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SYNTHESIS AND CHARACTERIZATION OF CARBOXYMETHYLCELLULOSE–SERICIN HYDROGEL REINFORCED WITH SILVER–BISMUTH BIMETALLIC NANOPARTICLES

Khilola B. Khabibullaeva, Foziljon M. Turakulov, Sardor S. Yarmatov, Javlon Z. Jalilov, Khaydar E. Yunusov

Institute of Polymer Chemistry and Physics, Academy of Sciences of the Republic of Uzbekistan, Tashkent 100128, Uzbekistan.

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Corresponding author
silver4727@yahoo.com

ABSTRACT

In this study, a biocomposite hydrogel was synthesized based on purified sodium carboxymethylcellulose (Na-CMC) with a degree of substitution (DS) of 0.88 and a degree of polymerization (DP) of 850, together with sericin obtained from natural silk. The physicochemical properties of the obtained hydrogel were investigated: electrostatic interactions between the carboxyl (–COO^-) groups of Na-CMC and the amide I and amide II groups of sericin macromolecules were identified in the FTIR spectrum within the range of 1600-1700 cm^{-1}. Rheological studies confirmed that intermolecular crosslinking between the functional groups of Na-CMC and sericin led to a proportional increase in the viscosity of the hydrogel. After lyophilization of the Na-CMC/sericin hydrogel, scanning electron microscopy (SEM) revealed a porous structure with pore sizes ranging from 20 to 110 μm. The synthesized Na-CMC/sericin biocomposites hydrogel containing stable Ag–Bi bimetallic nanoparticles with sizes of 5–160 nm demonstrate strong potential for application in medical practice as non-surgical therapeutic biomaterials for the treatment of gastric and duodenal ulcer disease.

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Introduction

Na-CMC is a simple ether of cellulose, a linear polysaccharide linked via intermolecular glycosidic bonds between the 1,4-carbon atoms of anhydrous glucose [1]. Na-CMC macromolecules are linear polymers whose solubility in water depends on the degree of substitution (DS) values and whose viscosity depends on the degree of polymerization (DP) values, as DS increases, the viscosity increases [2]. The hydrophilicity, adhesiveness, rheological properties, absence of harmful functional groups of Na-CMC, and due to the low cost of its manufacturing technology and the adequacy of its raw material base, it is widely used in the biomedical, pharmaceutical, textile, construction, food, cosmetics, paper manufacturing and oil drilling industries [3]. Particularly in the medical field, Na-CMC and its organic and inorganic composites are used in tissue and organ engineering, It is widely used in wound dressing, as a polymer matrix for biomimetic implants and artificial organs, as an antioxidant and for obtaining antibacterial biomaterials, and as a filler in drug manufacturing [4]. CMC-hydrogels are widely used in drug delivery systems due to their high biocompatibility, stability, pH sensitivity and ability to bind active substances such as drugs and enzymes [5]. The anionic carboxyl (–COO^-) and hydroxyl (–OH) groups in its molecule confer hydrophilicity and ensure good compatibility with body tissues [6]. Therefore, CMC-hydrogels have been shown to be effective in preclinical and clinical trials for

wound healing and the controlled delivery of drugs. Furthermore, the combination of Na-CMC with other water-soluble polymers has enabled the successful application of 3D hydrogels, films, micro-needles and nanofibrous biomaterials in medical practice [7]. By adding additional chemical reagents to the Na-CMC composition, a stable hydrogel with good mechanical properties can be produced [8]. It was found that the physicochemical properties of the Na-CMC hydrogel changed when polyethylene glycol, epichlorohydrin, diepoxy, dicarboxylic acid and sericin compounds were added to it. Silver nanoparticles (Ag NPs) and bismuth nanoparticles (Bi NPs) have individually shown therapeutic potential in the treatment of gastric ulcers, where Ag NPs provide strong broad-spectrum antibacterial activity (including against *Helicobacter pylori*), while Bi NPs contribute mucosal protection, anti-inflammatory effects, and ulcer healing promotion; however, when combined into bimetallic Ag-Bi NPs, a synergistic effect arises that enhances antimicrobial efficiency, improves stability, and enables more controlled ion release, thereby increasing therapeutic efficacy and reducing required dosage [9]. Furthermore, stabilization with Na-CMC offers additional advantages, as it prevents nanoparticle aggregation, improves dispersion in aqueous media, enhances biocompatibility, and provides a protective polymer matrix that can prolong nanoparticle activity and facilitate interaction with the gastric mucosa. In addition sericin the sticky protein that surrounds the fibroin fibres in silk, is widely used as a natural biomaterial in the pharmaceutical and biomedical fields [10]. Sericin has been found to possess various biological activities, including protection against ultraviolet rays, antioxidant, antibacterial and anti-tumour properties [11]. The development of hydrogels based on sericin and Na-CMC that enable controlled release and possess such unique properties, and their effective application in medical practice, is considered a promising avenue [12]. Due to its hydrophilic nature, sericin's ability to keep wounds dry, absorb discharging pus, its adhesion properties, and its anti-inflammatory and antibacterial characteristics are of significant practical importance.

