Supporting material

Development of Self-Healing Modified Pullulan-Based Active Coating Incorporating a Neem Oil-β-Cyclodextrin Inclusion Complex

Tamara Erceg [1,*], Sanja Rackov [1], Aleksandra Jovanović [2], Olja Šovljanski [1], Slavica Lazarević [3], Senka Popović [1], and Aleksandar Marinković [3]

[1] Faculty of Technology Novi Sad, University of Novi Sad, Bulevar cara Lazara 1, Novi Sad 21000, Serbia; sanja.rackov@uns.ac.rs (S.R.); oljasovljanski@uns.ac.rs (O.Š.), madjarev@uns.ac.rs (S.P.)
[2] Institute for the Application of Nuclear Energy INEP, University of Belgrade, Banatska 31b, Belgrade 11080, Serbia; ajovanovic@inep.co.rs
[3] Faculty of Technology and Metallurgy, University of Belgrade, Karnegijeva 4, Belgrade 11120, Serbia; slazarevic@tmf.bg.ac.rs (S.L.); marinko@tmf.bg.ac.rs (A.M.)
* Correspondence: tamara.erceg@uns.ac.rs

S1. Determining the Degree of Esterification (EV) by Potentiometric Titration

The degree of esterification (DE) of Pull-AMA and Pull-bet was determined via a two-stage potentiometric titration procedure comprising the quantification of free carboxylic acid moieties followed by basic saponification of the maleate ester linkages.

First, the acid value (AV) was determined to quantify free carboxyl groups in accordance with ASTM D3644. An accurately weighed quantity of dry modified Pull ($m_{\text{Pull-mod}}$, g) was completely dissolved in a deionized water/DMSO solvent mixture (1: 1 v/v). The solution was titrated potentiometrically against a standardized 0.1 M KOH solution. The AV (mg KOH/g) was calculated using Equation (S1):

$$AV = \frac{56.1 \times c_{KOH} \times V_{KOH}}{m_{Pull\text{-}mod}} \quad \text{(S1)}$$

where c_{KOH} is the exact concentration of the KOH solution (0.1 mol/L), V_{KOH} is the volume of KOH consumed at the potentiometric equivalence point (mL), $m_{\text{Pull-mod}}$ is the dry sample mass (g), and 56.1 corresponds to the molar mass of KOH (g/mol).

Subsequently, the total carboxylic groups (comprising both free and esterified maleate moieties) were evaluated through basic saponification according to ISO 3657:2023. The sample was treated with an excess of standard KOH solution under gentle reflux/stirring to achieve complete cleavage of the maleate ester bonds. The unreacted excess base was then back-titrated potentiometrically using standard 0.1 M HCl. The saponification value (SV, mg KOH/g) was determined via Equation (S2):

$$SV = \frac{56.1 \times c_{HCl} \times \left(V_{HCl,0} - V_{HCl}\right)}{m_{Pull\text{-}mod}} \quad \text{(S2)}$$

where c_{HCl} is the concentration of the standard HCl (0.1 mol/L), $V_{\text{HCl,0}}$ and V_{HCl} are the volumes of HCl (mL) required for the blank and sample titrations, respectively.

The ester value (EV, mg KOH/g), corresponding strictly to the esterified maleate content, was derived as:

$$EV = SV - AV \quad \text{(S3)}$$

The degree of substitution (DS, average number of maleate groups per anhydroglucose unit) and degree of esterification (DE, % of esterified hydroxyl groups) were calculated according to Equations (S4) and (S5):

$$DS = \frac{162.14 \times EV}{56100 - (Mr \times EV)} \quad \text{(S4)}$$

$$DE(\%) = \left(\frac{DS}{3}\right) \times 100 \quad \text{(S5)}$$

where 162.14 g/mol is the molar mass of an unsubstituted anhydroglucose unit (AGU) and Mr is 98.06 g/mol is for Pull-AMA (the net molar mass increase per introduced maleate group), and 99.13 g/mol for Pull-bet.

S2. Determination of Encapsulation Efficiency of Neem Oil in β-CD

The encapsulation efficiency (*EE%*) and surface oil content of the inclusion complex were determined gravimetrically, according to the method described by Bae and Lee, 2008 [15], by quantifying both the unencapsulated (free surface) oil and total oil contents. To extract the unencapsulated surface oil, an accurately weighed sample (0.5 g) of the dried inclusion complex was placed into a 30 mL screw-cap glass vial, followed by the addition of 15 mL of n-hexane. The mixture was agitated using a vortex mixer for 2 min at ambient temperature. The suspension was filtered through filter paper into a pre-weighed evaporating dish. To ensure complete recovery of surface oil, the retained powder on the filter paper was rinsed three times with 20 mL of n-hexane per wash. The collected filtrate was evaporated and dried in an oven to a constant weight (at 40–60 °C) to vaporize all residual solvent. The dish was reweighed to determine the mass of the extracted free surface oil. Alternatively, the washed residual powder was dried to a constant weight at 60 °C to verify the mass loss by weight difference before and after extraction.

For total oil determination, a separate accurately weighed sample of the complex was dispersed in an ethanol–water mixture and subjected to sonication to completely disrupt the matrix and release the entrapped oil. The liberated oil was subsequently extracted into n-hexane using a separating funnel. The organic layer was transferred to a pre-weighed dish, evaporated, and dried to a constant weight to calculate the total oil mass.

