Pickering emulsions co-stabilised by cellulose nanofibres and nicotinamide mononucleotide

Dehai Yu · Qi Luo · Jing Zhang · Qiang Wang · Huili Wang · Zhaoping Song · Shan Li · Wenzia Liu · Fengshan Zhang · Dandan Ji

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Abstract Emulsified solid particles adsorbed at the oil–water interface can stabilise Pickering emulsions by acting as a physical barrier to the coalescence of oil droplets. Cellulose nanofibres (CNFs) have been used in food-grade Pickering emulsions because of their excellent performance as a low cost and sustainable material. Nicotinamide mononucleotide (NMN) is a small-molecule zwitterion with polar functionality capable of interacting with the CNFs. The ability of cationic CNFs and NMN to co-stabilise sunflower oil Pickering emulsions was investigated under various conditions using methods such as contact angle measurement, creaming stability, rheology, microscopy, thermal stability, and water-holding capacity. Emulsions with ultra-high stability, good gelation, and high plasticity were obtained using single-step shear dispersion with 0.25 wt% CNFs, 0.05–0.3 wt% NMN, and near-neutral pH. NMN stabilised the oil–water interface through electrostatic interactions and hydrogen bonding with CNFs. NMN transferred into the aqueous phase and interacted with CNFs to form a complex with a three-dimensional network structure, which improved the bulk viscosity and steric hindrance of the emulsion and created more compact adsorption of CNFs at the oil–water interfaces. Overall, the synergistic effects of various factors allow NMN to effectively co-stabilise Pickering emulsions with CNFs, making it an exciting method that can be used to encapsulate oil-soluble substances.

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Various particles, including silica (Midmore 1998), modified starch (Chen et al. 2021), modified chitin (Sun et al. 2019), and graphene (Tang et al. 2014), have been used to prepare Pickering emulsions. However, most of these particles are not up to the standard of green materials after modification (Shi and Russell 2018). The increasing demand for environmentally friendly foodstuffs has further stimulated research into the naturally green components of Pickering emulsions. Biomass materials, such as cellulose (Kedzior et al. 2020), lignin (Rojas et al. 2007), xylan (Wang and Xiang 2021) and glucomannan (Kuncheva et al. 2007), were successfully applied to the preparation of Pickering emulsion. Cellulose consists of anhydroglucose units that are linearly connected by β-1,4-glycosidic bonds (Hieta et al. 1984). As the most abundant natural hydrophilic polymer, cellulose has a wide range of sources, including depleted wood, wheat, straw, cotton and coconut fiber (Jonoobi et al. 2011; Klemm et al. 2005). Cellulose nanofibres (CNFs) are ultra-fine with a diameter of approximately 1–100 nm. CNFs have shown excellent characteristics and performance as green, low-cost, and sustainable plant-based nanoparticles (Bai et al. 2018a, b; Eichhorn 2011). As the most abundant natural hydrophilic polymer, cellulose has a wide range of sources, including depleted wood, wheat, straw, cotton and coconut fiber (Jonoobi et al. 2011; Klemm et al. 2005). Cellulose nanofibres (CNFs) are ultra-fine with a diameter of approximately 1–100 nm. CNFs have shown excellent characteristics and performance as green, low-cost, and sustainable plant-based nanoparticles (Bai et al. 2018a, b; Eichhorn 2011). 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As the most abundant natural hydrophilic polymer, cellulose has a wide range of sources, including depleted wood, wheat, straw, cotton and coconut fiber (Jonoobi et al. 2011; Klemm et al. 2005). Cellulose nanofibres (CNFs) are ultra-fine with a diameter of approximately 1–100 nm. CNFs have shown excellent characteristics and performance as green, low-cost, and sustainable plant-based nanoparticles (Bai et al. 2018a, b; Eichhorn 2011).
CNF has been proved to inhibit lipid digestion and slow down glucose diffusion (Bai et al. 2019; Li et al. 2019).

Nicotinamide mononucleotide (NMN), as a extracted substance in some fruits and vegetables, is the most direct precursor of nicotinamide adenine dinucleotide (NAD), a coenzyme essential for metabolism in the human body (Fang et al. 2021; Uddin et al. 2017). Yoshino and Verdi’s work studied the anti-ageing effect of NMN on the body and found it to be an effective dietary additive for treating age-related diseases, such as senile degenerative diseases, diabetes, and retinal degenerative diseases (Eric Dickinson 2012; Habermann and Habermannova 1960; Yasuda et al. 2021; Yoshino et al. 2021). However, NMN is easily destroyed by gastric acid and enzymes in the stomach, making it generally necessary to increase the local drug concentration by means of enteric-coated capsules rather than adding NMN directly into food. Pickering emulsions could protect the materials in the inner phase from light, oxidation and other metamorphic processes. To the best of our knowledge, the use of NMN to develop food-grade Pickering-type emulsions has never been reported in the literature.

