

Synthesis, characterisation, and antifungal activity of salicylic acid-grafted chitosan and its nanoparticles against *Fusarium solani* and *Fusarium proliferatum*

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ABSTRACT

Chitosan (CS) possesses versatile biological activities, but its applicability is limited by poor water solubility. This study developed water-soluble salicylic acid-grafted CS (SA-g-CS) derivatives and their nanoparticles (SANP) and evaluated their physicochemical properties and antifungal activity. SA was grafted onto CS at different SA-to-CS mass ratios via free-radical grafting, followed by the formation of SANP from the selected SA-g-CS through ionic gelation with sodium tripolyphosphate. The grafting of SA onto CS was characterised using Fourier transform infrared (FTIR), ultraviolet-visible (UV-Vis), and proton nuclear magnetic resonance (¹H NMR) spectroscopy. The grafted SA content ranged from 102.0 to 324.1 mg/g, with SA-g-CS (0.75:1) exhibiting the highest grafting efficiency (43.2%) and grafted SA content (324.1 ± 7.5 mg/g). SA grafting also improved the water solubility of CS. Antifungal activity increased with concentration, and at 5.0 mg/mL, SA-g-CS (0.75:1) achieved 75.5% and 68.8% inhibition against *Fusarium solani* and *Fusarium proliferatum*, respectively. SANP prepared from SA-g-CS (0.75:1) exhibited an average size of 145.9 nm, a polydispersity index (PDI) of 0.125, and a ζ-potential of +16.4 mV. Despite its smaller particle size, SANP exhibited lower antifungal activity than SA-g-CS, which was associated with the reduction in ζ-potential from +34.7 to +16.4 mV following nanoparticle formation. These findings demonstrate that SA grafting improves the water solubility and antifungal performance of CS, whereas subsequent nanoparticle formation does not necessarily enhance antifungal activity, highlighting the importance of surface properties in the design of SA-g-CS-based antifungal materials.

1. Introduction

Plant fungal diseases are a major challenge to the global agricultural production, causing substantial crop losses and threatening food security. Fungal diseases have been estimated to cause annual crop yield losses of approximately 10 – 23% [1]. Among phytopathogenic fungi, *Fusarium* species comprise a diverse group of plant pathogens, some of which also produce mycotoxins [2]. *Fusarium proliferatum* is associated with several plant diseases and can produce mycotoxins, including fumonisins [3–5], whereas *Fusarium solani* is an important soil-borne pathogen associated with diseases such as root rot, wilt, and crown

rot [6,7]. Although chemical fungicides remain widely used for disease management, concerns regarding their environmental and health effects and the development of fungicide resistance have increased interest in alternative antifungal materials [8,9].

Chitosan (CS) is a biodegradable and chemically modifiable biopolymer with considerable potential for agricultural applications [10]. It has been investigated for promoting plant growth and defence responses and can be applied through foliar, soil, root, and seed treatments [11,12]. CS also possesses intrinsic antifungal activity [13]; however, its practical application is limited by its poor solubility at neutral and alkaline pH and the relatively high viscosity of its aqueous acidic solutions. Chemical modification of CS therefore represents an important strategy for im-

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proving its aqueous solubility and functional properties. In particular, grafting with phenolic acids can alter the physicochemical properties of CS while enhancing its water solubility and biological activities [14,15].

Salicylic acid (SA) is a naturally occurring phenolic acid that plays important roles in plant signalling, defence, and responses to environmental stress [16–18]. In addition to its physiological functions in plants, SA exhibits direct antifungal activity. For example, da Rocha Neto et al. [19] demonstrated that SA inhibited *Penicillium expansum* and investigated its possible mechanisms of antifungal action. These properties have prompted interest in grafting SA with CS. Shi et al. [20] prepared SA grafted CS (SA-g-CS) for grapefruit preservation and reported reduced green mould caused by *Penicillium digitatum*. Nevertheless, the relationship between the extent of SA grafting, the resulting physicochemical properties, and antifungal activity remains insufficiently understood.

Nanoparticle formation provides an additional approach for modifying the physicochemical characteristics of CS-based materials. CS nanoparticles have been investigated for agricultural applications, including the control of phytopathogenic fungi [21,22]. Their nanoscale dimensions and high surface-to-volume ratio can influence interactions with biological surfaces and the accessibility of functional groups [23]. Although CS derivatives and CS nanoparticles have been widely investigated as antifungal materials, the effects of SA grafting and subsequent nanoparticle formation on the physicochemical properties and antifungal performance of CS have not been systematically compared.

Therefore, this study developed water-soluble SA-g-CS derivatives at different SA-to-CS mass ratios and subsequently prepared nanoparticles (SANP) from the selected SA-g-CS formulation. The physicochemical, structural, and morphological properties of SA-g-CS and SANP were characterised, and their antifungal activities against *F. solani* and *F. proliferatum* were evaluated. Particular emphasis was placed on evaluating how SA grafting and subsequent nanoparticle formation influence grafting characteristics, aqueous solubility, particle size, surface charge, and antifungal performance. The direct comparison between SA-g-CS and its corresponding nanoparticles provides insight into the structure-property-activity relationships governing the antifungal performance of these phenolic acid-grafted CS materials.

2. Materials and methods

2.1. Materials

Low-molecular-weight chitosan (CS; catalogue no. 448869; experimentally determined degree of deacetylation, DD = 81%, manufacturer-specified molecular-weight range = 50 – 190 kDa), L-ascorbic acid ($\geq 99.5\%$), hydrogen peroxide (30% w/w), salicylic acid (SA; $\geq 98\%$), and sodium tripolyphosphate (STPP) were purchased from Sigma-Aldrich (USA). Potato dextrose agar (PDA; HiMedia, India) and mancozeb (80%; Halex, Thailand) were used for the antifungal assay. Other chemicals and solvents used were of analytical grade. *Fusarium solani* (Fs-8JD) and *Fusarium proliferatum* (Fp-2RN) were obtained from the Molecular Genetics Laboratory, Faculty of Resource Science and Technology, Universiti Malaysia Sarawak, as previously reported by Tan et al. [24].

2.2. Synthesis of SA-g-CS

SA-g-CS was prepared by free-radical grafting of SA onto CS, with slight modifications to a previously reported method [25]. CS (1.0 g) was dissolved in 100 mL of 0.5% (v/v) acetic acid and stirred at 25 °C until completely dissolved. A redox initiator comprising 0.6 g of ascorbic acid and 1 mL of 1 M hydrogen peroxide (H_2O_2) was prepared in an ice bath and added dropwise to the CS solution, followed by stirring for 2 h at 25 °C under a nitrogen atmosphere.

SA was dissolved in 1 mL of dimethyl sulfoxide (DMSO) and gradually added to the reaction mixture at SA-to-CS mass ratios of 0.25:1, 0.5:1, 0.75:1, and 1:1, corresponding to 1.8, 3.6, 5.4, and 7.2 mmol of

SA, respectively. The mixtures were stirred at 700 rpm for 24 h at 25 °C. Subsequently, the reaction mixtures were dialysed against distilled water using dialysis membranes (MWCO 10 – 16 kDa) for 1 day, with the water replaced every 4 – 6 h, to remove unreacted SA and other low-molecular-weight compounds. Removal of unbound SA was monitored by UV-Vis analysis of the dialysate. The purified SA-g-CS solutions were then freeze-dried (L-200 Lyovapor, Büchi, Switzerland). A physical mixture of CS, SA and the redox initiator, prepared without the grafting reaction, was used as a non-grafted control for comparison with SA-g-CS.

2.3. Preparation of SA-g-CS nanoparticles (SANP)

SA-g-CS nanoparticles (SANP) were prepared via ionic gelation with STPP according to Katas [26], with slight modifications. SA-g-CS (0.75:1) was selected for nanoparticle preparation because it exhibited the highest grafting efficiency and grafted SA content. SA-g-CS (0.75:1) was dissolved in 0.1% (v/v) acetic acid at a concentration of 1.0 mg/mL, and the pH was adjusted to 4.5 with 0.1 M NaOH. STPP (1.0 mg/mL) was separately prepared in ultrapure water. The STPP-to-SA-g-CS mass ratio was maintained at 1:4. The STPP solution was added dropwise to the SA-g-CS solution under continuous stirring at 25 °C for 2 h to induce nanoparticle formation. The resulting suspension was ultrasonicated at 20 kHz for 7 min in an ice bath to reduce agglomeration. SANP were collected by centrifugation at 14,000 rpm for 15 min at 4 °C, resuspended in ultrapure water, and washed twice. Finally, the SANP were freeze-dried and stored at 4 °C until further analysis.

2.4. Grafting properties and solubility of SA-g-CS

The pH of SA-g-CS samples was measured after dialysis using a pH meter, while the colour of the freeze-dried samples was visually assessed. Thin-layer chromatography (TLC) was performed to assess the presence of free SA in the selected SA-g-CS sample. Samples of SA-g-CS with the highest grafted SA content and free SA were spotted onto silica gel 60 pre-coated TLC plates and developed using chloroform/ethyl acetate/acetic acid (50:50:1, v/v/v) as the mobile phase. The spots were visualised under UV light at 254 nm.

