Controlling the processing of co-precipitated magnetic bacterial cellulose/iron oxide nanocomposites

Monthakarn Chanthiwong a, Wiyada Mongkolthanaruk b, Stephen J. Eichhorn c,⁎ Supree Pinitsoontorn a,d,⁎

a Materials Science and Nanotechnology Program, Department of Physics, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
b Department of Microbiology, Faculty of Science, Khon Kaen University, Khon Kaen 40002, Thailand
c Bristol Composites Institute, School of Civil, Aerospace, and Mechanical Engineering, University Walk, University of Bristol, Bristol, UK.
d Institute of Nanomaterials Research and Innovation for Energy (IN-RIE), NANOTEC-KKU RNN on Nanomaterials Research and Innovation for Energy, Khon Kaen University, Khon Kaen 40002, Thailand

HIGHLIGHTS
• The starting reactants are not important for the characteristics and properties of the Fe3O4 NPs without BC.
• When NPs are formed inside BC networks, the starting materials influence the nanocomposites characteristics and properties.
• Morphology, size, crystal structure, loading amount, and magnetic phase of Fe3O4 NPs are affected by the BC networks.
• The ability to alter and control the properties of the NPs enables one to direct towards specific target applications.

GRAPHICAL ABSTRACT

ABSTRACT

Hybrid nanocomposites of bacterial cellulose (BC) and magnetic iron oxide nanoparticles (NPs) are of interest due to their potential for novel applications. Magnetic NPs are typically synthesized by co-precipitation since it is facile, enabling control of their size and distribution. This work investigates the effect of using different starting reactants (Fe(II) and Fe(III) salts) in the fabrication and control of the properties of BC/iron oxide nanocomposites. It was found that the choices of starting reactants are not important for synthesizing NPs outside of the BC networks. However, the starting reactants do affect the formation of NPs when they are synthesized in the BC network. Significant differences in the morphologies, sizes, crystal structures, and magnetic phases of NPs occurs when in this environment. The nanopores of BC networks in some instances force the aggregation of the NPs, either within the pores, or on the surfaces of the fibrils. Nanocomposites from Fe(II) acetate and Fe(III) chloride, though exhibiting lower magnetization, preserve a porous structure. Thus, they have potential as adsorbents or for wound healing applications.

© 2020 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
1. Introduction

Bacterial cellulose (BC) is a fascinating and renewable natural nanomaterial, produced from the cultivation of gram-negative bacteria e.g. Gluconacetobacter xylinum. It has received a large amount of interest owing to its unique structural features and favorable properties, for example, remarkable mechanical properties, porosity, water absorbency, moldability, biodegradability, and excellent biological affinity [1,2]. Intensive research and exploration on BC nanomaterials have been conducted. This research has mainly focused on the biosynthetic process to achieve low-cost preparation [3–5], but also towards potential applications in a wide range of fields, such as paper making [6], food and food packaging [7], and medicine [8,9]. Nevertheless, BC lacks certain properties (antibacterial, electrically conducting, ferromagnetic), which limits its applications in various fields, such as an antimicrobial material, for electronic devices, batteries, sensors, or electromagnetic shielding. Therefore, the synthesis of BC nanocomposites, combining it with other materials, has been conducted to address these limitations [10–13].

In recent years, hybrid nanocomposites consisting of BC nanofibers and magnetic nanoparticles (NPs) have attracted much attention due to their potential novel applications, for instance as actuators [14,15], sensors [16,17], for electromagnetic shielding [18,19], information storage [20], anti-counterfeit materials [21], and heavy metal adsorption [22,23]. The most common magnetic NPs that have been incorporated into a BC nanostructure are magnetite (Fe₃O₄) forms; this is due to their relatively large magnetization and chemical stability [15,24–28]. There are several approaches for synthesizing magnetic BC nanocomposites, such as incubation of BC in the magnetite-dispersed culture medium [23,29], the immersion of BC in Fe₃O₄ solution [15,16,24], hydrothermal processing [30], and co-precipitation [19,20,25–28].

The co-precipitation method is one of the most popular choices for synthesizing BC/iron oxide nanocomposites [19,20,25–28]. This process is straightforward and can provide a good distribution of magnetite NPs. The approach also enables control of the size and distribution of sizes by adjusting appropriate processing parameters [31–34]. Moreover, the choices of the starting reactants in the process can be tuned. For co-precipitation of iron oxide (Fe₂O₃), Fe²⁺ ions (e.g. FeCl₂, FeSO₄) and Fe³⁺ ions (e.g. FeCl₃, Fe(NO₃)₃) are required. Either sodium hydroxide [31,32] or ammonia [33,34] are common alkaline agents for converting Fe(II), Fe(III) salts into Fe₂O₃ NPs. FeCl₂ and FeCl₃ are the most common starting reactants for preparing BC/Fe₂O₃ nanocomposites [20,25,27,35]. However, other forms of Fe(II), Fe(III) salts have also been used. For example, combinations of FeSO₄ and FeCl₃ [26,36], or FeSO₄ and Fe₃(SO₄)₂ [37], have also been explored. Furthermore, decomposition of iron acetylacetonate (Fe(acac)₃) under microwave irradiation has been used for the fabrication of magnetic BC film [38].

It is therefore known that various forms of iron precursors can be used for the synthesis of BC/Fe₂O₃ nanocomposites. Nevertheless, no systematic study has been reported on the effect of the starting reactants on the formation, morphology, size and distribution, and magnetic properties of magnetic BC composites, particularly where the NPs are systematically studied and discussed in terms of the properties they exhibit. The understanding of the structure and properties of the magnetic BC nanocomposites via the controlled synthesis using the right pairs of starting reactants would suggest implications for the design for target applications.

