═══════════ COMPOSITE MATERIALS ═══════════

Bio-Nanocomposite Hydrogel Beads Based on Cellulose and Biogenic Silver Nanoparticles Prepared by *ex-situ* and *in-situ* Methods for Antibacterial Applications

Mohammed F. Silwadi[a],*, A. H. Bhat[a], Alathraa Mubarak Alrahbi[a], Hala Qassim Albattashi[a], Fatma Ali Albattashi[a], and Hanan Saif Alabri[a]

*[a] University of Technology and Applied Sciences (UTAS), Applied Science Department,
Muscat, Sultanate of Oman
e-mail: mohammadsilwadi@utas.edu.om

Abstract—This study describes the fabrication and characterization of nanocomposite hydrogel beads based on Cellulose and synthesized silver nanoparticles (Ag-NPs) prepared by insitu and exsitu methods. The silver nanoparticles were synthesized by reducing silver nitrate ($AgNO_3$) by Sodium borohydrate ($NaBH_4$). Cellulose/Ag-NPs hydrogel beads were prepared using calcium chloride as the cross-linker. The hydrogel beads were characterized using FTIR and XRD. Moreover, swelling property of the cellulose /Ag-NPs hydrogel beads was investigated. The Ag release profile of the hydrogels was obtained for the amount of Ag released using Atomic Absorption spectroscopy (AAS). The cumulative release of Ag-NPs was estimated by measuring the absorbance at 405 nm of samples obtained at various time intervals. The exsitu and insitu nanocomposite hydrogels showed greater swelling behavior in comparison with virgin cellulose hydrogel. Both insitu and exsitu cellulose/Ag-NPs presented good antibacterial activities against Escherichia coli and Bacillus and with maximum zones of inhibition 12 ± 2 mm more than the pristine cellulose hydrogel thereby, have great pharmacological potential and a suitable level of safety for use in the biological systems.

Keywords: bionanocomposites, hydrogel, cellulose, silver, nanoparticles

DOI: 10.1134/S1070427224020149

INTRODUCTION

Presently, the threat of pathogen contamination has attracted great attention in the potable water [1], food [2], and medical and health industries [3, 4]. In particular, diseases and deaths caused by pathogen infection pose a great challenge to human health and public health safety, resulting in the loss of nearly 10 million lives worldwide every year.

Hydrogels are a type of polymer-based material that can absorb and retain large amounts of water or other biological fluids. They are composed of a three-dimensional network of hydrophilic polymer chains that can swell in the presence of water or other aqueous solutions. Hydrogels can be made from a variety of natural or synthetic polymers, including polyvinyl alcohol, polyethylene glycol, polyacrylamide, and polysaccharides like agarose or alginate. They can also be functionalized with different chemical groups to make them responsive to external stimuli such as temperature, pH, or light [5, 6].

Hydrogels have a wide range of applications in biomedical, environmental, and industrial fields. In medicine, they are used as drug delivery systems, wound dressings, and tissue engineering scaffolds. They can also be used as sensors, actuators, or membranes in various industries [7, 8]. Additionally, hydrogels have the potential to be used in water treatment and environmental remediation due to their ability to absorb and retain large amounts of water and contaminants [9].

Silver nanoparticles (AgNPs) have shown unusual physio-chemical properties, such as a large surface area for dispersion, high electrical and thermal conductivity, catalytic activity, non-linear behavior, and prominent
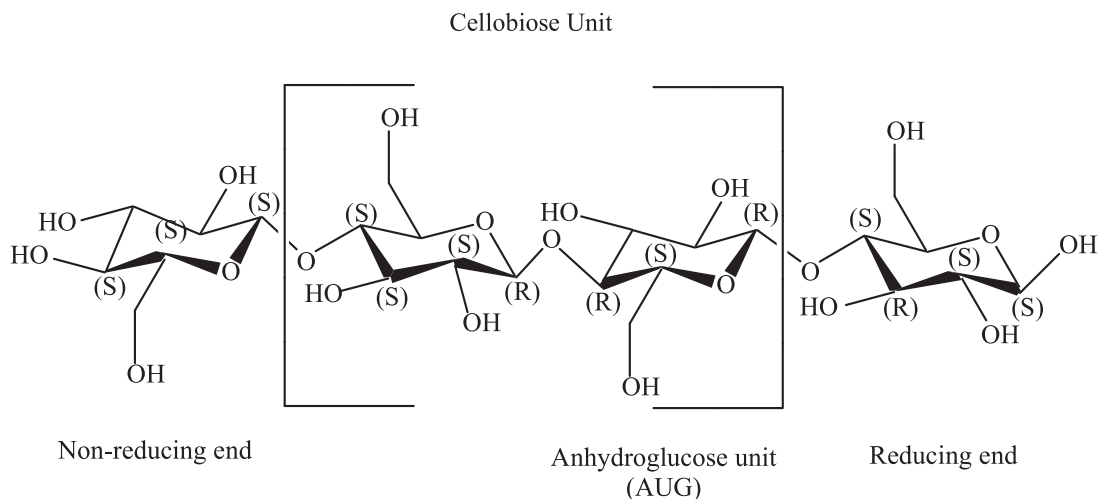
Cellobiose Unit

