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# Preparation of Antimicrobial Hydrogel Film Based on Chitosan, Poly Vinyl Poly Pyrrolidone, and Copper Ions

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**Abstract**

Easy operation, eco-friendliness, and high capacity are the advantages of using hydrogel-based membranes in water treatment, medicine, drug delivery, wound care products, and protective clothing. In the present work, we tried to prepare bio-films with antimicrobial ability by incorporating of Cu ions in chitosan and poly vinyl poly Pyrrolidone. The prepared film was characterized by the thermal gravimetric analysis (TGA), Fourier transforms infrared spectroscopy (FT-IR), scanning electron microscopy with X-ray microanalysis (SEM-EDS), and X-ray powder diffraction. The antimicrobial ability of the prepared film was confirmed due to the analysis of antibacterial assay results by using gram-positive and gram-negative bacteria. Based on the achieved data, the prepared multifunctional bio-film could be a considerable candidate in packing industry, especially in medical compounds, tissue engineering, and pharmacology.

**Keywords**: Antimicrobial, Biofilm, Polymer, Copper, Chitosan.

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## Introduction

The use of copper and silver as anti-microbial metals has been common for a long time. Most of the supplies and equipment used by scientists were made of brass-an copper-zinc alloy. In the recent years, organic materials containing metal ions such as copper, iron, zinc, silver, and titanium have been considered as antibacterial agents [1,2]. Nanocompounds and nanoparticles are the best candidates due to the reported antibacterial properties [3-5]. Nanometals have unique physical and chemical properties due to quantum sizes and special surface areas which distinguish them from their bulk state. The high surface area in nanocompounds allows the atoms and molecules to contact directly with the microbial cell membrane and destroy the microbial membrane during the metal ion release process [6,7]. The antifungal, antibacterial, and antimicrobial properties of copper make it useful in medical fibers and textiles such as sterile gauze, clothing, sutures, etc [8].

Some technology has recently succeeded in using Cu nanoparticles to produce antibacterial fibers [9]. Because copper nanoparticles are less expensive than silver nanoparticles (which have very high antibacterial properties), the researchers decided to replace silver nanoparticles with copper nanoparticles in antibacterial textiles [10]. Copper is an effective agent in killing bacteria, improve blood flow, and repair tissues [11]. Copper has excellent properties that endow it as an excellent active ingredient in medical products [12,13].

In the present research, we tried to prepare an antimicrobial hydrogel-based film using chitosan (CS), polyvinyl poly pyrrolidone (PVPP), and copper ion (Cu). PVPP has been applied as a polyphenol agent in adsorption process. Solvents do not easily affect this water-insoluble compound. Therefore, it is a good candidate in different medical situations [14,15]. Chitosan is used in clinical products as a drug carrier due to its biocompatibility, easy digestibility, non-toxicity, high adsorption capacity, and availability [16-18].

SEM, XRD, FT-IR, and TGA analysis have been applied to determine the morphology and chemical structure of the prepared bio-film. EDS results confirmed existence of Cu particles in the prepared bio-film. It demonstrated acceptable antibacterial activity against Gram-positive and Gram-negative bacteria. It is believed that the combination of a resistant polymer, a biodegradable hydrogel and antibacterial particles will open up new perspectives on the use of these substances in medical products.

## Experimental

*Materials and methods*

Chitosan (190,000-310,000 Da) (CS), PVPP (40000 Da), and copper nitrate ($Cu(NO_3)_2$) were prepared from Sigma-Aldrich Company and used without any purification. To identify, and analyze the morphology, structure, and thermal stability of the prepared film, scanning electron microscopy (SEM, Tescan model, Vega Company), X-ray diffractometer (XRD, Philips PW 1800 X'PERT), Fourier transform infrared spectrometer (FT-IR, Vertex model, Bruker Company), STA/TGA (Simultaneous Thermal Analysis), NETZSCH model, NETZSCH Company, Germany have been used.