The amino-group substitution (AS) of SA-g-CS was determined using the ninhydrin assay according to Chatterjee et al. [27]. SA-g-CS (10 mg/mL in 0.5% (v/v) acetic acid; 1 mL) was mixed with 0.25 mL ninhydrin reagent, 0.50 mL acetate buffer (pH 5.0), and 0.25 mL dimethylformamide (DMF). The mixture was heated at 100 °C for 15 min, cooled to 25 °C, and the absorbance was measured at 570 nm. D-glucosamine (10 – 50 mg/mL) was used as the standard, while unmodified CS and 0.5% (v/v) acetic acid served as controls. The AS (%) was calculated using Equation (1):

$$\text{AS (\%)} = \left(\frac{\text{Abs CS} - \text{Abs grafted CS}}{\text{Abs CS}} \right) \times 100\% \quad (1)$$

where Abs CS and Abs grafted CS are the absorbance values of CS and SA-g-CS, respectively.

The grafted SA content was estimated using the Folin-Ciocalteu assay according to Hu et al. [28]. The sample solution (0.5 mL) was mixed with 1.0 mL of 0.2 N Folin-Ciocalteu reagent and incubated in the dark for 15 min, followed by the addition of 2.0 mL of 8.5% (w/v) sodium carbonate (Na_2CO_3). After incubation at 25 °C for 2 h, the absorbance was measured at 760 nm. SA (1.4 – 7.0 mg/L) was used for calibration. The grafted SA content was expressed as milligrams of SA equivalents per gram of SA-g-CS (mg/g) and calculated using Equation (2):

$$\text{SA content (mg/g)} = \frac{(\text{Abs}_{760}/k) \times V \times df}{m} \quad (2)$$

where Abs_{760} is the absorbance at 760 nm, k is the slope of the calibration curve, V is the sample volume, df is the dilution factor, and m is the mass of SA-g-CS. The grafting efficiency (GE, %) was subsequently calculated according to Nagy et al. [29] using Equation (3):

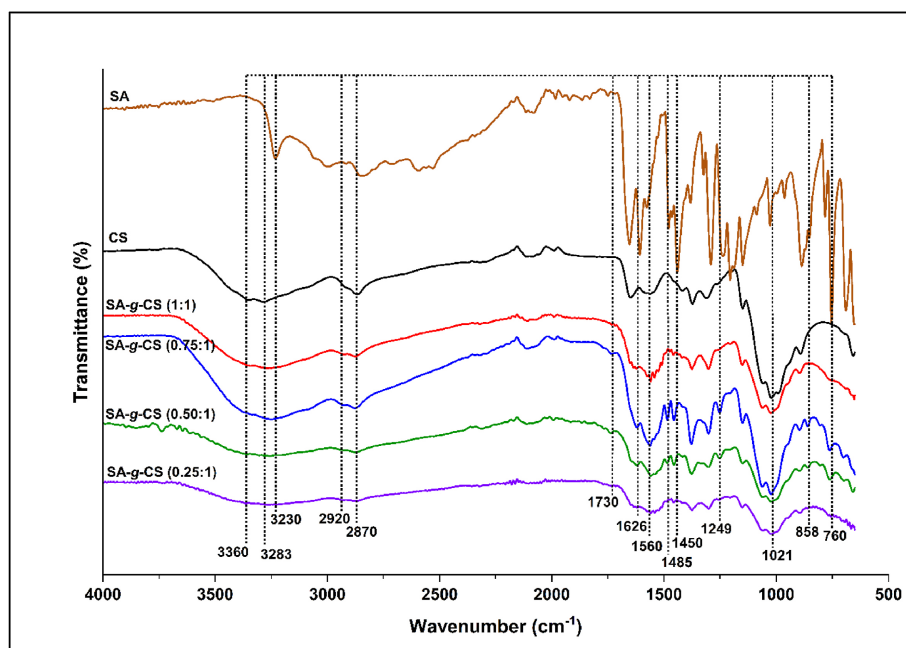


Fig. 1. ATR-FTIR spectra of SA, CS and SA-g-CS at different SA-to-CS mass ratios.

$$GE (\%) = \left(\frac{\text{Grafted SA (mg)}}{\text{Used SA (mg)}} \right) \times 100\% \quad (3)$$

The pH-dependent apparent solubility of SA-g-CS was evaluated using a turbidity method [30]. Each sample was dissolved in 0.5% (v/v) acetic acid at 1.0 mg/mL, and the pH was gradually adjusted from 5 to 13 using 1 M NaOH. Transmittance at 600 nm was measured at each pH, with higher transmittance indicating greater solution clarity and apparent solubility [31]. Water solubility was additionally assessed by directly dissolving freeze-dried SA-g-CS in distilled water at 0.1 mg/mL.

2.5. Characterisation of SA-g-CS and SANP

2.5.1. Fourier transform infrared (FTIR) analysis

FTIR spectra of CS, SA, SA-g-CS samples at different mass ratios, and SANP were recorded using an attenuated total reflection FTIR spectrometer (Cary 630 ATR-FTIR, Agilent Technologies) over the range of 4000 – 400 cm^{-1} to characterise changes in functional groups following SA grafting and nanoparticle formation. The DD of CS was determined from the FTIR spectrum based on the absorbance (Abs) values at 1320 and 1420 cm^{-1} according to Brugnerotto et al. [32] and calculated using Equation (4):

$$DD (\%) = 100 - \left(\frac{\text{Abs } 1320}{\text{Abs } 1420} - 0.3822 \times \frac{1}{0.03133} \right) \quad (4)$$

2.5.2. Ultraviolet-visible (UV-Vis) spectral analysis

UV-Vis spectra of CS, SA, SA-g-CS samples at different mass ratios, and SANP were recorded using a UV-Vis spectrophotometer (UV-2600i, Shimadzu, Japan) to evaluate changes in their absorption characteristics following SA grafting and nanoparticle formation. The samples were dispersed in 2.0 mL of deionised water and analysed over the wavelength range of 200 – 400 nm.

2.5.3. Elemental composition (CHN) analysis

The elemental compositions of CS and SA-g-CS samples at different mass ratios were determined using a CHN elemental analyser (Thermo Scientific FlashSmart Elemental Analyser, USA). Approximately 3 mg of each sample was used to determine the carbon (C), hydrogen (H), and nitrogen (N) contents.

2.5.4. Proton nuclear magnetic resonance (^1H NMR) spectroscopy

^1H NMR spectroscopy was performed to characterise the chemical structure of SA-g-CS and to assess the incorporation of SA onto the CS structure. The spectrum was recorded using a ^1H NMR spectrometer (JNM-ECZ500R, JEOL, Japan) operating at 500 MHz and 298 K, with D_2O as the solvent. The ^1H NMR spectrum of free SA was separately recorded in $\text{DMSO}-d_6$ for comparison.

2.5.5. Average particle size (Z-average), zeta potential (ζ), and polydispersity index (PDI)

The Z-average, ζ , and PDI of SA-g-CS and SANP were measured using a Zetasizer Nano ZS90 (Malvern Instruments, UK). SA-g-CS was dissolved, while SANP was dispersed, in distilled water at a concentration of 1.0 mg/mL. Measurements were performed in triplicate at 25 °C. The Z-average and PDI were determined at a scattering angle of 90°, with water used as the dispersant (refractive index = 1.33).

2.5.6. Morphological analysis

The morphologies of SA-g-CS samples at different mass ratios and SANP were examined using field-emission scanning electron microscopy (FESEM; JSM-6390LA, JEOL, Japan) at an accelerating voltage of 15 kV. SA-g-CS samples and SANP were examined at magnifications of 1000× and 20,000×, respectively. The morphology and particle size of SANP were further examined using transmission electron microscopy (TEM; Libra 120, Zeiss, Germany).

2.5.7. X-ray diffraction (XRD) analysis

The crystallinity structures of CS, SA-g-CS and SANP were analysed using XRD (PANalytical, Netherlands) with $\text{Cu K}\alpha$ radiation ($\lambda = 1.5406 \text{ \AA}$) operated at 45 kV and 40 mA. The diffraction patterns were recorded over a 2θ range of 5 – 80°.

2.5.8. Thermogravimetric analysis (TGA)

The thermal properties of CS, SA-g-CS, and SANP were evaluated using TGA (Shimadzu, Japan). Samples (3 – 6 mg) were heated from 30 to 650 °C at a heating rate 5 °C/min under a nitrogen flow of 60 mL/min.

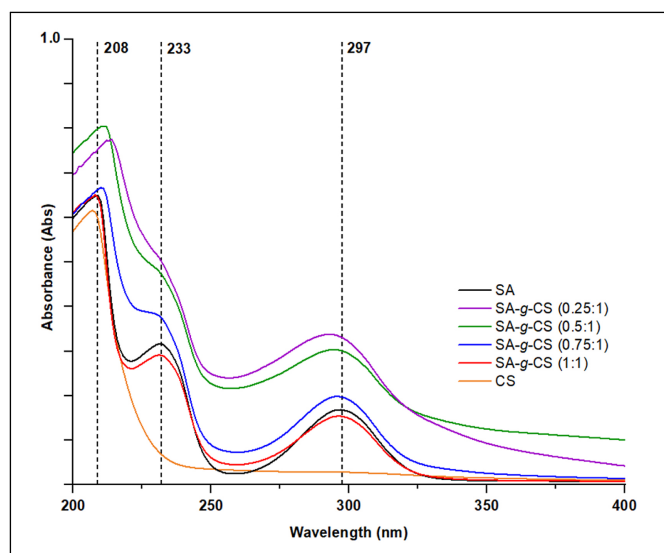


Fig. 2. UV-Vis spectra of CS, SA, and SA-g-CS synthesized at different SA-to-CS mass ratios.

