Cellulose nanocrystal (CNC)-stabilized Pickering emulsion for improved curcumin storage stability

Yuan Zhe Aw\textsuperscript{a,b}, Hui Peng Lim\textsuperscript{a,b}, Liang Ee Low\textsuperscript{a,c,d}, Charanjit Kaur Surjit Singh\textsuperscript{a,b}, Eng Seng Chan\textsuperscript{a,b}, Beng Ti Tey\textsuperscript{a,d,*}

\textsuperscript{a} Chemical Engineering Discipline, School of Engineering, Monash University Malaysia, Selangor, Malaysia
\textsuperscript{b} Monash-Industry Palm Oil Education and Research Platform (MIPO), Monash University Malaysia, Selangor, Malaysia
\textsuperscript{c} Biofunctional Molecule Exploratory (BMEX) Research Group, School of Pharmacy, Monash University Malaysia, Bandar Sunway, 47500, Selangor, Malaysia
\textsuperscript{d} Advanced Engineering Platform, Monash University Malaysia, Selangor, Malaysia

\begin{itemize}
\item \textbf{Keywords:} Curcumin, Cellulose nanocrystal, Pickering emulsion, Encapsulation, Storage stability
\end{itemize}

\begin{abstract}
Curcumin is a bioactive supplement known to have many medical benefits. However, it has low bioavailability due to its insolubility in aqueous solution and rapid degradation profile. In this paper, an oil-in-water (O/W) Pickering emulsion (PE) was fabricated ultrasonically with different cellulose nanocrystal (CNC) concentrations to investigate its effect on the physicochemical properties of PE and the storage stability of curcumin. The CNC-PE produced remained stable for at least one month. The highest encapsulation efficiency of curcumin in CNC-PE was found to exceed 99\%. For storage of curcumin containing CNC-PE under dark conditions, good stability of curcumin was observed with a half-life of 98.47 d, reflecting a nearly 20-fold improvement as compared to other existing PE systems. On the other hand, storage under visible light, UV light, and at elevated temperature (50 °C) increased the degradation rate of encapsulated curcumin following the order: 50 °C > UV light > visible light > dark. Our findings elucidated the promising potential of CNC-PE as a food-safe delivery system for sensitive lipophilic bioactive compounds.
\end{abstract}

1. Introduction

Curcumin (diferuloylmethane; 1,7-bis[4-hydroxy-3-methoxy-phenyl]-1,6-heptadiene-3,5-dione) (Fig. 1), a lipophilic bioactive ingredient found in turmeric, a flowering plant (i.e. \textit{Curcuma longa}) of the ginger family, is known to provide medicinal properties against anxiety, arthritis, and hyperlipidemia (Hewlings & Kalman, 2017). Research has also shown that curcumin possesses anti-microbial, anti-mutagenic, and anti-cancer properties (Wright et al., 2013). This encourages its application to be taken as a health supplement to promote health benefits (Gupta et al., 2013). Nevertheless, curcumin often exhibited poor bioavailability due to its inherent hydrophobic nature and rapid degradation profile (Kita et al., 2008).

Generally, there are two main degradation pathways of curcumin, i.e., oxidative degradation and photodegradation. Both degradation pathways generate a variety of degradation products, which have less potent antioxidant and anticancer activities as compared to the curcumin molecule itself, and thus undesirable (Jankun et al., 2016).

Oxidative degradation of curcumin occurs spontaneously in aqueous solutions due to the radical chain reaction (Gordon et al., 2015). It is generally induced by the oxidative stress acting on the curcumin in the presence of reactive oxygen species (ROS) that is generated by pro-oxidants. Two forms of pro-oxidants, namely oxygen and transition metals, are known to initiate the reduction reactions that form superoxide or hydroxyl radicals responsible for the inception of oxidative stress (Rahal et al., 2014). Photodegradation, on the other hand, occurs due to the strong light absorption of curcumin in the visible range which promotes the formation of triplet excited states and generation of singlet oxygen (Jankun et al., 2016).

