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

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

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Abstract

A poorly water-soluble anticancer drug, curcumin was loaded in to cellulose nanocrystals by dissolving it in a commonly used nonionic surfactant medium. Results showed that the drug loading capacity of nanocellulose increased with increasing the surfactant concentration of the medium.The drug loading capacity of nanocellulose in surfactant medium was significantly higher (7.73mg/g) when compared to the drug loading capacity (3.35mg/g) in methanolic medium. The nanocellulose drug loaded in surfactant medium (TW/CNC) showed higher drug 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 increased stability of curcumin in acidic medium, all the nanoparticles showed higher drug 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. UV-visible spectra revealed that the curcumin retained its chemical activity after in vitro release. From these findings, it is believed that the incorporation of curcumin into nanocellulose in surfactant medium provides a promising approach for delivery of curcumin to stomach and upper intestinal tract.

Key words: Nanocellulose, Curcumin, Tween 20, Bioavailability

Introduction

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 formulation. Today, nanotechnology is being applied in the areas of drug delivery and formulations known as 'Nanopharmaceutics'. As nanoparticles are accepted by cells more effectively than larger microparticles, they can be used as efficient transport and delivery systems. Nanostructured-based drug delivery systems offer many advantages over conventional drug delivery systems, including their ability to pass through the narrow capillary vessels due to their smaller size, ability to penetrate cells and tissue gap to arrive at target organs and to provide controlled drug release over a prolong period (Rizvi and Saleh 2017). Nanoparticles exhibit greater drug uptake compared to microparticles. The small dimensions of nanoparticles compared to their bulk counterparts bring drug more closer to the surface of the particle, which 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 exposure and the basics of the interaction of nanoparticles with living cells, organs and organisms is still limited. In this respect, materials and strategies which minimize the possibilities of causing adverse and toxic effectshave been developed for therapeutic delivery, in particular the choice of biodegradable nanoparticles with a limited life span which avoid the 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

beclomethasone, diproprionate, indomethacin and itraconazol from previous studies (Löbmann and Svagan 2017. The unique physico-chemical, rheological and barrier properties of nanocellulose provide them to stabilize air/water and oil/water interfaces (Löbmann and Svagan 2017). Also, their large surface area-to-volume ratios offer possibilities for positive molecular interactions with poorly-soluble drugs. The acid hydrolysis is an economical method that have been extensively used for nanocellulose extraction from various natural sources such as plant fiber, wood fiber, microcrystalline cellulose, algae, tunicate and bacteria. Nanocellulose synthesized using acid hydrolysis has a size range from 10 nm to 350 nm (Phanthong et al. 2015; 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 literature it was reported that the optimum size for nanoparticle as carriers for drug delivery is approximately 100 nm (Rizvi and Saleh 2017). Thereby, acid hydrolysis can be used to produce the nanocelluloses with optimal size which is suitable for drug delivery.

Curcumin is a polyphenol obtained from the plant *Curcumalonga*. Curcumin has received worldwide attention due to its promising anti-cancer properties (Ibrahim et al. 2018). Also, it can cause a high rate of *Helicobacter pylori* eradication which was identified as a group I 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 and low bioavailability. Several strategies have been developed to enhance the solubility and 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 method to overcome the challenges associated with poor water solubility of drugs. However the miscibility and stability of the dispersion are main limitations related with the development of

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 surfactant molecules are dissolved in water at a concentration greater than critical micelle concentration (cmc), they form spherical form of aggregates known as micelles. The solubility of hydrophobic drugs in nonionic surfactant solutions is greater than compared to solubility in ionic 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 their contact with water, and the hydrophilic heads region faces the outside surface of the micelle in order to maximize their contact with water.