The aim of this work is to synthesis and study physcochemical characteristics of biocomposites of Na-CMC/sericin containing stable Ag-Bi NPs, for the non-surgical treatment of gastric and duodenal ulcer disease.

Materials and Methods

Materials

In this work, industrial samples of Na-CMC purified of organic and inorganic additives, produced by Promxim Impex LLC (Uzbekistan) with DS=0.88 and DP=850, were used as the polymer matrix [13]. Silk fibrous waste (UzDSt 5618-80) is purified from organic and mineral impurities by treatment with organic solvents - carbon tetrachloride (Sigma-Aldrich, CAS No. 32488-50-9) and chloroform (Sigma-Aldrich, CAS No. 67-66-3) followed by triple washing with an ethanol/water mixture (70:30, v/v) at 50 °C. The presence of Cl^- ions in the filtrate is monitored by a qualitative reaction with Silver nitrate ($AgNO_3$) (Sigma-Aldrich, CAS No. 7761-88-8) and Bismuth(III) nitrate pentahydrate ($Bi(NO_3)_3\times 5H_2O$) (≥99.99%, Sigma-Aldrich, CAS No. 10035-06-0).

Methods

Subsequently, the cocoons were boiled in water at a mass ratio of 1:10 (w/v) in an autoclave at 110 °C for 1–24 h, allowing sericin to dissolve into the aqueous solution. The resulting sericin solution was then lyophilized at −80 °C for 6–7 h to obtain sericin powder [14]. A 2% aqueous solution of purified Na-CMC samples was prepared and subjected to centrifugation at 8000 rpm for 20 minutes using a CenLee 20K centrifuge (China), allowing separation of the soluble and gel fractions. The soluble fractions of the obtained 2% Na-CMC solutions were then mixed with a 2% aqueous solution of sericin (molecular weight 25 - 150 kDa), which was isolated from natural silk fibers by hydrolysis, in specified experimental ratios. The mixture was mechanically stirred at 120 rpm for 6 hours, resulting in the formation of a shape-retaining hydrogel (Figure 1).

Preparation of Bi NPs

The 3 mL of $Bi(NO_3)_3 \times 5H_2O$ was added dropwise to (100 mL) dissolved deionized water and nitric acid solution (pH = 2-3) was stirred on an electronic stirrer (MS-H280-Pro, DLAB, China) for 30 min. Afterward, 0.01 M citric acid solution was added for catching unstable Bi^{3+} sounded and 0.01 M NaOH solution dropped until pH 4.5 – 5. Finally for synthesis of Bi NPs, a (6 mL of 3 mM) of $C_6H_8O_6$ solution was added dropwise with a pipette at 60 - 70 °C, in order to reduce Bi^{3+} ions to elemental Bi^0, while being continuously mixed with an electronic stirrer system for 2 h, during which the solution turned dark colur, indicating the formation of Bi NPs [15] After that to increase the dispersion degree of NPs, the solution was processed in sonication for 15 min.

Synthesis of Ag-Bi NPs within Na-CMC/Sericin hydrogels

Purified samples of Na-CMC with were obtained from the cotton cellulose and used as polymer matrices [16]. To prepare Ag NPs within the solution of Na-CMC, various concentrations of aqueous $AgNO_3$ were utilized. Mainly previously prepared aqueous solutions of purified Na-CMC/Sericin solution mixed amounts of 0.01 M aqueous solutions of $AgNO_3$ and stirred until formation of homogeneous Ag^+CMC^- hydrogels. The chemical reduction of silver ions immobilized within the Na-CMC was performed at 25°C by ascorbic acid. After that previously prepared H_2O/Bi NPs solution added to Na-CMC/Ag NPs solution for synthesis of bimetallic Ag-Bi nanoparticles in Na-CMC/Sericin hydrogels. The dispersions of Ag-Bi NPs in the matrix of Na-CMC/Sericin hydrogels were prepared by ultrasonic treatment with the help of UZDN-1 and U-4.2 ultrasonic dispersers (Figure 1).