Pickering emulsion (PE) (i.e., emulsion stabilized by solid particles) is advantageous in delivering lipophilic bioactives due to its surfactant-free characteristic (Mwangi et al., 2020). It has strong resistance towards de-stabilization due to the irreversible adsorption of solid particles at the oil/water interface (Low et al., 2019). Many food-safe particles have been researched as Pickering emulsifiers, e.g., chitosan nanoparticles (Lim et al., 2020; Singh et al., 2021), starch nanocrystal (Azfaralariff et al., 2020), chitin nanofibers (Zhang et al., 2015), kafirin nanoparticles

\textsuperscript{*} Corresponding author. Chemical Engineering Discipline, School of Engineering, Monash University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, 47500, Selangor, Malaysia.

\textit{E-mail address:} tey.beng.ti@monash.edu (B.T. Tey).

https://doi.org/10.1016/j.lwt.2022.113249
Received 22 August 2021; Received in revised form 16 January 2022; Accepted 13 February 2022
Available online 14 February 2022
0023-6438/© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).
which is the research gap this paper intend to fill. We hypothesized that CNC would provide a strong Pickering barrier with high surface coverage, which prevents the transfer of curcumin from oil phase into aqueous phase. The pure and unmodified CNC could also provide abundant -OH functional groups to protect the curcumin against degradation via charge repulsion of ROS. Furthermore, the effects of different storage conditions (i.e., light and heat) on the stability of curcumin encapsulated in CNC-stabilized PE (CNC-PE) have been frequently overlooked. Therefore, in this study, we aimed to investigate the effect of unmodified CNC concentration on the storage stability of curcumin in CNC-PE formed using ultrasonication. Red palm oil was used as a model oil in this study, because it is commercially available in Malaysia. Besides, curcumin also exhibits the highest solubility in the red palm oil as compared to other types of oil (Low et al., 2019; Takenaka et al., 2013). The physicochemical properties of CNC-PE and stability of curcumin of the freshly prepared emulsions were evaluated. The CNC-PEs were stored under different conditions, i.e., in dark, visible light, UV light, and elevated temperature, and the storage stability of encapsulated curcumin were evaluated. These understanding are important to improve the stability of curcumin during transportation, application, and storage.

2. Materials and methods

2.1. Raw material

Curcumin [from Curcuma longa (turmeric) 65 g/100 g], calcificlor white, and Nile red were purchased from Sigma-Aldrich, Malaysia. Red palm superolein (275 ppm β-carotene, melting point 19 °C) was acquired from Sime Darby Jamalina Sdn. Bhd., Malaysia. Freeze-dried CNC (0.96 g/100 g sulfur content) was acquired from the University of Maine, USA. Ethanol (AR standard), sodium hydroxide (NaOH), hydrochloric acid (HCl), and sodium chloride (NaCl) were obtained from R&M Chemicals, UK. Ultrapure water (18.2 MΩ-cm, Millipore, USA) was used throughout this study. All chemicals used were of analytical grade.

2.2. Preparation and characterisation of CNC dispersion

The dispersion of CNC in water was prepared via ultrasonication. Firstly, a known weight of CNC and NaCl was added to water, and ultrasonication was performed for 1 min to obtain a CNC dispersion. The ultrasound treatment was carried out using a 20 kHz ultrasound generator (Qsonica, Q700, 220 V), equipped with a titanium probe and a replaceable tip (diameter = 1.27 cm), under pulse mode (15 s ON, 10 s OFF). The concentration of CNC and NaCl was varied from 0.1 to 1.5 g/100 mL and 0–100 mmol/L, respectively.

The zeta potential of CNC dispersion was measured using Zetasizer Nano ZS (Malvern Instruments, UK) at 25 °C. The surface morphology and particle size of CNC were viewed via a Hitachi SU8010 Field Emission Scanning Electron Microscope (FE-SEM) under scanning transmission electron microscopy (STEM) mode at 15 kV. The CNC sample was air-dried on a copper grid prior to viewing.

2.3. Preparation and characterisation of curcumin-containing CNC-PE

The oil phase consisting of 1 mg/mL curcumin was prepared by dissolving 8 mg of curcumin powder in 8 mL of red palm oil and magnetically stirred for 12 h at 30 °C in the dark. The curcumin-containing CNC-PE with oil/water ratio of 1:4 was prepared via a two-step method. Firstly, 8 mL of oil phase containing curcumin was added to 32 mL of ultrapure water and magnetically stirred at 750 rpm for 4 min. The mixture was then sent for ultrasonication using an ultrasonic processor (UP100Hd, Hielser Ultrasonics GmbH, Germany) at 80% intensity for 4 min. The CNC-PE was stored in a glass vial in the dark at room temperature for further analysis.

