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RESEARCH PAPER

# Characterizations and Antibacterial Activity of Ampicillin Loaded on Shellac-Chitosan Nanoparticles

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## ABSTRACT

This work aims to construct nanoparticles using different stabilizers of low-toxic natural polymers. These polymers derived from two natural substances, shellac (SH) and chitosan (CH) which formulated together by a linkage, then loaded with ampicillin. The dynamic light scattering (DLS) technique was used to calculate the size of amp- SH-CH NPs and was (60.63 ±1.87nm) with zeta potential of (+28.2 mV). The shape of amp-SH-CH NPs was evaluated using FESEM and TEM techniques. The formation SH-CH NPs loaded with ampicillin were characterized using FTIR and $^{1}$HNMR. According to the results, SH-CH NPs loaded with ampicillin can be exploited as potential Nano carriers with low toxicity and good antibacterial performance against positively and negatively bacteria. The nanocomposite's minimum inhibitory concentration (MIC) against *Staphylococcus aurius* and *Klebsiella pneumonia* was reported to be 0.001 mg.mL$^{-1}$, in comparison to 0.1 mg.mL$^{-1}$ for the antibiotic nonleaded ampicillin. Ampicillin loaded on shellac-chitosan nanoparticles (Amp-SH-CH NPs) have the opportunity to reduce bacterial resistance and thus increase the antimicrobial activity of ampicillin.

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## INTRODUCTION

A bactericidal antibiotic is ampicillin, which has a vast range and a semi-synthetic beta-lactam penicillin structure. The binding and inactivation of ampicillin involves the proteins that bind penicillin (PBPs) on the cell wall of the bacterial inner membrane. PBP inactivation inhibits peptidoglycan chain cross-linking, which is necessary for the strength and rigidity of bacterial cell walls. This disrupts cell wall of microorganisms' formation, as a result of which the bacterial cell wall becomes weakened and cell lysis occurs [1–3]. Because a wide range of beta-lactamases can hydrolyze ampicillin, it can be applied to treat a variety of diseases that are a result of gram-positive and gram-negative bacteria, such as infections of the respiratory tract, gastrointestinal tract, urinary tract, and meningitis [4,5]. as a result of *Escherichia coli*, *Plasmodium enterica*, *Enterococcus*, *Shigella*, *Typhus*, and other *Salmonella*, non-penicillinase-producing *Neccillins*, *Staphylococcus*, and *Streptococcus* [6]. Many antimicrobials that were previously used effectively have been phased out of clinical usage as a result of the appearance and spread of drug-resistant bacteria. Drug resistance is reducing the efficacy of commonly used antimicrobials. Pathogenic microorganisms now frequently

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exhibit multidrug resistance as a result of excessive antibiotic usage [7,8]. Because the amount of antibiotics commonly used for treatment is much higher than the dose required to kill pathogens, medication efficacy must be assessed. This, in turn, can produce a toxic effect. As a result, several crucial medications, including Ampicillin, lost their biological function. Currently, we are interested in enhancing the biological characteristics of ampicillin by using natural nanoparticles in this research [9–11]. Nanoparticles have many uses due to their unique properties, including biomolecule discovery, drug delivery, and release [12,13]. To ensure successful antibiotic therapy, the dosage should be lowered to minimize adverse effects while increasing stability, which was accomplished by loading ampicillin with natural nanoparticles (Fig. 1). Shellac is a natural substance composed of a complex combination of esters, polyesters, and poly hydroxy acids [14]. It is the hardened secretion of the microscopic parasitic insect *Kerria lacca*, also known as the lac bug in the area. It is the only commercial resin known to be of animal origin [15,16]. With the advancement of biological sciences, more emphasis has been placed on the limits of synthetic materials, such as their irritability, carcinogenicity, and so on. Shellac is the only animal resin that may be utilized in medicinal applications, so it has been employed as a natural drug carrier [17]. Shellac's surface carboxyl groups allow it to interact chemically or physically with nanoparticles, drugs, polymers, and cells. Shellac was used many times as nanocarrier loaded with different types of antibiotic such as chlorhexidine, vancomycin, and berberine [18–20]. On the other hand, chitosan is also a natural carbohydrate polymer that has been partially deacetylated from chitin, a natural biopolymer obtained from shellfish, crustaceans, microorganisms, fungi, and yeast. 2-Deoxy-2-acetylaminoglucose is the basic unit of the chitin polymer [21–23]. Chitosan was also used as nanocarrier with many antibiotics such as it is used to deliver intravenous medications to the ocular mucosa. Several chitosan-based nanocarriers with distinct curcumin delivery properties Chitosan was also modified with shellac NPs and loaded with ciprofloxacin to improve its anti-cancer and anti-bacterial activity. shellac-chitosan nanocarriers was used few times with some drugs [24,25]. Depending on the properties of the prepared carrier, the ampicillin-loaded carrier provides high stability, a low rate of release, and continuous drug delivery.