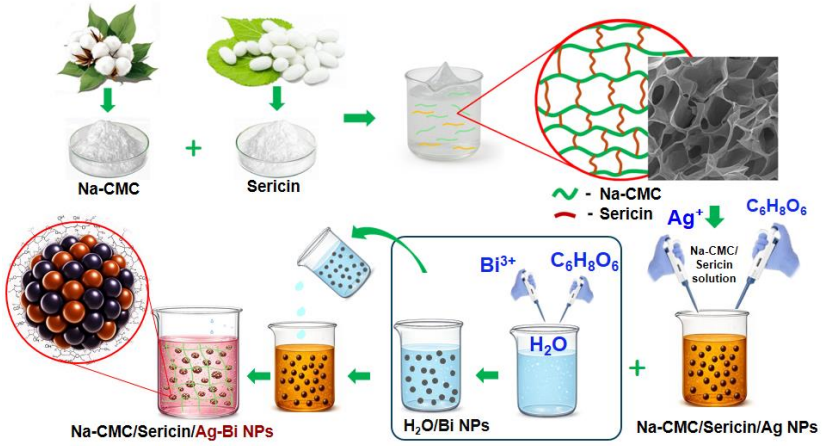

Figure 1. *Method for obtaining Na-CMC/sericin/Ag-Bi NPs biocomposites*

To determine the physicochemical properties of the prepared hydrogel, it was transferred into a Petri dish and frozen at −20 °C for 24 hours. Subsequently, it was lyophilized for 72 hours using a BK-FD12P lyophilizer (Biobase, China) and converted powder form.

Physicochemical characterization methods

The UV-vis absorption spectra of biocomposite solutions were measured in a Specord 210 UV spectrophotometer (Analytic Jena, Germany) at wavelength intervals of 190 - 900 nm. The structures and changes in functional groups of and biocomposites were studied using an (Inventio-S, Germany)

Rheological analysis. The rheological properties of Na-CMC and Na-CMC/sericin hydrogels were determined using an Anton Paar MCR 92 (Austria) rheometer at shear rate gradients ranging from 1 to 7000 s^{-1}, following the method described in [17].

FTIR spectroscopy. Changes in the functional groups of the samples were analyzed using an Inventio-S FTIR spectrometer (Germany) in the wavenumber range of 500–4000 cm^{-1}.

Scanning Electron Microscopy (SEM). The pore size of the lyophilized samples was examined using a JCM-6000PLUS Jeol (Japan) SEM instrument. The analysis was carried out at

different magnifications ranging from ×15.000 to ×35.000 and under an accelerating voltage of 20–30 kV, according to the method described in [18].

Results and discussion

The formation of stable biocomposite hydrogel samples, based on 2% of Na-CMC and 2% of sericin solutions prepared in various ratios, was investigated through rheological studies, The optimal ratios of Na-CMC and sericin solutions were selected, and the results are presented in Figure 2.

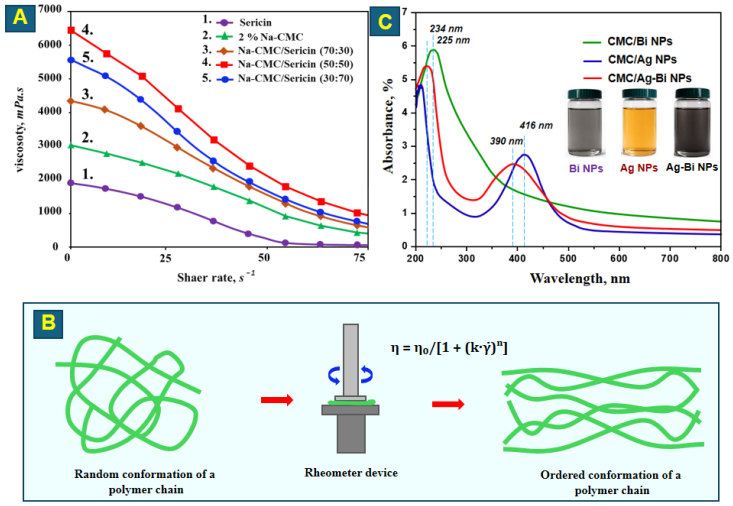

Figure 2. *Results of rheological studies (A), UV-Vis spectroscopy analysis (B) conformational states of the polymer chain dependent on the shear rate gradient (C)*