Fig. 1. Chemical structure of cellulose.

antibacterial activities. AgNPs have arisen as new antimicrobial agents because they are comparably more efficient and potent against several pathogens compared to the conventional antibiotics. The antimicrobial activity is due to the interaction with sulfur-containing proteins present in the bacterial cell membrane as well as with phosphorus-containing compounds like DNA. Moreover, AgNPs can be synthesized by using different synthetic methods with specific morphologies and unique characteristics [10, 11]. Currently, new strategies are being used to incorporate AgNPs into hydrogels without involving any toxic chemical as a stabilizing reagent, or complicated physical techniques such as sputtering and plasma deposition. One such technique include the incorporation of nanoparticles (NPs) into the matrix material either by insitu or exsitu methods [12].

Ex-situ method involves the synthesis of nanoparticles (NPs) in a separate step and then mixing them with matrix materials to develop the composite. This method typically involves the use of reducing agents or surfactants to stabilize the NPs. The resulting composite can be used in applications such as wound dressings, food packaging, and water filtration [13].

In-situ method involves the synthesis of NPs within the matrix. This method is typically achieved through chemical reduction or photochemical reduction. The resulting composite is typically more stable than ex-situ method and is less likely to cause the release of NPs into the environment. This method has been used in applications such as tissue engineering, drug delivery, and food packaging [14].

Both ex-situ and in-situ methods have been shown to be effective in creating nanoparticle composites with various properties. The antibacterial activity of the composite depends on various factors, such as the concentration of NPs, the size and shape of the nanoparticles, and the type of cellulose used [15, 16].

Cellulose, a high molecular weight carbohydrate polymer of β-1,4-linked anhydro-D glucose units is represented in Fig. 1 [17]. This structure is secured by an intramolecular hydrogen bonds between the hydroxyl groups and oxygen of adjacent molecules. Cellulose molecular chains are biosynthesiszed and self-assembled into microfibrils in which crystalline regions alternate with amorphous regions [18]. Cellulose microfibrils posseses diameter in the range of 10–30 nm and are made up of 30–100 cellulose molecules in extended chain confirmation and provide mechanical strength to the fiber [19]. The hydrophilic nature of cellulose macromolecules is due to the three alcoholic hydroxyl (–OH) groups [20]. Therefore, unbonded hydroxyl group that mainly presents in amorphous region of cellulose plays the major role for their reactive nature during hydrolysis process whereas crystalline region of cellulose remains intact [21].

Owing to the greater biomedical relevance, there is an increasing attention to develop the antibacterial hydrogels [8]. Among the antimicrobial hydrogels, the antibacterial inorganic-based nanocomposite hydrogels are especially favorable to inhibit the bacterial growth, consequently making them attractive in the field of biomedical and biotechnology [21]. Several types of inorganic overall, cellulose-silver nanoparticle composites have the potential

to be used in a variety of antibacterial applications due to their biocompatibility, biodegradability, and antimicrobial properties. The novelty of this study is preparation of in-situ and ex-situ silver nanoparticles and their use in the preparation of cellulosic bionanocomposites hydrogel beads in order to understand their pharmacological potential and a suitable level of safety for use in the biological systems.

EXPERIMENTAL

Materials

Cellulose powder, zinc chloride, 70% calcium chloride solution, silver nitrate, sodium borohydride, aqua regia were purchased from Sigma Aldrich.

Preparation of cellulose hydrogel. Cellulose hydrogel is prepared by taking 0.8 g of cellulose powder and adding 1.6 mL of deionizes water to it. In a different beaker, we took 16.24 g of $ZnCl_2$ with 6 mL of deionized water. At 60°C, the solutions were mixed together for 50 min, and at room temperature, the hot CZ gel was dropped into 70% (w/v) calcium chloride solution and left for 2 h, then the cellulose (CB) granules were rinsed three times in distilled water for 20 min at a time. Finally, it was air dried [22].

Ex-situ and in-situ preparation of cellulose based silver hydrogel.

Preparation of silver nanoparticles. As described by Solomon (2007) with some modification, the process is reducing $AgNO_3$ with $NaBH_4$, silver nanoparticles were created. This was made by titration 10 mL of 0.01 M aqueous solution of $AgNO_3$ agents 30 mL of 0.02 M aqueous solution of $NaBH_4$. After that, stirred with a magnetic stirrer for 20 min until the formation of particles.

Ex-situ method. Cellulose powder (0.8 g) and adding 1.6 mL of silver nanoparticles to it. In a different beaker, 16.24 g of $ZnCl_2$ with 6 mL of deionized water was taken. At 60°C, the solutions were mixed together for 50 min, and at room temperature, the hot CZ gel was dropped into 70% (w/v) calcium chloride solution and left for 2 h, then the cellulose (CB) granules were rinsed three times in volume in distilled water for 20 min at a time. After that, we allowed it to air dry.