2.6. Mycelial growth inhibition assay

The antifungal activities of SA-g-Cs and SANP against *F. solani* and *F. proliferatum* were evaluated using a mycelial growth inhibition assay. SA-g-CS samples at different mass ratios were dissolved, while SANP were dispersed, in sterile ultrapure water at concentrations of 1.0, 3.0, and 5.0 mg/mL. Each preparation was mixed with 15 mL of PDA, gently swirled, and poured into 90-mm Petri dishes. After solidification, a 5-mm mycelial disc obtained from a fresh fungal culture was placed at the centre of each plate. Untreated PDA served as the negative control, while mancozeb served as the positive control. Unmodified CS and SA were included as reference treatments. Owing to its limited water solubility, unmodified CS was prepared in 0.1% (v/v) acetic acid, whereas SA and mancozeb were dispersed in ultrapure water. The SA dispersion was heated to 50 °C during preparation. SA-g-CS, SANP, CS, SA, and mancozeb were evaluated at concentrations of 1.0, 3.0, and 5.0 mg/mL. The prepared solutions and dispersions were exposed to UV light for 15–20 min before being aseptically mixed with PDA under laminar air-flow. The plates were incubated at 25 °C in the dark, and radial mycelial growth was measured in two perpendicular directions on day 6. Each treatment was performed in triplicate. The percentage inhibition of radial mycelial growth (PIRG) was calculated according to Kheiri et al. [33] using Equation (5):

$$\text{PIRG (\%)} = 100 - \left(\frac{R_T}{R_C} \times 100\% \right) \quad (5)$$

where R_T and R_C represent the radial mycelial growth of the treated and negative control plates, respectively.

Table 1
Elemental composition of CS, SA, and SA-g-CS at different SA-to-CS mass ratios.

Sample	Elemental composition (%)			C/N ratio
	C	H	N	
CS	45.40	8.89	9.56	4.75
SA	60.80	4.41	0.00	-
SA-g-CS (0.25:1)	46.20	6.53	5.66	8.16
SA-g-CS (0.5:1)	46.50	6.57	5.69	8.17
SA-g-CS (0.75:1)	48.10	7.50	5.98	8.04
SA-g-CS (1:1)	53.70	7.28	6.36	8.44

2.7. Data and statistical analysis

Graphs were generated using GraphPad Prism (version 10.4.2, GraphPad Software, USA) and OriginPro 2025 (Learning Edition, OriginLab Corporation, USA). ^1H NMR spectra were analysed using MestReNova version 6.0 (Mestrelab Research, Spain), while particle diameters from microscopy images were measured using ImageJ (National Institutes of Health, USA). Chemical structures were drawn using ChemDraw Professional 15.0 (PerkinElmer, USA). Statistical analysis of the antifungal data was performed using GraphPad Prism. Two-way analysis of variance (ANOVA) followed by Tukey's multiple-comparisons test was used to evaluate differences among treatments and concentrations. Data are presented as mean $\pm$ standard deviation (SD) from three replicates ($n = 3$), with differences considered statistically significant at $p < 0.05$.

3. Results and discussion

3.1. Structural analysis of SA-g-CS

The FTIR spectra of CS, SA, and SA-g-CS prepared at different SA-to-CS mass ratios are shown in Fig. 1. SA exhibited characteristic absorption bands at approximately 3230 and 1653 cm^{-1} , attributed to phenolic O–H and carboxylic C=O stretching vibrations, respectively. Bands associated with aromatic C=C stretching were observed at 1450–1610 cm^{-1} , while those at approximately 1249 and 760–858 cm^{-1} were assigned to C–O stretching and aromatic C–H deformation vibrations, respectively. CS exhibited a broad band at 3283–3360 cm^{-1} associated with overlapping O–H and N–H stretching vibrations, together with bands at 2920 and 1651 cm^{-1} corresponding to C–H stretching and amide I vibrations, respectively. Following grafting, the SA-g-CS samples retained the characteristics bands of the CS backbone but exhibited several spectral changes. The amide I band shifted from approximately 1651 cm^{-1} in CS to 1626 cm^{-1} in SA-g-CS, while additional bands within 1450–1523 cm^{-1} were attributed to aromatic vibrations associated with SA. A band near 1560 cm^{-1} was also observed in the grafted samples and was assigned to amide II vibrations. These changes are consistent with interactions between the amino groups of CS and SA and support the incorporation of SA into the CS structure, in agreement with previous reports on CS-SA conjugates [34]. Additional bands at approximately 760–858, 1249, and 1450 cm^{-1} , associated with aromatic C–H deformation, C–O stretching, and aromatic C=C stretching, respectively, were particularly evident in SA-g-CS (0.5:1) and SA-g-CS (0.75:1). These features further support the presence of SA moieties in the modified CS structure [35]. Moreover, a band near 1738 cm^{-1} observed in SA-g-CS (0.5:1) and SA-g-CS (0.75:1) may be attributed to ester-type C=O stretching. Similar carbonyl bands have been reported for SA-modified polysaccharide systems [38,39]. Collectively, the observed FTIR spectral changes support the incorporation of SA into the CS structure.

The UV-Vis spectra of CS, SA, and SA-g-CS are shown in Fig. 2. SA exhibited absorption maxima (λ_{max}) at approximately 208, 233, and 297 nm, associated with electronic transitions of its aromatic structure ($\pi \rightarrow \pi^*$). The SA-g-CS samples exhibited characteristic absorption features of SA, including bands near 209 and 297 nm. Compared with free SA, the band at 297 nm shifted to approximately 292–295 nm in several SA-g-CS samples. This slight hypsochromic shift indicates changes in the electronic environment of the SA chromophore following its interaction with the CS matrix [37]. SA-g-CS (1:1) exhibited comparatively smaller spectral changes, consistent with its lower experimentally determined grafting efficiency. Together with the FTIR results, these spectral changes support the incorporation of SA into the modified CS.

Elemental analysis was performed to examine changes in the elemental composition following SA grafting onto the CS backbone (Table 1). Compared with unmodified CS, the SA-g-CS samples exhibited higher C contents, ranging from 46.20 to 53.70%, together with increased C/N ratios of 8.04–8.44. These changes are consistent with the incorporation of the carbon-rich aromatic SA moiety into the CS matrix. In contrast,

the H and N contents generally decreased following grafting, indicating changes in the chemical composition of CS associated with the modification process. Similar changes in the elemental composition of modified CS have been associated with reactions involving its amino groups and possible radical-induced changes during grafting [38]. The elemental composition did not vary proportionally with the initial SA-to-CS mass ratio. Notably, SA-g-CS (1:1) exhibited the highest C content among the

grafted samples despite its relatively low grafting efficiency (17.7%). This discrepancy suggests that increasing the initial SA concentration did not necessarily result in greater covalent grafting. In addition to grafted SA, non-covalent interactions between the amino groups of CS and the carboxylate groups of SA may also influence the composition of the resulting material, as such electrostatic interactions have been reported for CS-based systems [39].

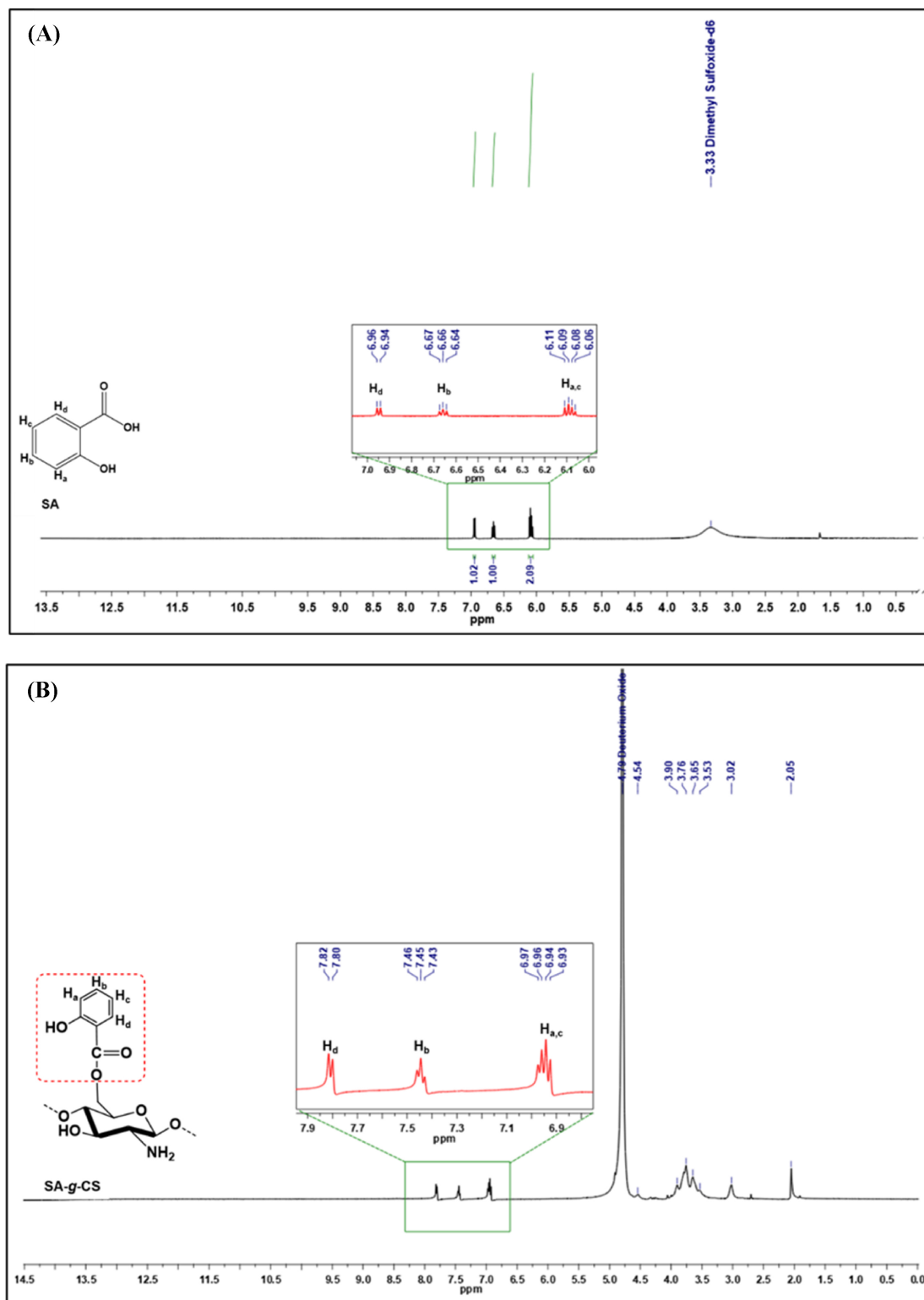


Fig. 3. ¹H NMR spectra of (A) SA (DMSO-*d*₆, 500 MHz) and (B) SA-g-CS (0.75:1) (D₂O, 500 MHz).