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 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 investigation on the enhancement of bioavailability of curcumin. From our previous study, curcumin and nonionic surfactant were incorporated into nanocellulose reinforced chitosan hydrogel and studied the drug delivery behavior. We observed that the cumulative drug release 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 hydrogel (Gunathilake et al. 2018). Furthermore, the addition of nanocellulose enhanced the mechanical strength and swelling behavior of the hydrogel (Sampath et al. 2017).However, the problems encountered in previous study were the decrease of encapsulation efficiency of the drug with increasing of surfactant concentration and incomplete drug release profiles. Polymer phase of chitosan hydrogel acted as a diffusion barrier against movement of drug in the previous

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

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

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 surfactant and the entrapment efficiency of drug delivery systems. Increasing the alkyl chain 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 hydrophobic drugs. This is due to the fact that the surfactants having lower HLB values are more lipophilic and less water soluble. But, the surfactants with higher HLB values such as Tween 20 (HLB= 16.7) helps to improve the release rates of hydrophobic drugs to a desired extent. According to the findings of this study, we envision that our proposed curcumin/nonionic surfactant-incorporated cellulose nanoparticle drug delivery system has great potential for enhancing the bioavailability of curcumin.

Experimental methodology

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).

Methodology

Extraction of curcumin from turmeric

Curcumin was extracted from turmeric (rhizomes of Curcuma longa) by solvent extraction 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.

FTIR study

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

X-ray diffraction

The crystallinity degrees of curcumin, nanocellulose and curcumin incorporated nanocelulosewere studied using X-ray diffractometer (PANalytical EMPYREAN diffractometer). The samples were dried and powdered before they were analyzed. The samples were exposed to Cu Kα radiation generated at 40 mA, 40 kV, a 2θ angle of 5-60°, and a scan rate of 6°/min.

Drug loading

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

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)).

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

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.

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

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

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

202

203

204 *Solubility studies of curcumin in distilled water*

205 To determine the solubility of curcumin in distilled water, an excess amount of curcumin extract
206 was added to 30 mL of distilled water and mixed with different concentrations of Tween 20
207 (0.8%, 1.6%, 2.4%, 3.2%, 4%, 4.8%,5.6% (w/v)). Then, the mixtures were stirred (350 rpm)
208 using magnetic stirrer for 12h. Samples were covered in order to prevent photo-degradation.
209 After that, the solutions were centrifuged (10,000 rpm for 10 min) to separate the undissolved
210 curcumin and the dissolved curcumin was determined using UV-Vis spectroscopic method.

211 *Drug activity*

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

Results and discussion

FTIR study

Fig. 1 displays the FTIR spectra of curcumin, nonionic surfactant, nanocellulose, drug loaded nanocellulose in surfactant medium and drug loaded nanocellulose in methanolic medium. The IR spectrum of curcumin derived from turmeric is more similar to the IR spectrum of crystalline curcumin derived from turmeric powder, which was reported in previous studies(Bich et al. 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 al. 2018). In the FTIR spectrum of curcumin, the highest frequency bands observed within 2700-3000 cm^{-1} region are assigned to the aromatic C−H stretches (Kolev et al. 2005). The IR bands at 815 cm^{-1} and 720 cm^{-1} belongs to the ν(C-H) out of plane vibration of the aromatic ring (Kolev et al. 2005). In the range of 700-500 cm^{-1}, we couldsee deformation vibrations of both benzene rings and the out of plane vibrations of both OH groups, which are at 607 cm^{-1} and 546 cm^{-1} (Bich et al. 2009). These aromatic groups provide much of the hydrophobicity to the curcumin 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^{-1} appeared due to the C=O vibrations (Bich et al. 2009). The most prominent band in the IR spectrum is at 1509 cm^{-1} can be attributed to highly mixed vibrations ($^{\nu}C=O$, $^{\delta}CC^{10}C$, $^{\delta}CC=O$)(Bich et al. 2009). These ketone group exhibits keto-enoltautomerism forming a predominant keto form in acidic and neutral media and stable enol form in alkaline medium. Therefore, it is insoluble in water under acidic or neutral pHs but dissolves in alkaline conditions(Jankun et al. 2016).