In-situ preparation of cellulose based silver hydrogel. The synthesized hydrogels have been applied to achieve hydrogel/silver nano hybrids. Using 0.8 g of cellulose powder and 1.6 mL of silver nitrate (0.1 M). We mixed 16.24 g of $ZnCl_2$ with 6 mL of deionized water in a separate beaker. The solutions were combined for 50 min at 60°C, then the heated CZ gel was placed into a 70% (w/v) calcium chloride solution and left for 2 h at room temperature, after which the cellulose (CB) granules were washed three times in distilled water volume for 20 min each time. After that, we let it dry naturally.

Swelling studies. Accurately weighed pure hydrogel and in-situ and ex-situ hydrogel discs were equilibrated in 10 mL distilled water at 25°C for (10 min, 30 min, 1 h, 180 min, 24 h, 72 h). The degree of swelling (%) of the hydrogels was calculated using:

$$DS\ (\%) = (W_w - W_d/W_d) \times 100\%,$$

where W_w is the weight of the swollen hydrogel and W_d is the dry weight of the hydrogel.

Antibacterial Activity

The antibacterial activity of those hydrogel nanocomposites changed into execution on *E. coli* and *Bacillus* cultures in a nutrient agar medium. This nutrient agar medium becomes transferred into sterilized Petri dishes in laminar airflow. After solidification of the media, *E. coli* or *Bacillus* way of life becomes streaked on the stable floor of the media. To this inoculated petri dish, a small amount of pure hydrogel and in-situ and ex-situ hydrogel was added to the agar medium for one and three days at 37°C in an incubation chamber [23].

Release Study of Silver Content from Hydrogel Nanocomposites

Cellulose/silver nanoparticles hydrogel (50.0 mg) were weighed and liberated from the wet pellet, suspended in 1.5 mL distilled water, and incubated at 37°C at 100 rpm in a shaking apparatus and analysed for the amount of Ag released using atomic absorption spectroscopy (AAS). The cumulative release of Ag-NPs was estimated by measuring the absorbance at 405 nm of samples obtained at various time points. The release profile of Ag nanoparticles from the cellulose hydrogel nanocomposites was studied by release kinetics Ritger–Peppas model. A semi-empirical power low described by equation:
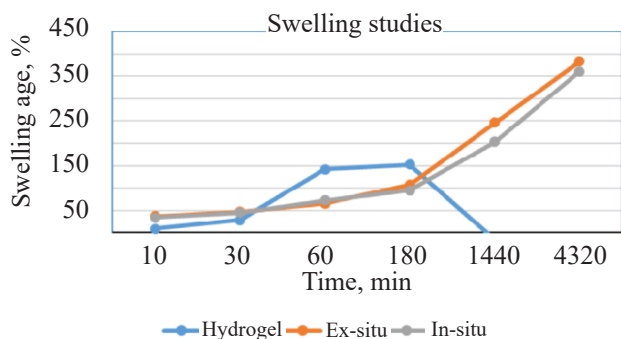
Fig. 2. Illustrates the swelling capacity of cellulose hydrogel (H1), ex-situ (H2) and in-situ (H3).

Fig. 3. Effect of calcium chloride concentration on crosslinking of cellulosic network.

$$\frac{C_t}{C_{eq}} = Kt^n,$$

where C_t and C_{eq} are cumulative concentration of Ag release from the hydrogel at specific time and at equilibrium , respectively , K is a characteristic constant of the hydrogel and n is the diffusional coefficient used to interpret the release mechanism.

Silver Content Determination

The fixed quantity of nanocomposite hydrogel pieces were placed in glass bottles with 5 ml aqua regia (HCl : HNO_3 3 : 1); the bottles were sealed and kept at room temperature until the gels were completely dissolved. Atomic absorption spectrometry was used to quantify the amount of silver ions in the dissolved acid solution (AAS).

Characterization and Analysis

X-ray diffraction analysis. X-ray hygiene is a nanotechnology molecule that was detected using XRD in the hydrogel Nanocomposite. These measurements were taken on the Siemens diffractometer scale in the range of 35 kV using Cu-K radiation.

FTIR studies. The FTIR was used in order to analyze the possible biomolecules present in the synthesized in-situ nano-silver loaded cellulose hydrogel. KBr pellets were used at the wavelength range of 4000–400 cm^{-1}.

RESULTS AND DISCUSSION

Swelling Measurements

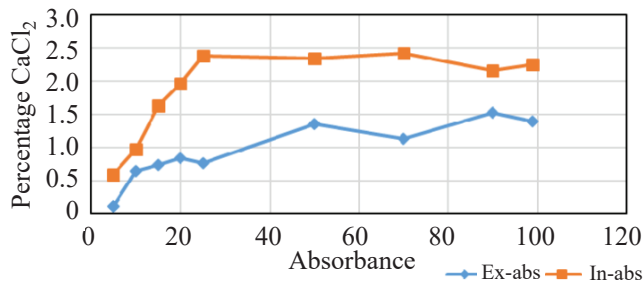Cellulose-based hydrogels have the advantages of strong water retention, good biodegradability, good biocompatibility, highly modifiable, low cost, and good mechanical properties. The swelling ratio highlights the superabsorbent capacity of the hydrogels.