*Preparation of the PVPP/ CS/Cu film*

In the first step, a solution containing 1.5 g polymer was prepared. Due to the low solubility of the polymer in water, the solution was stirred for 10 hours and then sonicated for 30 min. To prepare chitosan solution, 5 g of chitosan in 20 ml of 2% acetic acid solution was solved. Dissolve 4 g of copper nitrate (MW = 187.56 g/mol) in a suitable solvent and dilute with solvent to a total volume of exactly 20 mL in order to get the desired concentration of 1.06 M. The mixture of polymers and copper nitrate was stirred for one hour to obtain a uniform solution. In the next step, the solution containing the polymer and chitosan was gently mixed together and then casted into the petri dish to form a membrane and evaporate the solvent[17,18].The image of obtained film is given in Figure 1.

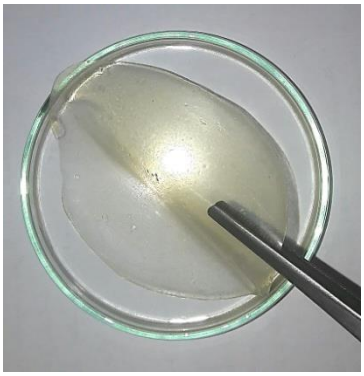


**Figure 1.** Image of prepared bio-film.

*Antibacterial sensitivity test*

Four microbial species including two Gram-positive bacteria, *S. aureus* (ATCC 25923); *Bacillus cereus* (ATC 11788); and two Gram-negative bacteria, *E. coli* (ATCC 35218); and *Pseudomonas aeruginosa* (ATCC 49189) were used. The microorganisms were cultured in

tryptic Soy Broth (TSB) media and incubated at 37 °C for 24 h. The antibacterial properties of the prepared copper nanoparticles against the aforementioned bacteria was carried out using the Disk Diffusion Susceptibility Test method [19]. The bacterial culture suspension was adjusted to an optical density of 0.1 at 600 nm (approximately $1.5 \times 10^8$ CFU/mL) using the McFarland standard turbidity which were later spread on Mueller-Hinton agar (MHA) (Merck, Germany). Blank sterilized paper disks were loaded with freshly prepared sample and were applied to the surface of agar plates incubated with tested pathogenic strains. Standard antibiotic disc (tetracycline 30 μg) was used as a control group. The inhibition zone measured after 24 hours of incubation at 37 °C [20].

**Results and discussion**

Figure 2 shows the scanning electron microscopy of the prepared bio-film. The surface of the film is uniform and the distribution of copper microparticles is similar. The aggregation of Cu micro particles could not be seen in the prepared film. The observation of some peaks in the energy dispersive spectrum (EDS) in Figure 2b confirmed the presence of the elemental form of Cu in the film. Compared to the carbon and nitrogen EDS peaks, the peaks in the prepared bio-film are around 8-9 keV, with very weak intensity, which are due to the low amount of Cu. The content of Cu in the film was 0.16 % by weight. Moreover, the EDS result demonstrates that nearly all the Cu were successfully immobilized on the surface of film.