## METHODS AND MATERIALS

### *Materials*

Alkaline solution of shellac ammonium was provided from (Stroever Schellack, Bremen, Germany). It was employed as a Shellac ammonium's alkaline solution was made available by (25 wt%) soluble ammonium salt at pH > 7. Sigma-Aldrich delivered the ampicillin medication with purity of 100%. Chitosan (95.5%) was obtained from Shaanxi Sangherb Bio-Teching. Fluka (Switzerland) company supplied glutamic acid, while Gainlan Chemical Company (UK) supplied the DMSO solvent. Bacteria were cultivated and analyzed in the biology department of the College of Science at the University of Babylon. In all experiments, deionized water was utilized.

### *Preparation of ampicillin-loaded shellac-chitosan Nano carriers (Amp-SH-CH NPs)*

The method of the preparation used this study was based on the method used by Kerset. Pet [26,19] with some modifications. A shellac-chitosan-based nanocarrier with a stable salt bridge of glutamic acid was created using the escalation method. In a 1:1 molar ratio, shellac, chitosan, and glutamic acid was dissolved in 2.0 mL DMSO. The mixture was put in a round (50 mL) beaker. to re-condensate the prepared mixture for 60-65 $^{\circ}$C for one hour. After that, the SH-CH nanocarrier precipitate was rinsed with 100% ethanol and filtered. The ampicillin medication is next loaded using thionyl chloride in excess ($SOCl_2$) and stirred. Ampicillin was given an equal quantity of the produced nanocarriers (SH-CH NPs). This combination was reheated at 60–65 $^{\circ}$C for roughly an hour. The precipitate of Amp-SH-CH NPs was then washed with 100% ethanol and filtered. Fig. 2. Shows the process for loading ampicillin into SH-CH NPs.

### *FTIR and $^1$HNMR Studies*

Using the KBr technique on a Shimadzu 8400 spectrophotometer (400–4000 $cm^{-1}$), Fourier transform infrared (FT-IR) analysis was performed (Japan). As a result of dissolving loaded medication in DMSO and utilizing a Varian Inova 500 MHz spectrometer, the nuclear magnetic resonance ($^1$HNMR) approach was also carried out (USA). These investigations were used to characterize the production of shellac-chitosan NPs loaded with

ampicillin and to confirm cross-linking between anionic nanocarrier molecules and cationic ampicillin molecules [27].

*Amp-SH-CH NPs Morphology Measurements*

Light scattering (DLS), employing Zeiss equipment for TEM analysis (Germany, EM10C, 100Kv) and field emission scanning electron microscopy (FESEM-Tescan, Mira3) were used to investigate the size, charge, and shape of amp-SH-CH NPs in suspension at pH 4.

*Amp-SH-CH NPs Release Rate Study*

The ampicillin release rate was determined by dissolving 0.01 gram of loaded ampicillin on shellac-chitosan NPs 1:1 DMSO:$H_2O$ in 10.0 ml (pH 7.2). Medication was placed in a porous dialysis bag with a width of 2.5 nm. MWCO and immersed in a buffer solution of 50 mL. (at pH 2 and 7.2). A magnetic stirrer with a speed of 100 rpm was used. Every 2 hours for 12 hours, at 37 ˚C, the drug's absorbance in acidic and basic solutions was determined. The equation 1 was used to compute the amount of medication released [28].

M released denotes the quantity of Amp-SH-CH NPs released from shellac-chitosan nanoparticles at time t, whereas M total denotes the total amount of Ampicillin medication which loaded on SH-CH NPs.