In this work, we developed a novel Pickering emulsion co-stabilized by the cellulose nanofibres and NMN without any surfactants and modification. We explored the performance of CNFs in stabilising sunflower oil Pickering emulsions with different NMN concentrations and pH. We also examined the structure–activity relationships to provide simple principles for the design of novel CNF-based emulsifiers for Pickering emulsions. In general, the Pickering emulsion stabilizing method presented in this study is simple, general, and green, offering a new platform for the construction of food-grade emulsions for CNFs and NMN.

Materials and methods

Materials

NMN was gifted from by Effepharm Pharmaceutical Technology Co., Ltd. (Uthever, Shanghai, China). Cationic CNFs were purchased from Tianjin Woodelf, Biotechnology Co., Ltd. (Woodelf, China), the detailed specification of cationic CNF used in this work is shown in Table S1 (Supporting Information). Sunflower oil was purchased from a local supermarket and used directly. Nile Red and Nile Blue were supplied by Aladdin Chemistry Co. Ltd. (Shanghai, China). The water used in the experiment was deionised water with a resistivity of 18.2 MΩ cm. All other reagents were of analytical grade.

Preparation of NMN/CNF complexes

The CNF stock solution was prepared as a 0.25% suspension with deionised water and then fully stirred to obtain the aqueous phase of the emulsion (concentration were determined through pre-experiments). Subsequently, a certain quantity of NMN was added to the CNF solution and the mixture was magnetically stirred in an ice bath for 12 h. NMN that was not bound to the CNFs was removed using a dialysis bag with a molecular weight of 500. The NMN/CNF complex dispersion was frozen at − 50 °C for 12 h, and then put in a vacuum freeze-dryer for 24 h.

Characterisation of NMN/CNF complexes

Zeta potential and particle size

A Zetasizer Nano (Malvern Instruments, Worcestershire, UK) was used to measure the zeta potentials and droplet diameters of the samples. To avoid multiple scattering effects, the samples were diluted with deionised water before measurement. All samples were freshly prepared in duplicate and tested three times.

Interface behaviour

The interfacial tension was measured by the drape method using an automatic video optical contact angle meter (OCA25, Germany). Sunflower oil was added to a cuvette, and the transmittance of the oil was checked before the measurement. In brief, suspension droplets in 5 µL solution containing pure NMN or NMN/CNF complexes were formed at the terminal of the low-retention pipette tip immersed in the oil phase. Thereafter, the formed droplets were balanced at ambient temperature for 10 min. The interfacial tension value was accurately calculated according to the droplet shape recorded during the balancing process. Using air as the continuous phase,
surface tension was measured using the same procedure (Liu et al. 2016). All samples were freshly prepared in duplicate and tested three times.

**Fourier transform infrared spectra**

The NMN and NMN/CNF samples were mixed with KBr and then tableted using a tableting machine. The samples were analysed and tested using a Fourier transform infrared spectrometer (ALPHA Bruker, Germany) in the range of 4000–400 cm⁻¹.

**X-ray diffraction patterns**

The dried samples were placed in the diffraction groove of the instrument. The samples were scanned by D8 ADVANCE X-ray diffractometer (Bruker, Billerica, Massachusetts, USA). The test conditions were as follows: Cu target (λ = 0.1542 nm); scanning range of 2–10°; scanning speed of 2°/min (Yang et al. 2019). The crystallinity degree of different samples was calculated by Segal method:

\[
CrI = \frac{(I_{200} - I_{am})}{I_{200}} \times 100
\]

In this equation, where \(CrI\) expresses the relative degree of crystallinity, \(I_{200}\) is the maximum intensity (in arbitrary units) of the \(I_{200}\) lattice diffraction and \(I_{am}\) is the intensity of diffraction in the same units at \(2\theta = 18°\).