2. Materials and methods

2.1. Chemicals

The chemicals used in this work were iron (II) chloride tetrahydrate (FeCl₂·4H₂O, purity ≥99.0%, Sigma-Aldrich), iron (II) sulfate heptahydrate (FeSO₄·7H₂O, Reagent grade, Ajax Finechem), iron (II) acetate (Fe(C₂H₃O₂)₂, ≥99.99%, Sigma-Aldrich), iron (III) chloride hexahydrate (FeCl₃·6H₂O, Reagent grade, Sigma-Aldrich), iron (III) nitrate nonahydrate (Fe(NO₃)₃·9H₂O, Reagent grade, Ajax Finechem), sodium hydroxide (NaOH, 99%, RCI Labscan), Yeast Extract Powder (HiMedia) and D-Glucose (anhydrous AR, Ajax Finechem), and ammonia solution (28% NH₃ in H₂O, Grade AR, QRC).}

2.2. Biosynthesis of bacterial cellulose

Bacterial cellulose (BC) pellets were prepared by cultivation of the bacterial strain Komagataeibacter nataicolus (strain TISTR 975), supplied from the Microbiological Resources Centre, Thailand Institute of Scientific and Technological Research (TISTR). The culture was grown in a medium consisting of 100 g of anhydrous d-glucose (Ajax Finechem) and 10 g of yeast extract powder (HiMedia) in 1 L of de-ionized (DI) water. This combination was cultivated for 24 h at 30 °C in a shaker incubator (150 rpm). Then, 1 ml of the cell suspension was introduced into a container containing 50 ml of a fresh liquid culture medium. The cultivation continued at 30 °C under static conditions for 14 days. After that, the BC pellet was harvested. It was purified by boiling in distilled water, following by soaking in NaOH solution for 24 h. Finally, it was rinsed with DI water several times until a pH of 7 was reached. The purified BC hydrogel was transformed into a BC aerogel by freeze-drying which was kept at room temperature before further use.

2.3. Synthesis of BC/Fe₂O₃ nanocomposites and Fe₂O₃ nanoparticles

For the synthesis of the BC/iron oxide nanocomposites, firstly, the starting reactants of Fe⁺² (0.01 mol) and Fe⁺³ (0.02 mol) were separately dissolved in 200 ml of DI water at 50 °C with continuous stirring for 10 min, before mixing together. These processes were carried out in an ambient atmosphere. Then, the freeze-dried BC aerogels were then immersed in the iron ion solution and soaked for 90 min with continuous stirring. The color of BC pellets changed from opaque white to orange during this process. Argon gas was then purged into the solution for 10 min. Subsequently, 400 ml of aqueous NH₃ was slowly added to the solution for the co-precipitation of iron oxide NPs. During this process, the BC pellets gradually turned black. When the process was completed, the products were rinsed with water several times under ambient conditions to remove unwanted materials until a pH of 7 was obtained. The BC nanocomposites in the form of a hydrogel were oven-dried at 80 °C overnight to obtain the magnetic BC sheets.

To investigate the effect of the starting reactants, iron (II) chloride (C), sulfate (S), and acetate (A), and iron (III) chloride (C) and nitrate (N), were used. Thus, a total of 6 pairs of chemicals were studied. The chemical reactions for the co-precipitation of each pair are as follows:

\[
\text{C} + \text{C} + \text{FeCl}_2 + 2\text{FeCl}_3 + 8\text{NH}_4\text{OH} \rightarrow \text{Fe}_2\text{O}_3 + 8\text{NH}_4\text{Cl} + 4\text{H}_2\text{O} \tag{1}
\]

\[
\text{S} + \text{C} + \text{FeSO}_4 + 2\text{FeCl}_3 + 8\text{NH}_4\text{OH} \rightarrow \text{Fe}_2\text{O}_3 + 6\text{NH}_4\text{Cl} + (\text{NH}_4)_2\text{SO}_4 + 4\text{H}_2\text{O} \tag{2}
\]

\[
\text{A} + \text{C} + \text{Fe(C}_2\text{H}_3\text{O}_2)_2 + 2\text{FeCl}_3 + 8\text{NH}_4\text{OH} \rightarrow \text{Fe}_2\text{O}_3 + 6\text{NH}_4\text{Cl} + 2\text{NH}_4\text{C}_2\text{H}_3\text{O}_2 + 4\text{H}_2\text{O} \tag{3}
\]
The BC/Fe3O4 nanocomposites were studied using Fourier transform infrared (FTIR) spectroscopy (Bruker, TENSOR27, Germany) within the wavenumber range of 600–4000 cm⁻¹. The magnetic properties of the samples were studied using a vibrating sample magnetometer (VSM) option in the VersaLab instrument (Quantum Design, USA) in the 2θ range of 10–80°. Magnetic property measurements are shown in Fig. 4. The data for all samples are similar. The magnetization (M) versus magnetic field (H) curves follow a typical S-shape for the ferrimagnetism of magnetic iron oxide (XAS) of the Fe K-edge was conducted at BL1.1 W: Multiple X-ray Techniques at the Synchrotron Light Research Institute (SLRI), Thailand.

3. Results and discussion

The results on the synthesis of the iron oxide NPs prepared by co-precipitation from different starting reactants, but in the absence of the BC networks, were firstly presented. Fig. 1 presents SEM images showing the morphology of the iron oxide NPs. No obvious differences can be found among the samples. Clusters of irregular-shaped NPs were observed with sizes in the range 30–38 nm (Table 1). The TEM images in Fig. 2 show a distribution of sizes of Fe3O4 NPs. The boundaries of each particle are more visible, but, again, there are no distinct features between the samples synthesized using different starting reactants. The particle sizes of each sample varied in the range of 9–11 nm (labeled in Fig. 2 as well as in Table 1), which in comparison to the SEM images suggests a smaller ultimate NP size. This smaller size may be because of clustering of the NPs in the SEM images making it difficult to resolve individual particles.