In the FTIR spectrum of the nonionic surfactant, the peak around 3522 $cm^{-1}$is due to the hydrogen bonded O-H stretching vibrations. The peaks at 2935 cm^{-1} and 2883 cm^{-1} are due to the asymmetric and symmetric methylene stretching vibrations. The 1741 cm^{-1} peak represents the carbonyl group from R-CO-O-R and the peak at 1667 cm^{-1} attributed to the carbonyl stretching. The most prominent peak at 1103 cm^{-1} 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 cm^{-1} is due to the characteristic –OH stretching from vibrations in the intra-and intermolecular hydrogen-bonded hydroxyl groups (Gunathilake et al. 2017). The band at 2901 cm^{-1} is corresponded to aliphatic saturated CH-stretching in the glucose units. Other peaks detected include the adsorption band at 1636 cm^{-1} which is due to the absorption of water onto cellulose, the peak at 1428 cm^{-1} associated with CH_2 symmetrical bending and scissoring motion in cellulose, the peak at 1159 cm^{-1} is due to the asymmetrical bridge C–O–C stretching from theglycosidic bond, the band at 1029 cm^{-1} representing stretching of the glucopyranose unit and the peak at 896 $cm^{-1}$is typical of the β-glycosidic linkage in cellulose (Ching & Ng 2014, Sahlin et al. 2018).

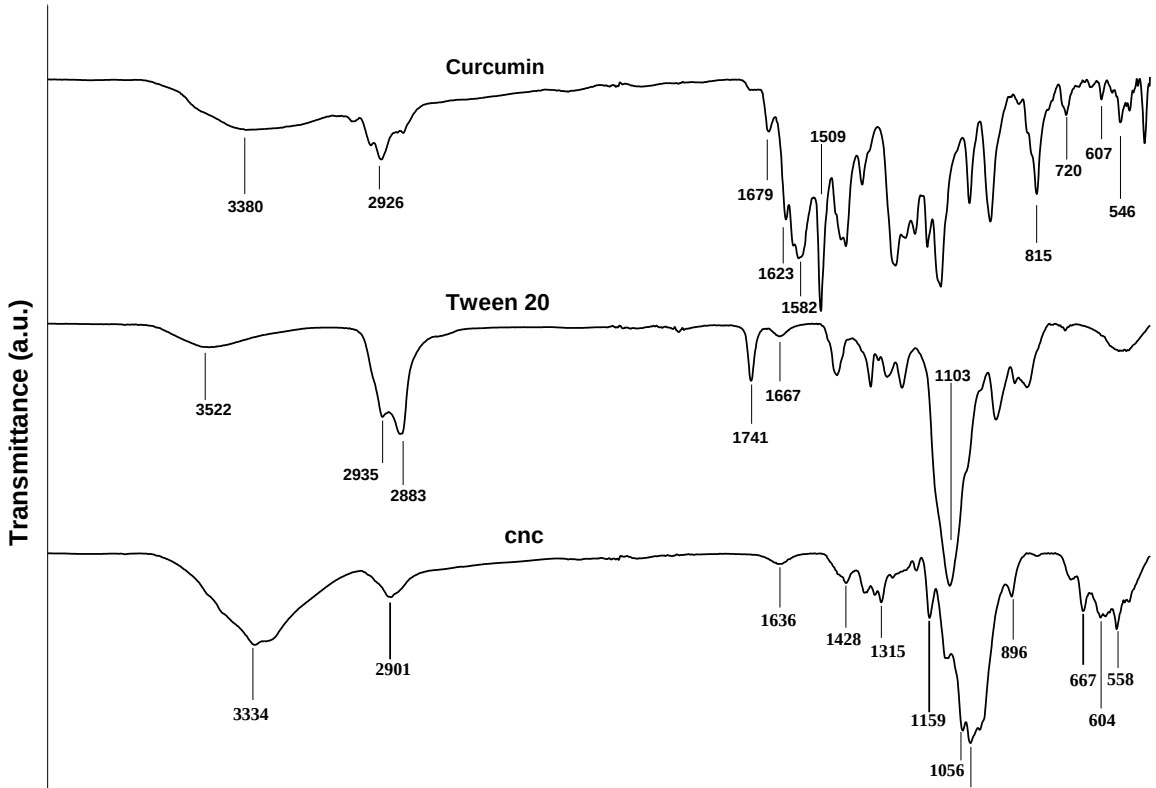

Fig. 1 FTIR spectra of curcumin, nonionic surfactant and nanocellulose (CNC)