*Amp-SH-CH NPs' Antibacterial Effectiveness*

Ability of Amp-SH-CH NPs to combat microorganisms against two types of bacteria, positive and negative gram, was investigated. The minimum inhibition concentration (MIC) was employed to quantify the inhibitory impact of the compounds generated on these bacteria. This procedure is based on past work [29,30]. To reach a cell density of 3 * 106 colony-forming units, all microbial cultures were cultivated in nutrient broth medium at 37 ˚C (CFU). Mueller-Hinton Broth was used to fill the wells of 96 well plates (MHB). MHB concentration in the first column of 96 well plates was altered. This first column was filled with an equal volume of test samples, namely ampicillin and SH-CH-amp NPs. To generate a decreasing concentration gradient, the loaded test samples

$$\%In\ vitro\ Amp-SH-CH\ NPs\ release = \frac{M_{released}}{M_{total}} * 100 \quad (1)$$

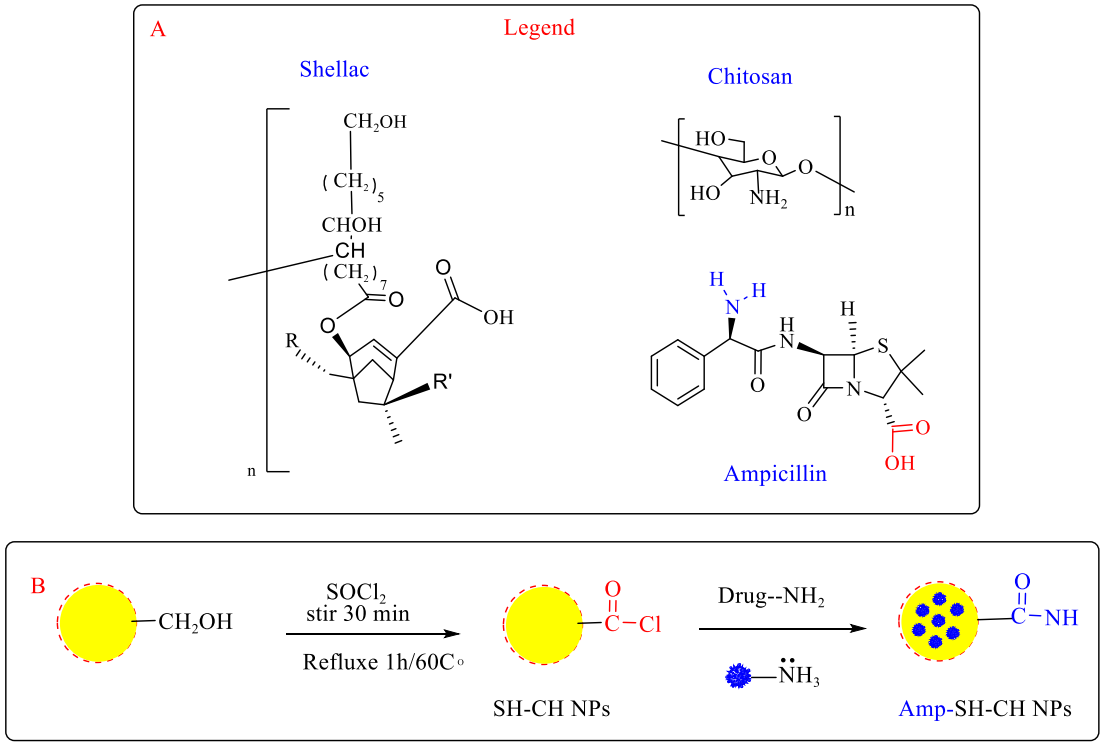


Fig. 1. loading ampicillin on natural SH-CH nanoparticles (Amp-SH-CH NPs)

were serially diluted with a multi-channel pipette. 10 mL of activated microbial cultures were added to each well. For 24 hours at 37˚C, the plates were incubated.. The MIC was determined by witnessing complete suppression of the bacterial population at varied concentrations. The minimal concentration at which full suppression of the bacterial population seen was recorded as the MIC of that specific drug.

## RESULTS AND DISCUSSION

*Characterization of Ampicillin Loaded Shellac-Chitosan NPs (Amp-SH-CH NPs)*

In the presence of a salt bridge facilitating the dissociation of the nanocarrier prepared by the modified ion crosslinking method, ampicillin is loaded onto a nanocarrier prepared from two natural polymers (chitosan and shellac). Fig. 3(A and B) depicts the colloidal particle size range (60.63 ±1.87) nm with zeta potential of surface charge of the loaded nanocarrier (+28.2 mV) for Amp-SH-CH. TEM was used to verify the features. Morphology of the ampicillin-loaded nanoparticles, according to Fig. 3 B. The drug-loaded nanoparticles have a spherical shape at nano size.