The emulsion droplet size of CNC-PE was measured using a Master-
sizer (Mastersizer 3000, Malvern Instrument, UK). The morphology and size of CNC-PE were viewed using an inverted light microscope (Nikon Eclipse Ti-E, Nikon Instruments Inc., USA) at 20× magnification. The fabricated CNC-PE was stained with calcofluor white (to stain CNC particles) and Nile red (to stain oil phase) to visualize the adsorption of CNC solid particles at the water/oil interface of CNC-PE. The stability, size homogeneity, and creaming of CNC-PE were monitored throughout the storage duration of 2 months. The creaming index, CI (%) of CNC-PE were calculated using the equation below:

\[ CI = \frac{\text{Height of Serum Layer}}{\text{Total Emulsion Height}} \times 100\% \] (1)

2.4. Encapsulation efficiency of curcumin in CNC-PE

Curcumin content in the CNC-PE sample was extracted using ethanol. In brief, 10 mL of ethanol was added to one mL of CNC-PE and the mixture was subjected to vigorous shaking using a vortex mixer (Vortex 3, IKA, Germany) for 1 min. The mixture was then centrifuged at 5000 rpm for 5 min. The curcumin content in the supernatant was measured using UV–Vis spectrophotometer (Genesys 10s UV, Thermo Fisher Scientific, USA) at 425 nm. Ethanol solution was used as blank. Encapsulation efficiency, EE (%) of curcumin in CNC-PE was calculated using the equation below:

\[ \% \text{EE}_{\text{encapsulation}} = \frac{\text{Curcumin content in emulsion}}{\text{Initial Curcumin content added}} \times 100\% \] (2)

2.5. Storage stability of curcumin in CNC-PE

The CNC-PEs containing curcumin were stored under different environmental conditions, namely: in dark, visible light, UV light, and elevated temperature. For storage in dark, the CNC-PE samples were stored at room temperature for up to 3 months. The photodegradation of curcumin was studied by exposing the CNC-PE samples to visible light (\(\lambda = 400-700\) nm) or UV light (\(\lambda = 100-400\) nm) at room temperature. The thermal sensitivity of curcumin in the CNC-PE sample was determined by storing the CNC-PE samples at an elevated temperature of 50 °C in the oven under dark condition. The curcumin content of CNC-PE samples stored under all the different conditions was measured periodically. The half-life of curcumin was calculated by estimating the time required for the curcumin content to reach 50% of the initial curcumin content via linear regression analysis.

2.6. Statistical analysis

All the experiments were conducted in duplicates, and results were expressed as means ± standard deviations.

3. Results and discussion

3.1. Preparation and characterization of cellulose nanocrystal (CNC) dispersion

The CNC used in this study had a rod-like shape (see Fig. 2a) with a length of approximately 200-300 nm and a width of around 5-10 nm. The surface charge of CNC dispersion without the addition of NaCl salt was highly negatively charged (−55 mV) due to the presence of hydroxyl and sulfate groups (Varanasi et al., 2018). The high surface charge resulted in the strong electrostatic repulsion between CNC particles, thereby retarding their adsorption at the oil/water interface. Due to the high surface charge, it is unlikely to obtain a stable emulsion regardless of the cellulosic source, emulsification technique, or the energy intensity applied during the emulsification process (Low et al., 2020). Therefore, salts are usually introduced in the formulation to screen the surface charge of CNC, thus reducing the electrostatic repulsion between the negatively charged CNC nanoparticles, which is essential to ensure that CNC can adsorb irreversibly at the oil-water interface (Kalashnikova et al., 2012). In this study, the effect of salt (i.e., NaCl) addition on the zeta potential of the CNC dispersion was investigated to select a suitable NaCl concentration for the emulsification process. Fig. 2b shows the zeta potential of CNC dispersion with CNC concentration of 0.1 to 1.5 g/100 mL, at various NaCl concentrations (i.e., 0 to 100 mmol/L NaCl). The zeta potential of CNC dispersion was found to decrease linearly from −55 mV to −28 mV at increasing NaCl concentration from 0 to 40 mmol/L. However, a plateau of zeta potential of CNC dispersion was observed for salt concentrations above 40 mmol/L due to the limited charge screening ability provided by NaCl. The salt concentration of 40 mmol/L was therefore used in the subsequent formation of curcumin-containing CNC-PE.