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

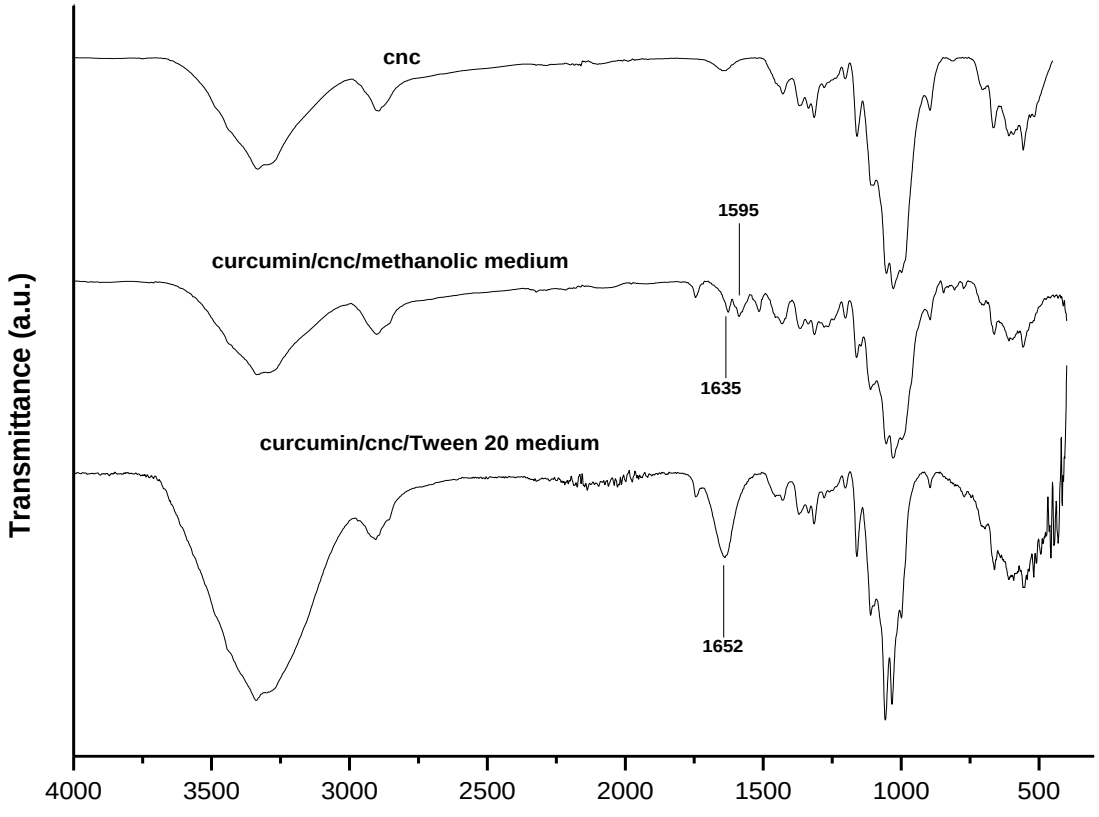

Fig. 2 FTIR spectra of cellulose nanoparticles, curcumin loaded cellulose nanoparticles in methanolic medium and curcumin loaded cellulose nanoparticles in surfactant medium

X-ray diffraction analysis

The X-ray diffraction (XRD) patterns of the curcumin, nanocellulose and curcumin incorporated nanocellulose were investigated via powder XRD analysis (Fig. 3). As shown in the diffractogram, the CNC exhibited peaks around 2θ = 15°, 16.5°, 20.5°, 22.5°, 34.5°, which respectively represent the (1-10), (110), (102), (200), and (004) crystallographic planes of a typical cellulose I structure (Novo et al. 2015).