Typical XRD patterns are presented in Fig. 3. These patterns enabled the phase and crystal structures of the NPs to be determined. The XRD pattern of each sample was compared to the standard Fe3O4 reference (ICDD: 00-019-0629). No secondary phases were observed. This indicates that the co-precipitation method, whether using any pair of starting reactants, can form a magnetite phase, with a negligible change in the lattice parameters (8.314–8.350 Å). The crystallite size of each NP, calculated by using the Scherrer equation, is in the range of 10–13 nm (Table 1), which is within the same range as values obtained from TEM analysis. It should be noted that the XRD pattern for the magnetite (Fe3O4) phase is similar to the maghemite (Fe2O3) phase (ICDD: 00-039-1346) (also shown in Fig. 3). Therefore, the synthesized NPs in this work could possibly be either the Fe3O4 phase or the Fe2O3 phase, or possibly a combination of both phases. The fact that it is very difficult to distinguish between these two phases has been previously noted in the literature [39,40].

The magnetic property measurements are shown in Fig. 4. The data for all samples are similar. The magnetization (M) versus magnetic field (H) curves follow a typical S-shape for the ferrimagnetism of magnetic iron oxide.
oxides. An absence of a hysteresis loop, and the near-zero coercivity \((H_c)\), imply superparamagnetism, which is thought to be due to the small sizes of the NPs. The magnetic susceptibility (slopes of the \(M-H\) curves) of all samples are almost identical, and the saturation magnetization \((M_s)\) varies only slightly from 55 emu/g for P-SC to 61 emu/g for P-CC (Table 1). Since the morphology, size, and crystal structures of the NPs from each pair of reactants are similar, it is not unexpected that the magnetic properties of each sample are virtually unchanged.

From the above results, it can be concluded that using different starting reactants in preparing iron oxide NPs by co-precipitation does not significantly affect the morphology, size, phase and crystal structure, or magnetic properties of the synthesized particles. Whether it is P-CC or P-AN, or other samples, they show no distinct characteristics. Any kind of ferrous or ferric salts can be employed in the co-precipitation process of iron oxide NPs. These findings are however contradictory to previous reports which found differences in the size and \(M_s\) values for iron oxide NPs using various Fe reactants [41]. The discrepancy between the present work and others [41] could be attributed to several factors related to the details of the co-precipitation synthesis. Firstly, the alkaline agent in the present work (\(NH_4OH\)) is different from NaOH used for reference [41]. The kinetics for the chemical reactions between \(NH_4OH\) and NaOH may be different and, thus, the rate for converting iron ions into iron oxides is likely to vary. Dissimilar alkaline reagents would lead to resultant iron oxide NPs with differing morphologies and sizes. Moreover, the concentration of precursors, the time and temperature of co-precipitation all have an effect on the morphology and size of the magnetic iron oxides. Nabiyoui et al. found that increasing the co-precipitation time resulted in a larger particle size [42]. They also found that by lowering the magnetic ion concentration, larger NPs were observed, while heating during the process increased both size and \(M_s\) of the NPs. Therefore, the results in the present work cannot be directly compared with other studies since the processing parameters were not systematically controlled for a direct comparison.

On the other hand, the morphology, phases, functional groups, and magnetic properties of the BC/iron oxide nanocomposites, prepared by co-precipitation in the presence of BC, show some variations when using different starting reactants. These differences are unexpected since the reactants are the same, however the medium in which they are produced has changed to a BC network. This environment of a fibrous network may present a particular pore size that inhibits the growth of the NPs during co-precipitation. Fig. 5 shows representative SEM images of the BC/iron oxide samples. Unlike the SEM images for the NPs, the nanocomposite samples show an obvious difference between samples. For instance, the iron oxide NPs appear to be densely packed within the 3D network of the BC nanofibers for BC-CC whereas the NPs are loosely formed on the surface of the BC nanofibers for BC-AC. The formation mechanism of the iron oxide NPs in the BC template is thus dependent on the starting reactants. The porous networks of the BC also form an environment in which the NPs are synthesized. It could be that given this environment, there is an alteration of the clustering and distribution of the NPs depending on the starting reactants. The size of the NP clusters in each sample is estimated from the SEM images as shown in Table 2. It is observed that the cluster sizes of the BC-CC, BC-SC and BC-CN are larger (75–86 nm) compared to the cluster sizes for BC-AN, BC-SN, and BC-AC (55–65 nm). It is especially noted for

![Fig. 2. Typical TEM images of the \(Fe_3O_4\) NPs prepared from co-precipitation using different starting reactants: (a) P-CC, (b) P-SC, (c) P-AC, (d) P-CN, (e) P-SN, (f) P-AN. c.s. = crystallite size.](image)
been reported to be in the range 10–100 nm [43–45], which by a consideration of their stochastic geometry yields pore sizes less than 0.1 μm [43]. Within this confined geometry, it would be expected that the NPs would cluster, or given the right size range, coat the surface of the BC fibrils. It is expected that smaller NPs will have a higher surface to volume ratio, and therefore couple to the BC fibrils through increased van der Waals interactions, or interactions between the positive charge of free Fe ions, and the partial negative charge of the –OH groups on the cellulose during co-precipitation.