As can be seen from the results obtained, the viscosity of the samples decreased with an increase in the shear rate gradient in sericin, CMC-hydrogel, and Na-CMC/Sericin hydrogels at various ratios (Figure 2A), The dependence of viscosity on the shear rate gradient indicates that the samples are non-Newtonian fluids, and they exhibit pseudoplastic behaviour, with viscosity returning to its initial value as the shear rate gradient decreases. If the pseudoplastic property is explained in terms of Na–CMC macromolecules, then with an increasing shear velocity gradient the macromolecules are aligned along the flow direction. At the same time, the number of hydrogen bonds formed via the –OH groups in the glucoside ring and of intermolecular electrostatic interactions decreases, causing the macromolecule to adopt an ordered conformation (Figure 2B) [19]. As the shear rate gradient decreases, hydrogen bonds and electrostatic interactions between macromolecules are re-established, resulting in a reduction of the polymer chain's oriented state and a return to its original random conformation. As is known from the literature, such rheological properties of polymer solutions are consistent with the Williamson model:

$$\eta = \eta_0/[1 + (k\cdot\dot{\gamma})^n]$$

Here: η – the current viscosity; η_0 – the viscosity at a zero shear rate gradient; k – the empirical time parameter (s); $\dot{\gamma}$ – the shear rate gradient (s^{-1}); n – the empirical index [20].

At a zero shear rate gradient, the 2% sericin solution exhibited the lowest viscosity compared to the Na-CMC solution, which may be attributed to the molecular weight of sericin (Figure 2A, curves 1 and 2). In hydrogels prepared from Na-CMC and sericin solutions at ratios of 70:30 and 50:50, an increase in sericin content led to a corresponding increase in viscosity up to 4200 mPa·s and 6500 mPa·s, respectively, compared to the initial 2% Na-CMC solution (Figure 2A, curves 3 and 4). This indicates that the viscosity of hydrogels obtained from 2% Na-CMC and sericin solutions is higher than that of the initial solutions. This behavior can likely be explained, as shown

in Figure 2B, by intermolecular crosslinking formed due to electrostatic interactions and hydrogen bonding between the functional groups of Na-CMC and sericin macromolecules. However, for hydrogels prepared at a Na-CMC/sericin ratio of 30:70, the viscosity decreased to 5500 mPa·s (Figure 2A, curve 5). This can be explained by the increase in the amount of low-molecular-weight sericin molecules, which leads to an increase in the distance between polymer chains and a decrease in the proportion of high-molecular-weight Na-CMC in the system, ultimately reducing the viscosity of the resulting hydrogel [21]. The UV-Vis spectroscopy studies were observed of Na-CMC/Ag NPs, Na-CMC/Bi NPs and Na-CMC/Ag-Bi NPs samples in the wavelength range of 200-800 nm (Figure 2C). The colourful CMC/Bi NPs solution exhibited absorption peak in the range of λ = 224 nm, illustrating the formation Bi NPs. After UV–irradiation of Na–CMC solutions containing Ag^+ a stable colloidal system of nanosilver (light brown color) is formed with absorbance maximum at λ_{max} = 416 nm and size of Ag NPs 20–80 nm, .The colourful CMC/Ag-Bi NPs solution exhibited absorption peak in the range of λ = 390 nm, illustrating the formation Bi NPs associated with surface Plasmon resonance (SPR) [22] Because of SPR, the collective vibration of free surface electrons leaded to the observation of a characteristic absorption spectrum of NPs. On the other hand, the shift of the characteristic peaked of Bm NPs to λ_{max} = 274 nm suggested the increasing of viscosity during the CMC/Ag-Bi NPs combination [23].

To identify modified functional groups in Na-CMC/sericin-based hydrogel macromolecules and to evaluate the chemical interactions between Na-CMC and sericin, FTIR spectroscopy studies were carried out on Na-CMC, sericin, and Na-CMC/sericin/Ag-Bi NPs samples, and the obtained results are presented in Figure 2.