The diffraction pattern of curcumin exhibited peaks at angles 8.59°, 11.24°, 17.35°, 18.19, 19.46, 21.28°, 23.44°, 24.58°, 25.59°, 26.17°, 26.79°, 27.39°, 28.27°, 28.97°,31.6° and 36.23° which correspond to curcumin polymorph 1, indicating that curcumin sample exists as form 1(Poornima et al. 2016; Sanphui et al. 2011). Curcumin exhibited well-defined sharp, narrow diffraction peaks between 10° and 30°, indicating the high crystalline structure (Cheng et al. 2017; Singh et al. 2014). The diffractogram of curcumin incorporated nanocellulose showed peaks which corresponded to both nanocellulose and curcumin. However, the peaks intensity of CNCs decreased after the incorporation of curcumin as shown in Fig. 3 which indicates a low crystallinity of the curcumin incorporated nanocellulose relative to the pure CNCs. From these results, it can be concluded that the interaction of the hydroxyl groups of CNCs with curcumin breaks the intermolecular and intramolecular hydrogen bonds of the CNCs and modifies the

crystalline regions of the CNCs. Therefore, the crystallinity of CNCs decreases by the interaction of curcumin with both the inner and surface hydroxyl groups.

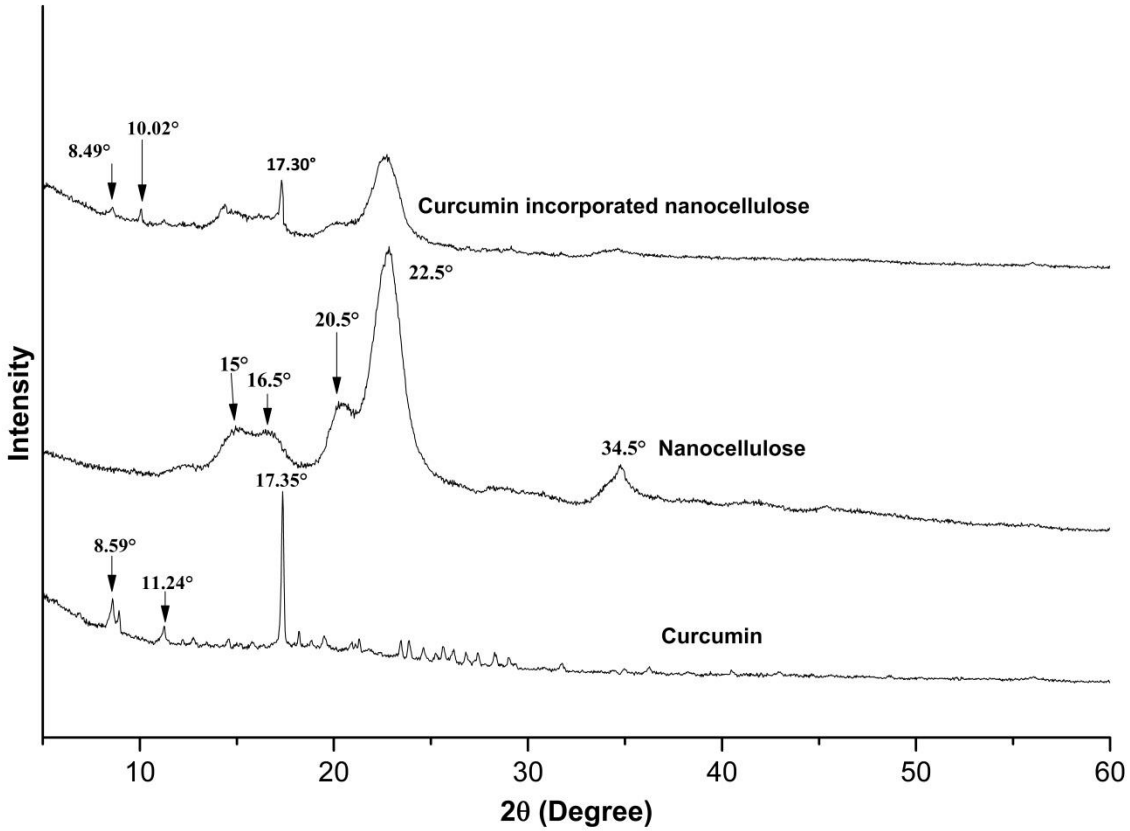

Fig. 3 X-ray diffraction (XRD) patterns of curcumin, nanocellulose and curcumin incorporated nanocellulose