*FTIR and $^{1}$HNMR Studies*

FT-IR spectra of the loaded carrier (Amp-SH-CH), which revealed these groups of bands: (3211,3323,3340,3352,3394) N-H Amide, (1516) N-H Amine, (2933, 2886) Alkyl-CH, , (1689, 1654)

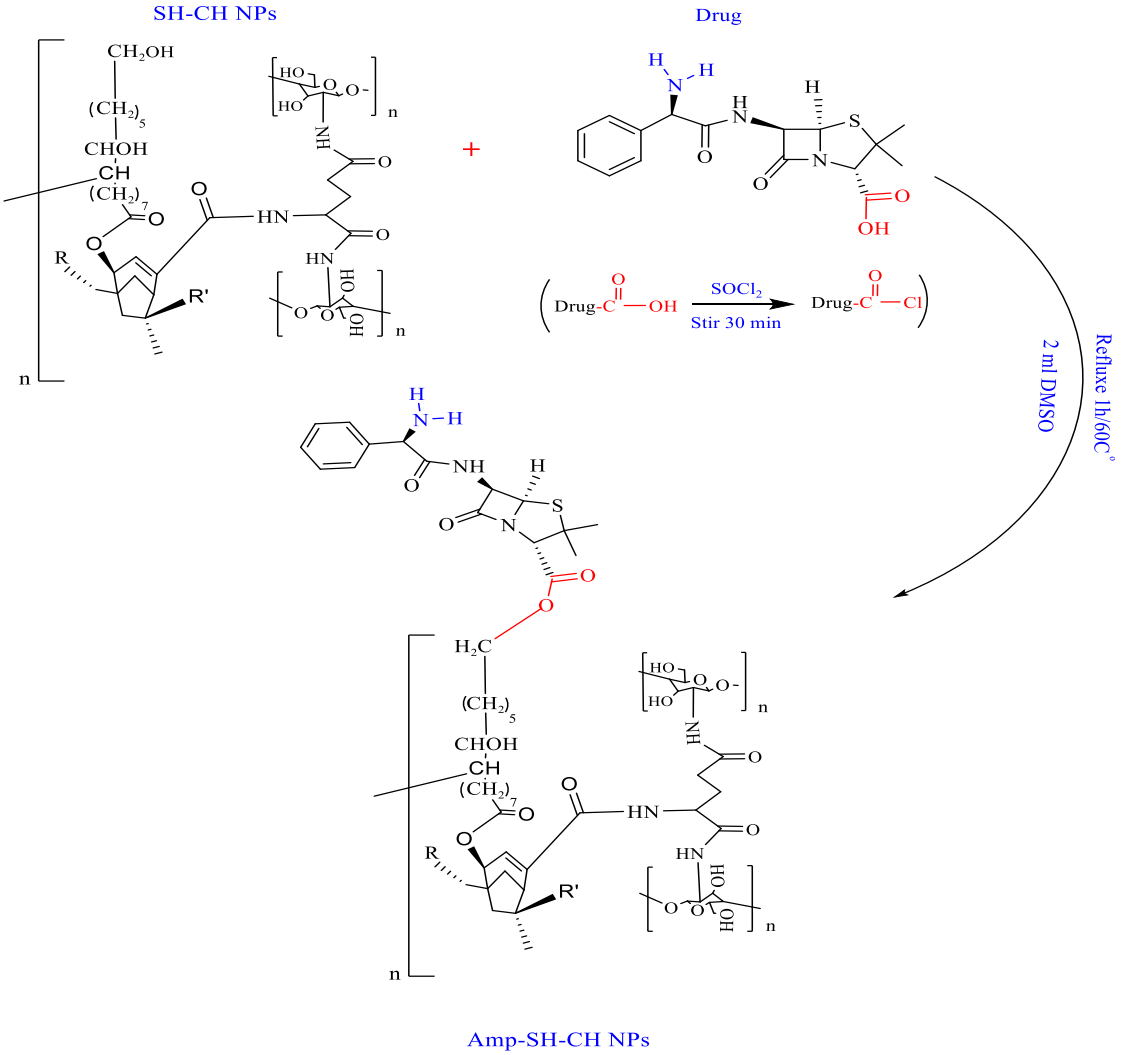


Fig. 2. The preparation steps of Amp-SH-CH NPs .