The mechanism for the formation iron oxide NPs in the BC nanofibril network starts from the attraction of Fe$^{2+}$, Fe$^{3+}$ ions to the hydroxyl (–OH) group of the BC fibrils. When the alkaline agent (in this case NH$_4$OH) was introduced in the system, the partial negative charge on the OH groups interact with the Fe$^{2+}$, Fe$^{3+}$ ions, converting them into iron oxide NPs on the surface of the BC fibrils. However, there are other anions (Cl$^-$, NO$_3^-$, C$_2$H$_3$O$_2^-$, SO$_4^{2-}$), which are present in Eqs. (1)–(6), which are also involved in the co-precipitation process. In the absence of the BC networks, these anions are dispersed loosely in the solution, and hardly affect the co-precipitation of iron oxides. This is the reason why the characteristics and properties of the NPs are mostly independent of the starting reactants for the synthesis of iron oxide NPs alone. In contrast, for the synthesis of nanocomposites, the porous networks of BC form an environment in which the NPs are synthesized. The chemical reaction occurs in a very limited space inside the open structure of the BC aerogels. The conversion of iron ions into iron oxide NPs will depend on the OH groups interacting with the Fe$^{2+}$, Fe$^{3+}$ ions. The existence of the anions (Cl$^-$, NO$_3^-$, C$_2$H$_3$O$_2^-$, SO$_4^{2-}$) in the proximity of BC fibrils might inhibit or slow the rate of the conversion due to repulsive forces from the same negative charges between the anions and OH groups. Depending on how strong the electrostatic forces between anions and the corresponding cation, the rate of the chemical reactions to form iron oxide NPs occurs differently by using various starting materials. The different rate in co-precipitation causes the difference in the growth of the NPs and clustering. For instance, for BC-CC, BC-SC, and BC-CN, the rate of their NP formation is likely to be increased compared to other samples. Thus, the NPs appear to be more densely packed in those samples, and the clustering of NPs are also higher. This interpretation is supported by the larger cluster sizes of NPs for BC-CC, BC-SC, and BC-CN (55–65 nm) compared to BC-AN, BC-SN, and BC-AC (55–65 nm). In other words, if the kinetics of the NPs formation in the porous BC networks is high for the particular pairs of starting reactants, it promotes the growth of NPs, resulting in the high density of NPs and the large cluster sizes.

The phase and crystal structures of the BC/iron oxide nanocomposites have been determined by XRD analysis, as shown in Fig. 6. The XRD pattern of the pristine BC is also presented. This shows the presence of Bragg peaks located at 14.3°, 16.6° and 22.3° corresponding to the (110), (111) and (200) diffraction planes, in agreement with previous studies [21,46]. For nanocomposites, when the iron oxide NPs were introduced into the systems, the diffracted peaks at 2θ = 30.0°, 35.4°, 43.1°, 57.2°, 62.6°, equivalent to (220), (311), (400), (511), and (440) reflections of the magnetite structure, are observed. This confirms the formation of iron oxide NPs in the BC structure. On the other hand, the XRD peaks of BC are mostly suppressed, which is due to the presence of the intense XRD peaks from the crystalline iron oxide phases. The observed reduction of the BC diffraction peaks is commonly observed in the BC/NPs composites in the literature [15,25,37,47]. However, some samples still consist of secondary phases, such as BC-CC (at 2θ = 32.4°), or BC-AC (at 2θ = 44.5°). The secondary phase for BC-CC was identified as NH$_4$Cl which is a salt byproduct from Eq. (1). Similarly, the secondary phase observed for BC-AC could also be from byproducts from Eq. (3). It should be noted that these secondary phases were not observed for P-CC or P-AC. This is because, in the case of NP synthesis, the byproducts can be easily removed through centrifugation and washing. For BC nanocomposites, they are grown in the open structure of the

BC-SN and BC-AC, that the NPs only coat the surface of the BC nanofibers but do not fill the interfibrillar pores. It is noted that the cluster sizes of the NPs in the nanocomposites are relatively larger than in the case of the pure NPs. This might be due to the open structure of the BC aerogels which allow the clustering of NPs to occur more readily in the co-precipitation process. The lateral sizes of BC fibrils have previously...
BC network and attach to the BC nanofibrils. This makes them more difficult to be removed by washing. This point is discussed again in the FTIR section.

The crystallite size of the NPs in BC, calculated by using Scherrer equation, is in the range of 10–16 nm (Table 2). These sizes are very close to the crystallite sizes for the pure NPs without BC. This indicates that although the NPs are found to cluster, with much larger overall sizes in the BC networks, the sizes of the iron oxide crystals are still relatively unchanged. It is noted however, that due to peak broadening, because of the small size of the NPs, that these figures are subject to some error when determined using the Scherrer equation. As noted, since the XRD patterns of Fe3O4 and Fe2O3 are very similar, the nanocomposites could be either BC/Fe3O4 or BC/Fe2O3, or the combination of both phases. The presence of mixed phases could potentially influence the magnetization of the nanocomposites, as discussed later.