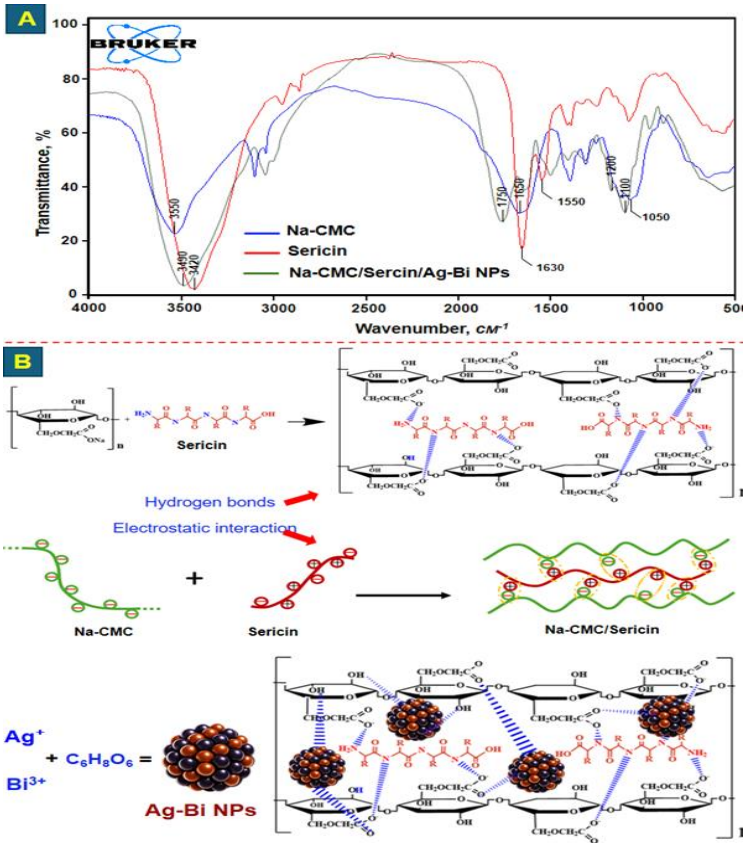

Figure 3. *IQ-Fourier spectroscopy analyses of the Na-CMC, sericin and Na-CMC/sericin samples (A), and the proposed probability-based interaction mechanism between Na-CMC, sericin and Ag-Bi NPs (B)*

According to the FTIR spectroscopy results, the spectra of Na-CMC hydrogels showed peaks in the regions of 600 and 1200 cm^{-1} corresponding to the glycosidic ring vibrations of –C–O, C–CH, and C–O–C groups, respectively. The shift observed from 1422 cm^{-1} to 1419 cm^{-1} is associated with an increase in the bond length of C–H bonds [24]. In the spectra of sericin samples, characteristic peaks of amide I were observed in the range of 1700-1600 cm^{-1} and amide II in the range of 1600-1500 cm^{-1}.These regions corresponding to amide I and amide II groups allow the identification of various amino acids. In particular, the peaks appearing at around 1630 cm^{-1} correspond to C=O groups, while those at around 1550 cm^{-1} characterize the stretching vibrations of N–H groups [25]. In addition, the vibrations observed at around 1050 cm^{-1} in the spectra may be attributed to the stretching vibrations of C–C bonds (Figure 3A). In the spectra of Na-CMC/Sericin/Ag-Bi NPs samples, the peaks corresponding to the $–COO^-$ groups of Na-CMC shifted from 1650 cm^{-1} to 1750 cm^{-1} compared to the initial Na-CMC, which may be due to strong electrostatic interactions between the negatively charged $–COO^-$ groups and the positively polarized amide I and amide II groups of sericin molecules [26,27]. After mixed of CMC/Ag NPs the H_2O/Bi NPs current peak changed to 3426 cm^{-1}, 3424 cm^{-1} distinct peak, respectively, because of hydrogen bonds between –OH groups of CMC with Bm NPs. The stretching vibration belonging to $–COO^-$ groups were shifted from 1606 cm^{-1} to 1601 cm^{-1} and 1589 cm^{-1} after chemical reduction of precursor ions.

