

## Curcumin/Tween 20-incorporated cellulose nanoparticles with enhanced curcumin solubility for nano-drug delivery: characterization and in vitro evaluation

Article

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solubility for nano-drug delivery: characterization and in vitro evaluation
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Curcumin/Tween 20-incorporated cellulose nanoparticles with enhanced curcumin

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16

#### 28 Abstract

A poorly water-soluble anticancer drug, curcumin was loaded in to cellulose nanocrystals by 29 dissolving it in a commonly used nonionic surfactant medium. Results showed that the drug 30 loading capacity of nanocellulose increased with increasing the surfactant concentration of the 31 medium. The drug loading capacity of nanocellulose in surfactant medium was significantly 32 higher (7.73mg/g) when compared to the drug loading capacity (3.35mg/g) in methanolic 33 medium. The nanocellulose drug loaded in surfactant medium (TW/CNC) showed higher drug 34 35 release compared to the nanocellulose drug loaded in methanolic medium (METH/CNC). It was 8.99 mg/L for TW/CNC and 2.65 mg/L for METH/CNC in simulated gastric fluid. Due to the 36 increased stability of curcumin in acidic medium, all the nanoparticles showed higher drug 37 38 release in simulated gastric fluid compared to phosphate buffered saline solution. The maximum dissolution of curcumin was 2.13 mg/mL in distilled water containing 4% (w/v) of surfactant. 39 UV-visible spectra revealed that the curcumin retained its chemical activity after in vitro release. 40 From these findings, it is believed that the incorporation of curcumin into nanocellulose in 41 surfactant medium provides a promising approach for delivery of curcumin to stomach and upper 42 43 intestinal tract.

44 Key words: Nanocellulose, Curcumin, Tween 20, Bioavailability

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- 47

#### 49 Introduction

50 Nanotechnology is playing a key role in a broad range of applications. Since its introduction, it is a key component of advancing almost all areas of science, particularly, drug delivery and 51 formulation. Today, nanotechnology is being applied in the areas of drug delivery and 52 formulations known as 'Nanopharmaceutics'. As nanoparticles are accepted by cells more 53 54 effectively than larger microparticles, they can be used as efficient transport and delivery systems. Nanostructured-based drug delivery systems offer many advantages over conventional 55 drug delivery systems, including their ability to pass through the narrow capillary vessels due to 56 their smaller size, ability to penetrate cells and tissue gap to arrive at target organs and to provide 57 58 controlled drug release over a prolong period (Rizvi and Saleh 2017). Nanoparticles exhibit greater drug uptake compared to microparticles. The small dimensions of nanoparticles 59 compared to their bulk counterparts bring drug more closer to the surface of the particle, which 60 61 results in faster drug release (Rizvi and Saleh 2017). With the increase in potential usefulness of nanoparticles in therapeutics delivery, knowledge on the health effects of the nanoparticle 62 exposure and the basics of the interaction of nanoparticles with living cells, organs and 63 organisms is still limited. In this respect, materials and strategies which minimize the 64 possibilities of causing adverse and toxic effectshave been developed for therapeutic delivery, in 65 particular the choice of biodegradable nanoparticles with a limited life span which avoid the 66 67 accumulation in the liver and spleen(Gustafson et al. 2015).

Nanocellulose is a biodegradable nanomaterial which is obtained by most abundant natural polymer on earth. Nanocelluloses and their derivatives are attractive candidates for controlled drug delivery systems due to their biocompatibility, biodegradability and stimuli-responsiveness. It has been investigated for the delivery of protein, poorly water soluble drugs such as 72 beclomethasone, diproprionate, indomethacin and itraconazol from previous studies (Löbmann and Svagan 2017. The unique physico-chemical, rheological and barrier properties of 73 nanocellulose provide them to stabilize air/water and oil/water interfaces (Löbmann and Svagan 74 2017). Also, their large surface area-to-volume ratios offer possibilities for positive molecular 75 interactions with poorly-soluble drugs. The acid hydrolysis is an economical method that have 76 77 been extensively used for nanocellulose extraction from various natural sources such as plant fiber, wood fiber, microcrystalline cellulose, algae, tunicate and bacteria. Nanocellulose 78 synthesized using acid hydrolysis has a size range from 10 nm to 350 nm (Phanthong et al. 2015; 79 80 Sadeghifar et al. 2011; Sampath et al. 2017). Numerous studies have reported relationship between nanoparticle size and biological adverse effects (Gustafson et al. 2015). From the 81 literature it was reported that the optimum size for nanoparticle as carriers for drug delivery is 82 approximately 100 nm (Rizvi and Saleh 2017). Thereby, acid hydrolysis can be used to produce 83 the nanocelluloses with optimal size which is suitable for drug delivery. 84

Curcumin is a polyphenol obtained from the plant Curcumalonga. Curcumin has received 85 worldwide attention due to its promising anti-cancer properties (Ibrahim et al. 2018). Also, it can 86 cause a high rate of *Helicobacter pylori* eradication which was identified as a group I 87 88 carcinogenic agent of human gastric cancer (De et al. 2009; Santos et al. 2018). However, the complete potential of curcumin has not been successfully utilized due of its poor water solubility 89 and low bioavailability. Several strategies have been developed to enhance the solubility and 90 91 bioavailability of curcumin such as formation of micelles, nanosuspensions, nanoparticles and nano-emulsions are some of them (Kamaraj et al. 2018). Solid dispersion is one of efficient 92 method to overcome the challenges associated with poor water solubility of drugs. However the 93 94 miscibility and stability of the dispersion are main limitations related with the development of