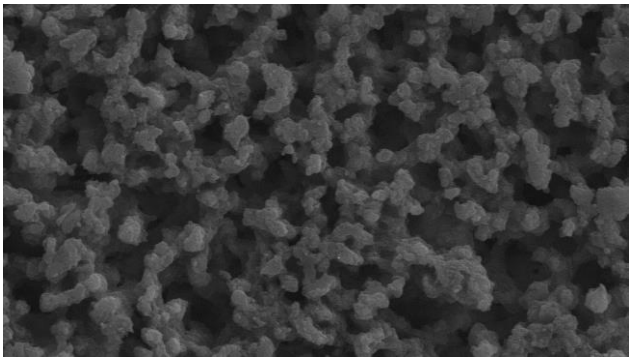
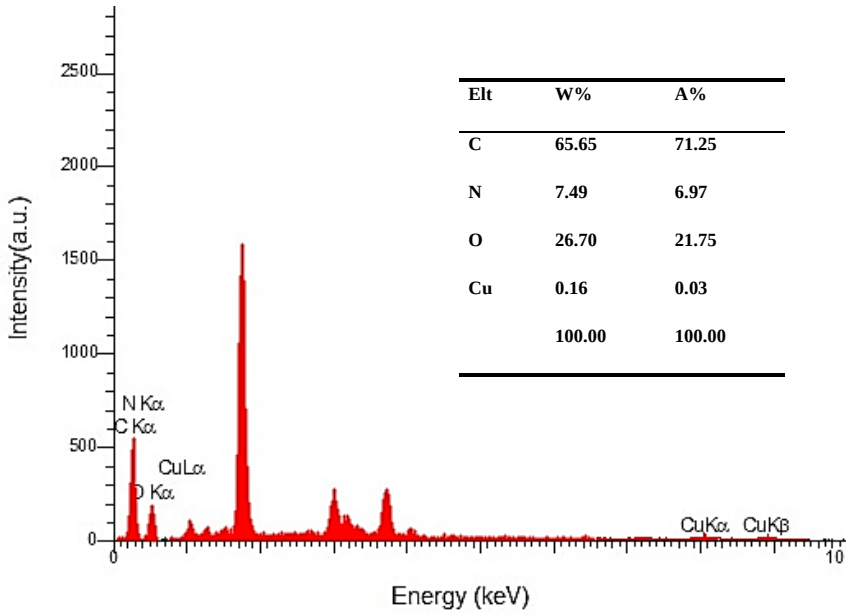


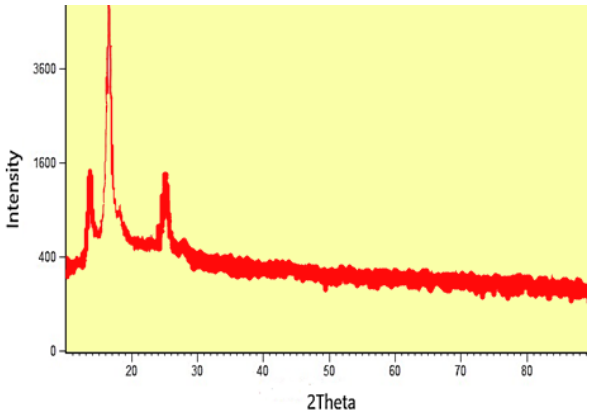
**Figure 2**a. SEM imageof PVPP/CS/Cu film.




| Elt | W% | A% |
|---|---|---|
| C | 65.65 | 71.25 |
| N | 7.49 | 6.97 |
| O | 26.70 | 21.75 |
| Cu | 0.16 | 0.03 |
| | 100.00 | 100.00 |

**Figure 2b.** EDS of PVPP/CS/Cu film.

The XRD pattern of the PVPP/CS and PVPP/CS/Cu films are given in Figure 3. Based on the reported data in the literature usually all of polymeric compounds with amorphous structure show a broad peak at 2θ = 10-30°. Our result in Figure 3 confirmed the amorphous structure of used PVPP and chitosan in the film. In addition, the intensity of the peaks between 10-30 is may be due to the incorporation of Cu particles onto film. The presence of some peaks at about 2θ= 32° and 50° pointed to the (111) and (200) planes of the fcc structure of the copper, respectively [21-25].