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

nonionic surfactant (0.8%,1.6%, 2.4%, 3.2%, 4%, 4.8%, 5.6% (w/v)) for the drug loading process. For thecomparison of drug loading capacity, curcumin was incorporated into nanocellulose in methanolic medium. (Here, the same concentration of curcumin and same amount of nanocellulose were used as similar to those used in surfactant solutions). As shown in Fig. 4, the drug loading capacity of nanocellulose increased with increasing the surfactant concentration of the medium.It increased from 0.1mg/g to 7.73 mg/g with increasing the surfactant concentration from 0% to 4%. The highest drug loading capacity of nanocellulose was given at which the surfactant concentration showed the highest solubility of curcumin. After that, the loading capacity of nanocellulose was not increased with increasing the surfactant concentration. For curcumin dissolved in methanolic medium,the maximum drug loading capacity was 3.35 mg/g. It showed almost two fold increases in the drug loading capacity of nanocellulose in surfactant medium compared to methanolic medium. This may be due to the formation of micelles facilitating the incorporation of drug into nanoparticles.

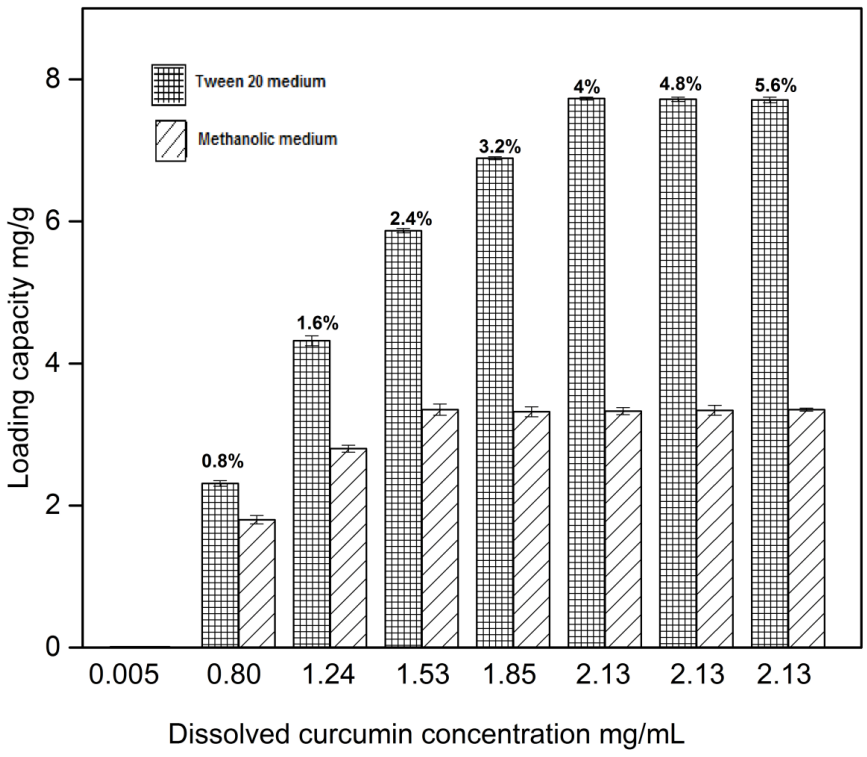

Fig. 4 Drug loading capacity of nanocellulose in surfactant and methanolic medium

Surfactant micelles are formed at the critical micelle concentration in aqueous medium due to the 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 shape and contain around 60-100 surfactant molecules. It has the hydrophobic oil-like core 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 aqueous medium, which result in an increase in the apparent aqueous solubility of the drug. There are number of possible locations of solubilization for a drug in a micelle, depending on 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).