Figure 2 illustrates the swelling capacity of cellulose hydrogel (H1), ex-situ (H2) and in-situ (H3) with same concentration of $CaCl_2$ (cross-linker) in distilled water at 25°C. As shown in Fig. 2, initially, swelling sharply increased up to 142.23% after 60 min for H1 hydrogel and increased up to 383.3, 361.36% after 4320 min for H2 and H3 hydrogels, respectively. The water within the swollen hydrogel contained free water, bound water and half bound water, and free water which had greater mobility than other forms of water and thus could be released more easily. Therefore, after the swelling of the hydrogel, the dehydration process occurred, which means that the free water in the hydrogel structure is released after the maximum swelling of the hydrogel structure. As shown in Fig. 2, the degassing of the H1 hydrogel occurred after 180 min.

Crosslinking Effect

In order to understand the role of calcium ions on the retention of beads, beads were prepared with 0 to 3% of $CaCl_2$ concentration.

The observation was that as the $CaCl_2$ concentration increases, beads turn out to be whiter and harder. It has been reported that upon dissolving the cellulose in the $ZnCl_2$ solution, the polymer chains could be cross linked via calcium ions [24] resulting in the formation of interpenetrating cellulosic network. Thus, it appears that the presence of more calcium ions in the solution, the more synergistic interactions will be among the dissolved cellulose chains, which results in harder beads. The introduction of the cross-linking points caused an appreciable change in the physical properties as can be seen in Fig. 3.

Effect of Silver Content

The effect of silver nanoparticles (AgNPs) concentration on the absorbance can vary depending on the method of synthesis (ex-situ vs. in-situ).

Ex-situ method involves the synthesis of AgNPs in a separate step and then mixing them with a solution or substrate. In this method, the absorbance of the solution or substrate increases with increasing concentration of AgNPs due to the resonance effect. As the concentration of AgNPs increases, the absorbance peak shifts towards longer wavelengths, resulting in an increase in absorbance at those wavelengths as shown in Fig. 4. However, at higher concentrations, the peak can become broader and less intense due to particle aggregation, which leads to a decrease in absorbance.

In-situ method involves the synthesis of AgNPs within a substrate or matrix. In this method, the absorbance can also increase with increasing concentration of AgNPs due to the resonance effect as shown in Fig. 5. However,
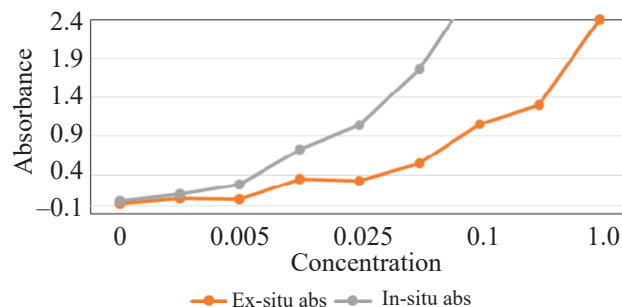

Fig. 4. Effect of silver nanoparticles (AgNPs) concentration on absorbance.

the absorbance can also depend on the thickness and uniformity of the substrate or matrix. Thicker substrates can result in lower absorbance due to the attenuation of light, while non-uniform matrices can lead to variations in the absorbance across the sample.

Overall, the effect of AgNPs concentration on absorbance can be different depending on the method of synthesis used. It is important to optimize the synthesis
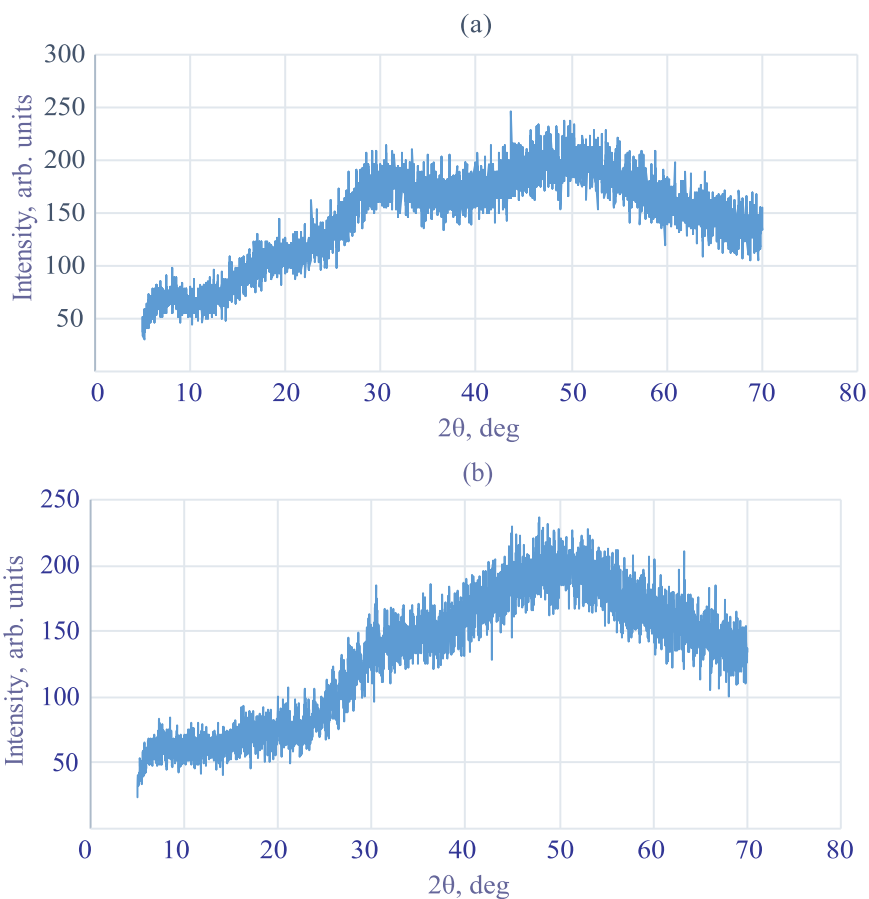