95 solid dispersions. The use of surfactant in solid dispersion can overcome these limitations (Chaudhari and Dugar 2017). Surfactants can be cationic, anionic, nonionic or amphoteric. When 96 surfactant molecules are dissolved in water at a concentration greater than critical micelle 97 concentration (cmc), they form spherical form of aggregates known as micelles. The solubility of 98 hydrophobic drugs in nonionic surfactant solutions is greater than compared to solubility in ionic 99 100 surfactant solutions, because of their lower cmc values (Rangel-Yagui et al. 2005). In a micelle, the hydrophobic tails of several surfactant molecules flock into oil-like core in order to minimize 101 their contact with water, and the hydrophilic heads region faces the outside surface of the micelle 102 103 in order to maximize their contact with water.

104 Although research on nanocellulose based drug delivery systems are exponentially growing, there are a few reports that have been published on nanocellulose/curcumin drug delivery 105 106 systems (de Castro et al. 2018; Mohan Yallapu et al. 2012; Ntoutoume et al. 2016). Inspired by our previous work (Gunathilake et al. 2018; Udeni Gunathilake et al. 2017), we continued our 107 investigation on the enhancement of bioavailability of curcumin. From our previous study, 108 109 curcumin and nonionic surfactant were incorporated into nanocellulose reinforced chitosan hydrogel and studied the drug delivery behavior. We observed that the cumulative drug release 110 111 of the hydrogel increased with increasing the nonionic surfactant concentration. However, the drug loading efficiency of the hydrogel decreased with the incorporation of the surfactant to the 112 hydrogel (Gunathilake et al. 2018). Furthermore, the addition of nanocellulose enhanced the 113 mechanical strength and swelling behavior of the hydrogel (Sampath et al. 2017). However, the 114 problems encountered in previous study were the decrease of encapsulation efficiency of the 115 drug with increasing of surfactant concentration and incomplete drug release profiles. Polymer 116 117 phase of chitosan hydrogel acted as a diffusion barrier against movement of drug in the previous

118 study. Due to the hydrophilic property of the surfactant (high HLB value), the intake of water has 119 narrowed the diffusion barrier. Hence the entrapment efficiency decreased with increasing of surfactant. In this study, the increase of surfactant concentration will improve the micelle 120 121 formation and it will facilitate incorporation of drug into nanocellulose and hence, result in improvement of encapsulation efficiency. Previous study showed incomplete drug release 122 profiles over the time of monitoring. This is due to the fact that the embedded drug released 123 slowly over a longer period of time by diffusion through the hydrogel matrix. In this study, the 124 small dimensions of nanoparticles bring drug closer to the surface of the nanocellulose particles 125 which results in faster drug release. This will lead to complete drug release during shorter 126 residence time of the dosage form in the stomach. Therefore, the curcumin/Tween 20 127 incorporated cellulose nanoparticle system will provide a better platform to overcome the 128 problems associated with the curcumin/nanocellulose reinforced chitosan hydrogel system as 129 described in our previous study (Gunathilake et al. 2018). 130

In this study, we dissolved hydrophobic drug (curcumin) in a commonly used nonionic surfactant 131 132 solution and incorporated in to nanocellulose, which synthesized from microcrystalline cellulose by sulphuric acid hydrolysis method. Depending on the arrangement of hydrophobic and 133 134 hydrophilic groups of surfactant molecules in the micelle structure, there may be different interactions canoccur with drug molecules and cellulose nanocrystals. Hydrophobic drug 135 (curcumin) may be located in the inner core of the micelle of nonionic surfactant and the 136 hydrophilic groups of nonionic surfactants may have affinity for adsorption to cellulose, because 137 of its hydroxyl groups. Tween is a non-ionic surfactant commonly used in drug delivery 138 applications for dispersing of hydrophobic drugs. They consist of two different groups: a 139 140 hydrophilic head group and a hydrophobic alkyl chain. Based on the alkyl chain length, there are

141 different types of Tween surfactants namely, Tween 20, 40, 60 and 80. The differences of the alkyl chain length of surfactants influence the hydrophile-lipophile balance (HLB) value of the 142 surfactant and the entrapment efficiency of drug delivery systems. Increasing the alkyl chain 143 144 length (the surfactants with lower HLB values) is leading to higher entrapment efficiency. The higher the chain length (surfactants with lower HLB values), would cause lower release rates of 145 hydrophobic drugs. This is due to the fact that the surfactants having lower HLB values are more 146 lipophilic and less water soluble. But, the surfactants with higher HLB values such as Tween 20 147 (HLB= 16.7) helps to improve the release rates of hydrophobic drugs to a desired extent. 148 According to the findings of this study, we envision that our proposed curcumin/nonionic 149 surfactant-incorporated cellulose nanoparticle drug delivery system has great potential for 150 enhancing the bioavailability of curcumin. 151

152

#### 153 Experimental methodology

154 Materials

Microcrystalline cellulose, Tween 20 and phosphate-buffered saline were supplied by R&M chemicals (Essex, UK). Sodium chloride, hydrochloric acid, methanol and sulfuric acid were purchased from Friendemann Schmidt Chemicals (Parkwood, Australia). The drug curcumin was provided by HIMEDIA laboratories Pvt Ltd. (Mumbai, India).

