



Design and characterization of an almond shell-based nanocellulose/chitosan nanocomposite as a bio-derived modifier

Athraa Basheer Radhi

Department of Laboratory and Clinical Sciences, College of Pharmacy, University of Anbar, Anbar, Iraq.

Email corresponding author's: ph.athraa.radhi@uoanbar.edu.iq

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Abstract

The increasing production of agro-industrial residues requires sustainable strategies that minimise waste and generate high-value materials. Cellulose nanocrystals (CNCs) were obtained from almond shell waste and integrated into a fully bio-based nanocomposite with chitosan and folic acid using aqueous, low-impact processing. Almond shells were purified through alkaline treatment and bleaching, hydrolysed with sulfuric acid to obtain the CNCs, and then oxidised using a TEMPO/NaBr/NaClO system to introduce carboxylate groups onto the surface. Chitosan nanoparticles were prepared by ionic gelation with tripolyphosphate, and folic acid, used as a model bioactive compound, was loaded into hybrid CNC/chitosan dispersions. Field-emission scanning electron microscopy revealed a porous lamellar structure with nanometric cellulose sheets (80–250 nm thick) embedded in a continuous chitosan-rich matrix, providing a high-surface-area scaffold. X-ray diffraction confirmed that the cellulose I polymorph and nanocrystalline character were maintained, while a moderate decrease in the Segal crystallinity index was observed upon composite formation; this decrease was attributed to the dilution effect of amorphous chitosan and folate rather than to lattice disruption. Crystallite sizes estimated using the Scherrer equation were in the range of 10–15 nm. ATR-FTIR spectroscopy indicated extensive hydrogen bonding and electrostatic interactions among CNCs, chitosan, and folic acid, and successful folate incorporation was confirmed by diagnostic aromatic and carbonyl bands. Zeta potential measurements for 6 days revealed dynamic interfacial charge evolution with time, from a cationic surface (+26.80 mV on Day 0) to intermediate values (+16.26 mV on Day 2 and +7.50 mV on Day 4) and finally to a weakly negative potential (–4.59 mV on Day 6, indicating progressive interfacial rearrangement and electrostatic equilibration within the ternary network.

Keyword : Almond shell waste, Cellulose nanocrystals (CNCs), Chitosan–TPP nanoparticles, TEMPO oxidation, Sustainable environmental applications

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INTRODUCTION

The rising accumulation of agro-industrial residues has increased global awareness of the resource inefficiency, climate impact, and environmental burden associated with traditional disposal methods, including open burning and landfilling. A typical example in the tree-nut industry is almond processing, where shells and hulls can account for about one-third of the fruit mass yet are still predominantly burned as low-value waste despite their lignocellulosic richness and large annual tonnage [1,2]. At the same time, increasing pressure to reduce dependence on petrochemical plastics has spurred the development of bio-based, biodegradable materials that can deliver similar performance with a substantially improved environmental profile [3].

In this context, valorization of almond shell waste into functional nanostructured materials is a particularly appealing strategy to reduce waste streams, close carbon loops and produce high-value products according to circular bioeconomy principles [2,4].

Cellulose nanocrystals (CNCs) have emerged as key building blocks in sustainable materials engineering because of their high specific strength and stiffness, low density, tunable surface chemistry, and inherent biodegradability [5]. CNCs also offer an additional environmental advantage when derived from lignocellulosic residues, as they valorise underused biomass into advanced functional components rather than relying on dedicated feedstocks [3,6]. Almond shells are particularly promising in this respect: recent work has shown that they can yield cellulose-rich fractions with crystallinity indices above 50–60% after suitable pretreatments, and that subsequent acid hydrolysis or TEMPO-mediated oxidation can produce well-defined nanocellulose suitable for assembling films, aerogels, and foams with high surface area and tailored porosity

[2,7]. These developments highlight the potential of almond shell nanocellulose as a viable platform for next-generation bio-based materials while providing considerable scope to optimise composite architectures and functionalities for specific environmental or biomedical applications. The combination of nanocellulose with other polysaccharides yields nanobiopolymer composites that are highly successful in bridging structural performance with processability and functional versatility [3]. Chitosan is a cationic biopolymer, obtained from chitin from the crustacean shell waste, which forms films. It is well known for its biocompatibility, antimicrobial activity and ability to form nanoparticles by ionotropic gelation with multivalent anions [8].

Blending of chitosan with anionic nanocellulose results in the formation of a network of electrostatic and hydrogen-bonding interactions which can improve mechanical properties, change surface charge and form interconnected porous structures suitable for adsorption, encapsulation and controlled release [3,8]. Folic acid also extends this design space: apart from its nutritional value, folate has many carboxyl and aromatic groups that can participate in hydrogen bonding and ionic interactions, and its strong binding to folate receptors is often utilized in targeted delivery systems [9]. Hybrid systems based on chitosan nanoparticles and CNCs have already demonstrated improved encapsulation efficiency and protection against degradation upon co-encapsulation of folic acid, highlighting the potential of multicomponent, fully bio-derived matrices to host and stabilize sensitive active compounds [10].

Despite these advances, a clear gap remains in the literature regarding ternary nanocomposites that simultaneously combine multi-source waste valorisation (almond shells for nanocellulose and marine shell waste for chitosan), aqueous and low-impact processing, and full characterisation as modular platforms for environmental and prospective biomedical applications. Previous work on almond shell nanocellulose has largely focused on its production and physical structuring into aerogels or films. The incorporation of almond shell nanocellulose into hybrid carriers that provide mechanical reinforcement, tunable interfacial chemistry, and the ability to encapsulate and present small bioactive molecules such as folic acid has been comparatively less investigated [2,7,10]. At the same time, reviews of nanocellulose/biopolymer composites and biopolymer-derived nanomaterials for environmental remediation consistently emphasise the need for systems that integrate scalable waste-derived feedstocks, mild processing routes, and multifunctionality, including adsorption, barrier properties, and controlled release [11]. In this work, we aim to address this gap by designing and characterising a fully bio-derived nanocomposite based on TEMPO-oxidised cellulose nanocrystals obtained from almond shell waste, ionotropically crosslinked chitosan, and folic acid as a model small bioactive compound. This work combines detailed physicochemical characterisation with a clear emphasis on waste valorisation and green processing to advance almond shell-derived CNC/chitosan/folic acid assemblies towards a versatile nanoplatform supporting a range of environmentally focused applications.