C=O Amide, (1462, 1421, 1408) C=C Aromatic, (1388, 1381, 1346) C-O Ester, (1732) Ester C=O, (1242, 1165,1082,1014) C-N Amine, (3740) Amine N-H, Aromatic C-H (947, 709), Carboxylic acid C=O(1708,1701) Fig. 4(A). Fig. 4(B) depicts $^{1}$HNMR spectrum of loaded nanocarrier (Amp-SH-CH) in a solvent (DMSO d6,499.42 MHZ), which revealed a mono at signal 1.2 ppm for R-$CH_3$ protons, a multiple at signal 1.9 ppm for C-H Alkyl, and a polysignal at 2 ppm R-S-C-H and a polysignal at 2.1ppm for R-N-C-H protons, a polysignal R-OH (SH) Alcohol at 2.3,2.4,2.5 ppm, a monosignal at

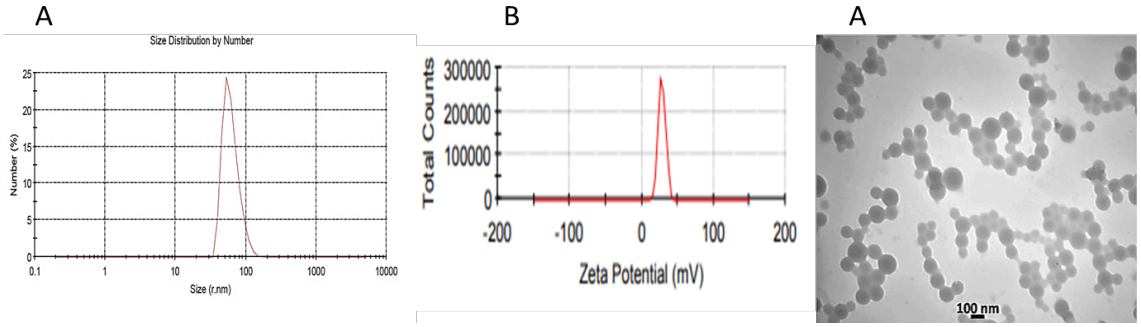


Fig. 3. (A and B) the size and the surface charge of Amp-SH-CH NPs, (C) TEM of Ampicillin Loaded Shellac- Chitosan NP

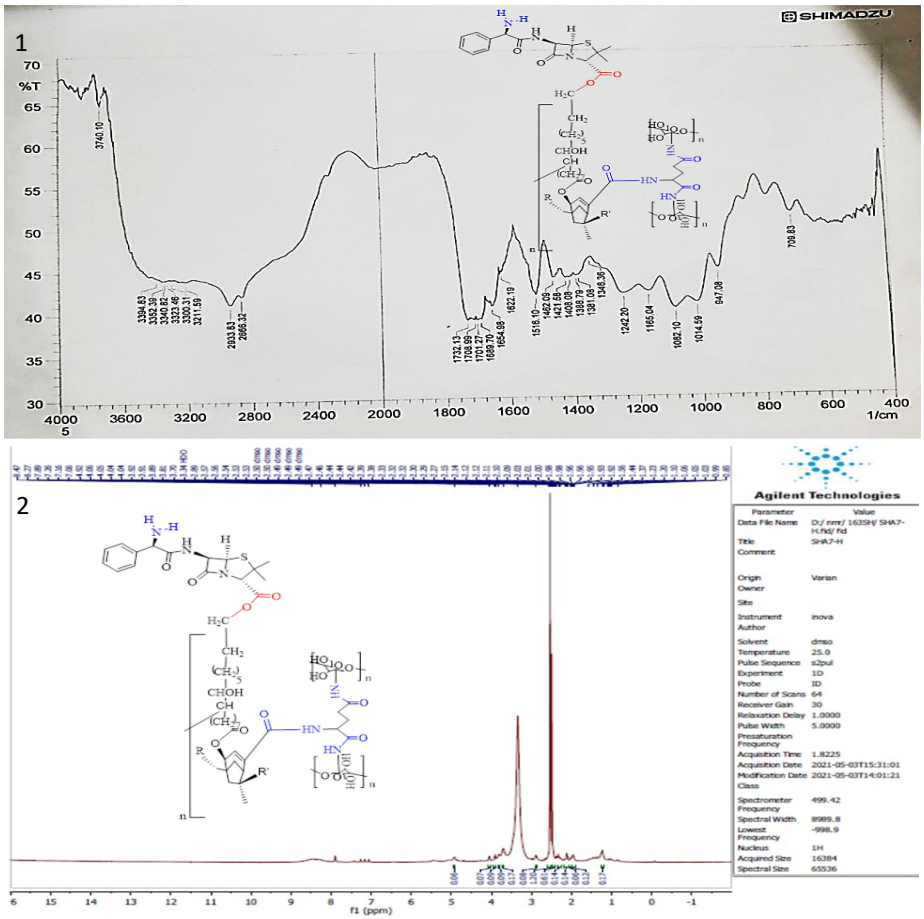


Fig. 4. FT-IR spectra of ampicillin loaded with the Nano carrier, FT-IR1 Amp-SH-CH and $^{1}$HNMR 2spectrum of the loaded Nano carrier Amp-SH-CH.