The ^1H NMR spectra of SA and SA-g-CS (0.75:1) are shown in Fig. 3. Free SA, analysed in $\text{DMSO}-d_6$, exhibited four characteristic signals in the aromatic region corresponding to the four protons (H_a to H_d) of the phenyl ring (Fig. 3(A)). The signals at $\delta = 6.06, 6.08, 6.64$, and 6.94 ppm were assigned to $\text{H}_a, \text{H}_c, \text{H}_b$, and H_d , respectively. In comparison, the ^1H NMR spectrum of SA-g-CS (0.75:1) (Fig. 3(B)), recorded in D_2O , exhibited the characteristic proton signals of the CS backbone together with additional signals in the aromatic region. The multiplet at $\delta = 6.93 - 6.97$ ppm was assigned to H_a and H_c , while the signals at $\delta = 7.43$ and 7.80 ppm were assigned to H_b and H_d , respectively. The appearance of these SA-associated aromatic proton signals in the SA-g-CS spectrum supports the incorporation of the aromatic SA moiety into the modified CS structure. The proposed radical-mediated grafting mechanism is illustrated in Fig. 4. Under mildly acidic conditions ($\text{pH} \approx 4.1$), ascorbic acid is converted to its anionic form, ascorbate [40], which reacts with H_2O_2 to generate ascorbate ($\text{Asc}^\bullet$) and hydroxyl ($\bullet\text{OH}$) radicals. These radical species can abstract hydrogen atoms from the $-\text{NH}_2$ and $-\text{OH}$ groups of the CS backbone, generating CS macroradicals [41,42]. The resulting reactive sites subsequently facilitate the incorporation of SA into the CS structure through the proposed formation of amide and/or ester linkages. Based on the findings, the major structure of SA-g-CS produced in this study was proposed to involve an ester linkage between the C-6 hydroxyl group of CS and the carboxyl group of SA. This is supported by the strong ester absorption band observed at approximately 1738 cm^{-1} in the FTIR spectrum of SA-g-CS.

The surface morphologies of unmodified CS and SA-g-CS prepared at different SA-to-CS mass ratios are shown in Fig. 5. Unmodified CS exhibited a relatively compact and slightly rough surface with limited visible porosity. Following SA grafting, distinct changes in surface morphology were observed. SA-g-CS (0.25:1) exhibited a denser and rougher surface with noticeable aggregation, whereas SA-g-CS (0.5:1) displayed a more porous, network-like structure with interconnected voids. In comparison, SA-g-CS (0.75:1) exhibited a more continuous and compact surface with reduced visible porosity. Fibril-like features were also observed in the grafted samples, particularly in SA-g-CS (0.5:1). These morphologi-

cal changes may arise from disruption of the intermolecular interactions and structural organisation of CS during the radical-mediated grafting process. Radical-induced modification of CS has been reported to cause changes in its polymeric structure, including possible cleavage of β -1,4-glucosidic linkages [42]. However, the FESEM observations primarily demonstrate that SA grafting altered the surface morphology of CS, with the extent and nature of these changes varying with the SA-to-CS mass ratio.

3.2. Physicochemical properties and solubility behaviour of SA-g-CS

The physicochemical properties of SA-g-CS prepared at different SA-to-CS mass ratios are summarised in Table 2. The DD values of the SA-g-CS samples ranged from 75.7% to 77.9%, slightly lower than that of unmodified CS (80.8%). This decrease indicates a reduction in the relative availability of free amino groups following SA modification and is consistent with the involvement of amino groups in the grafting process [42]. The SA-g-CS samples also exhibited a light-yellow colour compared with the white appearance of unmodified CS (Supplementary Materials, Table S1). The colour change may be associated with the incorporation of phenolic SA moieties [43] and possible oxidative changes occurring during the grafting process [44]. Following dialysis, the pH values of the SA-g-CS solutions ranged from 5.20 to 5.75. The variation in pH among the grafted samples may reflect differences in the relative amounts and ionisation states of the remaining amino and carboxylic groups following SA modification. Phenolic acid grafting can alter the chemical environment of the amino and hydroxyl groups of CS and consequently influence its physicochemical behaviour in aqueous solution [45,46]. However, no simple relationship was observed between the initial SA-to-CS mass ratio and the final pH, indicating that solution pH was influenced by the overall chemical environment of the modified CS rather than solely by the amount of SA initially introduced. TLC was further used to assess the presence of residual free SA in the purified SA-g-CS (0.75:1) sample (Supplementary Materials, Fig. S1). No spot corresponding to free SA was detected in the SA-g-CS sample, whereas

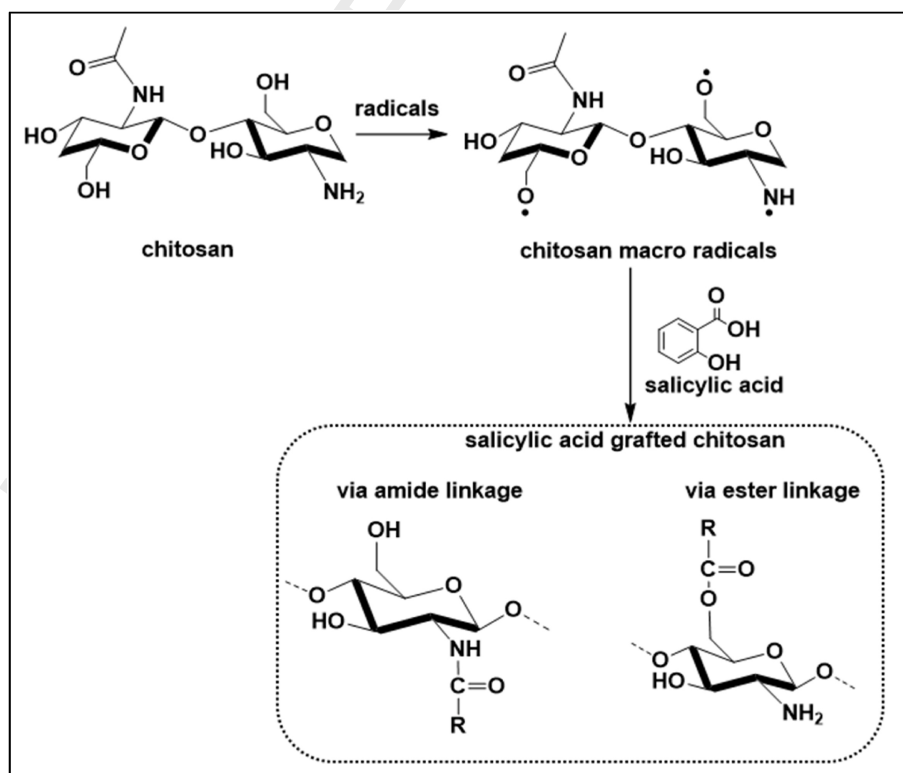


Fig. 4. Proposed free-radical grafting mechanism and structural representation for the formation of SA-g-CS, where R is SA moiety.