MATERIALS AND METHODS

Extraction of cellulose from almond shells

The extraction procedure was adapted from recent procedures for the separation of the cellulose from almond shell biomass [12]. The raw almond shells were washed thoroughly with distilled water to remove soluble contaminants and adhering dust, air dried at room temperature, and ground to a fine powder by a laboratory grinder. The resulting powder was sieved to a homogeneous fraction, and stored in sealed containers until used.

Alkaline treatment. Alkali treatment removed hemicellulose, residual proteins and part of lignin from the almond shell powder. In brief, the powder was suspended in a 2% (w/v) NaOH solution at a solid to liquid ratio of 1:20 (g/mL), heated at 80 °C for 4 h with constant stirring, and then cooled down to room temperature. The suspension was filtered and washed several times with distilled water until the filtrate was neutral (≈ 7.0). The alkali-treated solid was filtered and dried at 50 °C in a hot air oven to constant weight.

Bleach. The alkali-treated, cellulose-rich fraction was bleached by hypochlorite treatment as described for almond shells and similar nut waste to further delignify and decolorize the material. The dried product was stirred in an alkaline solution of commercial sodium hypochlorite (available chlorine, 4–5%) as bleaching agent at room temperature for 24–48 h. After bleaching, the material

was filtered and repeatedly washed with distilled water until a neutral pH was achieved, dried in an oven at 50 °C, and then processed further to obtain purified almond shell cellulose powder.

Almond shell cellulose-based cellulose nanocrystals preparation

Hydrolysis of sulfuric acid. Cellulose nanocrystals (CNCs) were prepared by controlled acid hydrolysis of bleached almond shell cellulose following literature procedures for lignocellulosic residues. Typically, 10 g of dried cellulose powder was added slowly to 100 mL of 30 % (v/v) H₂SO₄ pre-heated to 30 °C with vigorous mechanical stirring. The suspension was held at this temperature for 30 min to preferentially hydrolyze amorphous domains, whilst preserving crystalline regions. The mixture was diluted 10-fold with cold distilled water after the reaction was complete to stop hydrolysis. The mixture was centrifuged and washed with distilled water repeatedly until the conductivity of the supernatant was significantly reduced. The resulting CNC suspension was dialyzed against deionized water until a constant neutral pH was obtained.

Ultrasonic dispersing

To minimize residual aggregates and to obtain a stable colloidal dispersion, the CNC suspension was sonicated using a probe-type ultrasonicator (e.g., SONOPULS HD 2070, 20 kHz) at an appropriate amplitude for a total effective sonication time of 80 min in an ice bath to avoid overheating. The final dispersion was stored at 4 °C and utilized as the nanocellulose stock for subsequent oxidation and composite preparation.

Oxidation of almond shell nanocellulose using TEMPO mediation

The almond shell CNCs were oxidized on the surface with 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) according to the classical TEMPO/NaBr/NaClO system, described for cellulose nanofibers and nanocrystals [12]. 1 g freeze-dried CNC was dispersed in 100 mL deionized water by magnetic stirring. The 50 mL of water was added to dissolve 0.06 g of TEMPO and 0.10 g of NaBr, and then the CNC suspension was mixed with it. The oxidation was started by slow addition of 10% (w/v) NaClO solution and the reaction pH was kept at 10–11 with 0.5 M NaOH. The pH was monitored by a pH-meter and adjusted, if necessary, during the reaction [13]. The mixture was stirred at room temperature until the consumption of NaOH was constant, indicating complete oxidation of primary hydroxyl groups at C6 to carboxylate groups. Then, the reaction was quenched by adding a small volume of 0.5 M HCl to adjust the pH to neutral (≈ 7.0). The oxidized CNC suspension was washed thoroughly with deionized water by centrifugation or dialysis to remove salts and residual reagents. The final volume was adjusted to 200 mL, followed by high-shear homogenisation at 10,000 rpm and a brief (10 min) ultrasonication step to ensure a well-dispersed TEMPO-oxidised CNC colloid. The dispersion was stored at 4 °C until composite fabrication.

Preparation of chitosan nanoparticles and CNC/chitosan composite dispersion

Chitosan nanoparticles (ChNps) were prepared by the ionic gelation method using sodium tripolyphosphate (TPP) as a crosslinker, as previously reported [14]. To ensure complete solubilisation, medium-molecular-weight chitosan (2 g/L) was dissolved in 1% (v/v) acetic acid under continuous stirring for 16 h. The chitosan:TPP mass ratio was maintained at 3:1, a ratio reported to be ideal for nanoparticle formation and electrostatic complexation, by dropwise addition

of the TPP solution (0.6 g L^{-1}) at a rate of $1.0 \text{ mL} \cdot \text{min}^{-1}$ under magnetic stirring (Okamoto-Schalch et al. 2020). The resulting opalescent dispersion was then agitated for a further 30 min to allow complete formation of the ChNps.

For preparation of the CNC/chitosan composite (ChNp–CNC), the chitosan solution was mixed with an appropriate amount of TEMPO-oxidised CNC dispersion before the addition of TPP and homogenised by magnetic stirring for 5 min. The TPP solution was then added dropwise as described above. The final concentrations in the composite dispersion were 0.6 g/L TPP, 0.3 g/L TEMPO-oxidised almond shell CNC, and 2 g/L chitosan. The dispersion was stirred gently to allow complete formation of the hybrid network driven by electrostatic interactions between cationic chitosan and anionic CNC carboxylates.