159 Methodology

160 *Extraction of curcumin from turmeric* 

161 Curcumin was extracted from turmeric (rhizomes of Curcuma longa) by solvent extraction162 method Rhizomes were dried, crushed and soaked in methanol for 3 days. After that, the extract

was filtered with Whatman filter paper (pore size 0.2 µm). Finally, the filtrate was evaporated
under vacuum to obtain semi-dry oily mass.

165 *FTIR study* 

166 The FTIR spectra of curcumin extracted from rhizomes of curcuma longa, Tween 20, 167 nanocellulose and curcumin loaded nanocellulose were obtained using PerkinElmer spectrum 168 400 FTIR spectrometer over the range  $4000-400 \text{ cm}^{-1}$ .

169 *X-ray diffraction* 

170 The crystallinity degrees of curcumin, nanocellulose and curcumin incorporated nanocelulosewere X-ray (PANalytical **EMPYREAN** 171 studied using diffractometer 172 diffractometer). The samples were dried and powdered before they were analyzed. The samples were exposed to Cu Ka radiation generated at 40 mA, 40 kV, a 20 angle of 5-60°, and a scan rate 173 of 6°/min. 174

175 Drug loading

Cellulose nanocrystals (CNC) were synthesized by sulfuric acid hydrolysis of microcrystalline 176 cellulose, as reported in our previous study (Sampath et al. 2017). To prepare curcumin loaded 177 nanocellulose in Tween 20 medium, excess amount of curcumin were mixed with different 178 concentrations of Tween 20 (0.8%, 1.6%, 2.4%, 3.2%, 4%, 4.8%, 5.6% (w/v)) and stirred for 24 179 h. Then, the curcumin solutions (in Tween 20 medium) were centrifuged (10,000 rpm for 10 180 min) and supernatants were collected. After that, a constant amount of nanocellulose was mixed 181 182 with each curcumin solution (in Tween 20 medium) and stirred for 24 h. To prepare curcumin loaded nanocellulose in methanolic medium, constant amount of nanocellulose (similar to use in 183

Tween medium) was mixed with different concentrations (similar to the dissolved amount of curcumin present in Tween 20 solutions) of curcumin solutions (prepared by dissolving curcumin in methanol and diluting with distilled water) and stirred 24 h. After that, the suspension was centrifuged 6000 rpm for 20 min and supernatant was decanted and the remaining amount ofcurcumin was determined using UV-Vis spectroscopic method. The drug loading capacity was calculated based on the ratio of the absorbed amount of the drug from the solution to the weight of the nanocellulose (Eq. (1)).

191 .Drug loading capacity =  $\frac{Absorbed \ amount \ of \ drug \ from \ the \ solution}{Weight \ of \ nanocellulose}$ (1)

192 Drug release

In vitro drug release from drug loaded nanocellulose was studies in simulated gastric fluid (SGF) (prepared by dissolving 2 g NaCl in 7.0 mL HCl and water up to 1000 mL) and phosphate buffer saline solution (PBS) at 37 °C. In order to study the release, at prefixed time intervals, 3 mL of medium was withdrawn and returned it back to the solution after the analysis. The concentration of curcumin was determined using UV-Vis spectroscopic method. The experiments were replicated three times and average values were taken. The types of nanocellulose used for drug release studies are as per the details mentioned in Table 1.

- 200 Table 1 Types of nanocelluloseused for the drug release studies (based on the drug loading and
- 201 releasing medium).

Types of	Weight of	Amount of drug	Drug loading	Drug releasing
nanocellulose	nanocellulose (g)	(curcumin)	medium	medium
(based on the		loaded per 1 g of		
drug loading and		nanocellulose		
releasing		(mg)		
medium)				
TW/CNC-SGF	1	7.73	Aqueous solution	Simulated gastric
			of Tween 20	fluid

TW/CNC-PBS	1	7.73	Aqueous solution	Phosphate
			of Tween 20	buffered saline
				solution
METH/CNC-	1	3.35	Methanolic	Simulated gastric
SGF			medium	fluid
METH/CNC-	1	3.35	Methanolic	Phosphate
PBS			medium	buffered saline
				solution

203

#### 204 Solubility studies of curcumin in distilled water

To determine the solubility of curcumin in distilled water, an excess amount of curcumin extract was added to 30 mL of distilled water and mixed with different concentrations of Tween 20 (0.8%, 1.6%, 2.4%, 3.2%, 4%, 4.8%, 5.6% (w/v)). Then, the mixtures were stirred (350 rpm) using magnetic stirrer for 12h. Samples were covered in order to prevent photo-degradation. After that, the solutions were centrifuged (10,000 rpm for 10 min) to separate the undissolved curcumin and the dissolved curcumin was determined using UV-Vis spectroscopic method.