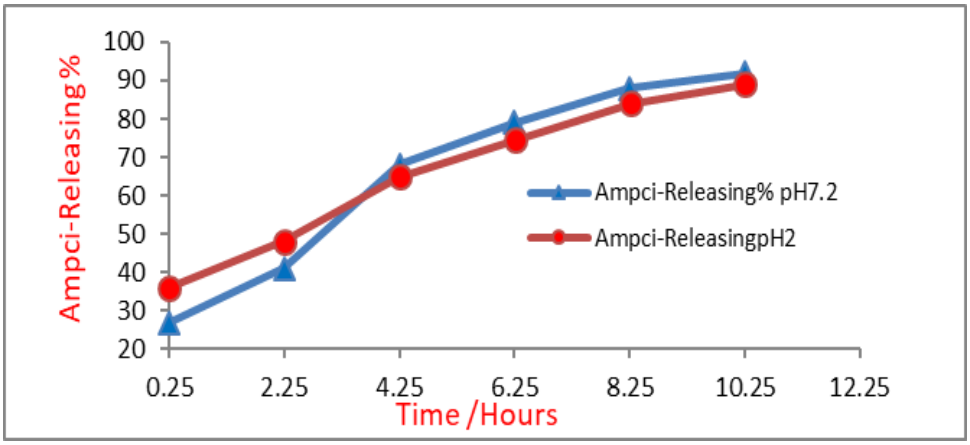


Fig. 5. In-Vitro Amp-SH-CH Evaluation of the Release

Table 1. Amp-SH-CH NPs' effectiveness against microbiological strains.

| Microbial strain | Amp-SH-CH mg/mL | Ampicillin mg/mL | SH-CH mg/mL |
|---|---|---|---|
| *Staphylococcus aurius* | 0.00 1 | 0.1 | - |
| *Klebsiella pneumoniae* | 0.001 | 0.1 | - |

2.6,2.9 ppm C-$CH_2$-C (SH) and a monosignal at 3.5 R-C=O-O-C-H (SH), monosignal at 3.7 ppm R-$NH_2$ Amine, monosignal at 3.8 ppm Ph-OH, multiple signal at 3.9,4,4.6 ppm Ph-OH, and monosignal at 4.9,7.2,7.4ppm R-C=O-N-H Amide and monomeric signal at 7.9 ppm Ar-H [31–33].

*The In-Vitro Amp-SH-CH Release Evaluation*

Release of Amp-SH-CH NPs in an acidic environment (pH 2) is larger than in a basic environment at a maximum wavelength of 350 nm, based on the type of medication (weak acid) placed on the nanoshellac-chitosan. This leads us to conclude that the rate of drug release at the end of the half-day release procedure is faster, reaching 100% in an acidic medium compared to a basic medium, where the highest drug release was about 80% after 12 hours (Fig. 5). The majority of medicines can be accessed as weak acids or weak bases. Due to the absence of ionization and the breakage of the ester bond, the weakly acidic medication is released more rapidly in the acidic environment compared to the basal medium (the intestines), and the release of the drug starts gradually.

*Amp-SH-CH NPs' Antibacterial Effectiveness*

The antibiotic action of the loaded nanocarrier was increased against both *Staphylococcus aurius* and *Klebsiella pneumoniae* bacterial strains. The Amp-SH-CH NPs minimum inhibitory concentration (MIC) against *Staphylococcus aurius* and *Klebsiella pneumonia* was reported to be 0.001 mg/mL, in comparison to 0.1 mg/mL for the antibiotic ampicillin against both kinds of bacteria. The nanocarrier proved to be non-toxic.

## CONCLUSION

By boosting the inhibitory effects of already available antibiotics and administering them using a non-toxic formulation, our results provide a potential method for treating drug-resistant bacteria. The effect of the surface charge of the nanocarrier loaded with different antimicrobials as electrostatic attractions between the antimicrobial agents loaded on the prepared nanocarrier and the negatively charged bacterial surfaces, which lead to their adhesion to the surface of the bacteria with high efficiency. Therefore, electrostatic attraction appears to play an important role in the binding probability of the prepared nanocarrier

to the surface of bacteria, thus increasing the delivery potential of ampicillin.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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