3.2. Preparation and characterization of curcumin-containing CNC-stabilized PE

In an attempt to form CNC-PE without any NaCl, no stable emulsion was obtained. This showed that charge screening was essential for PE formation. Fig. 3a shows the images of CNC-PE produced at increasing CNC concentration from 0.1 to 1.5 g/100 mL when 40 mmol/L NaCl was added into the formulation. CNC-PE with a CNC concentration higher...
than 1.5 g/100 mL was not included in this study due to the formation of highly viscous and gel-like PE. Fig. 3b shows the creaming indices of CNC-PEs as a function of different CNC concentrations. The error bars represent the standard deviation of the mean of duplicate samples.

For CNC-PE with CNC concentration of 0.1 to 1.5 g/100 mL, the aqueous layer (i.e., the continuous phase) of the CNC-PE appears completely transparent, consisted of only water, due to the creaming effect of PE (Tang et al., 2018). The creaming indices decreased as the concentration of CNC used to form the CNC-PE increased, owing to the higher availability of solid particles for the PE formation. At higher CNC content, more emulsion droplets of smaller droplet size were formed, which gave rise to a larger emulsion layer (i.e., the dispersed phase) (see Fig. 3a) (Bai et al., 2019). The smaller emulsion droplets which have lower buoyancy effect also resulted in less creaming observed (Ho et al., 2016).

The increased CNC concentration in the continuous aqueous phase could also retard the upward movement of droplets due to the gel-like network formed with slightly increased viscosity. Similar finding has been reported in a study involving PE stabilized by chitosan self-aggregated nanoparticles (Mwangi et al., 2016).

Fig. 4a shows the emulsion droplets size of CNC-PE produced at different CNC concentrations. The mean droplet size of CNC-PE decreased from 8.0 μm to 2.5 μm, as the concentration of CNC increased.
increased from 0.1 to 1.5 g/100 mL. All CNC-PEs possessed monomodal size distribution (see Fig. 4b), which were also verified qualitatively by light microscopy (see Fig. 5a–e). As CNC concentration increased, more solid particles were available to form more emulsion droplets. Since the total amount of oil phase was constant, when more emulsion droplets were formed, each emulsion droplet is smaller with a smaller volume of oil phase (Fujisawa, Togawa & Kuroda, 2017). Intriguingly, the hydrodynamic diameters of CNC-PEs at all CNC concentrations remained the same over a storage period of 1 month (see Fig. 4c), suggesting the high stability of the CNC-PE against droplets coalescence.

Fig. 5f shows a spherical emulsion droplet surrounded by rod-shaped CNC particles, with a droplet size of 2.8 μm. Fig. 6 shows the fluorescent microscopy images of CNC-PE fabricated at 0.1 g/100 mL CNC. The CNC-PE was stained with calcofluor white dye, which bound to polysaccharide to show a blue halo (see Fig. 6a), and Nile red, which stained the oil phase (see Fig. 6b). The combined image (Fig. 6c) displayed the PE morphology and CNC distribution, where the outer edge of the emulsion droplets was more bluish in colour, while the centre appears to be redder. This implied that the centre of emulsion droplets consisted of oil phase, while the edge consisted of CNC.

3.3. Encapsulation efficiency of curcumin in CNC-PE

The encapsulation efficiency of curcumin in CNC-PE was nearly 100% (see Table 1). This demonstrates that curcumin was completely dissolved in oil phase prior to the emulsification process, which was expected due to curcumin being an oil-soluble bioactive. All the dissolved curcumin was well encapsulated in the PE droplets due to the complete emulsification of the entire oil phase volume without the presence of non-emulsified oil (see Fig. 5a). The adsorption of curcumin by CNC can be neglected, as past studies suggested that complex surface modifications and loading processes were required to load curcumin onto CNC (Ching et al., 2019; Mohan Yallapu, Ray Dobberpuhl, Michele Maher, Jaggi, & Chand Chauhan, 2011; Sajjad et al., 2020; Subtaweesin et al., 2018). A control experiment was also performed prior to the Pickering emulsification, whereby the curcumin content in the oil phase was determined to be consistent even after in contact with CNC, showing no loading of curcumin onto CNC. A slight loss in EE can be explained by the extraction process using ethanol, which may not be 100% efficient since some curcumin would remain in the extracted oil phase.