**a**

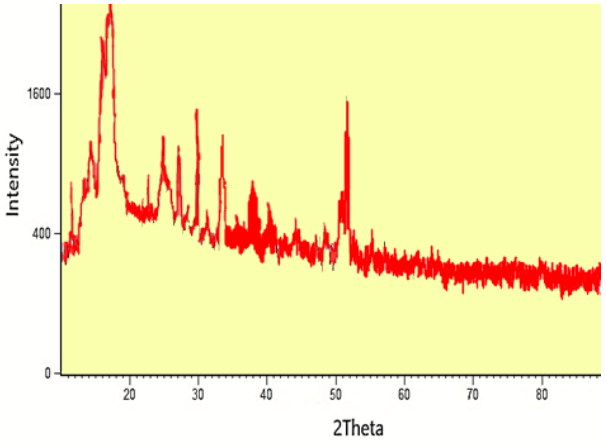



**Figure 3.** XRD patterns of ***a***:PVPP/CS and **b**: PVPP/CS/Cu film.

Variation in the position and strength of the chemical bonds in CS/Cu, and PVPP/CS/Cu film using FTIR analysis is studied based on Figure 4. For CS/Cu a characteristic peak in the region 3443 $cm^{-1}$ is due to the amine and hydroxyl group stretching, and the intermolecular hydrogen interaction. The absorption peaks between 2900-2800 $cm^{-1}$ show C-H symmetric and asymmetric stretching, of polysaccharide molecules. The N-acetyl groups showed the bands in 1644 $cm^{-1}$ and 1325 $cm^{-1}$, respectively. The bands at 1413 and 1339 $cm^{-1}$, confirmed the presence of $CH_2$ bending and $CH_3$ symmetrical vibration. The bands at 1073 $cm^{-1}$are related to C-O stretching [26,27]. The peak at 1021 $cm^{-1}$ indicated the C-O-C of the ether group in the chitosan structure. The bands are shown at 1644 $cm^{-1}$ and 2924 $cm^{-1}$ are due to the amino groups. The stretching vibration of the hydroxyl groups and hydrogen bonds was observed in 3464 $cm^{-1}$ regions [27,28].

The spectrum of the PVPP/CS/Cu film is also shown in Figure 4. The intensity of peaks in regions 3100-3500 $cm^{-1}$ reduced due to the interaction between chitosan and PVPP functional groups. Furthermore the characteristic peak at 1413 $cm^{-1}$ attributed to the pyrrolidinyl group present in PVPP [29]. In general, the position and intensity of the peaks do not change significantly in PVPP/CS/Cu film. This small change can also be attributed to the interaction of the two polymers. Furthermore, all of the spectrums in Figure 4 are the same and there is not any important variation in lication and intensity of bands.