The encapsulation efficiency (*EE%*) and free surface oil percentage (*FO%*) were calculated using Equations (S6) and (S7):

$$EE\ (\%) = \frac{Total\ oil\ mass - Free\ Surface\ oil\ mass}{Total\ oil\ mass} \cdot 100\% \quad \text{(S6)}$$

$$Free\ oil\ (\%) = \frac{Mass\ of\ extracted\ surface\ oil}{Initial\ mass\ of\ powder\ sample} \cdot 100\% \quad \text{(S7)}$$

Measurements were carried out in triplicate, and average values are presented as mean ± standard deviation.

S3. Statistical Analysis

Physical properties (MC, TSM, SD, and WVTR) and mechanical parameters (TS and EB) were evaluated using one-way ANOVA followed by Tukey's post hoc test. Differences between mean values were considered statistically significant at $p < 0.05$.

S4. Ftir Analysis Of Neem Oil, β/Cd, And β/Cd/No Inclusion Complex

The FTIR spectrum of neem oil exhibits a broad band at 3329 cm^{-1}, attributed to O–H stretching vibrations, which may arise from hydroxyl-containing constituents (β-sitosterol). A weak band at 3011 cm^{-1} corresponds to =C–H stretching vibrations, indicat-

ing the presence of unsaturated fatty acid chains (linoleic and oleic acid), further supported by a band observed at 1650 cm^{-1} associated with C=C stretching. This C=C stretching can arise from terpenoids. The strong absorption bands at 2922 and ~2850 cm^{-1} are assigned to asymmetric and symmetric C–H stretching vibrations of $–CH_2$ groups, characteristic of long aliphatic chains in fatty acids and triglycerides. A prominent peak at 1742 cm^{-1} is attributed to ester carbonyl (C=O) stretching, confirming the triglyceride structure of the oil. Additionally, the band at 1457 cm^{-1} corresponds to CH_2 bending (scissoring) vibrations, while weak bands in the 1370–1380 cm^{-1} region are assigned to CH_3 bending modes.

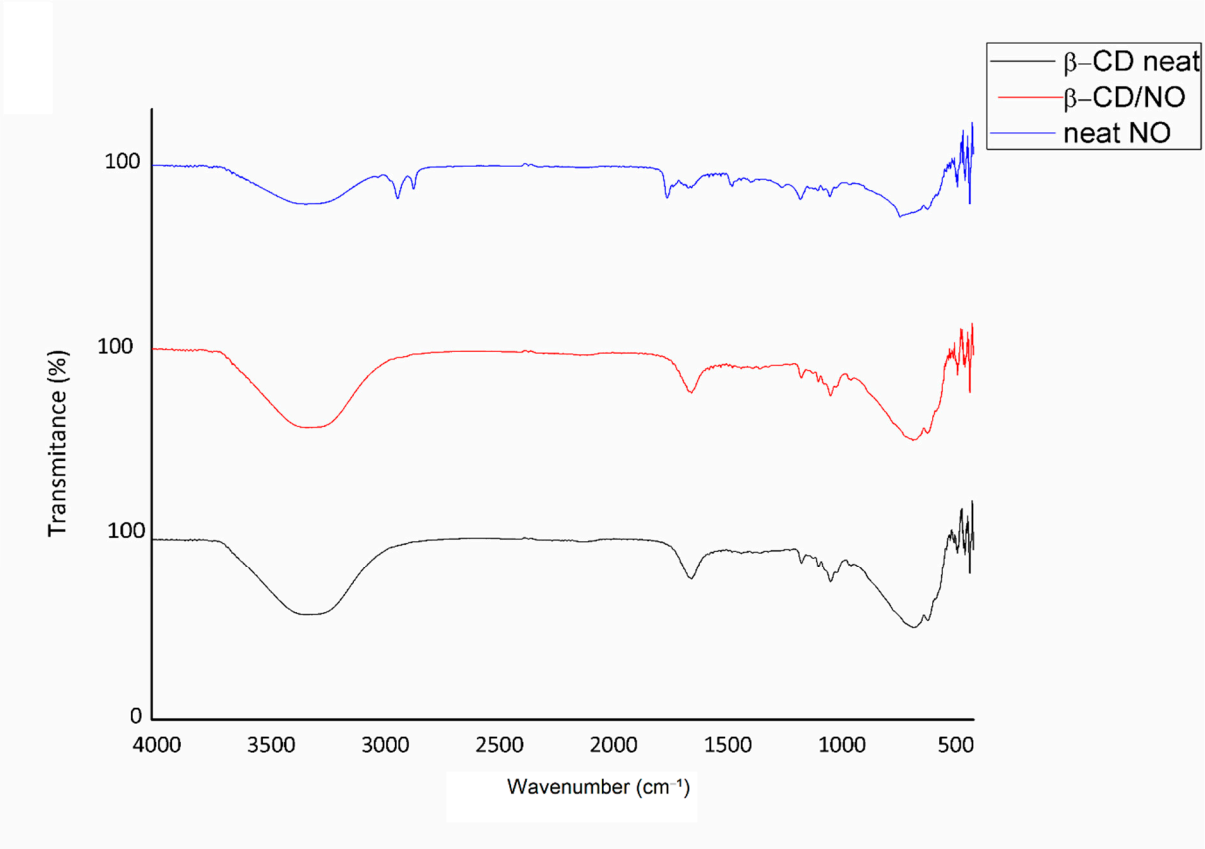


Figure S1. FTIR spectra of β-CD, the *β*-CD/NO inclusion complex, and neat NO.