#### 211 *Drug activity*

The drugs are often inevitable and therefore, the chemical reactivity related to biological activity 212 213 of the drug is most important parameter to be concerned when selecting a drug delivery carrier. The UV-Vis spectra of pure drug and released drug can be used to determine if any deterioration 214 215 reaction happened due to the destructive interactions between drug and carrier molecules (Bashir et al. 2016). The UV-visible spectra of pure drug and the drug released from the cellulose 216 217 nanoparticles were obtained by scanning the drug solutions using UV-visible spectrophotometer (scan range 300-800 nm). Drug activity was determined by comparing the spectra (the absorption 218 maxima ( $\lambda$ max)) of pure drug and drug released from nanocellulose. 219

#### 220 Results and discussion

#### 221 FTIR study

Fig. 1 displays the FTIR spectra of curcumin, nonionic surfactant, nanocellulose, drug loaded 222 nanocellulose in surfactant medium and drug loaded nanocellulose in methanolic medium. The 223 IR spectrum of curcumin derived from turmeric is more similar to the IR spectrum of crystalline 224 curcumin derived from turmeric powder, which was reported in previous studies(Bich et al. 225 226 2009; Fugita et al. 2012). From our previous study, we have compared and characterized the FTIR spectra of extracted curcumin and curcumin purchased from Himedia Co. (Gunathilake et 227 al. 2018). In the FTIR spectrum of curcumin, the highest frequency bands observed within 2700-228 3000 cm<sup>-1</sup> region are assigned to the aromatic C-H stretches (Kolev et al. 2005). The IR bands at 229 815 cm<sup>-1</sup> and 720 cm<sup>-1</sup> belongs to the v(C-H) out of plane vibration of the aromatic ring (Kolev et 230 al. 2005). In the range of 700-500 cm<sup>-1</sup>, we could see deformation vibrations of both benzene 231 rings and the out of plane vibrations of both OH groups, which are at 607  $cm^{-1}$  and 546  $cm^{-1}$ 232 (Bich et al. 2009). These aromatic groups provide much of the hydrophobicity to the curcumin 233 234 molecules. Due to these hydrophobic groups, curcumin molecules are located in the core of the surfactant micelles, when it dispersed in surfactant solution. The peak at 1679 cm<sup>-1</sup> appeared due 235 to the C=O vibrations (Bich et al. 2009). The most prominent band in the IR spectrum is at 1509 236 cm<sup>-1</sup> can be attributed to highly mixed vibrations ( $^{v}C=O$ ,  $^{\delta}CC^{10}C$ ,  $^{\delta}CC=O$ )(Bich et al. 2009). 237 These ketone group exhibits keto-enoltautomerism forming a predominant keto form in acidic 238 and neutral media and stable enol form in alkaline medium. Therefore, it is insoluble in water 239 under acidic or neutral pHs but dissolves in alkaline conditions(Jankun et al. 2016). 240

In the FTIR spectrum of the nonionic surfactant, the peak around 3522 cm<sup>-1</sup> is due to the hydrogen bonded O-H stretching vibrations. The peaks at 2935 cm<sup>-1</sup> and 2883 cm<sup>-1</sup> are due to the asymmetric and symmetric methylene stretching vibrations. The 1741 cm<sup>-1</sup> peak represents the carbonyl group from R-CO-O-R and the peak at 1667 cm<sup>-1</sup> attributed to the carbonyl stretching. The most prominent peak at 1103 cm<sup>-1</sup> is due to the stretching vibration of  $-CH_2-0-CH_2-(Ortiz-$ Tafoya and Tecante 2018).

In the FTIR spectrum of nanocellulose, the broad band at 3334  $\text{cm}^{-1}$  is due to the characteristic – 247 OH stretching from vibrations in the intra-and intermolecular hydrogen-bonded hydroxyl groups 248 (Gunathilake et al. 2017). The band at 2901 cm<sup>-1</sup> is corresponded to aliphatic saturated CH-249 stretching in the glucose units. Other peaks detected include the adsorption band at 1636 cm<sup>-1</sup> 250 which is due to the absorption of water onto cellulose, the peak at  $1428 \text{ cm}^{-1}$  associated with 251  $CH_2$  symmetrical bending and scissoring motion in cellulose, the peak at 1159 cm<sup>-1</sup> is due to the 252 asymmetrical bridge C–O–C stretching from the glycosidic bond, the band at 1029 cm<sup>-1</sup> 253 representing stretching of the glucopyranose unit and the peak at 896 cm<sup>-1</sup> is typical of the  $\beta$ -254 glycosidic linkage in cellulose (Ching & Ng 2014, Sahlin et al. 2018). 255



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#### 265

#### Fig. 1 FTIR spectra of curcumin, nonionic surfactant and nanocellulose (CNC)

FTIR spectrum of nanocellulose drug loaded in methanolic medium 266 From the (curcumin/cnc/methanolic medium), it can be seen that the **bands corresponding** to the functional 267 groups of CNC are more prominent (Fig. 2). However, small peaks at 1635cm<sup>-1</sup> and 268  $1595 \text{ cm}^{-1}$  are appeared due to the v(C=C) of the benzene ring, mixed v(C=C) and v(C=O) groups 269 of curcumin(Bich et al. 2009; Mohan et al. 2012). Also in the FTIR spectrum of nanocellulose 270 drug loaded in surfactant medium(curcumin/cnc/surfactant medium), the bands corresponding to 271 the functional groups of CNC are prominent. In this spectrum a sharp peak appeared at 1652 272 cm<sup>-1</sup>which represents the carbonyl group from R-CO-O-R of Tween 20(Ortiz-Tafoya and 273 Tecante 2018). However, no new peak appeared in both spectrum of curcumin/cnc/methanolic 274 275 medium and curcumin/cnc/surfactant medium.



