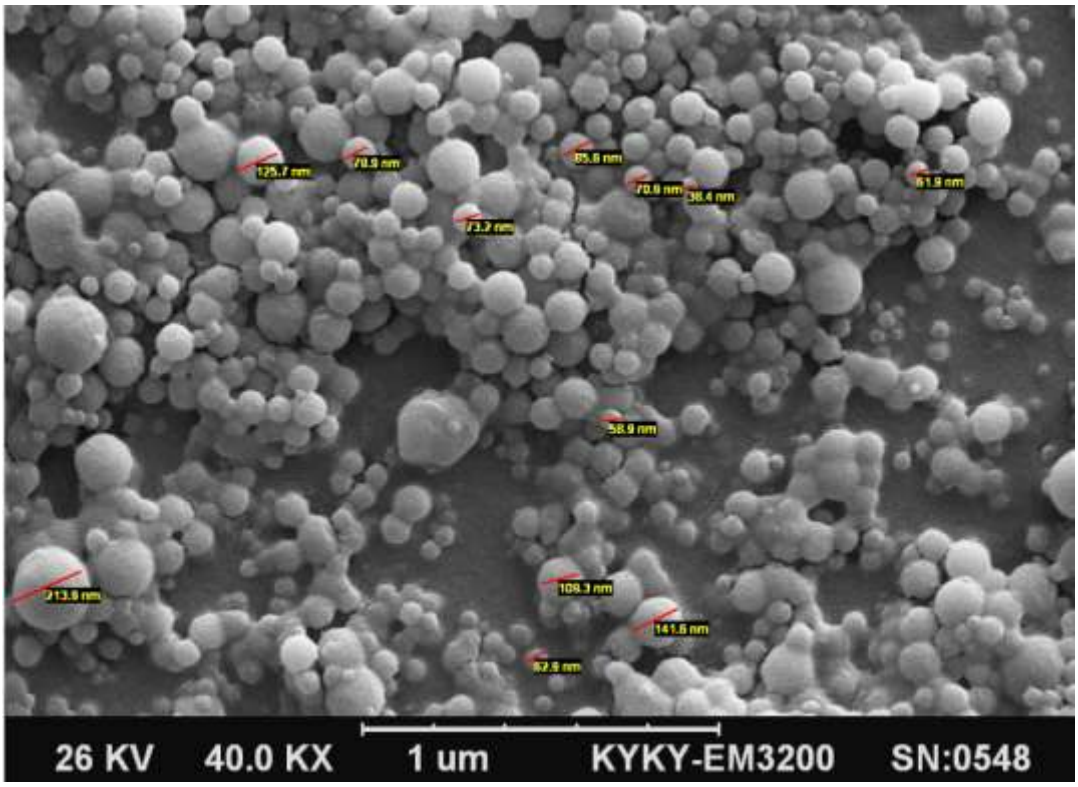

Figure 3a . Scanning electron microscopy picture of optimized Amipicillin solid lipid nanoparticles.

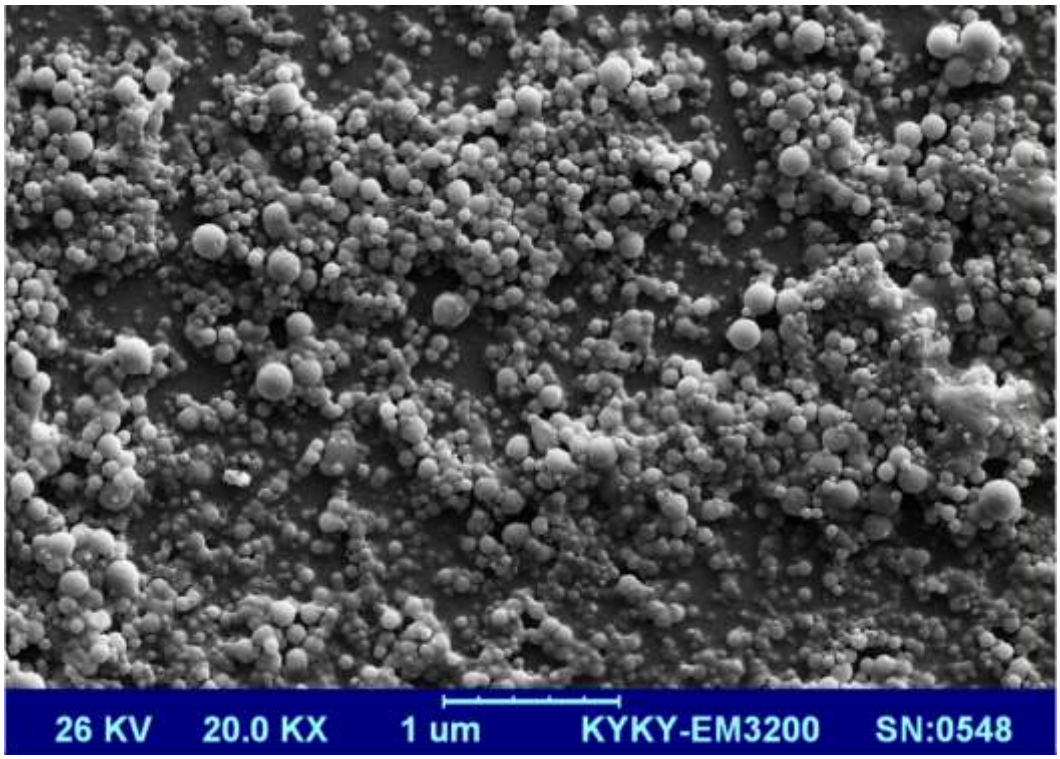

Figure 3b . Scanning electron microscopy picture of optimized Amipicillin solid lipid nanoparticles.