**Cryoscanning electron microscopy (cryo-SEM)**

A Hitachi SU3500 scanning electron microscope (Hitachi High-Tech Corporation, Tokyo, Japan) equipped with a cryo-SEM preparation system was used to observe the morphology of the Pickering emulsion. An appropriate volume (5–10 μL) was placed into the sample rack and immediately frozen in liquid nitrogen. The frozen sample was transferred to the low-temperature preparation chamber through the airlock under vacuum and then fractured at on the sample table (−150 °C). The fractured sample was subsequently etched for 10 min at −90 °C for ice sublimation. After sputtering and etching the surface at 170 °C and coating it with Au for 60 s, the sample was analysed with the scanning electron microscope at −150 °C.

**Preparation of NMN/CNF Pickering emulsion**

The CNFs stock solution was prepared as a 0.25% suspension with deionised water and then fully stirred to obtain the water phase of the emulsion. A certain quantity of NMN was added to the sunflower oil, and the mixture was magnetically stirred for 12 h to obtain the oil phase of the emulsion.

The o/w ratio was set to 4:5, and the NMN/CNF Pickering emulsion was obtained by ultrasonic treatment of a mixture of the two phases in an ice bath for 5 min using an ultrasonic cell disruptor (KQ-700 V, Kunshan, China). Before characterisation, photographs and micrographs of the emulsion were taken within 6 h of preparation.

**Characterisation of NMN/CNF-Pickering emulsion**

**Microstructure and droplet size**

Optical microscopy of the microstructures of the NMN/CNF-Pickering emulsion was performed using an optical microscope equipped with a camera (Leica, Germany). Moreover, the diameter distribution of the emulsion droplets was measured using the image analysis software ImageJ (National Institute of Health, Bethesda, Maryland, USA).

**Stability analysis**

The creaming stability of the emulsions was measured using Turbiscan equipment (LUMiSizer, LUM Jiangsu Instruments Co., Ltd., China). In the process of creaming, dynamic changes were appraised by the backscattering intensity of an optical laser as a function of test tube height position. After high-speed centrifugation (895 g) for 10 min, the sample was placed in a rectangular plastic tube (inner \(L \times W \times H = 10 \times 10 \times 100 \text{ mm}\)) and monitored at 25 °C.

**Rheological measurements**

All rheological measurements were performed with a strain-controlled rheometer (ARES-G2, TA Instruments, New Castle, Delaware, USA) using a cone
and plate clamp. The cone had a diameter of 50 mm. The gelation of the NMN/CNF Pickering emulsion was detected by frequency scanning (0.1–100 rad/s) and shear scanning (shear rate of 0.01–100 s⁻¹). The relationship between the elastic modulus, loss modulus, and frequency was recorded (Gao 2018; Li et al. 2020a, b). During the measurement, the temperature was maintained at around 25 °C.

Confocal laser scanning microscopy

To observe the aggregation of emulsion droplets, the dyed NMN/CNF Pickering emulsion was observed using a laser confocal microscope (SP8-X, Leica Microsystems GmbH, Wetzlar, Germany). Before observation, 2 mL of oil droplets were stained with 0.2 mL Nile Red solution. An analogous procedure was used for staining the aqueous phase with Nile Blue. After mixing and equilibrating at ambient temperature for 10 min, 6 µL of the stained sample was placed on a microscope slide and covered with cover glass. The respective peak excitation and emission are 488 and 539 nm for Nile Red and 633 and 672 nm for Nile Blue. The optical microscope images the NMN/CNF complexes and sunflower oil simultaneously with 63 oil-immersion objective lenses (Yan et al. 2019).

Thermogravimetric analysis

The thermal stability of the NMN/CNF complexes was analysed using a thermal analyser (TA Instruments, New Castle, Delaware, USA). During the measuring process in a nitrogen atmosphere, the heating rate of each sample was 10 °C/min and the end point was 600 °C.

Fourier transform infrared (FT-IR)

Fourier transform infrared spectroscopy (FT-IR) spectra were measured in absorbance mode using a FTIR spectrophotometer (Bruker Vertex V70) with the attenuated total reflection (ATR) diamond sensor. Spectra were collected at ambient conditions from an accumulation of 32 scans at a resolution of 4 cm⁻¹ over 400–4000 cm⁻¹ regions. All the NMN/CNF suspension samples were filtered by a B-GLM22 syringe filter (BKMAN, China) with 0.22 µm of membrane pore size before measurement.