Fig. 5 shows the FTIR spectra of the BC as well as the BC/iron oxide nanocomposites. The FTIR spectra of the Fe3O4 and Fe2O3 references are also included for comparison. Under the measuring range, Fe3O4 and Fe2O3 do not exhibit any distinct features, since the FTIR band for Fe–O stretching is between 400 and 600 cm−1 [48,49]. Thus, the presence of the iron oxide NPs should not obstruct the spectra of the BC nanocomposites. The characteristic absorption bands for the pristine BC were observed; namely, the hydroxyl (–OH) group at ~3350 cm−1, C–H stretching at ~2900 cm−1, and C-O-C bond stretching at ~1060 cm−1 [24,46]. The BC/iron oxide nanocomposite samples show unique changes in the positions of bands representing functional groups. The bands representing –OH groups of all samples were observed to shift to a lower wavenumber position in the range 3020–3350 cm−1. In co-precipitation, the Fe2+ and Fe3+ ions are thought to anchor to the –OH groups of the cellulose, before being converted to iron oxide NPs; this is believed to be the cause of a shift in the position of the band representing –OH groups [19]. The characteristics of the band representing C–O–C bonds in the pristine BC were also modified. Depending on the functional groups of the starting reactants, the characteristic bands for the nanocomposites can be found in the wavelength range 1000–1500 cm−1. These bands can be attributed to the by-products of the co-precipitation process from the chemical reactions (1)–(6). For example, the characteristic band of ammonium chloride (NH4Cl) at ~1390 cm−1 [50] is found in the spectrum of BC-CC, which corresponded well with the XRD secondary phase observed for this sample. Similarly, the bands located at ~1425 cm−1 and ~1050 cm−1 for BC-SC are assigned to the NH bending and the sulfate group of ammonium sulfate (NH4)2SO4 respectively. The band located at ~1300 cm−1 for BC-AC is associated with ammonium acetate (NH4C2H3O2)[51]. The NO3 asymmetric stretching band located at ~1315 cm−1 from ammonium nitrate can be found in the BC-SN sample. The observation of these functional groups indicates that even after thoroughly washing the BC nanocomposites in water several times, these salt by-products were not totally eliminated from the samples. Additionally, the presence of these functional groups could be

### Table 2

Comparison of the particle size, crystallite size, Ms, wt% of NPs, and fraction of Fe3O4 and Fe2O3 of the BC/Fe3O4 nanocomposites.

| Nanocomposite samples | Particle size (nm) | Crystallite size (nm) | Ms (emu/g) | TGA analysis wt% of NPs | XAS analysis fraction of Fe3O4 | XAS analysis fraction of Fe2O3 |
|-----------------------|-------------------|----------------------|-----------|-------------------------|-----------------------------|-------------------------------|
| BC-CC                 | 86 ± 25           | 15.4 ± 2.3           | 45.1 ± 2.3| 65.0 ± 1.9              | 0.12 ± 0.01                 | 0.88 ± 0.04                   |
| BC-SC                 | 75 ± 25           | 16.5 ± 2.5           | 56.6 ± 2.8| 74.9 ± 2.2              | 0.51 ± 0.03                 | 0.49 ± 0.02                   |
| BC-AC                 | 63 ± 20           | 13.0 ± 2.0           | 25.7 ± 1.3| 38.0 ± 1.1              | 0.34 ± 0.02                 | 0.66 ± 0.03                   |
| BC-CN                 | 85 ± 18           | 16.7 ± 2.5           | 35.4 ± 1.8| 40.5 ± 1.2              | 0.56 ± 0.03                 | 0.44 ± 0.02                   |
| BC-SN                 | 55 ± 15           | 10.1 ± 1.5           | 34.2 ± 1.7| 41.9 ± 1.3              | 0.43 ± 0.02                 | 0.57 ± 0.03                   |
| BC-AN                 | 65 ± 18           | 14.5 ± 2.2           | 45.6 ± 2.3| 49.9 ± 1.5              | 0.33 ± 0.02                 | 0.67 ± 0.03                   |

Fig. 5. Typical SEM images of the BC/Fe3O4 nanocomposites prepared from co-precipitation using different starting reactants: (a) BC-CC, (b) BC-SC, (c) BC-AC, (d) BC-CN, (e) BC-SN, (f) BC-AN.
another reason for the shift of the -OH groups of the BC since they can form strong hydrogen bonds with these groups.

The existence of the salt byproducts in the BC nanocomposites could play an important role in the formation of iron oxide NPs in the BC network. As explained earlier, the difference in the morphology and sizes of NPs in BC is due to the NP formation mechanism which involves the presence of various anions according to each chemical reaction. These anions may impede the reaction between the partial charge on the OH groups and the iron ions. Alternatively, they may react with NH₄⁺ ions and form salt byproducts coated on the surface of BC fibrils, as evidenced from FTIR. These byproducts may also hinder the formation of iron oxide NPs in the BC network. Since the byproducts of each reaction are different, depending on the starting reactants, their effects on the formation of NPs are not the same. Such effects would result in the difference in the morphology, cluster sizes, and distribution of NPs in the BC nanocomposite, as shown in Fig. 5. This in turn may also influence the magnetic properties of the nanocomposites.

The magnetic M-H curves of the nanocomposites are presented in Fig. 8. The characteristic curves of the superparamagnetism of the iron oxide NPs still persist. However, there are variations in the magnetizations of the BC/Fe₃O₄ nanocomposites prepared from different reactants, as tabulated in Table 2. The Ms values for each sample are ranked in the following order BC-SC (~57 emu/g) > (BC-AN, BC-CN) > (BC-CN, BC-SN) > BC-AC (~26 emu/g). This suggests that the Fe(II) sulfate and Fe(III) chloride salts are the best candidates for forming BC/Fe₃O₄ nanocomposites if the magnetization is the most important requirement. This particular nanocomposite could be potentially utilized in several industrial applications e.g. flexible sensors, actuators, or electromagnetic shielding. In contrast, although the BC-AC nanocomposite exhibits a lower Ms, the SEM image (Fig. 5) clearly shows a more open structure, with high porosity and surface area of the nanofibers. These features make this nanocomposite an excellent candidate for adsorption or wound healing applications. Thus, by adjusting the starting
reactants for the co-precipitation of Fe$_3$O$_4$ in BC structure, the characteristics and properties of the BC nanocomposites can be controlled towards an application.