Fig. 2 FTIR spectra of cellulose nanoparticles, curcumin loaded cellulose nanoparticles in
methanolic medium and curcumin loaded cellulose nanoparticles in surfactant medium

286 X-ray diffraction analysis

287	The X-ray	diffraction	(XRD)	patterns	of the	curcumin,	nanocellulose	and	curcumin	incorp	orated

- 288 nanocellulose were investigated via powder XRD analysis (Fig. 3). As shown in the
- diffractogram, the CNC exhibited peaks around  $2\theta = 15^{\circ}$ ,  $16.5^{\circ}$ ,  $20.5^{\circ}$ ,  $22.5^{\circ}$ ,  $34.5^{\circ}$ , which
- respectively represent the (1-10), (110), (102), (200), and (004) crystallographic planes of a
- 291 typical cellulose I structure (Novo et al. 2015).
- 292 The diffraction pattern of curcumin exhibited peaks at angles 8.59°, 11.24°, 17.35°, 18.19, 19.46,
- 293 21.28°, 23.44°, 24.58°, 25.59°, 26.17°, 26.79°, 27.39°, 28.27°, 28.97°, 31.6° and 36.23° which
- 294 correspond to curcumin polymorph 1, indicating that curcumin sample exists as form
  295 1(Poornima et al. 2016; Sanphui et al. 2011). Curcumin exhibited well-defined sharp, narrow
- 296 diffraction peaks between  $10^{\circ}$  and  $30^{\circ}$ , indicating the high crystalline structure (Cheng et al.
- 297 2017; Singh et al. 2014). The diffractogram of curcumin incorporated nanocellulose showed
- 298 peaks which corresponded to both nanocellulose and curcumin. However, the peaks intensity of
- 299 CNCs decreased after the incorporation of curcumin as shown in Fig. 3 which indicates a low
- 300 crystallinity of the curcumin incorporated nanocellulose relative to the pure CNCs. From these
- 301 results, it can be concluded that the interaction of the hydroxyl groups of CNCs with curcumin
- 302 breaks the intermolecular and intramolecular hydrogen bonds of the CNCs and modifies the



Fig. 3 X-ray diffraction (XRD) patterns of curcumin, nanocellulose and curcumin incorporated
nanocellulose

317 *Drug loading capacity* 

Since the solubility of curcumin in water is significantly low, we used nonionic surfactant (Tween 20) to dissolve the curcumin in aqueous medium. After that, the surfactant drug solution was stirred with cellulose nanocrystals to incorporate the drug into nanoparticles. (We used excess amount of curcumin, constant amount of nanocellulose with different concentration of 322 nonionic surfactant (0.8%, 1.6%, 2.4%, 3.2%, 4%, 4.8%, 5.6% (w/v)) for the drug loading 323 For the comparison of drug loading capacity, curcumin was incorporated into process. nanocellulose in methanolic medium. (Here, the same concentration of curcumin and same 324 amount of nanocellulose were used as similar to those used in surfactant solutions). As shown in 325 Fig. 4, the drug loading capacity of nanocellulose increased with increasing the surfactant 326 concentration of the medium. It increased from 0.1 mg/g to 7.73 mg/g with increasing the 327 surfactant concentration from 0% to 4%. The highest drug loading capacity of nanocellulose was 328 given at which the surfactant concentration showed the highest solubility of curcumin. After that, 329 330 the loading capacity of nanocellulose was not increased with increasing the surfactant concentration. For curcumin dissolved in methanolic medium, the maximum drug loading 331 capacity was 3.35 mg/g. It showed almost two fold increases in the drug loading capacity of 332 nanocellulose in surfactant medium compared to methanolic medium. This may be due to the 333 formation of micelles facilitating the incorporation of drug into nanoparticles. 334



346

#### Fig. 4 Drug loading capacity of nanocellulose in surfactant and methanolic medium

Surfactant micelles are formed at the critical micelle concentration in aqueous medium due to the 347 348 attainment of minimum free energy state. The critical micelle concentrations of nonionic surfactants are lower when compared to other ionic surfactants. Most micelles are spherical in 349 shape and contain around 60-100 surfactant molecules. It has the hydrophobic oil-like core 350 351 formed from the hydrocarbon chains surrounded by hydrophilic head groups of surfactant molecules. Formation of micelle facilitates the incorporation of poorly water- soluble drug in 352 aqueous medium, which result in an increase in the apparent aqueous solubility of the drug. 353 There are number of possible locations of solubilization for a drug in a micelle, depending on 354 355 their hydrophobicity.