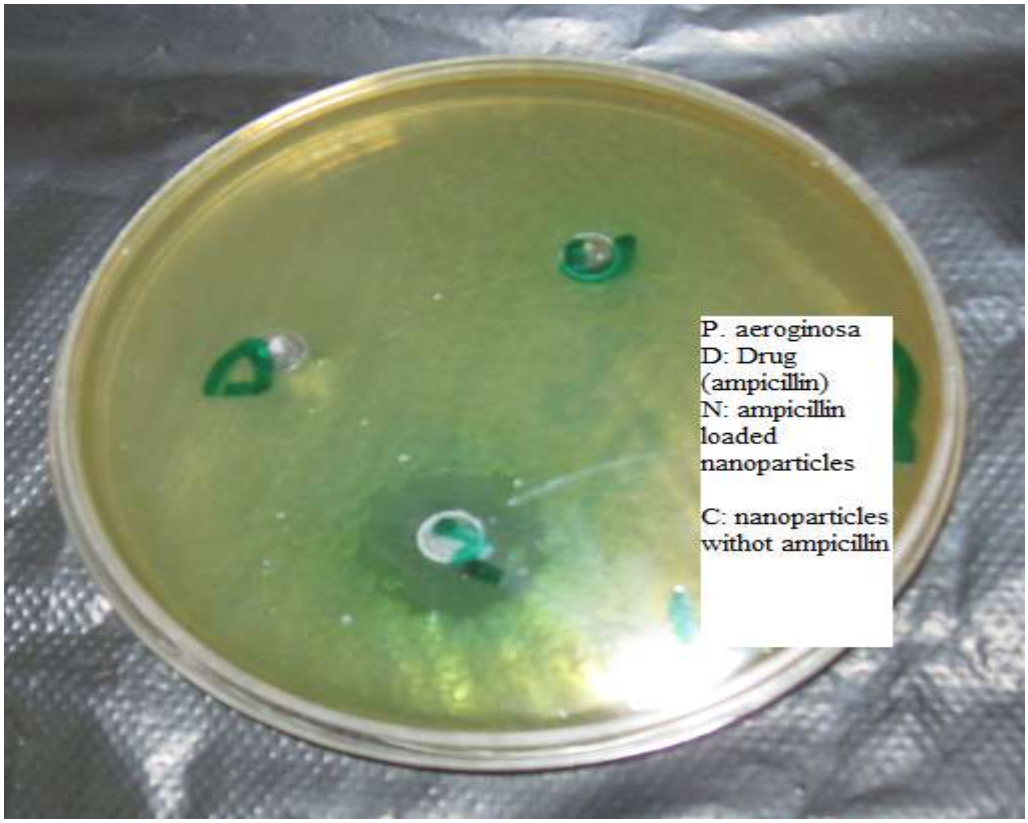

Figure 4(a).

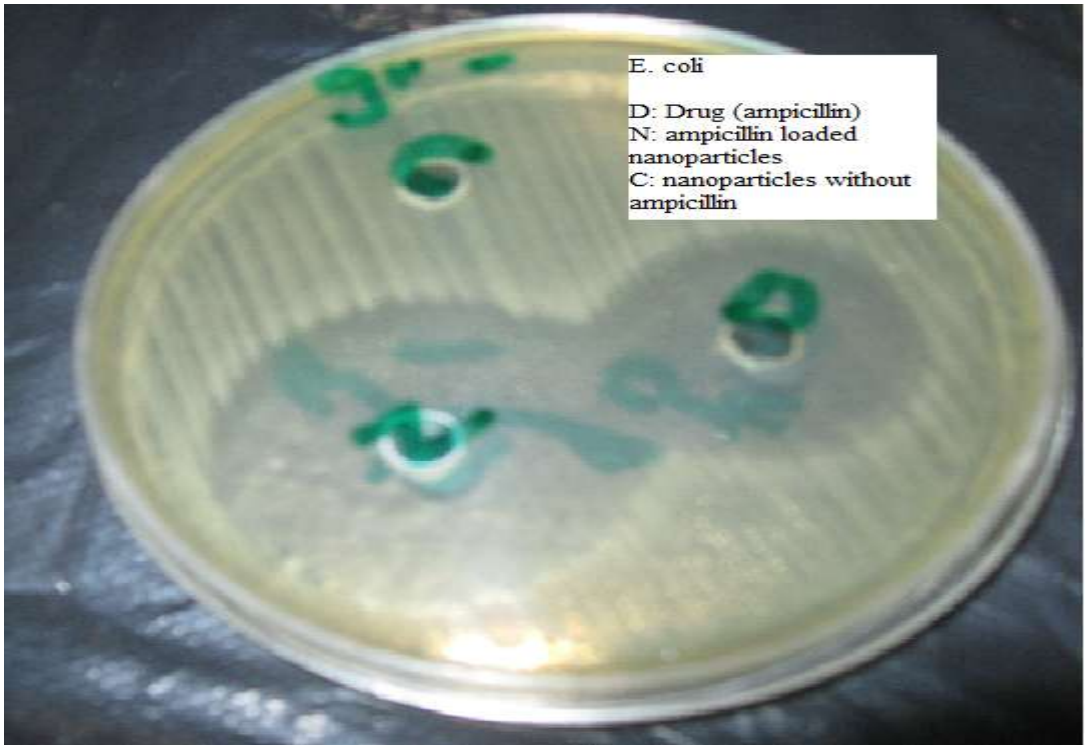

Figure 4(b).

Figure 4(c).

Figure 4(a, b and c): Photographs of the zone of inhibition produced by Ampicillin SLNs and free Ampicillin.

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