One of the reasons for the change in $M_s$ could be attributed to the content of the magnetic phases in the nanocomposite. In general, the higher the content of the magnetic phase, the larger the magnetization. To determine the content of the magnetic phases, the BC and the nanocomposite samples were subjected to TGA analysis. As shown in Fig. 9, BC, without the presence of NPs, has a prominent weight loss in the temperature range 250–350 °C. This loss is typically associated with the degradation of BC, including decomposition, dehydration, and depolymerization of the glycosidic units [24,53]. Above 600 °C, the residual weight of the sample is stable. For the BC/ironoxide nanocomposite samples, weight losses initiate at around 200 °C. The weight loss profiles are different, depending on the samples. For example, BC-AC and BC-CN exhibited significant weight losses of 55–60% at a temperature of ~250 °C, whereas the weights of other samples gradually decrease up to temperatures in the range 600–700 °C. The difference in the weight loss profiles is believed to be because of the retained functional groups in each nanocomposite, as shown in Fig. 7. To determine the content of the magnetic Fe$_3$O$_4$ phase, the weights of the pristine BC at 600 °C were subtracted from the weight of the nanocomposites at the same temperature. At this temperature, the retained functional groups are decomposed, and the remainder is the iron oxide residue. The weight percentages of the iron oxide NPs are presented in Table 2. From this analysis, it is noted that the largest value of $M_s$ for BC-SC correlates with the highest iron oxide NPs content. Conversely, BC-AC has the lowest Fe$_3$O$_4$ content, resulting in the lowest $M_s$ value. Besides, BC-CN and BC-SN show similar weight retentions and nearly the same $M_s$ values. The exceptions are for BC-CC and BC-AN, which exhibit similar $M_s$ values but the contents of iron oxide are quite different. Thus, other factors need to be considered, such as the sizes, and the crystal phases of the NPs.

The mean size of the magnetic NPs also has an influence on the $M_s$ value. It was found that the smaller NPs exhibit lower $M_s$ due to a surface disordering effect [54]. The surface of the NPs is composed of some canted or disordered spins that prevent the core spins from aligning along the magnetic field direction [55]. Since the smaller NPs consist of a higher fraction of atoms at the surface, the $M_s$ values of these NPs is proportionally lower. In the present work, from the XRD analysis, iron oxide NPs with small crystallite sizes (10–13 nm) are observed for BC-AC and BC-SN, which are relatively smaller than for other samples. These observations correlate well with the magnetic measurements, with the lowest $M_s$ values being recorded for these two samples. Another factor that could contribute to magnetism is the crystal structure. Only the crystalline phase of magnetite or maghemite can contribute to ferrimagnetism. A secondary phase or amorphous structure cannot enhance the magnetization. From Fig. 6, the XRD pattern of BC-CC shows a prominent peak for the secondary phase (located at 32.4°), which is likely to be non-magnetic, thereby suppressing the magnetization of the nanocomposite. This is probably the reason that the BC-CC sample exhibits a not so large $M_s$ despite a relatively substantial amount of the iron oxide NPs in the BC structure. Furthermore, the secondary phase (at 2θ = 44.5°) is also observed for the BC-AC sample. Its presence reduces the magnetization, and thus contributes to the lowest $M_s$ value of this sample.

Finally, the form of magnetic phases of NPs could lead to a variation in the magnetization. As mentioned earlier, the XRD patterns of magnetite (Fe$_3$O$_4$) and maghemite (Fe$_2$O$_3$) are very similar, and it is challenging to distinguish between these two phases from the XRD results. The BC nanocomposites likely contain both phases in different proportions. However, these two phases exhibit different $M_s$ values. The bulk $M_s$ value for Fe$_3$O$_4$ (92 emu/g) [56] is significantly higher than that of Fe$_2$O$_3$ (76 emu/g) [57], which means that the nanocomposites with a higher fraction of Fe$_3$O$_4$ are likely to possess higher magnetization. To determine the fraction of Fe$_3$O$_4$ and Fe$_2$O$_3$, the XAS analysis for Fe K-edge of the BC nanocomposite samples was carried out. The normalized XAS data were processed and analyzed after background subtraction in the pre-edge and post-edge region using the ATHENA software, which is included in the IFEFFIT package [58]. The XAS spectra of the BC nanocomposites, as well as the Fe$_3$O$_4$ and Fe$_2$O$_3$ references, are shown in Fig. 10. Since the absorption edge and peaks, and the characteristic features of Fe$_3$O$_4$ and Fe$_2$O$_3$ are different, the linear combination fit method in the ATHENA software was used to quantify the fraction of each phase, as represented in Table 2. As expected, all the nanocomposite samples comprise both magnetic oxide phases. In every sample, the

![Fig. 9. TGA measurements of the BC/Fe$_3$O$_4$ nanocomposites prepared from coprecipitation using different starting reactants: (a) BC-CC, (b) BC-SC, (c) BC-AC, (d) BC-CN, (e) BC-SN, (f) BC-AN.](image)

![Fig. 10. XAS analysis for Fe K-edge of the BC/Fe$_3$O$_4$ nanocomposites prepared from coprecipitation using different starting reactants: (a) BC-CC, (b) BC-SC, (c) BC-AC, (d) BC-CN, (e) BC-SN, (f) BC-AN.](image)
proportion of the FeO$_2$ phase is relatively high with respect to the total iron oxide content. The presence of the high proportion of FeO$_2$ could be due to the fabrication process under ambient conditions. The Fe$^{2+}$ ions were possibly oxidized to the Fe$^{3+}$ ions during the co-precipitation process. Furthermore, the FeO$_2$ NPs inside the BC structure could be partially oxidized after rinsing and drying the BC composite hydrogels under ambient conditions. Comparison among all samples, the fraction of Fe$_3$O$_4$ in BC-CC is noticeably lower than the other samples. It implies that using Fe(II) and Fe(III) chloride salts as starting reactants is likely to promote the formation of the Fe$_3$O$_4$ phase. The possible reason is that, according to the chemical reactions, only the Cl$^-$ anion was created when using Fe(II) and Fe(III) chloride salts (Eq. (1)), whereas two types of anion were generated for the other reactions (Eqs. (2)–(6)). This might be associated with the ease of oxidation of Fe$^{2+}$, having just one type of anion, and thus contributing to the conversion of FeO$_2$ to Fe$_3$O$_4$ in the case of BC-CC. It is also a part of the reason for the reduced M$_s$ of BC-CC due to the presence of a large proportion of the FeO$_2$ phase. On the other hand, BC-SC comprises a relatively large fraction of Fe$_3$O$_4$ (0.509) which correlates with a high M$_s$ value for this sample.