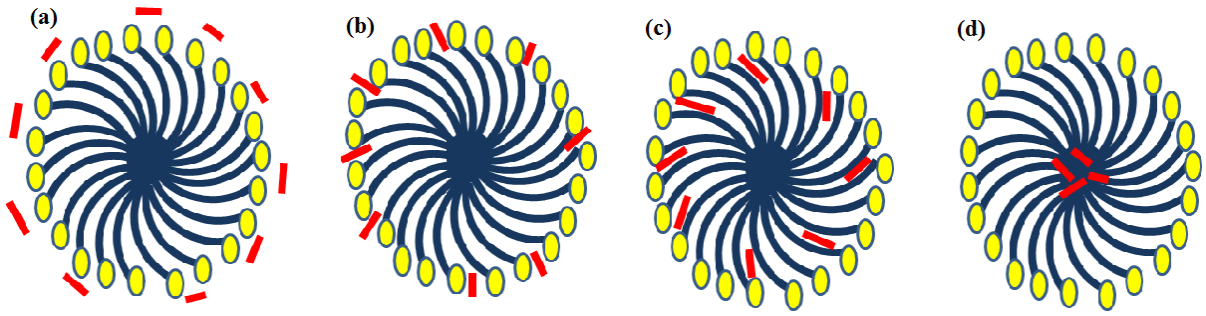

Fig. 5 Possible locations of drug in surfactant micelles, based on the drug hydrophobicity.The red bold lines represent the drug molecules, yellow circles display the surfactant heads and blue curved lines represents the hydrophobic tails of surfactants

Many researches have been discussed chemical strategies for surface modification of 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. In suchscenarios, physical adsorption such as charge–charge interactions, association of hydrophobic groups, and hydrogen bonding plays a key role in their association behavior. Adsorbing nonionic surfactantsis a feasible methodto enhance the compatibility of the hydrophilic cellulosic material with the typically hydrophobic materials. The hydrophilic groups of nonionic surfactants may have affinity for adsorption to the cellulose due to the hydroxyl groups of cellulose (as shown in Fig. 6). However, not all the hydroxyl groups present in cellulose nanocrystals are accessible. This is because some of them are oriented towards the 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 primary hydroxyl on C6 is most reactive(Gan et al. 2017).

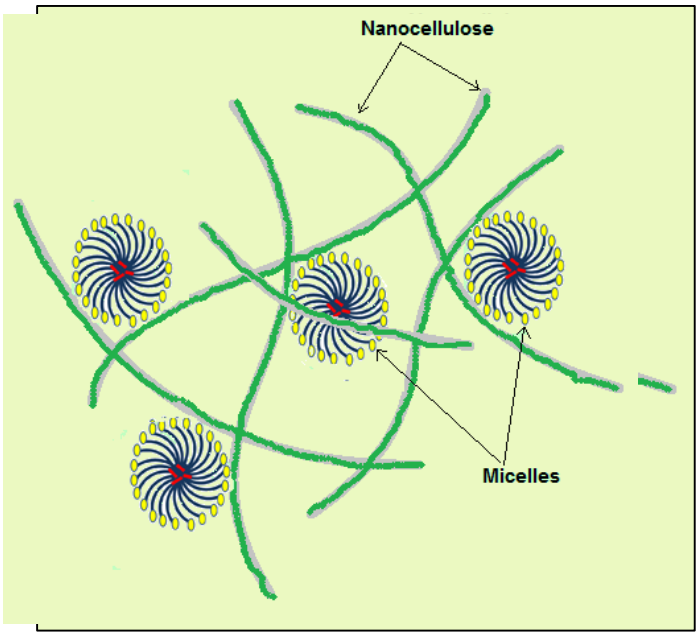

Fig. 6 Possible interactions of surfactant micelles with nanocellulose

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

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 medium (METH/CNC).The nanoparticles drug loaded in surfactant medium reached to equilibrium drug release stage at around 270 minutes (in SGF medium) and 350 minutes (in PBS 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 drug loaded in both surfactant and methanolic medium showed lower drug release in PBS medium. The reason for this may be due to the less stability of curcumin in neutral or pHs above neutral. In addition, previous studies indicated that the stability of curcumin can be strongly improved by lowering the pH of the medium (Khalkhali et al. 2015; Rao and Rao 2011; Wang et al. 1997).