3.4. Curcumin degradation study in CNC-PE

3.4.1. In dark

The curcumin degradation profile at different CNC concentration took place linearly when stored in dark environment (see Fig. 7a). From Table 2, it can be seen that the half-life of curcumin increased from $81.31 \pm 7.96$ to $98.47 \pm 3.08$ d, and then decreased to $58.26 \pm 5.53$ d, as the CNC concentration increased from 0.1 to 1.5 g/100 mL. A parabolic profile was observed for curcumin degradation rate in CNC-PE.
stored under dark condition (see Fig. 7e). At low CNC concentration (i.e., from 0.1 to 0.5 g/100 mL), the surface coverage of CNC on the droplet interface is low, which indicates that the PE was not entirely covered by solid particles. A portion of the oil phase remains in contact with the aqueous phase, thus causing a faster curcumin diffusion from the PE, thereby increasing curcumin degradation (Kalashnikova, Bizot, Cathala, & Capron, 2011). However, at higher CNC concentration (i.e., from 0.5 to 1.5 g/100 mL), although there was high surface coverage of CNC on the PE, the size of PE decreased as CNC concentration increased from 0.5 to 1.5 g/100 mL. This resulted in a greater contact area between the PE and the aqueous phase, thus causing a higher degree of curcumin diffusion from the PE, thereby increasing curcumin degradation (Kharat et al., 2020).

It is worth noting that the CNC-PE stabilized by 0.5 g/100 mL CNC revealed a nearly 20-fold improvement in protecting the curcumin from degradation, as compared to previous studies involving PE stabilized by other solid particles, such as chitosan-triopolyphosphate, silica, and whey protein isolate nanoparticles (Liu et al., 2019; Shah et al., 2016; Zhao et al., 2014). Several past studies had reported on antioxidant activity provided by CNC (Criado et al., 2015; Leite et al., 2021), which are due to the abundant -OH functional groups present on the unmodified CNC backbone. These functional groups provided protection via charge repulsion against the negatively charged ROS, such as superoxide (\(\cdot\)O\(_2\)) and hydroxyl anion (OH\(^-\)), which are responsible for inducing oxidative stress on curcumin (Touyz & Schiffrin, 2004). Besides, the supreme performance of CNC-PE in inhibiting curcumin degradation in this study is also attributable to the Pickering barrier provided by CNC at the oil-water interface. Moreover, the surfactant-free nature of the current PE delivery system using only biocompatible and biodegradable CNC further suggested its potential as a food-grade emulsion system for bioactive encapsulation.

### 3.4.2. Under visible light

Fig. 7b shows the curcumin degradation profile for CNC-PE storage under visible light, which occurred at a slightly faster rate than under dark conditions. From Table 2, the half-life of curcumin increased from 59.11 ± 7.39 to 63.89 ± 5.11 d, then decreased to 41.12 ± 4.65 d, as CNC concentration increased from 0.1 to 1.5 g/100 mL. A parabolic profile was also observed for curcumin degradation rate.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Encapsulation efficiency of curcumin into CNC-PE at various CNC concentrations.</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNC concentration (g/100 mL)</td>
<td>Encapsulation Efficiency, EE (%)</td>
</tr>
<tr>
<td>0.1</td>
<td>97.90 ± 2.10</td>
</tr>
<tr>
<td>0.25</td>
<td>98.66 ± 1.34</td>
</tr>
<tr>
<td>0.5</td>
<td>99.16 ± 0.84</td>
</tr>
<tr>
<td>1.0</td>
<td>99.14 ± 0.86</td>
</tr>
<tr>
<td>1.5</td>
<td>97.05 ± 2.95</td>
</tr>
<tr>
<td>Control*</td>
<td>0.00</td>
</tr>
</tbody>
</table>