Encapsulation of folic acid by ionic gelation

Folic acid (FA) was encapsulated in the ChNps and ChNp–CNC systems using ionic gelation as a mild, aqueous encapsulation strategy [10]. Briefly, 5 mg of FA was dissolved in 50 mL of 0.1 M anhydrous sodium phosphate buffer (pH 6.8). This FA solution was mixed with the chitosan solution and homogenised by magnetic stirring for 5 min, yielding final concentrations of 0.1 g/L FA and 2 g/L chitosan. The TPP solution was then added dropwise as described above to form FA-loaded ChNps (ChNp_FA).

For preparation of FA-loaded CNC/chitosan composite particles (ChNpCNC_FA), the FA solution was first combined with the TEMPO-oxidised CNC dispersion and chitosan solution, and the mixture was stirred for 5 min before TPP addition. The final composition of the ChNpCNC_FA dispersions was 0.1 g/L FA, 2 g/L chitosan, 0.3 g/L CNC, and 0.6 g/L TPP, mirroring conditions successfully employed in previous CNC/chitosan/FA hybrid systems, as illustrated in Figure 1.

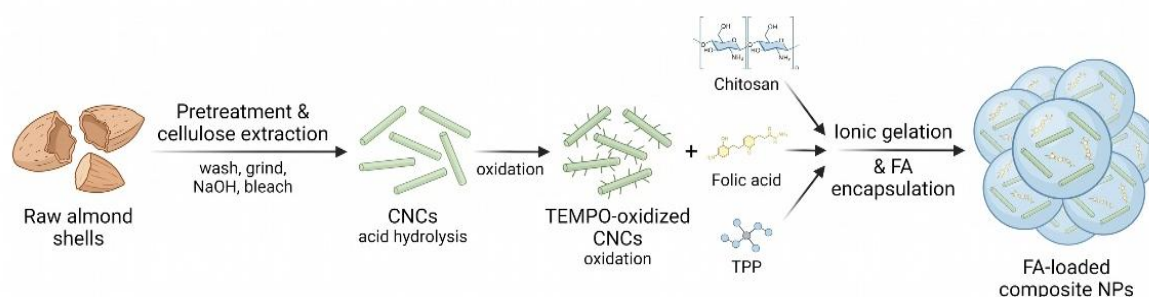


Figure 1. Schematic illustration of the preparation of FA-loaded CNC/chitosan nanoparticles from almond shell waste (created using AI).

Characterisation

Scanning electron microscopy (SEM)

The surface morphologies of almond shell-derived CNCs and the CNC/chitosan/folic acid composite were investigated by field-emission scanning electron microscopy. Dried samples were carefully ground, mounted on aluminium stubs using double-sided carbon tape, and sputter-coated with a thin layer of gold to minimise charging.

X-ray diffraction (XRD)

The diffraction behaviour of the final almond shell-derived CNC/chitosan/folic acid composite was examined by wide-angle X-ray diffraction using a powder diffractometer equipped with Cu K α radiation ($\lambda = 1.5406 \text{ \AA}$), operated at 40 kV and 8 mA. The final composite sample was scanned over a 2θ range of $7.01\text{--}99.99^\circ$.

Fourier transform infrared spectroscopy (FTIR)

Molecular structure and interactions among CNCs, chitosan, and folic acid were examined by attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). Dried samples were analysed over the range of $400\text{--}4000 \text{ cm}^{-1}$ using an appropriate resolution and number of scans.

Zeta Potential

The surface charge of the CNC/chitosan/folic acid nanocomposite dispersions from almond shell was determined by Zeta Potential Analyzer by electrophoretic light scattering (ELS) at 25°C . Samples were diluted with deionized water before measurement and treated in ultrasonic bath for 10 min to obtain homogeneous dispersion. Measurements were performed on Day 0 (freshly prepared), Day 2, Day 4 and Day 6 to check the stability and charge changes over time. All measurements were performed in triplicate and the results were expressed as mean $\pm$ SD.

RESULT AND DISCUSSION

High-vacuum scanning electron microscopy revealed a hierarchically organised morphology for the almond shell-derived CNC/chitosan/folic acid composite across multiple length scales. As shown in Figure 2, at low magnification ($\approx 4,000\times$), the sample exhibited a continuous, compact background matrix decorated with numerous thin, plate-like fragments distributed over the surface, suggesting a heterogeneous but well-integrated polysaccharide network in which larger chitosan-rich domains and residual cellulose microfibrils are embedded [17]. At intermediate magnifications ($\sim 16,000\times\text{--}30,000\times$), the surface was resolved into irregular flakes of lamellae with sharp fractured edges and submicrometre lateral dimensions, consistent with progressive delamination of the lignocellulosic framework into nanoscale cellulose sheets under the combined action of alkaline pretreatment, sulfuric acid hydrolysis and TEMPO-mediated oxidation [2,6]. The interlamellar voids and gaps between these flakes indicate a porous three-dimensional structure, favorable for subsequent diffusion and loading of low molecular weight active compounds.

At higher magnifications ($\approx 30,000\times\text{--}70,000\times$), individual lamellae could be directly measured, yielding thickness values of approximately 78.64 nm for the thinnest sheets and around 254.3 nm for thicker stacked fragments [17]. These nanometric dimensions confirm that the applied processing route successfully converted the almond shell biomass into nanostructured cellulose lamellae and are within the range reported for cellulose nanocrystal/nanofibril aggregates derived from comparable agro-industrial residues [2]. The coexistence of relatively thin and thick plates reflects the inherent polydispersity associated with top-down acid hydrolysis and the partial restacking of CNC units during drying. Compared with the rod-like or whisker-shaped CNCs typically obtained from milder hydrolysis of purified pulps, the predominantly lamellar morphology observed here can be attributed to the greater hydrolysis severity and the simultaneous presence of chitosan and folic acid, which favour lateral association and sheet formation during composite assembly and solvent removal [6]. Overall, the SEM observations confirm the formation of a nanostructured, lamellar composite in

which almond shell-derived cellulose provides a high-surface-area scaffold embedded in a continuous chitosan-rich phase.