As shown in Fig. 5a, hydrophilic drugs can be adsorbed on the surface of hydrophilic head region of the micelle. Drugs with intermediate solubility can be adsorbed between the hydrophilic head groups of surfactant micelle or in the palisade layer which is in between the hydrophilic and the first few carbon atoms of the hydrophobic tail (Fig. 5b,c). Drugs which are highly hydrophobic may be located in the inner core of the micelle. In this case, curcumin is highly hydrophobic and therefore, it may be adsorbed to the inner core of the micelle (Fig. 5d).









Fig. 5 Possible locations of drug in surfactant micelles, based on the drug hydrophobicity. The red bold lines represent the drug molecules, yellow circles display the surfactant heads and blue curved lines represents the hydrophobic tails of surfactants

Many researches have been discussed chemical strategies for surface modification of 369 370 nanocelluloses. The main aspect to be considered here is that in contrast to complex chemical modification of cellulose, simple addition of surfactants is an appealing alternative. 371 In such scenarios, physical adsorption such as charge-charge interactions, association of 372 hydrophobic groups, and hydrogen bonding plays a key role in their association behavior. 373 374 Adsorbing nonionic surfactantsis a feasible methodto enhance the compatibility of the hydrophilic cellulosic material with the typically hydrophobic materials. The hydrophilic groups 375 of nonionic surfactants may have affinity for adsorption to the cellulose due to the hydroxyl 376 groups of cellulose (as shown in Fig. 6). However, not all the hydroxyl groups present in 377 378 cellulose nanocrystals are accessible. This is because some of them are oriented towards the 379 inner part of nanoparticle. Previous literature reported that only one half of hydroxyl groups present in cellulose chains of nanoparticle are reactive (Ching et al. 206). It also reported that the 380 primary hydroxyl on C6 is most reactive(Gan et al. 2017). 381



388

#### Fig. 6 Possible interactions of surfactant micelles with nanocellulose

From previous studies, different types of surfactants have been used to improve the release of 389 390 drugs in different types of drug delivery systems. Pandav et al. (2013)reported that the encapsulation efficiency of microparticulate drug delivery system of propranolol 391 hydrochloridewas directly proportional to surfactant concentration. Furthermore, Tween80was 392 shownto be themore effective surfactant as compared to Span 60 for loading of curcumin onto 393 starch nanoparticles(Chin et al. 2014). In our previous study, we observed that the encapsulation 394 efficiency of curcumin in chitosan hydrogel decreased with increasing the surfactant 395 concentration. This was due to the fact that the higher concentration of the emulsifier increases 396 397 the partition of the drug from internal to external phase due to the increased solubility of the drug 398 in the external phase. But, in this study, the loading capacity of nanocellulose for curcumin increased with increasing the surfactant concentration. This is due to the increase of the number 399 of micelle formation and hence, facilitating the incorporation of drug into nanocellulose 400 401 particles.

#### 402 Drug release

Drug release studies were carried out in simulated gastric fluid (SGF) and phosphate buffered saline (PBS) solution. Constant weight of nanoparticles which adsorbed highest amount of curcumin in methanolic medium and Tween 20 medium were selected for drug release studies. All the nanoparticles showed initial burst release during first 30 min due to the fraction of drug which is weekly bound to the large surface area of nanoparticles. As shown in Fig. 7 and Fig.8, among all the nanoparticles, the highest drug release shown by the nanoparticles drug loaded

insurfactant medium (TW/CNC), compared to the nanoparticles drug loaded in methanolic 409 medium (METH/CNC). The nanoparticles drug loaded in surfactant medium reached to 410 equilibrium drug release stage at around 270 minutes (in SGF medium) and 350 minutes (in PBS 411 412 medium) while the nanoparticles drug loaded in methanolic medium reached to that stage at around 150 minutes and 270 minutes in SGFand PBS medium respectively. The nanoparticles 413 drug loaded in both surfactant and methanolic medium showed lower drug release in PBS 414 medium. The reason for this may be due to the less stability of curcumin in neutral or pHs above 415 neutral. In addition, previous studies indicated that the stability of curcumin can be strongly 416 improved by lowering the pH of the medium (Khalkhali et al. 2015; Rao and Rao 2011; Wang et 417





media



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# Fig. 8 Drug release from: a W/CNC into SGF medium; b METH/CNC into SGF medium; c TW/CNC into PBS medium; d METH/CNC into PBS medium ande control

434 According to our previous study, the nanoparticles prepared by sulphuric acid hydrolysis method were within the range of 200-300 nm in length and 40-50 nm in width(Sampath et al. 2017). As 435 436 the particle size gets smaller, their surface area to volume ratio gets larger. This would imply that 437 more of the drug is closer to the surface of the nanoparticle compared to a larger molecule. Being at or near the surface would lead to faster drug release (Rizvi and Saleh 2017). Average gastric 438 emptying times for healthy individuals at 1, 2, and 4 h are >90%, 60% and 10%, 439 respectively(Jobe et al. 2013). Due to the highest drug release shown in SGF medium, and faster 440 drug release, these drug delivery systems are more suitable for stomach delivery of curcumin. 441

The higher drug release by TW/CNC-SGF and TW/CNC-PBS nanoparticles, which immersed in 442 443 SGF and PBS media, is due to the presence of adsorbed nonionic surfactants into the nanoparticles. These surfactants facilitate the dispersion of curcumin in aqueous medium. They 444 445 improve the solubility of poorly water soluble curcumin by formation of micelles and adsorbing the hydrophobic drug into the core of the micelle structure. Hydrophilic head groups show more 446 affinity towards aqueous medium and thereby improve the solubility of the drug in SGF and PBS 447 448 medium. In contrast, for METH/CNC-SGF and METH/CNC-PBS conditions, the interactions 449 between nanocellulose and curcumin molecules are most probably by hydrogen bonds. Since, there is no micelle formation or facilitation for the dispersion of this poorly water soluble drug, 450

the release amount of drug to the SGF and PBS medium is very low compared to TW/CNC-SGFand TW/CNC-PBS conditions.