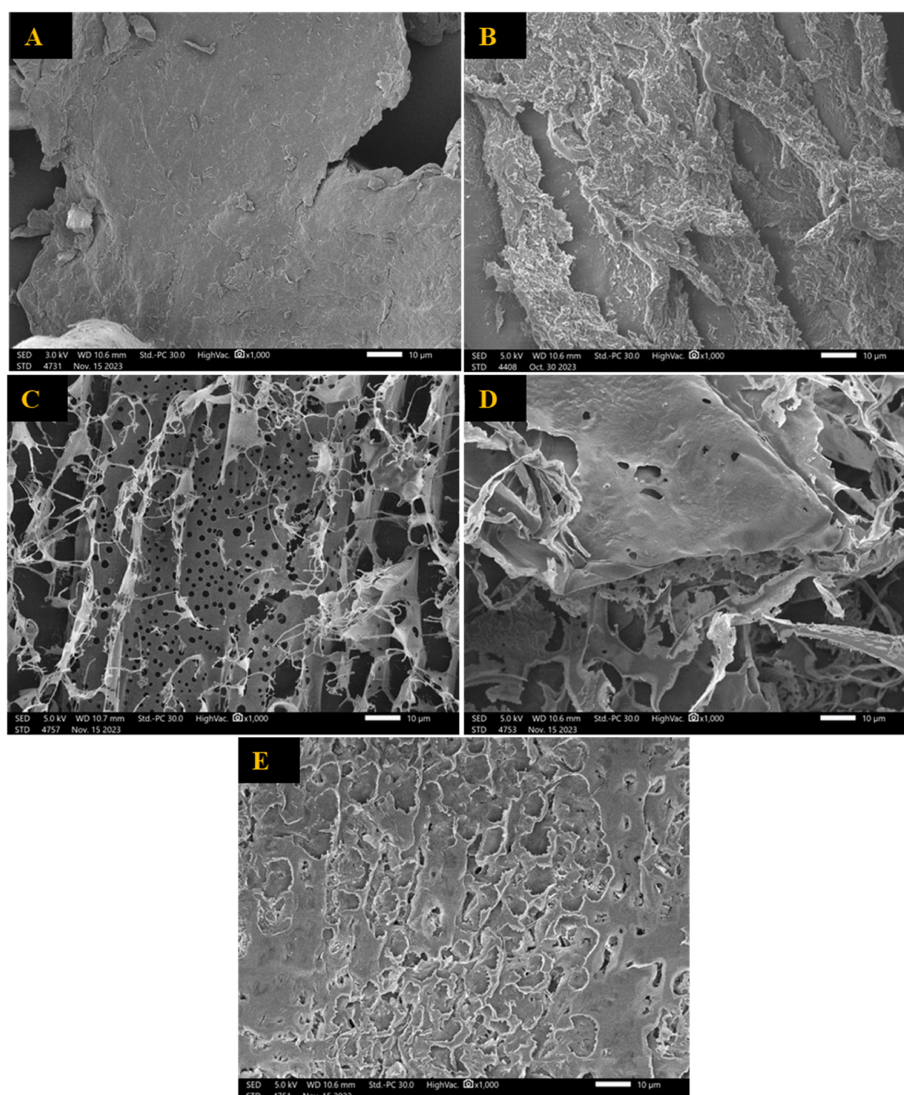


Fig. 5. FESEM micrographs of (A) CS; (B) SA-g-CS (0.25:1); (C) SA-g-CS (0.5:1); (D) SA-g-CS (0.75:1); and (E) SA-g-CS (1:1) at 1000× magnification.

Table 2

DD, solution pH, and appearance of CS and SA-g-CS solutions at different SA-to-CS mass ratios.

Sample	DD (%)	pH	Appearance
CS	80.8 ± 0.05	2.10	Cloudy white
SA-g-CS (0.25:1)	77.6 ± 0.02	5.20	Light yellow
SA-g-CS (0.50:1)	76.3 ± 0.01	5.56	Light yellow
SA-g-CS (0.75:1)	75.7 ± 0.03	5.75	Light yellow
SA-g-CS (1:1)	77.9 ± 0.01	5.62	Light yellow

Table 3

Amino-group substitution, SA content, and grafting efficiency of SA-g-CS prepared at different SA-to-CS mass ratios.

Sample	Amino group substitution (%)	SA content (mg/g)	Grafting efficiency (%)
SA-g-CS (0.25:1)	42.1 ± 0.8	102.0 ± 6.9	40.8
SA-g-CS (0.5:1)	37.8 ± 0.8	197.7 ± 7.4	41.4
SA-g-CS (0.75:1)	33.7 ± 1.5	324.1 ± 7.5	43.2
SA-g-CS (1:1)	26.6 ± 3.2	176.7 ± 11.2	17.7

free SA produced a distinct spot under the same chromatographic conditions. This result indicates that no detectable free SA remained after dialysis, supporting the effectiveness of the purification procedure and providing complementary evidence that the SA detected in the purified material was associated with the modified CS rather than present as readily detectable free SA.

The extent of SA modification was further evaluated based on amino-group substitution, grafted SA content, and grafting efficiency (Table 3). The amino group substitution decreased progressively from 42.1% for SA-g-CS (0.25:1) to 37.8%, 33.7%, and 26.6% for SA-g-CS prepared at mass ratios of 0.5:1, 0.75:1, and 1:1, respectively. The higher amino group substitution observed at the lower SA-to-CS ratio suggests greater involvement of the available amino groups of CS in the modification process. This observation is also consistent with the FTIR spectrum of SA-g-CS (0.25:1), which showed changes associated with the amide region without a prominent ester-type carbonyl band near 1738 cm⁻¹. As the amount of SA increased, the lower amino group substitution may indicate that modification was not restricted to the amino groups and that hydroxyl groups could also participate in the grafting reaction [41]. In contrast to the trend in amino-group substitution, both the grafted SA content and grafting efficiency initially increased with increasing SA-to-CS ratio. The grafted SA content increased from 102.0 mg/g for SA-g-CS (0.25:1) to 197.7 mg/g for SA-g-CS (0.5:1), reaching a maxi-

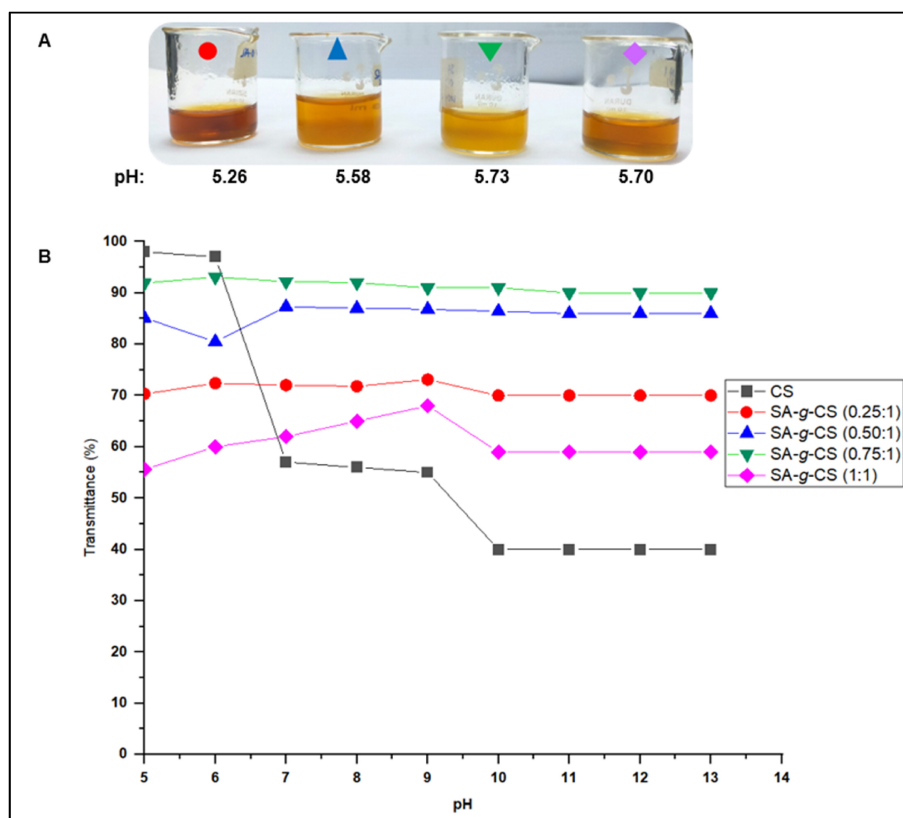


Fig. 6. (A) Dissolution of SA-g-CS samples in distilled water (0.1 mg/mL); (B) Transmittance profiles of SA-g-CS synthesized at different SA-to-CS mass ratios and pH values, measured as percent transmittance at 600 nm.

Table 4

Z-average, polydispersity index (PDI), and zeta potential (ζ) of SA-g-CS (0.75:1) and SANP.

Samples	Z-average (nm)	PDI	ζ -potential (mV)
SA-g-CS (0.75:1)	430.0 $\pm$ 9.1	0.278 $\pm$ 0.017	+34.7 $\pm$ 0.2
SANP	145.9 $\pm$ 1.3	0.125 $\pm$ 0.028	+16.4 $\pm$ 0.7

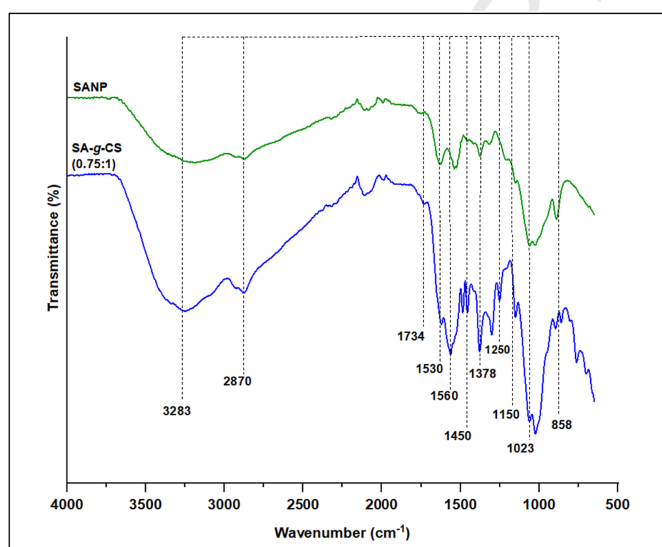


Fig. 7. ATR-FTIR spectra of SANP and SA-g-CS (0.75:1).

mum of 324.1 mg/g for SA-g-CS (0.75:1). Correspondingly, the grafting efficiency increased from 40.8% to 41.4% and 43.2%, respectively, in-

dicating that increasing the SA-to-CS ratio up to 0.75:1 promoted SA incorporation. However, further increasing the ratio to 1:1 reduced the grafted SA content to 176.7 mg/g and markedly decreased the grafting efficiency to 17.7%. This decline may reflect limited accessibility of reactive sites at higher SA concentrations, possibly due to saturation and steric effects. Hu et al. [41] similarly reported a decrease in phenolic acid grafting beyond an optimum concentration, suggesting saturation of the available reactive sites. The extend of grafting is also influenced by the reaction chemistry. For example, SA contents of 40.7 to 105.7 mg/g have been reported at SA-to-CS ratios of 0.09 to 1 using carbodiimide hydrochloride (EDC) and *N*-hydroxysuccinimide (NHS) as coupling reagents [34]. Overall, SA-g-CS (0.75:1) provided the optimum grafting condition in the present system, demonstrating that SA incorporation depends on both the reactant ratio and grafting conditions rather than the initial SA concentration.