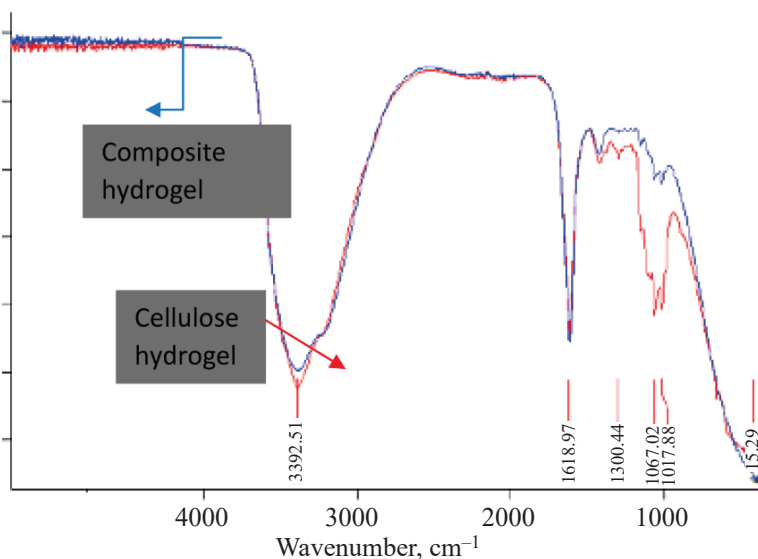**Fig. 5.** Silver nanoparticles prepared by in-situ and ex-situ method.

Fig. 6. FTIR spectrum of cellulose and composite hydrogel.

conditions to achieve the desired absorbance and properties of the composite material. Additionally, other factors such as the size and shape of the AgNPs, the type of substrate or matrix used, and the stability of the composite material should also be considered.

X-Ray Diffraction Analysis

The XRD analysis was performed in order to find the crystalline structure and the crystallite size of the NPs.

The XRD pattern of bio-synthesized AgNPs typically shows several sharp peaks, indicating that the particles have a crystalline structure. The peak positions and intensities can be used to identify the crystal structure of the AgNPs. For example, the most commonly observed crystal structure for AgNPs is face-centered cubic (fcc), which is characterized by sharp peaks at 2θ values of approximately $38.2°$, $44.3°$, and $64.5°$. The peaks correspond to Ag were almost the same as that of the bio-synthesized of Ag-NPs.

The Scherrer equation was used to calculate the average crystallite size of the AgNPs from the peak broadening. The equation relates the peak broadening to the particle size, crystallite size, and the wavelength of the X-rays used for the analysis. The size of Nanoparticles was found to be 23.86 nm by using Scherrer formula confirming that the sample is in nano range. The sharpness of the peaks concludes that the material is crystalline in nature. This information is used to optimize the synthesis

conditions and to understand the properties of the synthesized AgNPs for various application

Generally, cellulosic fibers are composed of crystalline and amorphous regions in their molecular structure. XRD diffraction pattern of Silver nanoparticles prepared by in-situ and ex-situ method is shown in fig. In the ex-situ method, AgNPs are synthesized separately and then mixed with cellulose to form the composite material while in the in-situ method, AgNPs are synthesized within the cellulose matrix. XRD pattern are observed to have no sharp peaks and may be due to the presence of cellulosic biomolecules which were introduced on the surface of silver nanoparticles or due to the encapsulation of silver nanoparticles by cellulose hydrogel.

Fourier Transform Infrared (FTIR) Studies

Fourier transform infrared (FTIR) spectroscopy is another commonly used technique to characterize cellulosic silver nanoparticle (AgNP) biocomposites. FTIR analysis was carried out to identify the functional groups of cellulose, as well as any changes in these groups resulting from the incorporation of AgNPs as shown in Fig. 6. The presence of AgNPs in the composite can also be confirmed by the appearance of characteristic absorption bands related to AgNPs.