Similar drug delivery systems are also reported with enhanced solubility of curcumin using 453 Tween 20. The drug delivery studies on chitin beads incorporated with curcumin, carried out by 454 455 Ratanajiajaroen and Ohshima (2012)reported that the solubility of curcumin increased up to 0.767 mg/mL with the presence of 2% (v/v) Tween 20 in acetate buffer medium (pH 5.5). They 456 also found that the drug release rate from chitin beads was proportional to the surfactant 457 concentration. Furthermore, the drug delivery studies on submicrometer spray-dried 458 chitosan/Tween 20 particles byO'Toole et al. (2012) showed that the curcumin can be 459 460 completely release from the matrix in both phosphate buffered saline solution and 1% acetic acid 461 over a 2 h period. In addition, it showed 12.7-fold increase in curcumin solubility with 0.05 w/v% of surfactant in 1% acetic acid solution. From our previous study of curcumin delivery 462 463 using chitosan/nanocellulose/surfactant hydrogel, we achieved 3.98mg/L of curcumin release in SGF medium after 7.5h. In this study, we achieved 8.99 mg/L of curcumin release in SGF 464 medium at around 270 minutes. Therefore, these curcumin loaded cellulose nanoparticles 465 provide more effective approach for delivery of curcumin to stomach and upper intestinal tract. 466

467 *Drug activity* 

The drugs are often inevitable and therefore, the chemical reactivity related to biological activity of the drug is most important parameter to be concerned when selecting a drug delivery carrier. For some drug delivery systems, drugs deteriorate due to the destructive interactions with the carrier molecules. In order to prevent this, the carrier should be prone to destructive interactions with the drug and able to be delivered into the body without any chemical deterioration. The UV-

Vis spectra of pure drug and released drug can be used to determine if any deterioration reaction happened due to the destructive interactions between drug and carrier molecules (Bashir et al. 2016). Curcumin has three reactive functional groups, namely one diketone moiety, and two phenolic groups which associated with its different biological activities. The diketone moiety involves in nucleophilic addition reactions and C-4 participates in hydrogen donation reactions leading to oxidation of curcumin; which are most important chemical reactions associated with its biological activities (Ahmed et al. 2017).

As shown in Fig.9a, UV-Vis spectrum of pure curcumin shows an absorption peak around 427 480 nm, which can be assigned to the low-energy  $\pi$ -  $\pi^*$  excitation of the chromophore, that formed 481 482 due to the enolization of the diketone group and conjugation between the  $\pi$ -electron clouds of the two vinylguaiacol (Zsila et al. 2004). In this study, curcumin is in contact with nonionic 483 surfactant, cellulose, simulated gastric fluid and phosphate buffer saline solution. Fig. 9b 484 represents the UV-Vis spectra of curcumin, which is released from nanocellulose (drug loaded in 485 surfactant medium) into simulated gastric medium and phosphate buffered saline solution. It is 486 487 clear that the absorption maximum of both these spectra is around 427 nm. It remains unchanged without shifting upward or downward regions of the spectrum. Fig. 9c represents the UV-Vis 488 spectra of curcumin, which is released from nanocellulose (drug loaded in methanolic medium) 489 into simulated gastric medium and phosphate buffered saline solution. Since the released amount 490 of drug from these nanoparticles is very low, the absorption peak around 427 nm is slightly small 491 compared to the absorption maximum of Fig. 9b. However, in this situation the absorption 492 maximum (427 nm) also remain unchanged (without shifting upward or downward direction of 493 494 the spectrum). Therefore, it revealed that the reactive functional groups, which are associated



with the biological activity of curcumin retained without any deterioration due to anydenaturation reaction with drug containing media or with carrier molecules (nanocellulose).