The aqueous solubility of SA-g-CS was evaluated by direct dissolution in water and by monitoring transmittance at 600 nm over a range of pH values (Fig. 6). All SA-g-CS samples readily dissolved in water at 0.1 mg/mL without heating or stirring (Fig. 6(A)), demonstrating improved water solubility compared with unmodified CS. The pH-dependent behaviour of SA-g-CS is shown in Fig. 6(B), where higher transmittance at 600 nm indicates greater solution clarity and apparent solubility. The SA-g-CS samples maintained relatively high transmittance over the investigated pH range, indicating improved aqueous behaviour beyond the acidic conditions normally required for unmodified CS. These improvements may be attributed to structural changes induced by SA grafting, which disrupt the intermolecular hydrogen-bonding network of CS and introduce hydrophilic functional groups that enhance polymer-water interactions and facilitate hydration of the polymer chains [47–50].

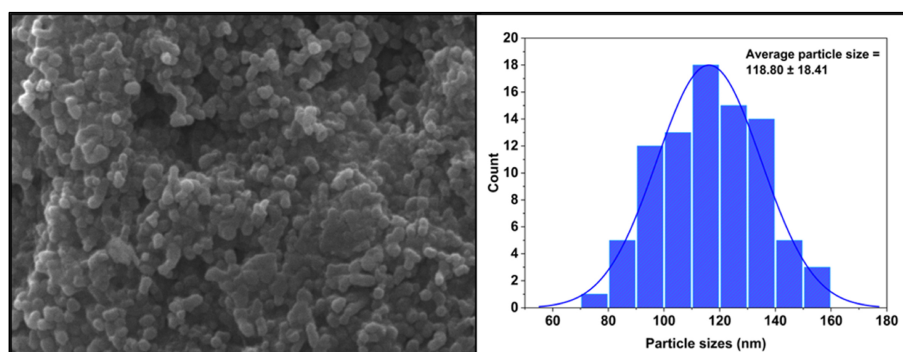


Fig. 8. FESEM micrograph of SANP captured at 20,000 $\times$ magnification, with corresponding particle size distribution histogram.

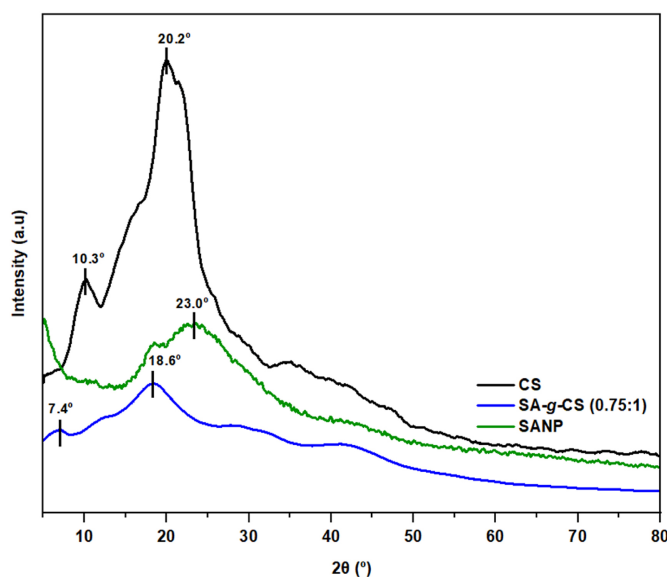


Fig. 9. X-ray diffractograms of SANP, SA-g-CS (0.75:1) and CS.

3.3. Physicochemical and morphological characterisation of SANP

The Z-average, PDI, and ζ -potential values of SA-g-CS and SANP are presented in Table 4. Following ionic gelation with STPP, the Z-average decreased from 430.0 nm for SA-g-CS to 145.9 nm for SANP, corresponding to an approximately 66% reduction in hydrodynamic size. This reduction is consistent with the formation of a crosslinked nanoparticle network through ionic interactions between the protonated amino groups ($-\text{NH}_3^+$) of SA-g-CS and the negatively charged phosphate groups of STPP [51]. The PDI also decreased from 0.278 for SA-g-CS to 0.125 for SANP, indicating a narrower particle-size distribution and greater size uniformity following nanoparticle formation. The ζ -potential decreased from $+34.7 \pm 0.2$ mV for SA-g-CS to $+16.4 \pm 0.7$ mV for SANP. The higher positive ζ -potential of SA-g-CS is associated with the presence of protonated amino groups on the polymer chains. Following ionic crosslinking, interactions between these positively charged amino groups and the negatively charged phosphate groups of STPP reduce the net positive surface charge [52], resulting in the lower ζ -potential of SANP. This reduction indicates that nanoparticle formation altered the surface-charge characteristics of SA-g-CS and may consequently influence its interactions with fungal cells.

The FTIR spectra of SANP and SA-g-CS (0.75:1) are shown in Fig. 7. SANP exhibited a spectral profile similar to that of SA-g-CS, reflecting the retention of the CS-based polymer structure following nanoparticle formation. A broad band at approximately 3200–3270 cm^{-1} was attributed to overlapping O–H and N–H stretching vibrations, while the band at approximately 2866 cm^{-1} corresponded to C–H stretching

[53]. Following ionic gelation with STPP, changes were observed in the amide region, including a shift of the amide I band from approximately 1651 cm^{-1} in CS to 1623 cm^{-1} in SANP and a shift of the amide II band from 1560 cm^{-1} in CS to 1530 cm^{-1} in SANP. These spectral changes are consistent with interactions between the protonated amino groups of SA-g-CS and the phosphate groups of STPP during ionic crosslinking [34,54]. In addition, SANP exhibited bands at approximately 1213 and 888 cm^{-1} , assigned to P=O and P–O–P stretching vibrations, respectively. The shifts in the amide-region bands and the presence of phosphate-associated bands support ionic interactions between SA-g-CS and STPP during SANP formation.

The UV-Vis spectra of SA-g-CS (0.75:1) and SANP are shown in Supplementary Fig. S2. SANP exhibited absorption bands at approximately 215, 230, and 296 nm, associated with electronic transitions of the aromatic ($\pi \rightarrow \pi^*$) and carbonyl groups of the SA moiety ($n \rightarrow \pi^*$) [55,56]. The retention of these characteristic absorption features in SANP indicates that the SA-associated chromophoric groups remained detectable following nanoparticle formation. Compared with SA-g-CS, slight shifts and broadening of the absorption peaks were observed in SANP, which may reflect changes in the local electronic environment of the SA-g-CS matrix following ionic interactions with STPP. Similar spectral changes have been reported by Esnaashari and Zahmatkesh [57] during the preparation of compound-loaded CS nanoparticles, where changes in the absorption characteristics were associated with nanoparticle formation. Thus, the observed spectral changes in current study are consistent with modifications in the local environment of SA-g-CS following ionic crosslinking with STPP.

Fig. 8 presents the FESEM micrograph of SANP together with the corresponding particle-size distribution. SANP exhibited predominantly spherical particles with relatively uniform morphology and an average particle size of 118.80 ± 18.41 nm, confirming their nanoscale dimensions. The TEM micrographs (Supplementary Fig. S3) further revealed well-defined, predominantly spherical particles with sizes ranging from approximately 45 to 150 nm, consistent with FESEM observations. Similar spherical morphologies have been reported for CS nanoparticles prepared via ionic gelation [58,59]. In contrast, irregular, dented, or rough morphologies have been reported in other CS nanoparticle systems, which may result from differences in formulation and preparation conditions, including the STPP-to-CS ratio, mixing conditions, and pH during ionic gelation [60]. Overall, the FESEM and TEM observations demonstrate the formation of discrete, predominantly spherical SANP following ionic gelation of SA-g-CS with STPP.

Fig. 9 presents the XRD patterns of SANP, SA-g-CS (0.75:1), and CS. Unmodified CS exhibited a characteristic diffraction peak at $2\theta \approx 10.3^\circ$, reflecting the partially crystalline organisation of the CS chains. Following SA grafting, changes in the diffraction pattern of SA-g-CS (0.75:1) indicated disruption of the ordered arrangement of the CS chains. This structural disorder became more pronounced following nanoparticle formation, with SANP exhibiting near disappearance of the peak at $2\theta \approx 10.3^\circ$ and a broad amorphous halo centred at approximately

$2\theta \approx 23.0^\circ$. The reduced structural order of SANP may be associated with ionic interactions between the protonated amino groups ($-\text{NH}_3^+$) of SA-g-CS and the phosphate groups of STPP, which disrupt the regular packing and intermolecular hydrogen bonding of the polymer chains [61–63]. These findings indicate that SA grafting and subsequent ionic crosslinking with STPP progressively altered the structural organisation of CS, resulting in a predominantly amorphous SANP structure.

Fig. 10 presents the TGA and DTG curves of SANP, SA-g-CS (0.75:1), and CS. SANP exhibited a two-step thermal degradation profile as shown in Fig. 10(A). The initial weight loss of approximately 13% between 35 and 150 °C was attributed to the loss of absorbed and bound water, corresponding to a DTG peak at approximately 65 °C (Fig. 10(B)).