Water-holding capacity

The Pickering emulsion was left at room temperature for two months to evaluate the long-term stability of the emulsion. Centrifugation at 4000 g was regarded as representative data, representing the long-term stability of the emulsion. Water-holding capacity (WHC) is a commonly used index for evaluating the stability of Pickering emulsions. Pickering emulsions (5 g) containing 60% oil were freshly prepared with the prepared water and oil phases and then centrifuged at 4 °C for 15 min at a speed of 4000 g. The weights of the bottom pellucid, middle emulsion, and upper sunflower oil phases were measured after centrifugation (Jouault et al. 2009; Wei et al. 2020). The WHC was calculated using the following formula:

\[ \text{WHC(%) = } \frac{W_T - W_F}{W_T} \times 100 \]

where \( W_T \) represents the total mass of water in each sample and \( W_F \) represents the mass of water released after centrifugation.

Antioxidant properties of emulsions

Lipid hydroperoxide content was measured to represent the oxidation of the emulsions. The emulsions (7.5 mL) oxidised in a test tube (10×50 mm) for 16 days (37 °C, in the dark). A portion of the emulsion (0.3 mL) was removed at specific time intervals, mixed with isoctane/2-propanol (1.5 mL, 3:1 v/v), swirled three times and centrifuged (1000 g) for 2 min to precipitate any protein nanoparticles that may exist in the test tube. The top organic layer (200 µL) was added to a mixture of methanol/1-butanol (2.8 mL, 2:1 v/v), ammonium thiocyanate (30 µL, 3.94 mol/L) and 30 µL of freshly mixed solution of BaCl₂ (0.132 mol/L) and FeSO₄ (0.144 mol/L). These chemicals were all dissolved in water. The resulting solution was allowed to rest at room temperature for 20 min, and a UV–vis spectrometer was used to measure the absorbance at 510 nm (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) (Tong et al. 2000). A standard curve for hydroperoxide was used.
to measure the lipid hydroperoxide concentration (µmol/g oil) of the emulsions.

Results and discussion

Appearance of Pickering emulsion co-stabilised by NMN/CNFs

A photograph of the emulsions stabilised by 0.25 wt% CNFs with different concentrations of NMN(0, 0.05, 0.1, 0.15, 0.2, 0.25 and 0.3 wt%) at an o/w volume ratio of 4:5 is shown in Fig. 1a. Uniform emulsions with no phase separation were obtained. Even after one day, no flocculation or delamination was observed in any of the samples, suggesting that CNFs could form a gel in the continuous water phase to stabilise the emulsions by providing a barrier to droplet coalescence (Kedzior et al. 2020). After the addition of NMN, the long-term storage stability of the prepared emulsions was improved. However, this does not mean that all the Pickering emulsions were stable. Figure 1b shows optical micrographs of the as-prepared emulsions stabilised by CNFs and NMN/CNF. Appearance and optical micrograph of Pickering emulsions solely stabilised by cationic CNFs are shown in Figs. S1 and S2 (Supporting Information). It can be seen that the particle size of emulsion stabilised by CNFs is larger than that stabilized by NMN/CNF complexes with the same particle concentration. Barely spherical droplets are obtained when the Pickering emulsion is stabilised by CNFs alone. Streaked emulsion droplets suggest imminent droplet coalescence and demulsification. With the addition of NMN (0.05–0.3 wt%), the uniformly spherical droplet diameters gradually decreased. When the concentration of NMN increased above 0.2 wt%, the droplet diameters were similar and relatively symmetrical. Clearly, the
addition of NMN plays a significant role in the stability of the NMN/CNF Pickering emulsion system. However, Pickering emulsion could hardly be formed and stabilised by NMN in the absence of CNF, which may be due to the strong lipophilicity and negative charge of NMN (Fang et al. 2021; Uddin et al. 2017).

Characterization of the NMN/CNF complex

To investigate the role of NMN in NMN/CNF-stabilised Pickering emulsions, the interfacial and surface tensions of NMN and CNFs were studied using a video contact angle measuring instrument using the hanging drop method. With an increase in the NMN concentration, the surface and interfacial tensions of the NMN/CNF complexes gradually decreased until they stabilised at a certain level (Fig. 2a). Before adding NMN, the interfacial layer formed by CNFs showed relatively weak coverage. NMN interacted with the CNFs and was adsorbed at the o/w interface, forming a relatively firm coating; hence, the interfacial and surface tensions were further reduced with more homogeneous emulsion droplets and smaller droplet sizes formed. NMN could enhance the noncovalent interactions among fibres, resulting in the formation of a multilayer interface structure and a stable emulsion gel. In addition, numerous studies have shown that

![Fig. 2  a](image1.png) Surface/interfacial tension and zeta potential of aqueous solutions containing CNF (0.25 wt%) with and without NMN as a function of initial surfactant concentration at 25 °C.