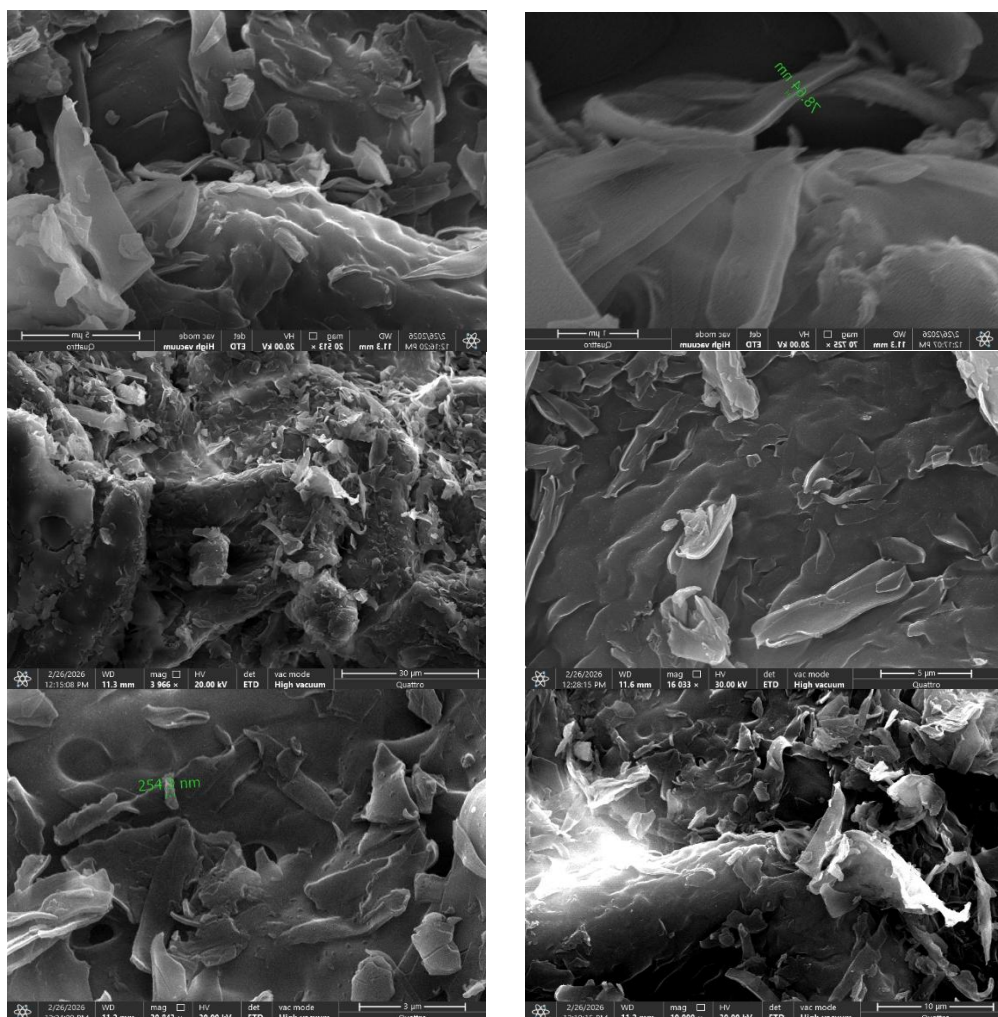


Figure 2. SEM micrographs of the almond shell-derived CNC/chitosan/folic acid nanocomposite at different magnifications, showing lamellar nanosheets (≈ 80 – 250 nm thick) embedded in a continuous polysaccharide matrix.

X-ray Diffraction (XRD)

The X-ray diffraction pattern of the final CNC/chitosan/folic acid composite is shown in Figure 3. The diffractogram was recorded over a 2θ range of 7.01 – 99.99° using Cu K α radiation ($\lambda = 1.5406$ Å). The pattern was characterised by broad diffraction scattering with a weak feature near $2\theta \approx 15.3^\circ$, while no intense and sharply resolved reflections corresponding to the individual starting components were observed. This profile indicates that formation of the ternary composite modified the long-range molecular organisation of the constituent materials and produced a structurally integrated hybrid assembly. The diffuse XRD profile is consistent with the intended interaction of TEMPO-oxidised CNCs, chitosan, and folic acid during ionic gelation. In this system, the negatively charged carboxylate groups of TEMPO-oxidised CNCs can interact with protonated amino groups of chitosan, while hydrogen bonding may occur among hydroxyl, amino, and carboxyl-containing groups. These interfacial interactions can bring the polymeric components into close contact, limit

regular chain packing, and reduce the resolution of the diffraction features observed for the individual constituents 3,10. Similar changes in diffraction behaviour have been reported for nanocellulose–chitosan composites following interfacial association and composite formation 3.

The weak feature near $2\theta \approx 15.3^\circ$ occurs within a region commonly associated with cellulose-related ordering 16. Its presence suggests that local ordered domains associated with the cellulose-derived component may remain embedded within the chitosan/folic acid network. The broad profile therefore supports a composite architecture that combines residual local order with a more flexible, interfacially associated matrix. Such structural organisation is relevant to the intended function of the material because it can provide accessible hydroxyl, amino, and carboxyl-containing groups at the interface, as independently supported by the FTIR spectrum. The XRD pattern should be interpreted together with the SEM and FTIR results. SEM revealed a lamellar nanostructured morphology, whereas FTIR indicated the coexistence and interaction of functional groups from the CNC, chitosan, and folic acid constituents. Taken together, these complementary findings support successful assembly of the final bio-derived CNC/chitosan/folic acid nanocomposite rather than the persistence of separate, independently ordered starting materials. Because the XRD measurement was performed only for the final composite, the present analysis is qualitative and does not separate the individual structural contributions of CNCs, chitosan, and folic acid. Future component-resolved XRD measurements could provide a more detailed quantitative evaluation of their individual diffraction contributions.