513 Fig. 9 UV-Vis spectra of: a pure curcumin; curcumin release from TW/CNC into PBS and SGF

514 media; **c** curcumin release from METH/CNC into PBS and SGF media

516 For the encapsulation of curcumin into nanocellulose, the curcumin was dissolved in surfactant distilled water medium. Therefore, the solubility studies of curcumin were carried out in distilled 517 water with the presence of nonionic surfactantat 37° C. As shown in Fig. 10 the dissolution rate 518 519 of curcumin increased with increasing thesurfactant concentration in dissolution medium and the 520 maximum dissolution of curcumin was 2.13 mg/mL in distilled water containing 4% w/v of surfactant. Addition of surfactant to the distilled water improves the dissolution of pure drug by 521 facilitating the dispersion of drug by micelle solubilization in the bulk medium. The amount of 522 surfactant needed depends on the critical micelle concentration and the solubilization capacity 523 for curcumin in surfactant micelles. It may be the reason for the fact that the amount of dissolved 524 curcumin did not increase above 4% of surfactant in distilled water. It reached a minimum 525 surface tension at 4% of surfactant with no significant change at higher concentrations. Similar 526 527 results were obtained from the studies on solubility of curcumin in aqueous polysorbate micelle, by Inchai et al. (2015). Their studies showed that the solubility of curcumin increased up to 2.7 528 mg/mL in 20% aqueous solution of Tween 20. From our previous study, we achieved the 529 solubility of curcumin with an upper limit of  $3.014 \pm 0.041$  mg/mL in the presence of 3.2% (w/v) 530 Tween 20 in simulated gastric medium. This may be due to the higher stability of curcumin in 531 acidic medium compared to neutral or alkaline medium. Previous studies have also confirmed 532 533 that the aqueous solubility and the stability of curcumin is higher in acidic medium compared to neutral or higher pH values (Khalkhali et al. 2015; Wang et al. 1997). 534

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- 540

Fig. 10Solubility of curcumin in distilled water with different concentrations of nonionic
surfactant

543 Conclusion

Cellulose nanocrystals were synthesized using microcrystalline cellulose via sulphuric acid 544 545 hydrolysis method. Curcumin was extracted using dried rhizomes of Curcuma longa following the methanolic extraction method. The incorporation of drug and surfactant into the cellulose 546 nanoparticles was verified through the FTIR characterization. The drug loading capacity of 547 548 nanocellulose increased from 0.1mg/g to 7.73 mg/g with increasing the surfactant concentration 549 from 0% to 4%. This may be due to the formation of micelles facilitating the incorporation of 550 drug into nanoparticles. The maximum drug loading capacity of nanocellulose in methanolic medium was 3.35 mg/g. Among all the nanoparticles, the highest drug release shown by the 551 552 nanoparticles drug loaded in surfactant medium (TW/CNC) compared to the nanoparticles drug 553 loaded in methanolic medium (METH/CNC). The nanoparticles drug loaded in both surfactant and methanolic medium showed lower drug release in PBS medium due to the less stability of 554 555 curcumin in neutral or pHs above neutral. Solubility of curcumin increased with an upper limit of 2.13 mg/mL with the presence of 4% (w/v) surfactant in distilled water. In addition, curcumin 556 retained its structural integrity after release to the SGF and PBS media, which is a critical 557

requirement for preserving drug activity. In conclusion, the enhancement of bioavailability of

559 curcumin using curcumin/nonionic surfactant-incorporated cellulose nanoparticles would

560 represent an important step forward in nanopharmaceutical field.

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694 Fig. 2 FTIR spectra of cellulose nanoparticles, curcumin loaded cellulose nanoparticles in

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**Fig. 10** Solubility of curcumin in distilled water with different concentrations of nonionic

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Table 1 Types of nanocellulose used for the drug release studies (based on the drug loading and
releasing medium)
releasing medium
releasi



Fig. 1 FTIR spectra of curcumin, nonionic surfactant and nanocellulose (CNC)





Fig. 2 FTIR spectra of cellulose nanoparticles, curcumin loaded cellulose nanoparticles in methanolic medium and curcumin loaded cellulose nanoparticles in surfactant medium





Fig. 3 X-ray diffraction (XRD) patterns of curcumin, nanocellulose and curcumin incorporated nanocelulose





Fig. 4 Drug loading capacity of nanocellulose in surfactant and methanolic medium



**Fig. 5** Possible locations of drug in surfactant micelles, based on the drug hydrophobicity. The red bold lines represent the drug molecules, yellow circles display the surfactant heads and blue curved lines represents the hydrophobic tails of surfactants



Fig. 6 Possible interactions of surfactant micelles with nanocellulose



Fig. 7 Drug release from cellulose nanoparticles (TW/CNC and METH/CNC) to SGF and PBS

media



**Fig. 8** Drug release from: **a** TW/CNC into SGF medium; **b** METH/CNC into SGF medium; **c** TW/CNC into PBS medium; **d** METH/CNC into PBS medium and **e** control





**Fig. 9** UV-Vis spectra of: **a** pure curcumin; **b** curcumin release from TW/CNC into PBS and SGF media; **c** curcumin release from METH/CNC into PBS and SGF media



Fig. 10 Solubility of curcumin in distilled water with different concentrations of nonionic surfactant

Table 1 Types of nanocellulose used for the drug release studies (based on the drug loading and

releasing medium)

Types of nanocellulose	Weight of	Amount of drug	Drug loading	Drug releasing
(based on the drug	nanocellulose (g)	(curcumin)	medium	medium
loading and releasing		loaded per 1 g		
medium)		of nanocellulose		
		(mg)		
TW/CNC-SGF	1	7.73	Aqueous	Simulated
			solution of	gastric fluid
			Tween 20	
TW/CNC-PBS	1	7.73	Aqueous	Phosphate
			solution of	buffered saline
			Tween 20	solution
METH/CNC-SGF	1	3.35	Methanolic	Simulated
			medium	gastric fluid
METH/CNC-PBS	1	3.35	Methanolic	Phosphate
			medium	buffered saline
				solution