![Fig. 2  b](image2.png) FTIR spectrum of the NMN, CNFs and NMN/CNF complexes.

![Fig. 2  c](image3.png) TG and ![Fig. 2  d](image4.png) DTG curves of original CNFs and NMN/CNF complexes.
the surface properties of particles can significantly influence the properties of Pickering emulsion systems (Azfaralariff et al. 2020; Bai et al. 2018a, b; Brinatti et al. 2016). After NMN was added into cationic CNFs solution, the zeta potential decreased from 51.0 to 17.4 mV, indicating that there is a strong electrostatic attraction between NMN and CNFs.

FTIR was employed to investigate the interaction between NMN and CNF. The results were shown in Fig. 2b. Typically, the peaks at 3394 cm\(^{-1}\) and 3282 cm\(^{-1}\) are characteristic peaks with –NH\(_2\). The bands at 1609 cm\(^{-1}\), 1521 cm\(^{-1}\) and 1478 cm\(^{-1}\) on the spectrum of NMN/CNF complex are related to the existence of aromatic rings of NMN. The peaks at 1146 cm\(^{-1}\) and 1060 cm\(^{-1}\) correspond to the asymmetric stretching vibration of –PO\(_4\) on NMN, and the peak at 663 cm\(^{-1}\) corresponds to the in-plane bending vibration of –PO\(_4\), respectively, in agreement with previous report (Johnson et al. 2011). The FTIR results show that NMN can be combined with CNF through long-range electrostatic attraction. X-ray diffraction (XRD) results also support the effective combination of NMN and CNF. Figure S3 shows XRD patterns of original CNFs and NMN/CNF complexes (Supporting Information). The peaks of samples at \(2\theta=22^\circ\) are the characteristic peaks of nanocellulose I\(\beta\). Compared with CNFs (49.21%), the crystallinity degree of NMN/CNF complexes (54.90%) is obviously increased, which may be related to the interaction between NMN and CNFs. The long-range electrostatic attraction and hydrogen bond between NMN and CNFs render the regularity of the arrangement of CNFs molecular chains. We evaluated the interaction between NMN and CNFs by thermodynamic measure (Fig. 2c, d). The initial degradation temperature of the CNFs is 226.6 °C, and its maximum thermal degradation rate appears at 328.7 °C. The thermal degradation rate of CNFs fluctuates in the temperature range of weightlessness, which can be explained by the decomposition of ammonium in CNFs. With the increase of NMN concentration, the initial degradation temperature of NMN/CNF complex decreased from 345.7 to 264.1 °C. The weight loss at 141.2 °C did not appear in the TG curve of the CNFs, which was caused by the decomposition of NMN. Moreover, the weight loss rate of CNFs grafted with NMN at 600 °C was much lower than that of the CNFs, and the addition of 0.3 wt% NMN/CNF complex decreased from 83.25 to 61.94%.

Cryo-SEM

The morphology of the NMN/CNF-stabilised Pickering emulsion was observed using cryo-SEM. The micrograph clearly shows that the CNFs loosely surround the emulsion droplets, and some CNFs adsorb to the sunflower o/w interface (Fig. 3), which is dominated by the charge attraction between NMN and CNFs. NMN in the sunflower oil phase and CNFs in the water phase are combined at the sunflower o/w interface through long-range electrostatic attraction. This increases the particle coverage on the surface of emulsion droplets, which has a positive effect on the stability of the Pickering emulsion. After the CNFs attach to the surfaces of the emulsion droplets, it becomes more difficult for the droplets to agglomerate, which enhances the stability of the Pickering emulsion. Moreover, CNFs formed a certain degree of network structure in the aqueous phase, which also limits the attraction and collision between emulsion droplets and stabilises the emulsion. In our study, the droplet diameter of the NMN/CNF-stabilised emulsion was much smaller than that of the CNFs-stabilised emulsion (see Fig. S4, Supporting Information), which could be due to the formation of enhanced interfacial layers after the addition of NMN. The synergy between CNFs and NMN improves the
effectiveness of both stabilisers. Owing to the enhancement of noncovalent interactions between the continuous phase and CNFs at the interface, a dense three-dimensional (3D) network structure was formed. This contributed to the stability of the emulsion because such tightly covered oil droplets have difficulty interacting. The 3D network structure may also facilitate gelation. This excellent stability even at a large droplet size may be due to the ability of NMN/CNFs to bridge the flocculation structure, the steric hindrance of NMN/CNFs to droplet coalescence, and the 3D network structure of CNFs in the bulk.