FTIR analysis typically involves the measurement of absorption spectra over a range of wavenumbers (cm^{-1}). The spectra were analyzed to identify characteristic absorption bands corresponding to specific functional
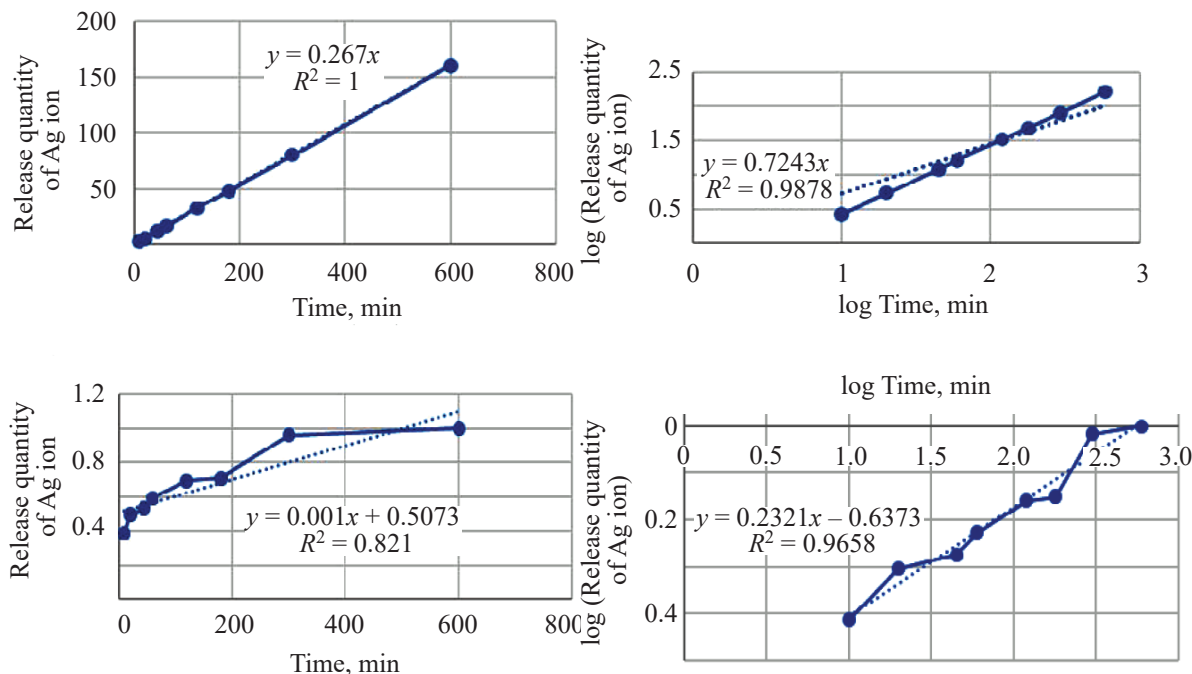
Fig. 7. AgNPs releasing properties of ex-situ and in-situ cellulose/Ag nano composites. (A) to (D): release quantity of silver ion with time.

groups and to determine the intensity of these bands, which reflects the concentration of the corresponding functional groups. Cellulose typically showed strong absorption bands in the region of 900–1200 cm^{-1}, corresponding to the C–O–C stretching vibration, and in the region of 3200–3600 cm^{-1}, corresponding to the O–H stretching vibration. The presence of AgNPs can be confirmed by the appearance of characteristic absorption bands in the region of 4000–600 cm^{-1}, corresponding to the Ag–O stretching vibration.

Overall, FTIR analysis can provide valuable information about the chemical composition and bonding of cellulosic AgNP biocomposites, including the presence of AgNPs and any changes in the functional groups of cellulose resulting from the incorporation of AgNPs. This information can be used to optimize the synthesis conditions and to understand the properties of the synthesized materials for various applications.

Release Study of Silver Content from Hydrogel Nanocomposites

A release study of silver content from a hydrogel composite involves investigating the rate at which silver ions are released from the hydrogel material into the surrounding environment. This type of study is important

for understanding the potential applications of hydrogel nanocomposites, particularly in areas such as drug delivery, wound healing, and antimicrobial coatings. In-situ release study involves monitoring the release of silver ions from the composite material in its actual or simulated application environment. Ex-situ release study involves monitoring the release of silver ions from the composite material in a controlled laboratory environment.

Silver releasing profiles of cellulose–Ag bio-composite in solution were presented in Figs. 7a and 7b. The power law exponent, from slopes of the logarithmical curves of C_t/C_{eq} as a function of time and fitting coefficient (R^2) calculated and listed in Table 1. The results show that n approximately is varied for samples which is indicative of different transport mechanism. The fact when n value is upto 0.5 means that the drug release mechanism is controlled by Fickian diffusion. The power law of Ag releasing rate of in-situ cellulose/Ag hydrogel is 0.2321 with $R^2 = 0.9658$, which seems the Ag release mechanism is controlled by Fickian diffusion [25].