Note: * referred to the control experiment to load curcumin into CNC, via mixing of curcumin-containing oil phase and CNC dispersion aqueous phase at 750 rpm for 24 h.
Fig. 7. Curcumin degradation profile at different CNC concentrations under (a) dark (b) light (c) UV light (d) elevated temperature (50 °C). Curcumin degradation rate at different CNC concentrations under (e) dark (f) light (g) UV light (h) elevated temperature (50 °C). The error bars represent the standard deviation of the mean of duplicate samples. Legend: (black line)- CNC-PE stabilized by 0.1 g/100 mL CNC; (red line)- CNC-PE stabilized by 0.25 g/100 mL CNC; (blue line)- CNC-PE stabilized by 0.5 g/100 mL CNC; (purple line)- CNC-PE stabilized by 1 g/100 mL CNC; (green line)- CNC-PE stabilized by 1.5 g/100 mL CNC. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)
against CNC concentration in CNC-PE stored under visible light (see Fig. 7f). This could be explained via the same reason as described in Section 3.4.1. The decreased half-life, when compared to storage in the dark, was due to the light-sensitive curcumin being excited by visible light into its degradation products. From the past literature, PE storage studies have been conducted in the dark to avoid light-induced curcumin degradation (Tikekar et al., 2013). Exposure to light (\(\lambda = 400\) to 510 nm) led to the formation of ferulic acid and ferulic aldehyde with radical intermediates. These two degradation products were formed by photochemical cleavage of \(\alpha\)-\(\beta\)-unsaturated ketone and \(\alpha\)-carbons (Tonnensen et al., 1986). In the prevention of light-induced curcumin degradation, CNC functions as a physical barrier to reduce the intensity of visible light emitted on the PE. Moreover, CNC has also been reported to be effective in quenching the peroxide radicals (i.e., the intermediates of curcumin photodegradation) to prevent further photochemical cleavage of curcumin (Wei et al., 2015).

### 3.4.3. Under UV light

The curcumin degradation profile for CNC-PE storage under UV light took place in a linear manner until a plateau was reached whereby all the curcumin has been degraded (see Fig. 7f). Storage under UV light yielded a significantly greater curcumin degradation rate when compared to visible light. From Table 2, at lower CNC concentration (i.e., from 0.1 to 0.5 g/100 mL), the half-life of curcumin was observed to be about 15 d. The half-life of curcumin decreased from 14.95 ± 1.41 to 8.12 ± 0.62 d as the CNC concentration increased from 0.5 to 1.5 g/100 mL. CNC is a strong UV absorber and initially acts as an UV blocker for curcumin, thus preventing curcumin degradation (Parit et al., 2018). However, prolonged UV exposure leads to photo-oxidation of CNC, which resulted in the formation of cellulose radicals (Sirvio et al., 2016). Cellulose radicals are stable because each monomer unit has five secondary carbons to form secondary radical. When the secondary radical abstracts proton from the adjacent carbon through hydrolysis, cellulose starts to fragment because of the breakage of \(\beta\)-1,4 glycosidic bond at C-1 or C-4 (Nakamura et al., 1985). As a result, the UV-absorbing properties of cellulose reduced, thus resulting in greater photodegradation of curcumin. The loss of CNC as a radical scavenger for intermediates generated from curcumin degradation synergistically decreased the concentration of curcumin in the sample. In the past study, a curcumin half-life of about 4 d in a PE system stabilized by ovotransferrin-gallic acid conjugates–carboxymethylxtran particles stored under UV light had been reported (Wei et al., 2019). Our results indicated that CNC-PE provided better protection for curcumin against UV radiation as compared to the past literature due to the role of CNC as a physical barrier.

The degradation profile as shown in Fig. 7g can be explained by the same reasons as those mentioned above. At low CNC concentrations, the protection provided by CNC solid particles and the greater creaming index (i.e., less UV light on emulsion layer) was dominant. On the contrary, at high CNC concentrations, CNC-PEs showed smaller droplet sizes and lower creaming indices. The greater degradation rate at high CNC concentration was contributed by the small droplet size (with larger droplet surface area) and the greater UV exposure over the higher emulsion layer. It is noteworthy that the curcumin degradation rate was higher when stored under UV light, as compared to that of visible light. This can be attributed to the fact that the high-energy UV rays accelerated cleavage of glycosidic bond in CNC, which reduced the ability of CNC to protect curcumin against photodegradation, as compared to visible light rays.