The interaction between NMN molecules and CNFs mainly depends on the charge attraction and hydrogen bonding (Fig. 4). There is an electrostatic attraction between the phosphate groups of NMN and the ammonium groups of CNFs at the o/w interface. In addition, the amide group of NMN can form intermolecular hydrogen bonds with the hydroxyl groups of CNFs, which also play a positive role in the formation of the NMN/CNF complex. Furthermore, NMN in the oil phase can transfer into the aqueous phase near the o/w interface to form a 3D network structure with CNFs. Thus, the NMN and CNFs act together to form a dense particle film that effectively prevents the collision and coalescence of oil droplets.

Confocal laser scanning microscopy

The location of the NMN/CNF complex was traced to further probe the stabilising principle of the Pickering emulsion. Figure 5 shows the microstructure of the NMN/CNF-stabilised Pickering emulsion. The dyed CNFs particles and oil droplets are shown in blue and red, respectively, in the microscope images. Confocal laser scanning microscopy imaging of the CNFs (dyed with Nile Blue) and sunflower oil (dyed with Nile Red) phases confirmed the formation of an o/w

**Fig. 4** Schematic representation of the interaction mechanism between NMN and CNFs (not drawn to scale)

**Fig. 5** Fluorescence micrographs of Pickering emulsions stabilized by NMN/CNFs (w/o = 5:4, 0.25 wt% CNFs) with different concentrations of NMN. The first and second rows are the images obtained from dyeing the oil phase and CNFs, respectively. The third row is a merging of the images from the other two rows. Nile Red is used for dyeing the oil phase and Nile Blue is used for dyeing the CNFs. All the scale bars correspond to 25 μm. All samples were stored at 25 °C.
emulsion and the attachment of CNFs to the interface. The droplets were spherical in shape and their size was dependent on the concentration of NMN used (Table S2, Supporting Information): the Pickering emulsion stabilised by 0.25 wt% NMN had the smallest emulsion droplets; non-spherical droplets were observed below 0.05 wt% NMN, which may be due to the unstable o/w interface from insufficient electrostatic repulsion provided by the NMN/CNFs. At intermediate concentrations of NMN (0.1–0.2 wt%), small and homogeneous droplets were obtained, suggesting that more CNFs were bonded to the o/w interface under the electrostatic attraction between CNFs and NMN. Not surprisingly, clear ‘blue rings’ were observed surrounding oil droplets, evidencing the adsorption of CNFs particles at the o/w interface. This could result in a dense interfacial layer, providing steric hindrance that could effectively prevent the coalescence of oil droplets (Wei et al. 2019). At high concentrations of NMN (0.25–0.3 wt%), NMN was difficult to completely dissolve in sunflower oil, and some large oil droplets appeared in the emulsion. Uneven dispersion of NMN may affect the stability of emulsion system.

Stability analysis

Figure 6 shows representative backscattering intensity distribution of emulsions with different concentrations of NMN. The transmittance curves in the figure transition from purple to red over the course of the experiment. Without the addition of NMN, the CNFs with super hydrophilicity would lead to poor Pickering emulsion stability, resulting in a large amount of water phase separated from the concentrated emulsion. The rapid growing transmittance suggested the poor stability of as-prepared Pickering emulsion based on only CNFs. The addition of NMN at low concentrations (0.05–0.1 wt%) had a slight influence on the stability, the transmittance increased rapidly at the beginning of centrifugation. The excess water was rapidly separated in vitro by centrifugation, forming a creaming layer. The addition of NMN at intermediate concentrations (0.2 wt%) showed a left-shift and narrowing of the transmittance curves, which indicates that a NMN/CNF-Pickering emulsion with low oil concentration can be obtained after centrifugation or storage with less cream and higher stability because of the continuous cellulosic network.

Fig. 6 Transmittance curves of NMN/CNFs Pickering emulsions (containing 0.25 wt% CNFs and 0–0.3 wt% NMN) after centrifugation of 50 cycles at 3000 rpm and the 50th transmittance curve of each sample as a function of the height position of the test tube. All samples were tested at ambient temperature.
When the concentration of NMN exceeded 0.3 wt%, an unstable emulsion with creaming was obtained, which may be due to the unstable interface affected by excessive NMN condensation in the oil phase. The test results indicate that the stability of each emulsion with NMN/CNF complexes was higher than that of the pure CNFs-stabilised emulsion, and its stability was enhanced with the increasing NMN concentration up to a limit. This effect was attributed to the formation of compact interfacial layers and steric hindrance from the NMN/CNF networks in a continuous phase that mitigated flocculation or coalescence of the oil droplets.