Anomalous transport mechanism, which is the contribution of both Non- Fickian diffusion and macromolecular relaxation is observed when $0.5 \leq n \leq 1$. In the ex-situ cellulose/Ag hydrogel nanocomposite, n increased to 0.7243, proposing a trend towards the

Table 1. Release quantity and release ratio of Ag from cellulose /Ag bio-composite

Sample	Release quantity								Slope of fitting n	Fitting coefficient R^2
	10	20	45	60	120	180	300	600		
Ex-situ	0.4089	0.2650	0.2752	−0.0834	0.5184	0.2535	0.9639	0.2290	0.7243	0.9175
In-situ	2.0711	2.6607	2.8587	3.1806	3.7261	3.8018	5.1542	5.3625	0.2321	0.9658

Table 2. Antibacterial activity of EX(A) & IN(B) silver Nano composite hydrogel

Bacteria	Zone of inhibition, mm		
	hydrogel	ex situ hydrogel	in situ hydrogel
Bacillus	37	48	52
E. coli	38	47	49

Fickian diffusion. Such cases, the agglomerated Ag-NPs in the hydrogel composites are very nearby to each other. Consequently, anion-anion electrostatic repulsion forces due to negative charges on their surface are generated, throughout the polymer network and the macromolecular relaxation [19]. Overall, the results of the release study of silver content from cellulose silver composite in both in-situ and ex-situ environments can provide important information about the potential environmental and health impacts of the material and guide the development of safer and more sustainable composite materials.

Antibacterial Activity

The results of antimicrobial activity of silver cellulose composite can vary depending on the environment in which the material is tested. In situ environments refer to the actual application of the material in a real-world setting, while ex situ environments refer to controlled laboratory conditions. The antibacterial effect of the neat hydrogel, Ag-NPs–hydrogel composites was investigated
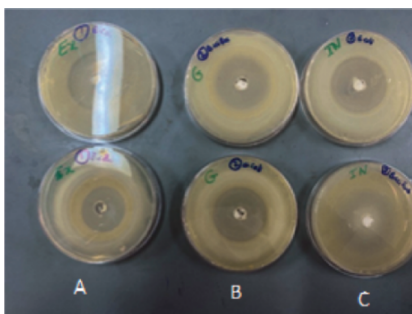
Fig. 8. Antibacterial action of ex (A), hydrogel (B) and in (C) silver nano composition hydrogel on *Bacillus* and *E. coli.*

by comparing the diameter of the growth inhibition zones against the urinary tract infection (UTI) pathogens (*Escherichia coli*, *Bacillus*) (Table 2). Paper discs soaked with neat hydrogels exhibited inhibition zone values of 38 and 37 mm against *E. coli*, and *Bacillus*, respectively. (Fig. 8). Ex-situ AgNPs–hydrogel composites (0.1 M) show inhibition zone values of 48 and 47 mm, which were measured against *Bacillus* and *Escherichia coli*, respectively. Discs soaked with the (0.1 M) of in-situ Ag-NPs–hydrogel composites displayed the highest inhibition zone value (55 mm) against *Bacillus*. These results suggest that in-situ Ag-NPs–hydrogel composites have better antibacterial activity than the neat hydrogels under similar test conditions against both *E. coils*, and *bacillus*.

CONCLUSIONS

The demand for cellulose-based products is growing due to several advantages: renewable, inexpensive and biodegradable. Herein, cellulose hydrogel beads have been prepared by first solubilizing cellulose in $ZnCl_2$ and then crosslinking the cellulose chains by calcium ions. The FTIR spectra was analyzed to identify characteristic absorption bands corresponding to specific functional groups and to determine the intensity of these bands, which reflects the effect of crosslinking on the concentration of the corresponding functional groups. The swelling capacity of cellulose hydrogel, ex-situ and in-situ with same concentration of $CaCl_2$ (cross-linker) in distilled water at 25°C was carried out. The prepared hydrogel composites showed an improved swelling capacity. The XRD pattern of bio-synthesized AgNPs typically shows several sharp peaks, indicating that the particles have a crystalline structure. The results of the release study of silver content from cellulose silver composites in both ex-situ and in-situ environments suggested both Fickian diffusion and non-Fickian diffusion release mechanism respectively. The antibacterial effect of the neat hydrogel, AgNPs–hydrogel composites was investigated by comparing the diameter of the growth inhibition zones against the urinary tract infection (UTI) pathogens. The

results suggest that in-situ AgNPs–hydrogel composites have better antibacterial activity than the neat hydrogels under similar test conditions against both E. coils, and *Bacillus*.

REFERENCES

1. Buenger, D., Topuz, F., and Groll, J., *Prog. Polym. Sci.,* 2012, vol. 37, pp. 1678–1719.
https://doi.org/10.1016/j.progpolymsci. 2012. 09. 001

2. Bhattarai, N., Gunn, J., and Zhang, M., *Adv Drug Del. Rev.,* 2010, vol. 62, pp. 83–99.
https://doi.org/10.1016/j.addr.2009.07.019

3. Glišić, S., Cakić, M., Nikolić, G., and Danilović, B., *J. Mol. Struc.,* 2015, vol. 1084, pp. 345–351.
https://doi.org/10. 1016/j.molstruc.2014.12.048

4. Bose, D. and Chatterjee, S., *Ind. J. Micro.,* 2015, vol. 55, pp. 163–167.
https://doi.org/10.1007/s12088-015-0512-1

5. Ahmed, E.M., *J. Adv. Res.,* 2015, vol. 6, pp. 105–121.
https://doi.org/10.1016/j.jare.2013.07.006

6. Ghorbani, P., Soltani, M., Homayouni-Tabrizi, M., Namvar, F., Azizi, S., Mohammad, R., and Boroumand, M.A., *Molecules*, 2015, vol. 2, pp. 12946–12958.
https://doi.org/10.3390/molecules200712946