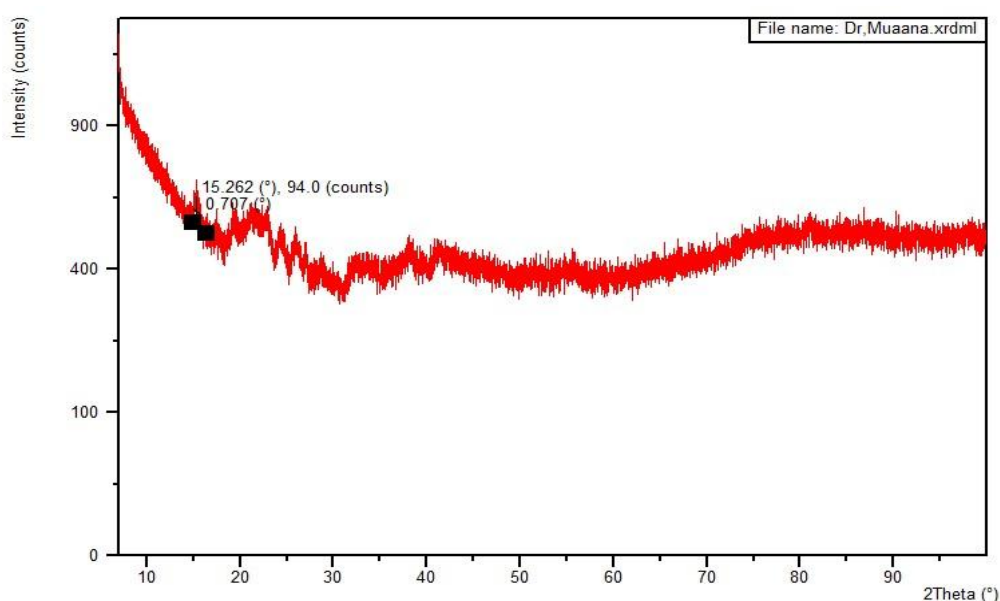


Figure 3. X-ray diffraction pattern of the final almond shell-derived CNC/chitosan/folic acid composite recorded over a 2θ range of $7.01\text{--}99.99^\circ$.

Fourier Transform Infrared (FTIR) Spectroscopy

The chemical structure and intermolecular interactions within the CNC/chitosan/folic acid composite were examined by ATR-FTIR spectroscopy over $400\text{--}4000\text{ cm}^{-1}$. The spectrum exhibited ten principal absorption bands at 3414 , 2922 , 1694 , 1605 , 1483 , 1412 , 1244 , 1190 , 1065 and 812 cm^{-1} , each corresponding to functional groups originating from the polysaccharide matrix and the loaded vitamin. The broad band centred at 3414 cm^{-1} arises from overlapping O–H stretching vibrations of cellulose and chitosan, together with N–H stretching from protonated amino groups and folic acid moieties; its breadth and intensity indicate a dense hydrogen-bonding network among these

functionalities, in agreement with strong interpolymeric association [6,7]. The weaker band at 2922 cm^{-1} corresponds to aliphatic C–H stretching in CH and CH₂ groups along the glucopyranose and glucosamine units, confirming retention of the saccharide backbones after the full processing route[20]. In the carbonyl and aromatic region, a distinct band at 1693 cm^{-1} is assigned to C=O stretching from folic acid carboxylic and amide groups, with additional contributions from carbonyl functionalities introduced by TEMPO oxidation on the CNC surface [5]. The position of this band slightly above the $\approx 1680 \text{ cm}^{-1}$ value typically observed for crystalline folic acid has been associated with ionic and hydrogen-bond interactions between folate carboxylate groups and protonated chitosan amino sites in comparable systems, indicating that the vitamin is not merely physically entrapped but participates in specific binding within the polysaccharide network. The band at 1605 cm^{-1} combines aromatic C=C stretching from the folic acid ring system with amide-related vibrations of chitosan, while the band at 1483 cm^{-1} corresponds to aromatic skeletal and C–H bending modes, together confirming that the aromatic framework of folic acid remains intact after encapsulation [17].

The absorption at 1412 cm^{-1} is attributable to superimposed CH₂ bending and symmetric COO[−] stretching, reflecting the presence of deprotonated carboxylate groups on both TEMPO-oxidised CNC and folic acid. Its appreciable intensity provides strong evidence for multiple carboxylate-bearing species and, when considered alongside the broad 3414 cm^{-1} band, supports a scenario in which electrostatic attraction between anionic CNC/folate sites and cationic chitosan domains plays a central role in composite formation. In the 1300–1000 cm^{-1} region, the bands at 1244 and 1190 cm^{-1} are associated with C–O and C–N stretching vibrations from both the polysaccharide glycosidic framework and folic acid heterocycle, while the intense band at 1065 cm^{-1} is characteristic of C–O–C and C–O stretching in β -1,4-glycosidic linkages of cellulose and chitosan, indicating that the fundamental saccharide connectivity is preserved [2]. At lower wavenumbers, the discrete band at 812 cm^{-1} , assigned to out-of-plane aromatic C–H bending of the folic acid ring system, is absent in unloaded CNC or chitosan–TPP spectra and therefore serves as a diagnostic marker confirming the successful incorporation of folate into the composite [6,10]. Collectively, the FTIR features demonstrate (i) structural integrity of the cellulose and chitosan backbones, (ii) retention of folic acid's key functional groups, and (iii) extensive hydrogen-bonding and electrostatic interactions among the three components, consistent with the formation of a structurally integrated ternary nanocomposite rather than a simple physical mixture.