We also evaluated the emulsifying activity of NMN/CNFs by different emulsifying methods (see Table S3). The results showed that the emulsifiers had different emulsification ability. When the oil was emulsified by gentle handshaking, the CNFs only formed a small amount of emulsion with 40% oil and 45% water separated, while NMN alone cannot form an emulsion. The emulsion can be prepared by NMN/CNFs with 65% emulsion volume fraction, but still separated 30% aqueous phase. When the emulsification method is changed to high-speed shear emulsification, CNFs and NMN stabilized 92% and 10% emulsion, respectively. However, 100% emulsion without phase separation was fabricated by NMN/CNFs, indicating that the interfacial NMN/CNF networks formed by the combination of NMN and CNFs at the oil-water interface can effectively improve the emulsifying ability of the composite particles.

Effect of pH

The pH of the Pickering emulsion has an appreciable impact on the wettability and coalescence stability of the particles (Scatena et al. 2001). The effect of pH on the stability of the NMN/CNF-Pickering emulsion was investigated using the conventional method of observing the appearance and droplet-size distribution of emulsions at different pH values, which are shown in Fig. 7 (Zhang et al. 2017). The emulsions showed great stability in different pH environments. The emulsions in the photo were very uniform with no oil-phase precipitation after 24 h. It can be clearly seen from the micrographs and droplet-size distribution that at pH 7 the emulsion droplets have the smallest average droplet size and the narrowest particle size distribution. At other pH values, the droplet size of the emulsion increased to a certain extent, and the droplet-size distribution became wider. At pH 3 and 13, the maximum diameter of the emulsion droplets was approximately 250 μm. Clearly, the emulsion has the greatest stability in a neutral environment. With the change in the hydroxyl concentration in the water phase, the hydrogen-bond network formed in the emulsion is destroyed. The amino group of the CNFs and the nicotine group of NMN can react with these hydroxyl ions, which changes the wettability of the NMN/CNF complexes (Chen et al. 2014; Scatena et al. 2001).

Rheological properties

In food emulsion processing, the rheological properties of emulsions have a significant influence on their application (Berton-Carabin and Schroën 2015; Bonnaud et al. 2010; Li et al. 2020a, b). The effect of the NMN concentration on the rheological properties of the NMN/CNF-Pickering emulsion is shown in Fig. 7. When the shear rate increases, the droplets deform and destroy each other during aggregation. All emulsion samples exhibited this shear-thinning behaviour (Fig. 8a). The viscosity of the emulsion was the highest when the concentration of NMN was 0.2 wt%. This may be related to the combination of NMN and CNFs strengthening the gel network structure because the Pickering emulsion system has the strongest stability in the 0–0.3 wt% NMN concentration range. The measurement of the oscillation frequency also supports the viscosity results (Fig. 8b). The $G'$ values of all emulsions were higher than the $G''$ values, which indicates that these emulsions have excellent gel properties and good elastic behaviour. With an increase in frequency, both $G'$ and $G''$ increased slightly, which indicates that physical interaction is beneficial to the formation of the NMN/CNF-Pickering emulsion. The values of $G'$ and $G''$ of the original CNFs-stabilised emulsion crossed at a high oscillation frequency, indicating that the gel network was destroyed and the flocculation worsened. These results show that the rheological properties of the Pickering emulsion improved after adding NMN.

The effect of different pH values on the rheological properties of the NMN/CNF-Pickering emulsions is shown in Fig. 9. In the shear rate range of 0.01–100 s$^{-1}$ (Fig. 9a), all emulsions show typical non-Newtonian pseudoplastic behaviour, that is,
shear-thinning behaviour. CNFs formed a large-scale network structure in the aqueous phase, and with an increase in shear rate, the network structure was destroyed and the viscosity significantly decreased (Xia et al. 2021). At pH 3 and 13, the viscosity of the emulsion at a low shear rate is significantly higher than that of the other samples. This may be due to the fact that the network structure formed by CNFs has a greater restrictive effect on larger diameter droplets. Within the pH range 3–9, the $G'$ of the emulsion is larger than $G''$ over the entire frequency range, which indicates that the rheological behaviour of the emulsion is mainly viscoelastic, and the droplets form a developed elastic network system. At pH 11 and 13,
$G'$ and $G''$ cross in a certain frequency range. This indicates that the gel network formed is unstable (Diamante and Lan 2014; Xia et al. 2021). Notably, $G'$ and $G''$ increase with increasing frequency and always maintained a large difference at pH 7, which indicates that the emulsion was more resistant to flocculation than those at other pH values. This is consistent with the results of droplet-size distribution.