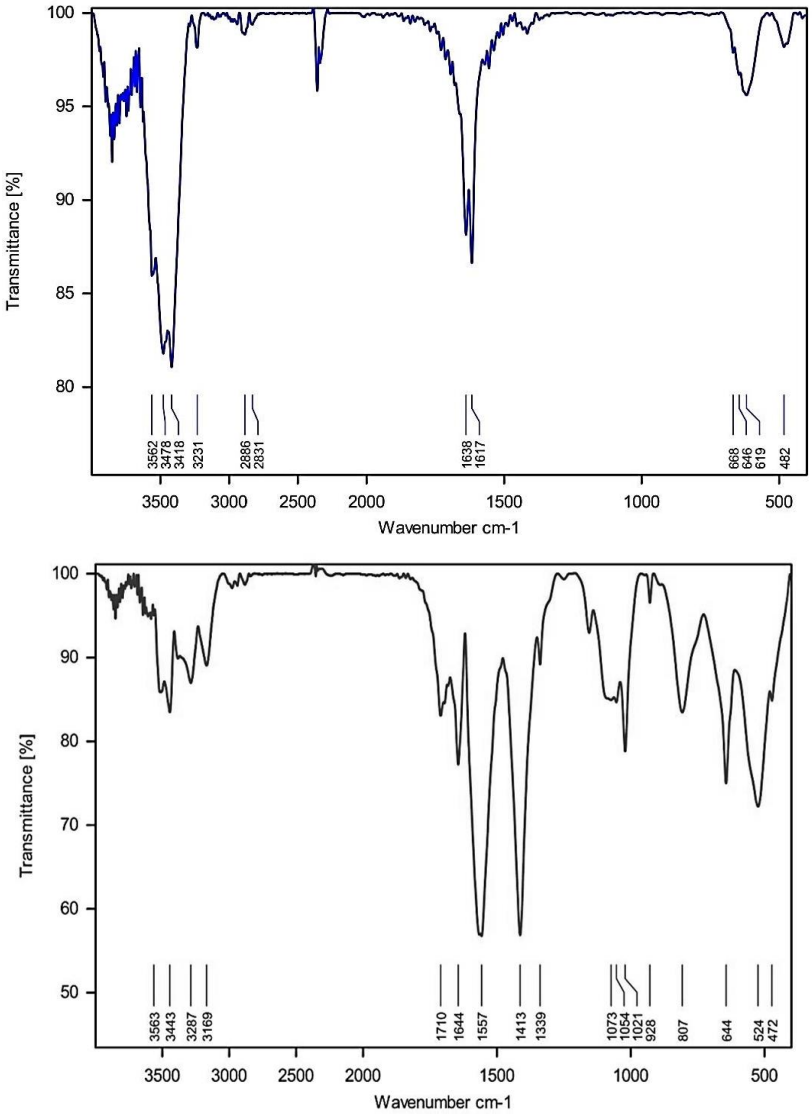



Figure 4. FT-IR spectrum of the prepared film: (blue line) CS/Cu; (Red line) PVPP/CS/Cu.

Thermal gravimetry analysis for chitosan (CS), PVPP/CS, and PVPP/CS/ Cu film are shown in Figure 5. As can be seen from Figure 5, chitosan showed two decomposition main stages. The first stage of decomposition (wt% 3.91) observed at 100 °C cause to the water loss. The other main stage at 220 ˚C with a maximum of 485 ˚C and 70% weight loss due to CS decomposition and dissociation of the polymer chain [30, 31]. PVPP/CS, and PVPP/CS/Cu film showed degradation in three steps. The first decomposition is due to the water loss whereas two decomposition that occurs at the next steps showed degraded form of CS and PVPP polymers. The hydrated form of polymeric compounds influenced by the primary and supramolecular structures. So, a shift in peaks situation and area correspond to the water loss could reveal physical variations due to the intermolecular interaction of polymers.

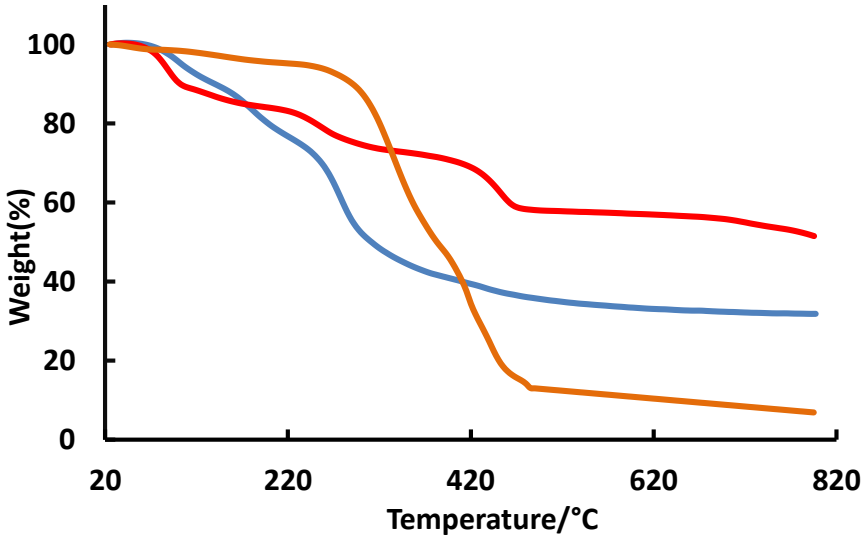



Figure 5. Thermogravimetric analysis (TGA) of (green line) chitosan; (blue line) PVPP/CS; (Red line) PVPP/CS/Cu film.

Figure 6 shows the antibacterial activity of the prepared film against four pathogenic bacteria, *E.coli* and *P.aeruginosa* (Gram-negative), *S.aureus* and *Bacillus cereus* (Gram-positive). The measured zones of inhibition diameter (in mm) were summarized in Table 1. According to the results the PVPP and PVPP/CS samples did not demonstrate any zone of inhibition. PVPP/CS/Cu film showed inhibition against all tested microorganisms. The zone of inhibition diameter was highest for *Bacillus cereus* (21 ± 3 mm), followed by *S. aureus* (14 ± 1mm), *E. coli* (11 ± 1), and then by *P. aeruginosa* (10 ± 2 mm) (Table 1).