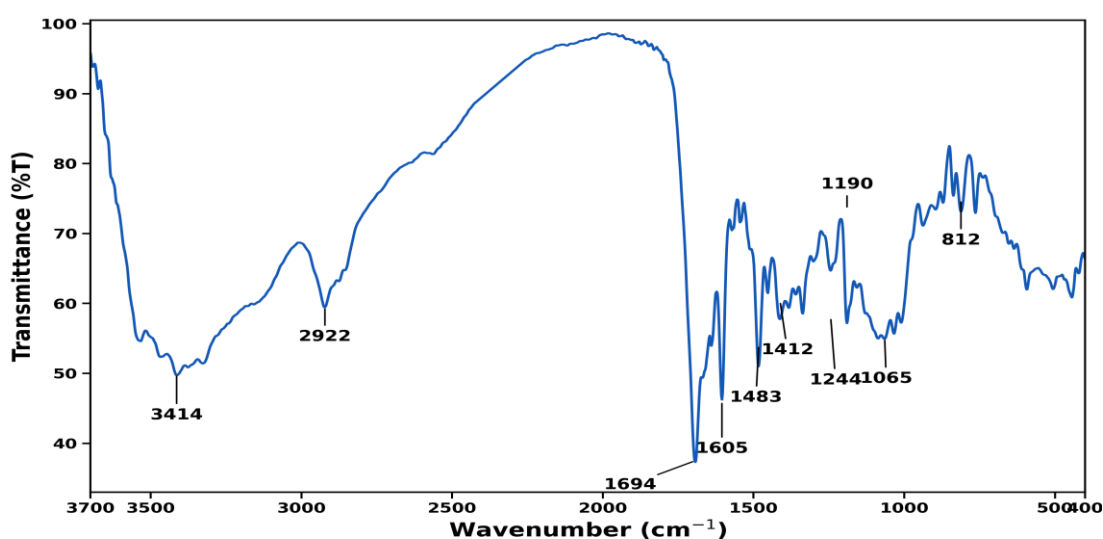


Figure 4. FTIR spectrum of the investigated sample with clearly labelled principal bands.

Zeta-Potential

The electrokinetic behavior of the formulation was evaluated by electrophoretic light scattering at Days 0, 2, 4 and 6. Day 0 +26.80 mV, Day 2 +16.26 mV, Day 4 +7.50 mV and Day 6 -4.59 mV. This series shows a gradual and systematic change of interfacial properties of the formulation during the experiment.

The freshly prepared nanocomposite on Day 0 has a positive zeta potential of +26.80 mV, which is indicative of a favorable initial electrokinetic state and significant electrostatic repulsion between the dispersed particles. This positive value suggested that the potential measured at the hydrodynamic shear plane was mainly determined by protonated amino groups ($-\text{NH}_3^+$) of chitosan. Such an interpretation is in agreement with the earlier reported studies of folic acid-loaded chitosan-TPP/cellulose nanocrystal systems where positive zeta-potential values are in the range of +23 to +25 mV (10).

The obtained potential is a sign of the interfacial equilibrium between the positively charged chitosan and the negatively charged species: phosphate groups of TPP, carboxylate groups of TEMPO-oxidized cellulose and ionizable groups of folic acid. TPP allows ionic cross-linking with the protonated amino groups in chitosan-based nanoparticles and affects particle formation and surface charge (22). Under the first preparation conditions, the contribution of the protonated chitosan groups was higher than that of the anionic components, resulting in a net positive surface potential.

+26.80 mV is slightly below the conventionally accepted limit of ± 30 mV, which is often used as a threshold value for strong electrostatic stabilization, but this is a good starting point for particle dispersion. However, zeta potential is dependent on pH, ionic strength, conductivity and composition of the medium, therefore this value should be considered as an indication of the initial electrostatic stabilization, but not as a definitive evidence of long term colloidal stability (22).

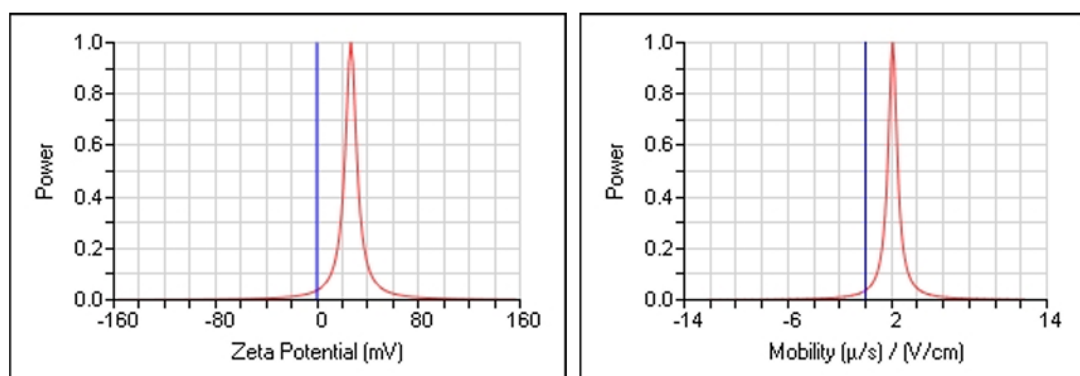


Figure 5. Electrokinetic profile of fresh prepared CNC/chitosan/folic acid nanocomposite dispersion (Day 0): (left) zeta potential distribution (+26.80 mV) and (right) electrophoretic mobility distribution.

Zeta potential of the nanocomposite dispersion on Day 2 was +16.26 mV and the particle interface remained mainly cationic. The decrease from +26.80 mV on Day 0 is consistent with a gradual change in the interfacial electrostatic environment rather than an abrupt change in surface character. This decrease is mainly due to the constant ionic interaction between the protonated amino groups of chitosan ($-\text{NH}_3^+$) and negatively charged species in the nanocomposite, mainly the phosphate groups of TPP and the anionic groups with the oxidized cellulose component and folic acid. In chitosan-TPP systems, the enhanced interactions with negatively charged TPP can partially

neutralize the amino groups of chitosan, leading to a reduction in the net positive zeta potential. Similar reduction in zeta potential with increase in TPP content has been observed in chitosan based nanoparticles. Counterion screening and compression of the electrical double layer also reduce the potential measured at the hydrodynamic slipping plane, which can contribute to the lower magnitude. Such electrokinetic changes depend on the ionic strength, pH, conductivity and composition of the dispersion medium. However, the positive value left on Day 2 indicates that cationic groups of chitosan were still playing a major role in the interfacial potential. Thus, +16.26 mV represents an intermediate electrokinetic state where the positive surface charge is preserved while the interfacial charge balance is being gradually compensated. This interpretation agrees with the progressive physicochemical evolution of a chitosan-TPP/cellulose-based nanocomposite during storage (23,24).