Compared to the initial Na-CMC sample, the increased intensity of the peaks characteristic of hydrogen bonding and their shift from 3550 cm^{-1} to 3420 cm^{-1} in Na-CMC/sericin samples indicate the formation of new hydrogen bonds between N–H groups and the $–COO^-$ and –OH groups of the glycosidic ring (Figure 3B). No changes were observed in the peaks corresponding to ether (C–O–C), carbonyl (C=O), and carbon–oxygen (C–O) bonds of the CMC-macromolecule, which are characterized in the regions of 1200 cm^{-1}, 1100 cm^{-1}, and 1050 cm^{-1}, respectively. The proposed stabilization mechanism as shown Figure 3B, the positively polarized the Ag-Bi NPs interact electrostatically with the highly negatively charged $–COO^-$ groups of the Na-CMC, leading to the formation of ion-dipole interactions between the NPs and the polymer chains. In addition, the –OH groups along the Na-CMC backbone can form hydrogen bonds with surface atoms of the Bi NPs, further enhancing their colloidal stability. Hydrogen bonds formed between $–COO^-$, -OH groups and amid groups, as determined by IQ-Fourier spectroscopy analyses, and the electrostatic interactions formed between the negative poles of Na-CMC and the positive poles of the sericin molecule, and the proposed structural mechanism of the resulting stable hydrogel, are shown in Figure 3B.

SEM analyses were carried out on powder samples obtained from Na-CMC, Sericin and Na-CMC/Sericin hydrogels, and the pore sizes formed in the Na-CMC/Sericin samples were calculated. The results are presented in Figure 4. The SEM images revealed that powders obtained from 2% Na-CMC solutions exhibited the formation of pores, and as can be seen from the histograms the pore sizes of Na-CMC/Sericin were determined to be in the range of 40 - 120 μm (Figure 4A, B).