In the present study, copper nanoparticles showed good antibacterial activity against all of the tested pathogens, but *Bacillus cereus* showed strong antimicrobial effects compared to other bacteria and the lowest amount of antimicrobial effect in *Pseudomonas aeruginosa* has been seen. These results may refer to differences in the cell wall of each strain. The normal mode of bacterial growth is in the form of a biofilm where the bacterial cells are adherent to a surface (or each other) and surrounded by a self-produced exopolymeric matrix comprising of polysaccharides, proteins and nucleic acids. Bacteria growing in a biofilm are challenging to remove, display increased resistance to antimicrobial agents compared to planktonic bacteria and are associated with medical device-related and tissue-related infections including urinary tract infections (UTIs), pneumonia and chronic wound infections [32-36].

The main mechanism of bactericidal activity is the generation of ROS, both dependent and independent from Fenton chemistry, and results in membrane damage. The principal mechanism of the activity of copper surface against bacteria was ion release leading to RNA degradation and membrane disruption in the case of enveloped viruses. For fungi, the uptake of copper ions and the physical deterioration of the membrane leading to copper influx are considered to be the primary mechanisms. Furthermore based on the literature, CuNPs results in significant damage, such as cell membrane damage and disruption of transport systems via plasma membrane and cell death. It can also generation of reactive oxygen species, protein oxidation, lipid peroxidation and DNA degradation in bacterial cells [32,33]. Ruparelia et al in 2008 reported Cu NPs compared to the silver particles were more efficient against *B. subtilis* which is suggested to be due to more affinity of the Cu NPs to surface amines and carboxyl groups of *B. subtilis* [34].

The antibacterial properties of the prepared film are very important in different fields from the wastewater treatment, wound healing and medicine due to the existence of Cu ions. The preparation of composite with multiple properties prevents the simultaneous use of different chemicals or toxic solvents. Therefore, the antimicrobial activity of the prepared film has made them strong candidates to be used as medical industry, water treatment, and packing of foods [35-39].