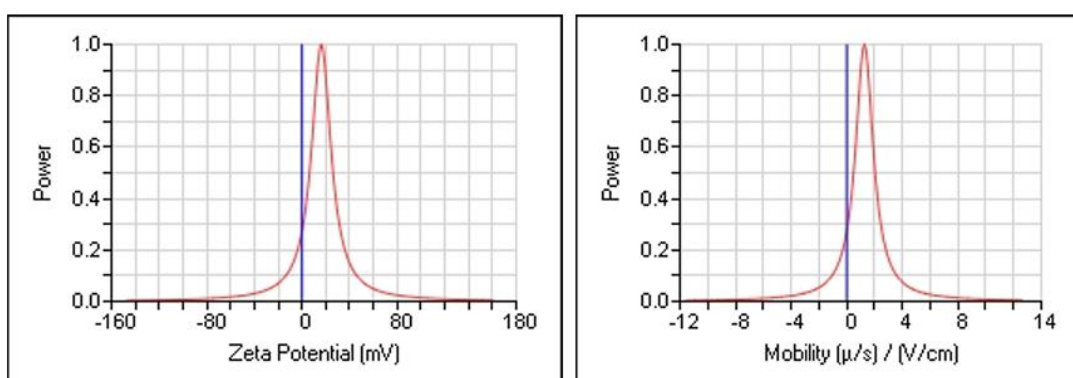


Figure 6. Electrokinetic profile of the CNC/chitosan/folic acid nanocomposite dispersion after 2 days of storage (Day 2). (Left) Zeta potential distribution (+16.26 mV) and (Right) Electrophoretic mobility distribution.

The zeta potential of the nanocomposite dispersion decreased to +7.50 mV on Day 4, indicating that the particle interface retained a weakly positive electrokinetic character. The decrease from the values recorded on Days 0 and 2 suggests a progressive modification of the interfacial charge balance rather than an abrupt change in the measured surface state.

This decrease may arise from gradual ionic interactions between the protonated amino groups of chitosan ($-\text{NH}_3^+$) and negatively charged species within the nanocomposite, particularly the phosphate groups of TPP and the anionic groups associated with the TEMPO-oxidized cellulose component and folic acid. Interactions between chitosan and anionic TPP can partially neutralize the protonated amino groups and, therefore, reduce the net positive zeta potential in chitosan-TPP systems (25).

The lower magnitude may also be attributed to counterion screening and compression of the electrical double layer, both of which can reduce the electrokinetic potential measured at the slipping plane. These effects are influenced by the pH, ionic strength, conductivity, and composition of the dispersion medium (26). Therefore, the observed decrease should be interpreted as a gradual change in the electrostatic environment surrounding the particles.

From a colloidal perspective, the +7.50 mV value indicates relatively weak electrostatic repulsion compared with the earlier measurements. Nevertheless, the potential remained positive, indicating that cationic chitosan groups continued to contribute to the interfacial potential. Therefore, Day 4 represents a transitional electrokinetic state in which partial charge compensation had occurred, but the surface had not yet reached the weakly negative state observed on Day 6.

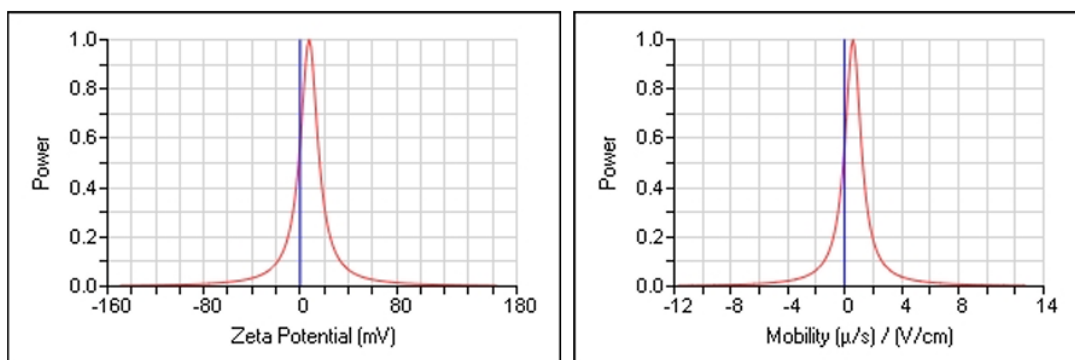


Figure 7. Electrokinetic profile of the CNC/chitosan/folic acid nanocomposite dispersion after 4 days of storage (Day 4) showing (left) zeta potential distribution (+7.50 mV) and (right) electrophoretic mobility distribution.

On Day 6, the formulation exhibited a zeta potential of -4.59 mV, shifting from the positive surface charge observed on Days 0, 2, and 4 to a weakly negative electrokinetic state. This change is primarily attributed to increased ionic interactions between the anionic phosphate groups of TPP and the protonated amino groups of chitosan, together with the increasing contribution of negatively charged carboxylate and ionizable groups associated with TEMPO-oxidized CNCs and folic acid. The low absolute value suggests weaker electrostatic repulsion and a lower electrostatic barrier to particle coalescence. However, a reduced zeta potential does not necessarily lead to substantial colloidal instability or complete aggregation, because many polysaccharide-based systems are also stabilized by steric and electrosteric effects. Therefore, the Day 6 result represents a weakly negative interfacial state reflecting progressive charge reorganization within the nanocomposite during storage (27,28).