The major degradation occurred between approximately 200 and 380 °C, with a weight loss of approximately 35%, and was associated with decomposition of the CS-based polymer network and disruption of ionic interactions between the protonated amino groups of SA-g-CS and the phosphate groups of STPP. The main DTG peak of SANP, observed at approximately 250 °C, shifted slightly to a higher temperature compared with SA-g-CS (0.75:1), suggesting that ionic crosslinking with STPP increased the thermal resistance of the polymer network by restricting polymer-chain mobility. The TGA/DTG results demonstrate that SA grafting and subsequent ionic crosslinking with STPP altered the thermal degradation behaviour of CS, with SANP exhibiting slightly greater thermal resistance than non-crosslinked SA-g-CS.

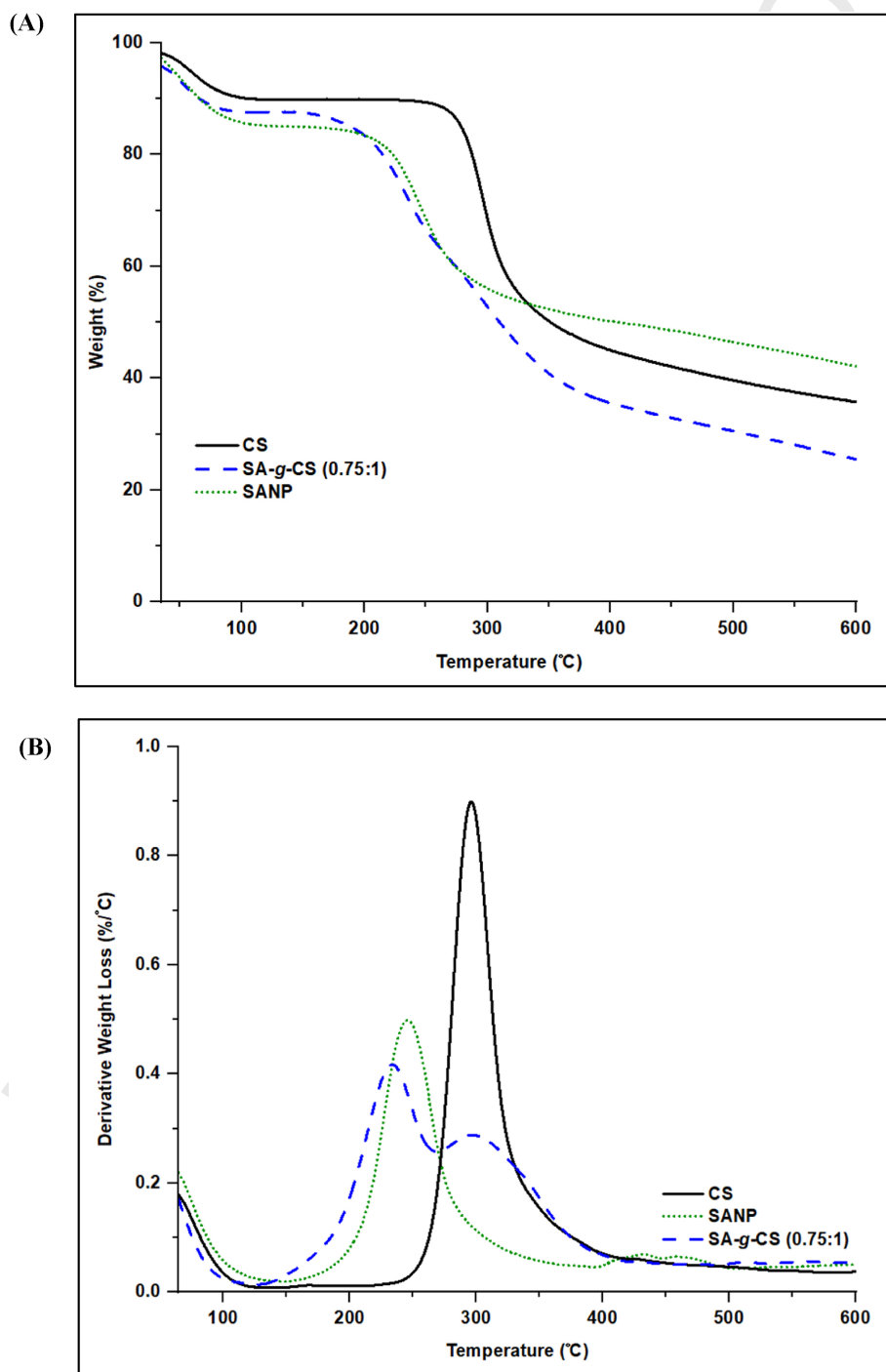


Fig. 10. (A) TGA and (B) DTG curves of SANP, SA-g-CS (0.75:1), and CS as a function of temperature.

3.4. Antifungal activity of SA-g-CS and SANP against *F. solani* and *F. proliferatum*

Fig. 11 shows the antifungal activity of SA-g-CS, SANP, unmodified CS, SA, and mancozeb against *F. solani*, while the corresponding mycelial growth after 6 days of incubation is presented in Fig. 12. The inhibitory activity of SA-g-CS generally increased with increasing concentration, indicating a concentration-dependent antifungal effect. At 1.0 and 3.0 mg/mL, all SA-g-CS samples exhibited less than 50% inhibition. A marked increase in activity was observed at 5.0 mg/mL, with inhibition ranging from $52.1 \pm 7.5\%$ to $75.5 \pm 1.5\%$. Among the grafted samples, SA-g-CS (0.75:1) exhibited the highest inhibition ($75.5 \pm 1.5\%$) and was significantly more effective than the other SA-g-CS formulations ($p < 0.05$). SA-g-CS (0.75:1) also exhibited significantly greater inhibition than unmodified CS but remained less effective than mancozeb ($p < 0.05$). Mancozeb was used as the positive control because of its established broad-spectrum antifungal activity [64]. Free SA exhibited greater antifungal activity against *F. solani* than the SA-g-CS samples across the tested concentrations, indicating that incorporation of SA into the CS structure did not enhance the antifungal activity relative to free SA. This difference may be associated with changes in the accessibility and chemical environment of the carboxylic and phenolic groups

of SA following grafting onto the CS backbone. SANP exhibited inhibition of 18.1%, 65.1%, and 72.2% at 1.0, 3.0, and 5.0 mg/mL, respectively. Although SANP approached the activity of SA-g-CS (0.75:1) at 5.0 mg/mL, nanoparticle formation did not provide a clear enhancement in antifungal activity against *F. solani*.

Fig. 13 shows the antifungal activity of SA-g-CS, SANP, unmodified CS, SA, and mancozeb against *F. proliferatum*, while the corresponding mycelial growth after 6 days of incubation is presented in Fig. 14. Similar to the results obtained for *F. solani*, the inhibitory activity generally increased with increasing concentration. At 5.0 mg/mL, SA-g-CS (0.75:1) exhibited the highest inhibition among the grafted samples ($68.8 \pm 3.0\%$), followed by SA-g-CS (0.5:1) ($65.6 \pm 1.5\%$). Both samples exhibited significantly greater inhibition than unmodified CS ($p < 0.05$). At the same concentration, mancozeb and free SA exhibited substantially higher inhibition, reaching $97.8 \pm 0.5\%$ and $97.5 \pm 0.7\%$, respectively. The high activity of free SA is consistent with its established antifungal properties, which have been associated with effects on fungal membrane integrity, ergosterol biosynthesis, and cellular metabolic processes [65]. SANP also exhibited concentration-dependent inhibition of *F. proliferatum*, increasing from 22.5% at 1.0 mg/mL to 37.0% at 3.0 mg/mL and 48.4% at 5.0 mg/mL. However, its activity remained lower than that of SA-g-CS (0.75:1), particularly at the highest concentration. Thus, de-

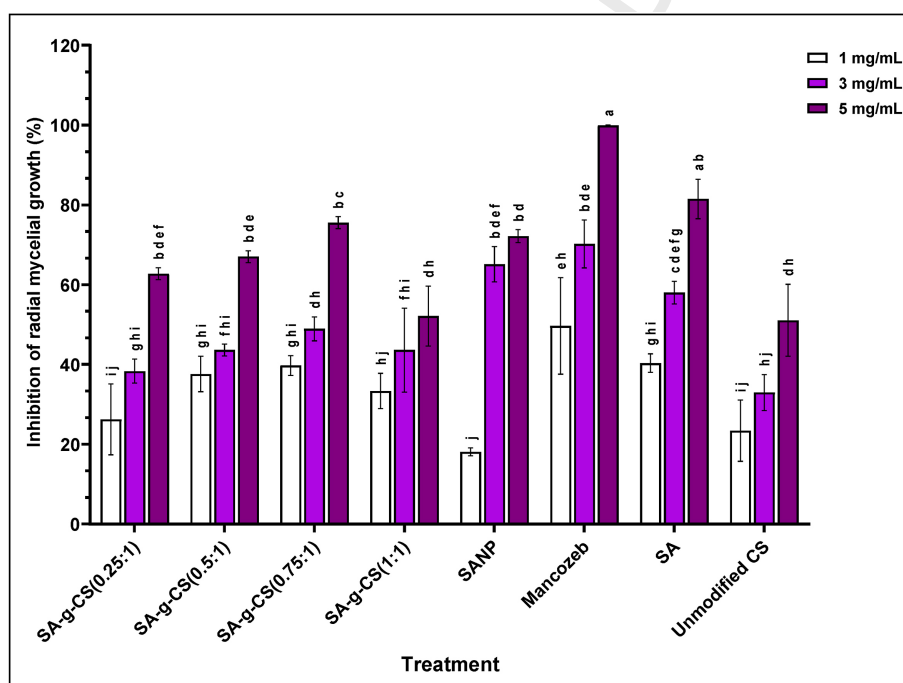


Fig. 11. Inhibition of *F. solani* growth by SA-g-CS at different mass ratios, SANP, mancozeb, SA, and unmodified CS at concentrations of 1.0, 3.0, and 5.0 mg/mL after 6 days of incubation.