### 3.4.4. At elevated temperature (50 °C)

The effect of elevated temperature on the degradation of curcumin was also studied (Fig. 7d). As the CNC concentration increased from 0.1 to 1.5 g/100 mL, the half-life of curcumin decreased from 13.44 ± 1.87 to 3.18 ± 0.27 d. The degradation rate of curcumin increased linearly with CNC concentrations (Fig. 7h). This observation is believed to be dominated entirely by the decreasing droplet size and increasing effective contact area between the carrier and the surrounding environment at high CNC concentration. In fact, among the different storage conditions, storage at an elevated temperature resulted in the greatest curcumin degradation rate. This can be explained by the Arrhenius rate law, where the rate of reaction increased exponentially with respect to temperature (Smith, 2008). Typically, the rate of all chemical reactions depends directly on the value of rate constant, which is influenced by the temperature in an exponential relationship, as shown in the equation below, where \(k\) is the rate constant, \(A\) is the pre-exponential factor, \(E_a\) is the activation energy, \(R\) is the ideal gas constant, \(T\) is temperature (Carvalho-Silva et al., 2019).

\[
k(T) = A \exp \left( \frac{-E_a}{RT} \right)
\]

At high temperature, there were greater collisions of molecules per unit time, which ultimately resulted in greater rate of curcumin degradation (Esatbeyoglu et al., 2015). Young et al. reported that only 66% of curcumin were recovered from a silica-stabilized PE stored at 70 °C for 30 min (Young et al., 2018). Our study demonstrated a significantly better retention of curcumin as compared to the literature, indicating the protective effect of CNC on degradation of curcumin.

### 4. Conclusion

In this study, the physicochemical properties of PE and curcumin storage stability were investigated for CNC-PE fabricated at different CNC concentrations. The addition of NaCl into the CNC dispersion allowed the screening of surface charge on CNC, which aided in the formation of PE via ultrasonication. The PEs exhibited good stability across all CNC concentrations. When CNC concentration increased from 0.1 to 1.5 g/100 mL, smaller emulsion droplets sizes with a homogenous micron-sized profile (i.e., about 2.5 to 8.0 \(\mu\)m) were obtained. The encapsulation efficiency of curcumin in CNC-PE was nearly 100%. When stored under dark condition, curcumin degraded linearly with a half-life ranging from 58.26 to 98.47 d. This study showed a great improvement in the storage stability of curcumin with a nearly 20-fold improvement as compared to past literature. Although the half-life of curcumin was found to decrease upon storage under light, UV, and at elevated temperature of 50 °C, it was worth noting that the obtained output in this study was superior to those reported in past literature. These results suggested the role of CNC in prolonging the half-life of curcumin in a PE system, whereby CNC acted as a physical and chemical barrier at the oil/water interface, thus limiting curcumin degradation. The surfactant-free nature of CNC-PE further reflects the promising potential of this PE system as an effective food-safe delivery system for efficient encapsulation and protection of lipophilic bioactives.

---

**Table 2**

Predicted half-life of curcumin for CNC-PE at various CNC concentrations under various environmental conditions.

<table>
<thead>
<tr>
<th>CNC concentration (g/100 mL)</th>
<th>Half-life of curcumin (d)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dark</td>
</tr>
<tr>
<td>0.10</td>
<td>81.31 ± 3.91</td>
</tr>
<tr>
<td>0.25</td>
<td>91.95 ± 4.90</td>
</tr>
<tr>
<td>0.50</td>
<td>98.47 ± 5.99</td>
</tr>
<tr>
<td>1.00</td>
<td>5.08 ± 1.11</td>
</tr>
<tr>
<td>1.50</td>
<td>88.82 ± 4.90</td>
</tr>
<tr>
<td></td>
<td>2.09 ± 1.33</td>
</tr>
<tr>
<td></td>
<td>58.26 ± 4.12</td>
</tr>
<tr>
<td></td>
<td>5.53 ± 4.65</td>
</tr>
</tbody>
</table>
CRediT authorship contribution statement

Yuan Zhe Aw: Methodology, Investigation, Validation, Writing – original draft, Writing – review & editing, Visualization. Hui Peng Lim: Writing – review & editing, Investigation, Validation. Liang Ee Low: Writing – review & editing, Investigation, Validation. Charanjit Kaur Surjit Singh: Writing – review & editing, Visualization. Eng Seng Chan: Supervision, Funding acquisition, Writing – review & editing. Beng Ti Tey: Conceptualization, Supervision, Funding acquisition, Writing – review & editing.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank the School of Engineering, Monash University Malaysia, for providing the Master studentship for Aw Yuan Zhe. This study was funded by FRGS Grant (FRGS/1/2020/STG01/MUSM/01/1) from Ministry of Higher Education, Malaysia.

References