WHC and antioxidant properties

The natural emulsification time of Pickering emulsions can be months or even years. Centrifugation can expedite the creaming of emulsions by droplets gathering and integrating with each other to form larger droplets. Subsequently, the water layer at the interface is removed from the emulsion, thus forming a tight filling pattern (Feng et al. 2019). As shown in Fig. 10a, the WHC of the Pickering emulsion first increased and then decreased with increasing NMN concentration, reaching a maximum value of 91.57% when 0.2 wt% NMN. It is worth noting that at this concentration, the WHC of the emulsion is 91.57%, which is 69.6% higher than that of the emulsion without NMN. This indicates that the addition of NMN enhances the strength of the emulsion gel network.
because the WHC of the emulsion mainly depends on the gel network (Lu et al. 2019). When the NMN concentration exceeds 0.2 wt%, the WHC of the emulsion decreases, which may be due to the decrease in the winding degree of CNFs and attenuation of the gel network. In addition, excessive NMN led to a slight decrease in the three-phase contact angle of NMN/CNF. This may be due to NMN shielding the hydroxyl groups on the surface of the CNFs, resulting in a decrease in the hydrophilicity of the complexes and thus reducing the WHC of the emulsion.

Lipid oxidation is a common phenomenon observed in emulsions (Begam et al. 2003; Song et al. 2020). The oxidation stability of Pickering emulsions with different NMN concentrations was evaluated by measuring lipid hydroperoxide (Fig. 10b). With the increasing storage time, the quantity of oil primary oxidation products in all samples (stored at 37 °C for 15 days) increased. The lipid hydroperoxide concentrations of the emulsions containing NMN were lower than that without NMN. At 0.2 wt% NMN, the lipid oxidation stability of the emulsion system was the best with only 11.64 µmol/g hydroxide formed after 16 days, which was 47.8% lower than that of the emulsion without NMN. This may be due to the strengthened gel network and ideal combination of NMN/CNF complexes to form a physical barrier to oxidation. The oil in the emulsion is mainly oxidised at the o/w interface. The adsorption of NMN/CNF complexes at the o/w interface acts as a physical barrier, which prevents the oxidation of the oil phase to a certain extent (Hu et al. 2020). The CNFs 3D-network structure formed around the emulsion droplets can also exclude the free radicals and pro-oxidants located in the contiguous areas of the oil droplets.

The synergistic effects of various factors allow NMN to effectively co-stabilise Pickering emulsions with CNFs, making it an exciting method that can be used to encapsulate oil-soluble substances. We believe that our study makes a significant contribution to the literature because it is the first time nicotinamide mononucleotide has been used to improve the Pickering emulsion stabilisation properties of cellulose nanofibres. This study presents a new structural reference of a co-stabilisation mechanism for the development of future Pickering emulsion stabilisers. However, the antioxidant properties of NMN in Pickering emulsions based on CNFs have not been systematically studied and discussed in this work. The anti-oxidative deterioration properties of Pickering emulsions containing NMN, which is an important indicator to influence NMN as an anti-aging drug or food ingredient, will be further investigated in our future work.

**Conclusion**

In this study, NMN was used for the first time as an efficient co-stabiliser of o/w Pickering emulsions...
with CNFs. A stable Pickering emulsion was successfully formulated by dissolving NMN in sunflower oil and mixing it with an aqueous dispersion of CNFs in an acohesive phase. CNFs play a significant role in the construction of a gel network, and NMN in the oil phase successfully combines with CNFs through electrostatic attraction and hydrogen bonds at the emulsion interface. The CNFs and NMN interact to form a dense interfacial film around the oil droplets, preventing their coalescence and providing stability to the Pickering emulsion. The strength of the gel network and lipid oxidation stability were the greatest at pH 7 and when the concentration of NMN was 0.2 wt%, resulting in an emulsion with meliorative rheology and stability for long-term storage. The NMN/CNF 3D-network structure described herein provides a practical structural reference and new ideas for the structural design, development, and application of Pickering stabilisers based on cationic particles and NMN. Considering the low cost, safety, effective antibacterial and antioxidant properties of CNFs, and the simplicity of the emulsification process, the developed stable Pickering emulsions containing NMN are expected to have diverse applications in the fields of food, medicine, cosmetics, and biomaterials.

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Declarations

Conflict of interest There are no conflicts of interest to declare.

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