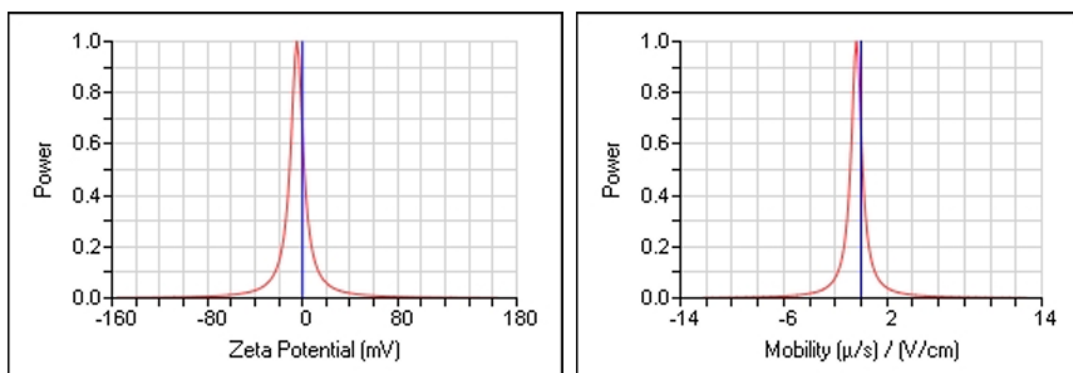


Figure 8. Electrokinetic profile of the CNC/chitosan/folic acid nanocomposite dispersion after 6 days of storage (Day 6), showing (left) zeta potential distribution (-4.59 mV) and (right) electrophoretic mobility distribution.

CONCLUSION

The present work establishes almond shell waste as an efficient precursor for TEMPO-oxidized cellulose nanocrystals and their integration into TPP-crosslinked chitosan/folic acid nanocomposites via a fully aqueous, low-impact route. X-ray diffraction of the final CNC/chitosan/folic acid formulation showed a broad diffraction profile with a weak feature near $2\theta \approx 15.3^\circ$, indicating the formation of a structurally integrated multicomponent biopolymeric assembly containing disordered and partially ordered domains. Consistent SEM observations of lamellar nanosheets with thicknesses in the 80–250 nm range, together Zeta potential measurements for 6 days revealed dynamic interfacial charge evolution with time, from a cationic surface (+26.80 mV on Day 0) to intermediate values (+16.26 mV on Day 2 and +7.50 mV on Day 4) and finally to a weakly negative potential (−4.59 mV on Day 6, indicating progressive interfacial rearrangement and electrostatic equilibration within the ternary network. , These features make the almond shell derived CNC/chitosan/folic acid nanocomposite a versatile, waste based platform for environmentally oriented applications, where nanostructured morphology, tunable surface chemistry and aqueous processability are essential design criteria.

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تصميم وتوصيف مادة نانوية مركبة (نانو-كومبوزيت) مكونة من نانو-السليولوز المشتق من قشور اللوز والكيوتوزان لاستخدامها كمُعِدِل حيوي المنشأ

المخلص

يتطلب تزايد إنتاج المخلفات الزراعية والصناعية تبني استراتيجيات مستدامة تهدف إلى تقليل النفايات وإنتاج مواد ذات قيمة عالية. في هذه الدراسة، تم استخلاص بلورات السليولوز النانوية (CNCs) من مخلفات قشور اللوز ودمجها في مادة مركبة نانوية حيوية بالكامل مع الكيوتوزان وحمض الفوليك، وذلك باستخدام عمليات مائية ذات تأثير بيئي منخفض. خضعت قشور اللوز لعمليات تنقية شملت المعالجة القلوية والتبييض، ثم التحلل المائي باستخدام حمض الكبريتيك للحصول على بلورات السليولوز النانوية، تلاها أكسدة باستخدام نظام (TEMPO/NaBr/NaClO) لإدخال مجموعات الكربوكسيلات على السطح. كما تم تحضير جسيمات الكيوتوزان النانوية عبر تقنية التجلط الأيوني باستخدام ثلاثي فوسفات البولي (TPP)، وتحميل حمض الفوليك كمركب نشط بيولوجياً نموذجي. داخل مشتتات هجينة تجمع بين بلورات السليولوز النانوية والكيوتوزان. وأظهر الفحص المجهر الإلكتروني الماسح (FE-SEM) بنية رقائقية مسامية تتضمن صفائح سليولوز نانوية (بسمكة تتراوح بين 80 و250 نانومتر) مغمورة في مصفوفة مستمرة غنية بالكيوتوزان، مما يوفر هيكلاً ذا مساحة سطحية كبيرة. وأكد حيود الأشعة السينية (XRD) الحفاظ على النمط البلوري للسليولوز من النوع الأول (Cellulose I) والخصائص البلورية النانوية، مع ملاحظة انخفاض معتدل في مؤشر "سيغال" (Segal) للتبلور عند تكوين المادة المركبة؛ وقد عزى هذا الانخفاض إلى تأثير التخفيف الناتج عن وجود الكيوتوزان والفولات (المواد غير المتبلورة) وليس إلى خلل في الشبكة البلورية. وتراوحت أحجام البلورات الدقيقة -المقدرة باستخدام معادلة شيرر (Scherrer)- بين 10 و15 نانومتر. كما أشارت تحليلات مطيافية الأشعة تحت الحمراء (ATR-FTIR) إلى وجود روابط هيدروجينية وتفاعلات إلكتروستاتيكية مكثفة بين بلورات السليولوز النانوية والكيوتوزان وحمض الفوليك، وتؤكد نجاح دمج الفولات من خلال ظهور حزم طيفية مميزة للمجموعات العطرية ومجموعات الكربونيل. وكشفت قياسات جهد "زيتا" (Zeta potential) على مدار 6 أيام عن تغير ديناميكي في الشحنة السطحية البينية بمرور الوقت، حيث تحولت من سطح موجب الشحنة (+26.80 مللي فولت في اليوم صفر) إلى قيم متوسطة (+16.26 مللي فولت في اليوم الثاني و+7.50 مللي فولت في اليوم الرابع)، وصولاً إلى جهد سالب ضعيف (-4.59 مللي فولت في اليوم السادس)، مما يشير إلى إعادة ترتيب تدريجية للواجهة البينية وتوازن إلكتروستاتيكي داخل الشبكة المكونة من ثلاثة عناصر.

الكلمات المفتاحية: مخلفات قشور اللوز، بلورات السليولوز النانوية (CNCs)، جسيمات الكيوتوزان-TPP النانوية، أكسدة TEMPO، تطبيقات بيئية مستدامة.