4. Conclusions

A systematic study on the synthesis of BC/iron oxide nanocomposites by a co-precipitation method has been presented. The effect of starting reactants on the physical properties of magnetic nanoparticles formed inside bacterial cellulose networks was reported. For these reactants, chloride, sulfate, and acetate salts were used as the source of Fe$^{2+}$ ions, whereas chloride and nitrate salts were used as the source of Fe$^{3+}$ ions. For the fabrication of iron oxide NPs, different starting reactants do not seem to significantly affect the morphology, size, phase, and magnetic properties of the synthesized NPs when formed outside of the bacterial cellulose environment. However, this does have an impact on the physical characteristics of the BC/iron oxide nanocomposites when formed in situ. Using different starting reactants leads to differences in the sizes of the NPs formed inside the BC structure, and various functional groups of the retained by-products. The main phases of Fe$_3$O$_4$ and FeO$_2$ were found in all nanocomposite samples, but the presence of secondary phases was also noted in some samples. Moreover, the loading amounts of the incorporated NPs were found to vary as the starting reactants were changed. Additionally, using different Fe(II) and Fe(III) salts as the starting reactants also yields a different ratio of the magnetite (Fe$_3$O$_4$) and maghemite (Fe$_2$O$_3$) phases. These ratios have significant influence on the measured magnetic properties. In summary, the BC/iron oxide nanocomposite synthesized from Fe(II) sulfate and Fe(III) chloride, BC-SC, yielded the most abundant amounts of NPs, pure phases of these materials, large particle sizes, and the highest Fe$_3$O$_4$/FeO$_2$ ratio. All these factors are thought to result in the highest magnetization value for the BC-SC nanocomposites compared to other samples. On the other hand, the BC-AC sample exhibited the lowest magnetization because of the low magnetic oxide content, small particle size, the existence of secondary phases, and a low proportion of the FeO$_2$ phase. The present study thus shows that the choices of the starting reactants have a direct implication on the design of the magnetic BC systems. If the magnetization is the most critical factor for an application, such as flexible sensors, actuators, or electromagnetic shielding, it is suggested that Fe(II) sulfate and Fe(III) chloride should be used as the starting reactants. On the other hand, though exhibiting lower magnetization, the BC-AC nanocomposite preserves a porous structure and a high surface area of the nanofibers, which makes it more suitable for applications such as adsorbents or wound healing.

Data availability statement

The raw/processed data required to reproduce these findings cannot be shared at this time as the data also forms part of an ongoing study.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

Acknowledgments

This work was supported by the Thailand Research Fund (TRF) in cooperation with Synchrotron Light Research Institute (public organization) and Khon Kaen University (RSA62800020), the Royal Society-Newton Advanced Fellowship (NA160147) in partnership with the Thailand Research Fund (TRF) (DBG6080002), the Research and Technology Transfer Affairs of Khon Kaen University, the Research Network NANOTEC (RNIN) program of the National Nanotechnology Center (NANOTEC), NSTDA, Ministry of Higher Education, Science, Research and Innovation, and the Research Fund for Supporting Lecturer to Admit High Potential Student to Study and Research on His Expert Program Year 2016, Graduate School Khon Kaen University.