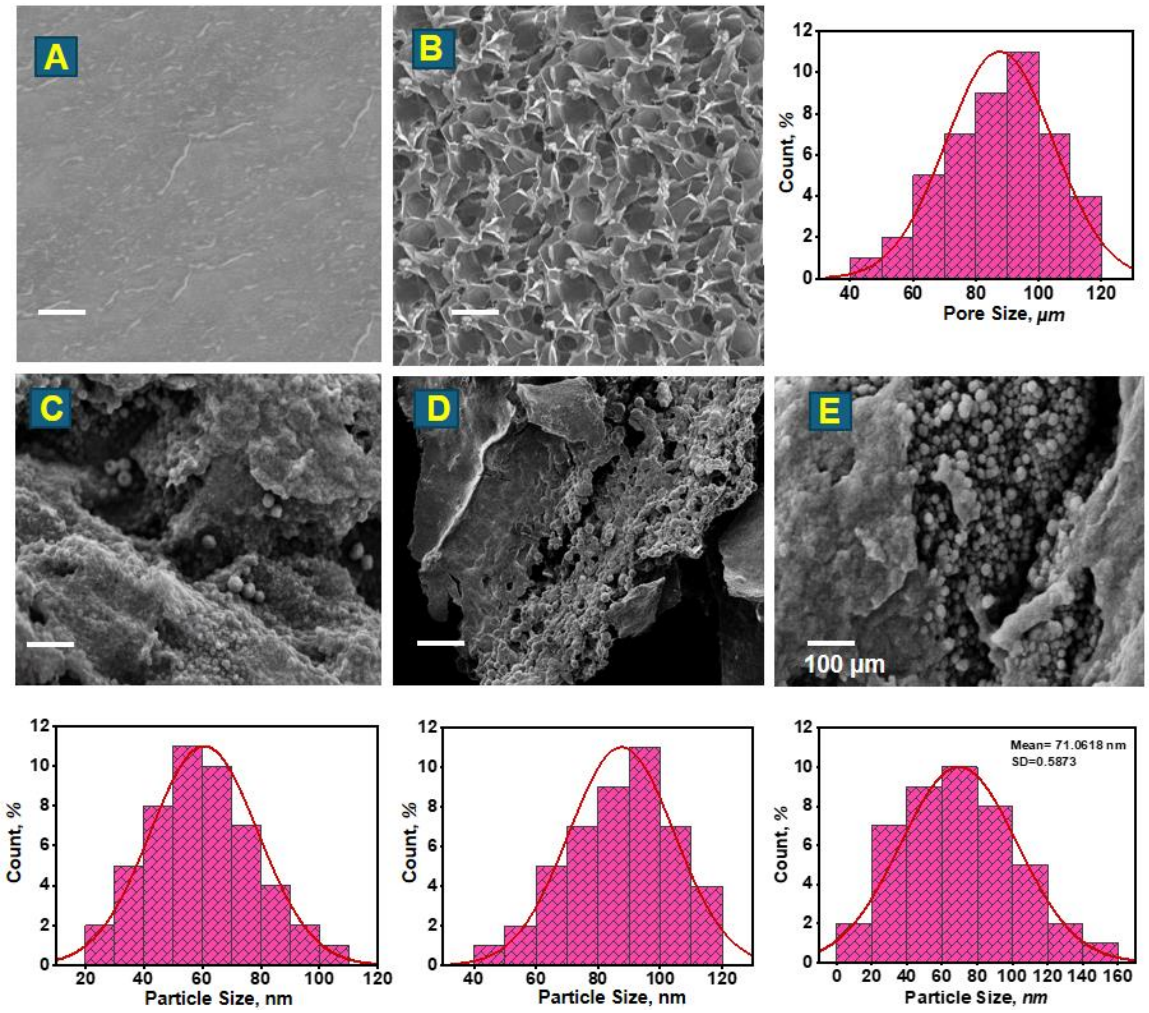

Figure 4. *SEM analysis results. Particle size histograms of Na-CMC (A), Na-CMC/Sericin with pore size histograms (B), CMC/Sericin/Bi NPs (C), CMC/Sericin/Ag NPs (D), CMC/Sericin/Ag-Bi NPs (E) and overall histograms of particle size distributions of Ag-Bi NPs.*

According to the histograms, the pore sizes in the microstructure were found to be in the range of 20–110 µm (Fig. 4F). The slightly smaller pore sizes in powders derived from Na-CMC/sericin hydrogels compared to those obtained from pure Na-CMC solutions may be attributed to the incorporation of sericin molecules into the Na-CMC matrix. This likely leads to a reduction in pore size, resulting in a denser and mechanically stronger Na-CMC/sericin hydrogel structure [28]. Furthermore, the sharp and size distribution of the monometallic nanoparticles (Bi NPs and Ag NPs) and bimetallic nanoparticles (Ag-Bi NPs) was evaluated by SEM analysis. The SEM images showed that, the all nanoparticles containing biocomposites took a clear spherical morphology, with various size distributions. Quantitatively, histogram analysis indicated that the main particle size did not change significantly between monometallic nanoparticles and bimetallic nanoparticles, which was promising confirming good long-term stability ranging from (20 – 110 nm) Bi NPs to (40 – 120 nm) Ag NPs and (5 – 160 nm) Ag-Bi NPs (Figure 4C-E). The increase in particle size from monometallic to bimetallic Ag–Bi NPs is maybe due to reduced nucleation and enhanced growth and coalescence during the formation process [29].

Conclusion

Based on the conducted studies, a stable hydrogel was obtained from a 2% solution of purified Na-CMC with a DS of 0.88 and a DP of 850, and a 2% aqueous solution of sericin with a molecular

weight of 25–150 kDa. FTIR spectroscopy confirmed that ionic coordination electrostatic interactions occur between the $–COO^-$ groups of Na-CMC macromolecules and the amide I and amide II groups of sericin macromolecules. In addition, hydrogen bonding interactions between N–H groups and $–COO^-$ and –OH groups were also identified. Rheological analysis revealed that the viscosity of the Na-CMC/sericin hydrogel increased compared to the initial 2% Na-CMC solution, which is attributed to the formation of intermolecular crosslinking and network structures. SEM analysis demonstrated that lyophilized Na-CMC, Na-CMC/sericin and Na-CMC/sericin/Ag-Bi NPs samples possess a porous morphological structure which increase in particle size from monometallic to Ag-Bi NPs bimetallic form with sizes of 5–160 nm. The high viscosity, network structure, and hydrophilic nature of Na-CMC/sericin hydrogels make them suitable for applications in wound healing, wound dressings, implant materials, and drug delivery systems. Furthermore, after comprehensive investigations of their biological activity, including antibacterial, antioxidant, and anti-inflammatory properties, these stable Na-CMC/sericin/Ag-Bi NP biocomposite hydrogels can be considered promising biomaterials with mucoadhesive and hemostatic properties for the non-surgical treatment of gastrointestinal and duodenal injuries.

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