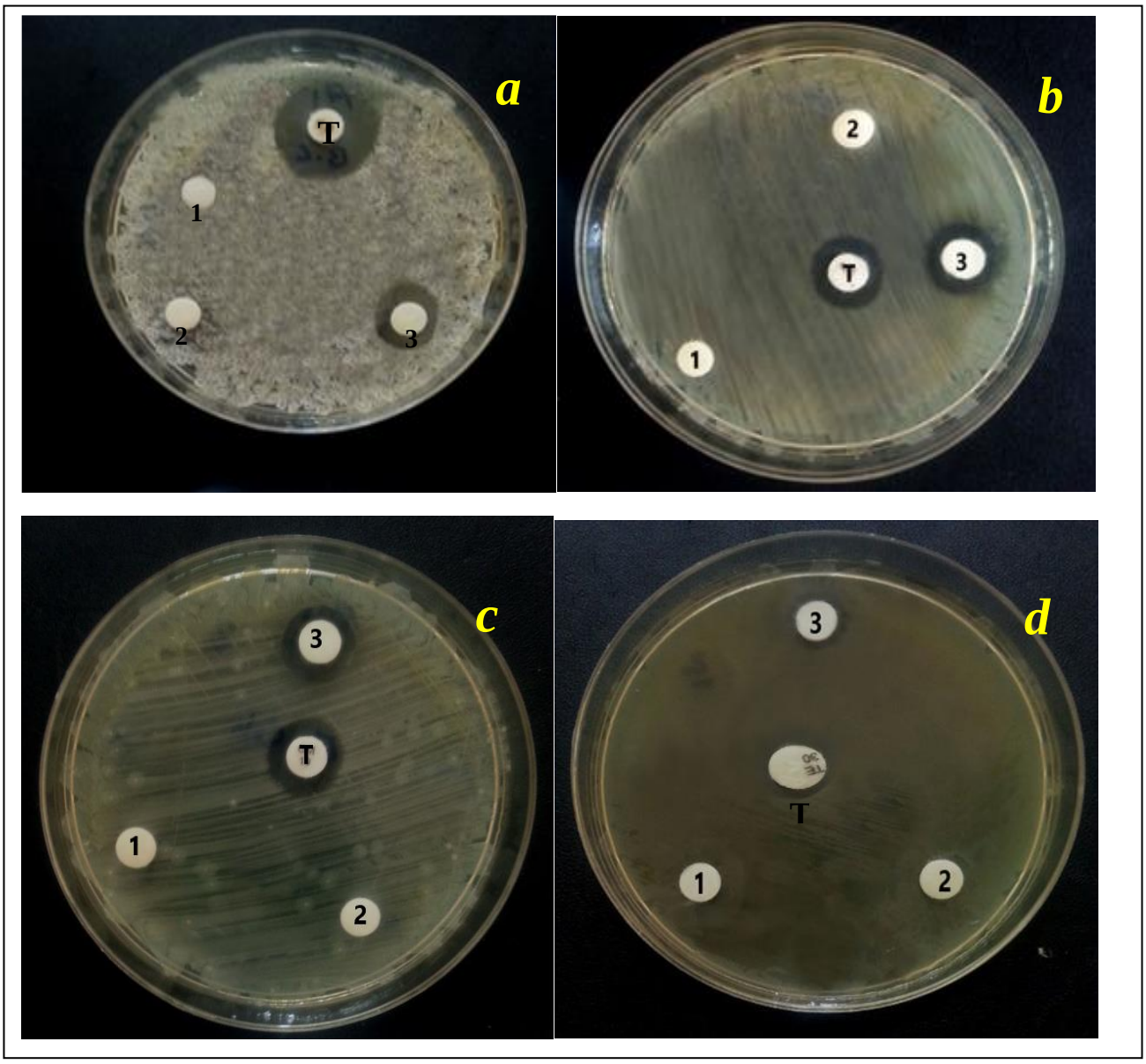



Figure 6. Antimicrobial susceptibility disk diffusion test.(T) Tetracycline, (1)PVPP ; (2) PVPP/CS, (3)PVPP/CS/Cu .(a)Bacillus cereus,(b) Staphylococcus aoureus, (c) Escherichia coli, (d) Pseudomonas aeruginosa.

**Table 1.** Disc diffusion analysis of Cu film. Each value is the mean±SD of triplicate analysis.

| Bacteria strains | Inhibition zone (mm) | | | |
|---|---|---|---|---|
| | PVPP | PVPP/CS | PVPP/CS/Cu | Tetracycline |
| Bacillus cereus | 0 | 0 | 21±3 | 32±5 |
| Staphylococcus aoureus | 0 | 0 | 14±1 | 14±2 |
| Escherichia coli | 0 | 0 | 11±1 | 13±2 |
| Pseudomonas aeruginosa | 0 | 0 | 10±2 | 11±1 |

## Conclusion

In the present research, a bio-composite of PVPP, chitosan, and the anti-bacterial agent have been fabricated by the solvent-cast method. The incorporating of Cu in hydrogel film was initially confirmed by EDS analysis. FTIR spectra revealed Cu atoms interact with surface functional group of chitosan provides the active sites and plays a significant role to destroy bacterial agents. Spherical Cu particles with smooth surface and without any coagulation in the prepared film is due to the neutralizes the surface charges of Cu nanoparticles and prevents the accumulation phenomena. This observation is essential in applying medical devices, such as wound dressing products and drug delivery research. The results from microbial tests confirmed the antibacterial effects of the prepared film, i.e. being highly adsorptive with great antibacterial activity. The application of eco-friendly and stable polymers reduces the risk of environmental pollution in wastewater treatment. Incorporation of Cu nanoparticles as antimicrobial agents and in the prepared film is the positive points due to the chemical operations in water treatment. It is hoped that by increasing the mechanical strength, porosity and selectivity, the presented biofilm can be applied in the medical products.

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