References

[1] Y. Huang, C.L. Zhu, JZ. Yang, Y. Nie, C.T. Chen, D.P. Sun, Recent advances in bacterial cellulose, Cellulose 21 (2014) 1–30.
[2] C. Sharma, N.K. Bhaward, Bacterial nanocellulose: present status, biomedical applications and future perspectives, Mater. Sci. Eng. C 104 (2019) 109983.
[3] S. Gorgiev, J. Troek, Bacterial cellulose: production, modification and perspectives in biomedical applications, Nanomaterials 9 (2019) 1352.
[4] J. Wang, J. Tavakoli, Y.H. Tang, Bacterial cellulose production, properties and applications with different culture methods - a review, Carbohydr. Polym. 219 (2019) 63–76.
[5] F.G.B. Parte, S.P. Santos, C.C. Chou, V. Verma, H.T. Wang, S. Imsadji, K.C. Cheng, Current progress on the production, modification, and applications of bacterial cellulose, Crit. Rev. Biotechnol. 40 (2020) 397–414.
[6] M. Slojch, Bacterial nanocellulose in papermaking, Cellulose 26 (2019) 6477–6488.
[7] H.M.C. Azrvedo, H. Barud, C.S. Farinas, V.M. Vasconcellos, A.M. Claro, Bacterial cellulose as a raw material for food and food packaging applications, Front. Sustain. Food Syst. 3 (2019) 2.
[8] R. Portela, C.R. Leal, P.L. Almeida, R.G. Sobral, Bacterial cellulose: a versatile biopolymer for wound dressing applications, Microb. Biotechnol. 12 (2019) 586–610.
[9] E.M. van Zyl, J.M. Coburn, Hierarchical structure of bacterial-derived cellulose and its impact on biomedical applications, Curr. Opin. Chem. Eng. 24 (2019) 122–130.
[10] W.L. Hu, S.Y. Chen, J.X. Yang, Z. Li, H.P. Wang, Functionalized bacterial cellulose derivatives and nanocomposites, Carbohydr. Polym. 101 (2014) 1043–1060.
[11] N. Shah, M. Ul-Islam, W.A. Khattak, J.K. Park, Overview of bacterial cellulose composites: a multipurpose advanced material, Carbohydr. Polym. 98 (2013) 1585–1598.
[12] F.G. Torres, J.J. Arroyo, O.P. Troncoso, Bacterial cellulose nanocomposites: an all-nano type of material, Mater. Sci. Eng. C 98 (2019) 1277–1293.
[13] N. Eslahi, A. Mahmoodi, N. Mahmoodi, N. Zandi, A. Simchi, Processing and properties of Nanofibrous bacterial-cellulose-containing polymer composites: a review of recent advances for biomedical applications, Polym. Rev. 60 (2020) 144–170.
[14] V. Thiruvengadam, S. Vitta, Flexible bacterial cellulose/permalloy nanocomposite xorganel sheets - size scalable magnetic actuator-cum-electrical conductor, AIP Adv. 7 (2017), 035107.
[15] N. Sripailai, W. Mongkolthanaruk, S.J. Eichhorn, S. Pinitsoontorn, Magnetically responsive and flexible bacterial cellulose membranes, Carbohydr. Polym. 192 (2018) 251–262.
[16] H.S. Barud, A. Tercjak, J. Gutierrez, W.R. Viali, E.S. Nunes, S.J.L. Ribeiro, M. Jafellici, M. Nalin, F.R.C. Marques, Biocellulose-based flexible magnetic paper, J. Appl. Phys. 117 (2015) 17B734.
[17] N. Sripailai, R. Mangayil, A. Pammoo, V. Santala, S. Tuukkanen, S. Pinitsoontorn, Enhancing piezoelectric properties of bacterial cellulose films by incorporation of MnFe$_2$O$_4$ nanoparticles, Carbohydr. Polym. 231 (2020) 115730.
[18] J.A. Marins, B.G. Soares, H.S. Barud, S.J.L. Ribeiro, Flexible magnetic membranes based on bacterial cellulose and its evaluation as electromagnetic interference shielding material, Mater. Sci. Eng. C 33 (2013) 3994–4001.
[19] M. Park, J. Cheng, J. Choi, J. Kim, J. Hyun, Electromagnetic nanocomposite of bacterial cellulose using magnetite nanoclusters and polyaniline, Colloids Surf. B 102 (2013) 238–242.
[20] Y. Zheng, J.X. Yang, W.L. Zheng, X. Wang, C. Xiang, L. Tang, W. Zhang, S.Y. Chen, H.P. Wang, Synthesis of flexible magnetic nanohybrid based on bacterial cellulose under ultrasonic irradiation, Mater. Sci. Eng. C 33 (2013) 2407–2412.
[21] N. Sripailai, P. Sirima, D. Palaporn, W. Mongkolthanaruk, S.J. Eichhorn, S. Pinitsoontorn, White magnetic paper based on a bacterial cellulose nanocomposite, J. Mater. Chem. C 6 (2018) 13827–13835.
[22] I.F. Nata, M. Sureshkumar, C.K. Lee, One-pot preparation of amine-rich magnetite/bacterial cellulose nanocomposite and its application for arsenate removal, RSC Adv. 1 (2011) 625–631.
F. Yazdani, W. Mongkolthanaruk, S.J. Eichhorn et al. Materials and Design 196 (2020) 109148

Y.M. Wang, X. Cao, G.H. Liu, R.Y. Hong, Y.M. Chen, X.F. Chen, H.Z. Li, B. Xu, D.G. Wei, T. Muthukumaran, J. Philip, Enhanced thermal stability of phosphate capped magnetite nanoparticles, J. Appl. Phys. 115 (2014) 224304.

W. Kim, C.Y. Suh, S.W. Cho, K.M. Roh, H. Kwon, K. Song, I.J. Shin, A new method for the identification and quantification of magnetite-maghemite mixture using conventional X-ray diffraction technique, Talanta 94 (2012) 348–352.

G. Nabiyouni, M. Julaee, D. Ghanbari, P.C. Alibadi, N. Safaei, Room temperature synthesis and magnetic property studies of Fe3O4 nanoparticles prepared by a simple precipitation method, J. Ind. Eng. Chem. 21 (2015) 599–603.

S. Tanpichai, W.W. Sampson, S.J. Eichhorn, Stress-transfer in microfibrillated cellulose reinforced poly(lactic acid) composites using Raman spectroscopy, Compos. Part A-App. 5 (2012) 1145–1152.

F. Quero, M. Nogó, H. Yano, K. Abdulsalami, S.M. Holmes, B.H. Sakakini, S.J. Eichhorn, Optimization of the mechanical performance of bacterial cellulose/poly(l-lactic acid) composites, ACS Appl. Mater. Interfaces 2 (2010) 321–330.

Y. Nishi, M. Uryu, S. Yamanaka, K. Watanebe, N. Kitamura, M. Iguchi, S. Mitsuhashi, The structure and mechanical properties of sheets prepared from bacterial cellulose, J. Mater. Sci. 25 (1990) 2987–3001.

P. Jeannettevanvich, D. Palaporn, N. Hanleke, Y. Poo-arpon, W. Mongkolthanaruk, S.J. Eichhorn, S. Pinitsoontorn, Carbon nanofiber aerogel/magnetic Core–Shell nanoparticle composites as recyclable oil sorbents, ACS Appl. Nano Mater. 3 (2020) 3939–3950.