Data represent mean $\pm$ SD ($n = 3$). Different lowercase letters indicate significant differences among treatments within the same concentration ($p < 0.05$).

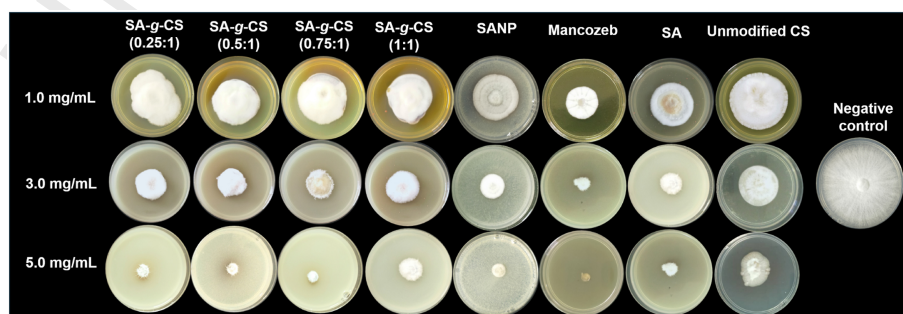


Fig. 12. Radial mycelial growth of *F. solani* on potato dextrose agar after treatment with SA-g-CS at various mass ratios, SANP, mancozeb, SA, and unmodified CS at concentrations of 1.0, 3.0, and 5.0 mg/mL, along with untreated PDA as the negative control, after 6 days of incubation at 25 °C.

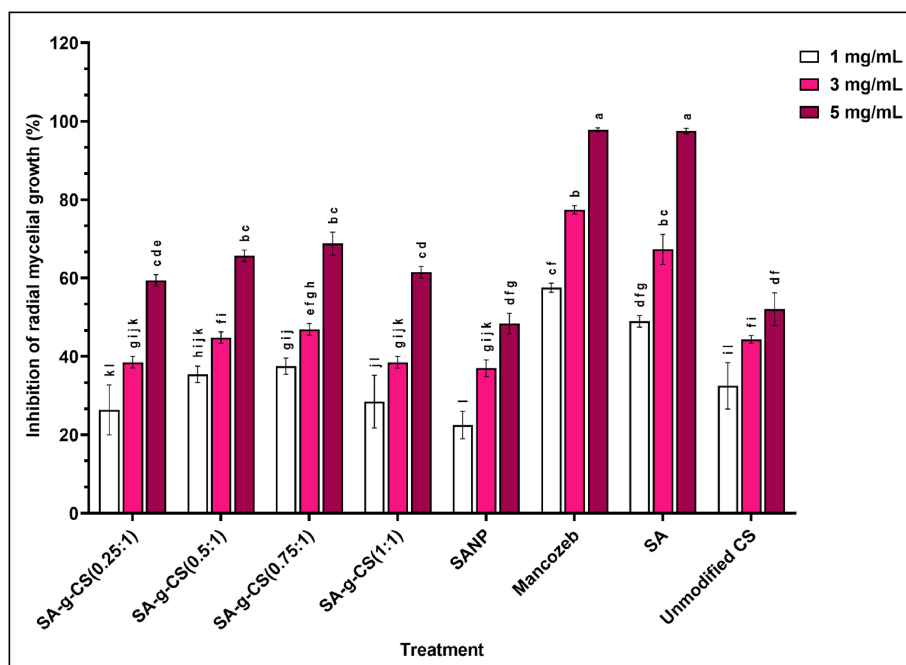


Fig. 13. Inhibition of *F. proliferatum* growth by SA-g-CS at different mass ratios, SANP, mancozeb, SA, and unmodified CS at concentrations of 1.0, 3.0, and 5.0 mg/mL after 6 days of incubation.

Data represent mean $\pm$ SD (n = 3). Different lowercase letters indicate significant differences among treatments within the same concentration ($p < 0.05$).

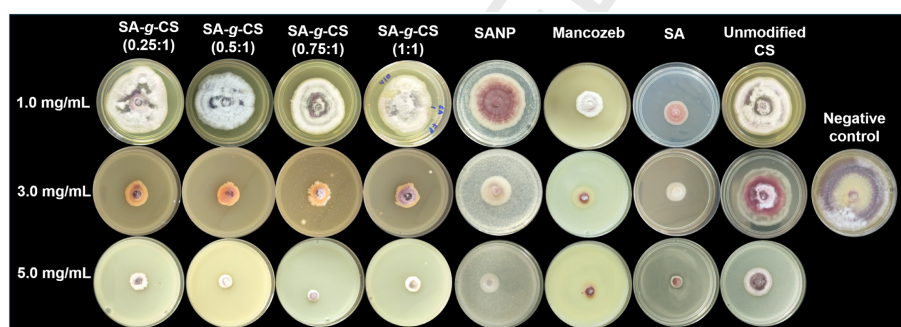


Fig. 14. Radial mycelial growth of *F. proliferatum* on potato dextrose agar after treatment with SA-g-CS at various mass ratios, SANP, mancozeb, SA, and unmodified CS at concentrations of 1.0, 3.0, and 5.0 mg/mL, along with untreated PDA as the negative control, after 6 days of incubation at 25 °C.

spite the reduction in particle size following ionic gelation, conversion of SA-g-CS into SANP did not enhance antifungal activity against *F. proliferatum*.

The non-grafted control mixture containing SA, CS, and the redox initiator exhibited lower antifungal activity than SA-g-CS over the investigated concentration range (Supplementary Fig. S4), indicating that the grafted materials exhibited different antifungal behaviour from the corresponding non-grafted mixture. Among the grafted samples, SA-g-CS (0.75:1), which exhibited the highest grafted SA content (324.1 mg/g) and grafting efficiency (43.2%), showed the greatest inhibition of both *F. solani* and *F. proliferatum*. Nevertheless, free SA exhibited greater antifungal activity than SA-g-CS. The high antifungal activity of free SA may partly be influenced by pH, as Amborabé et al. [66] reported that the inhibition of *Eutypa lata* by SA increased markedly under more acidic conditions. However, the limited aqueous solubility and tendency of SA to crystallise may restrict its direct application. Grafting SA onto the water-soluble CS derivative therefore provides an alternative strategy for incorporating SA into a polymeric material while improving its aqueous solubility. Conversion of SA-g-CS into SANP did not enhance antifungal activity despite the substantial reduction in particle size. This behaviour may be associated with changes in surface properties following ionic crosslinking with STPP. In particular, the ζ -potential decreased from

+34.7 mV for SA-g-CS to +16.4 mV for SANP. Because electrostatic interactions between positively charged CS and negatively charged components of fungal cell surfaces have been proposed to contribute to the antifungal activity of CS-based materials [67], the lower positive surface charge of SANP could reduce such interactions. Ionic crosslinking may also alter the accessibility of functional groups involved in interactions with fungal cells. Although CS nanoparticles have frequently been reported to exhibit enhanced antifungal activity because of their small particle size and high surface-to-volume ratio [33,68], the present findings indicate that particle-size reduction alone is insufficient to improve antifungal performance. Instead, the antifungal activity of SA-g-CS-based materials appears to depend on a combination of grafting characteristics, particle size, surface charge, and functional-group accessibility.

4. Conclusion

SA-g-CS derivatives were successfully prepared via free-radical grafting, with the incorporation of SA into the CS structure supported by FTIR, UV-Vis, and ^1H NMR analyses. Elemental analysis and morphological characterisation further demonstrated compositional and structural changes following SA modification. SA grafting improved the aqueous solubility of CS, with all SA-g-CS samples readily dissolving in water at

0.1 mg/mL without visible aggregation. Among the grafted samples, SA-g-CS (0.75:1) exhibited the highest grafted SA content (324.1 mg/g) and grafting efficiency (43.2%) and showed the greatest antifungal activity against both *F. solani* and *F. proliferatum*, reaching 75.5% and 68.8% inhibition, respectively, at 5.0 mg/mL. The antifungal activity generally increased with increasing concentration. Conversion of SA-g-CS (0.75:1) into SANP through ionic gelation with STPP reduced the particle size to a Z-average of 145.9 nm, with a PDI of 0.125. However, nanoparticle formation did not enhance antifungal activity relative to SA-g-CS. This behaviour was associated with changes in surface properties following ionic crosslinking, particularly the reduction in ζ -potential from +34.7 to +16.4 mV and possible changes in functional-group accessibility. These findings indicate that reducing particle size alone is insufficient to enhance antifungal performance and that surface properties should also be considered when designing SA-g-CS-based nanoparticles. Overall, SA grafting improved the aqueous solubility and antifungal performance of CS, supporting the potential of SA-g-CS as a bio-based material for further development in antifungal applications. Further studies should investigate the specific antifungal mechanisms, grafting sites, formulation stability, and biological and environmental safety of these materials.

Uncited references

[36].

CRedit authorship contribution statement

Wan Roslina Wan Yusof: Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Awang Ahmad Sallehin Awang Husaini:** Writing – review & editing, Validation, Supervision. **Enis Nadia Md Yusof:** Writing – review & editing, Validation. **Lee D. Wilson:** Writing – review & editing, Validation. **Sumiyyah Sabar:** Writing – review & editing, Visualization, Validation, Supervision, Resources, Methodology, Conceptualization.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work, the author(s) used ChatGPT in order to improve language and readability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.polymer.2026.130842>.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and/or its ESI.

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