7. Vimala, K., Varaprasad, K., Sadiku, R., Ramam, K., and Kanny, K., *Int. J. Biol Macromol*., 2014, vol. 63, pp. 75–82.
https://doi.org/10.1016/j.ijbiomac.2013.10.021

8. Shuqiang, L., Shujun, D.,Weiguo, X., Shicheng, T., Lesan, Y., Changwen, Z., Jianxun, D., and Xuesi, C., *Adv Sci (Weinh)*. 2018, vol. 5, p. 1700527.
https://doi.org/10.1002/advs.201700527

9. Haneda, M. and Towata, A., *Catalysis Today*, 2015, vol. 242, pp. 351–356.
https://jglobal.jst.go.jp/en/detail?JGLOBAL_ID=201402257682667764.

10. Lee, S.J., Heo, D.N., Moon, J.-H., Ko, W.-K., Lee, J.B., Bae, M.S., et al., *Carb. Polym*., 2014, vol. 111, pp. 530–537.
https://doi.org/10.1016/j.carbpol.2014.04.026

11. Maryan, A.S., Montazer, M., and Harifi, T., *Carb. Polym.,* 2015, vol. 115, pp. 568–574.
https://doi.org/10.1016/j.carbpol.2014.08.100

12. Li, C., and Yamauchi, Y., *Phys. Chemistry Chem. Phys.,* 2013, vol. 15(10), pp. 3490–3496.
https://doi.org/10.1039/C3CP44313B

13. Li, Y., Kim, Y.N., Lee, E.J., Cai, W.P., and Cho, S.O., *Nuc. Instr. and Meth. in Physics Res. Section B: Beam Inter. with Mater. and Atoms*, 2006, vol. 251, pp. 425–428.
https://doi.org/10.1016/j.nimb.2006.06.019

14. Qingchuan, G., Reza, G., Thomas, W., Andreas, A., Evgeny, L., Gurevich, C.E., Olaf, M., Wei, C., Chichkov, B., and Ostendorf, A., *Polym.,* 2014, vol. 6, pp. 2037–2050.
https://doi.org/10.3390/polym6072037

15. Haafiz, M.K.M., Eichhorn, S.J., Hassan, A., and Jawaid, M., *Carb. Polym*., 2013, vol. 93, pp. 628-634.
https://doi.org/10.1016/j.carbpol.2013.01.035

16. Nguyen, H.D., Mai, T.V.T., Nguyen, N.B., Dang, T.D.M., Le, L.P., and Dang, T.V., *Adv. in Natural Sci.: Nanosci. and Nanotech.,* 2013, vol. 4, p. 015016.
https://doi.org/10.1088/2043-6262/4/1/015016

17. Kalia, S., Dufresne, A., Cherian, B.M., Kaith, B., Avérous, L., Njuguna, J., et al., *Int. J. Polymer Sci.,* 2011, 2011.
https://doi.org/10.1155/2011/837875

18. Oksman, K., Mathew, A.P., Långström, R., Nyström, B., and Joseph, K., *Comp. Sci. and Tech*., 2009, vol. 69, pp. 1847–1853.
https://doi.org/10.1016/j.compscitech.2009.03.020

19. Azizi, S., Mohamad, R., Abdul Rahim, R., Mohammadinejad, R., and Ariff, A.B., *Int. J. Biol. Mac*., 2017, vol. 104, pp. 423–431.
https://doi.org/10.1016/j.ijbiomac.2017.06.010

20. Nie, G., Zang, Y., Yue, W., Wang, M., Baride, A., Sigde, A., and Janaswamy, S., *Carb. Poly. Tech. and Appl*., 2021, vol. 2, p. 100074.
https://doi.org/10.1016/j.carpta.2021.100074

21. Bacaita, E.S., Ciobanu, B.C., Popa, M., Agop, M., and Desbrieres, J., *Phys. Chem. Chem. Phys.,* 2014, vol. 16, pp. 25896–25905. https://doi.org/10.1039/C4CP03389B

22. González-Sánchez, M.I., Perni, S., Tommasi, G., Morris, N.G., Hawkins, K., López-Cabarcos, E., and Prokopovich, P., *Mat. Sci. and Eng*., 2015, vol. 50, pp. 332–340.
https://doi.org/10.1016/j.msec.2015.02.002

23. Murali, M.Y., Vimala, K., Thomas, V., Varaprasad, K., Sreedhar, B., Bajpai, S.K., and Mohana, R.K., *J. Colloid and Interface Science,* 2010, vol. 342, pp. 73–82.

24. Xu, M., Wang, Y., Zhang, L., and Dong, W., *Chem. Med. Chem.,* 2016, vol. 12, pp. 1600–1609.

25. Zahedi, S.M. and Mansourpanah, Y., *Plast., Rubb. and Comp.,* 2018, vol. 47, pp. 273–281.
https://doi.org/10.1080/14658011.2018.1475166