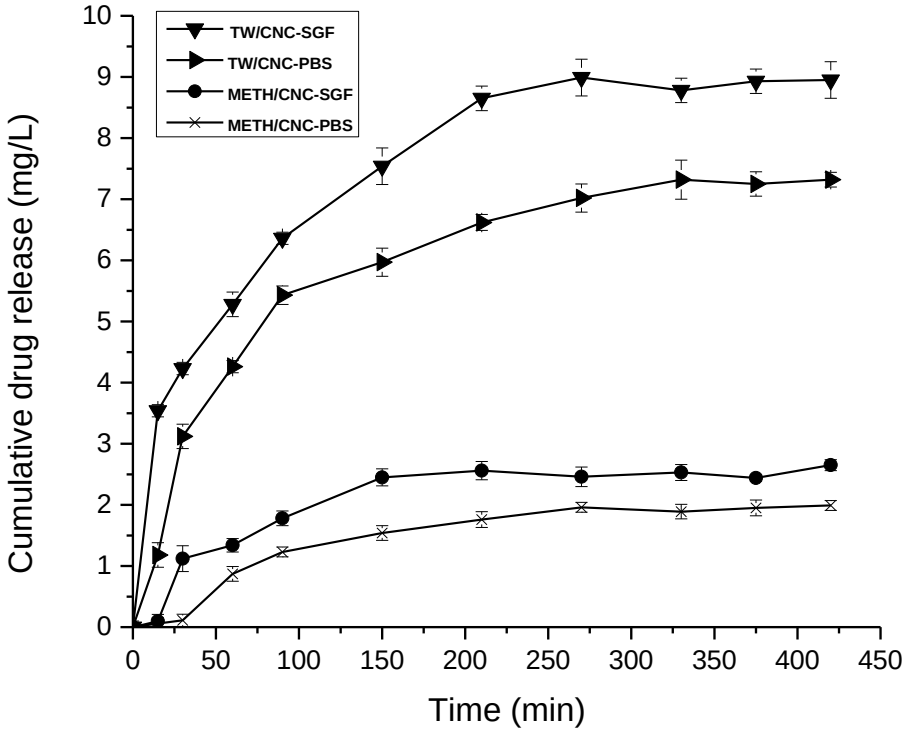

Fig. 7 Drug release from cellulose nanoparticles (TW/CNC and METH/CNC) to SGF and PBS media

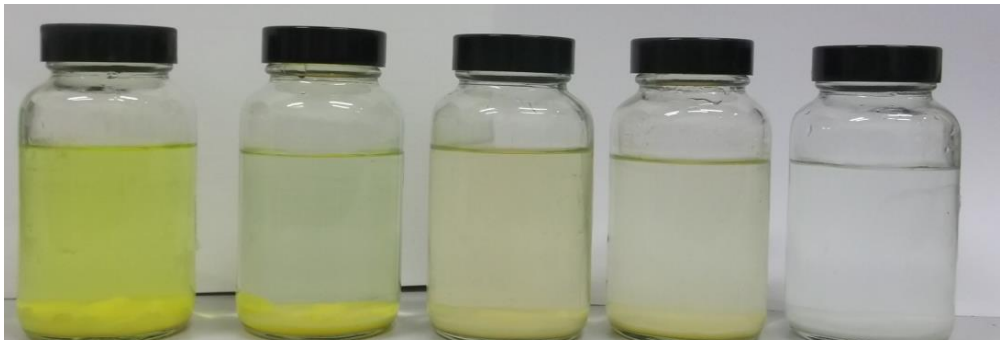

Fig. 8 Drug release from: **a** W/CNC into SGF medium; **b** METH/CNC into SGF medium; **c** TW/CNC into PBS medium; **d** METH/CNC into PBS medium and**e** control

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 the particle size gets smaller, their surface area to volume ratio gets larger. This would imply that more of the drug is closerto 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 emptying times for healthy individuals at 1, 2, and 4 h are >90%, 60% and 10%, respectively(Jobe et al. 2013). Due to the highest drug release shown in SGF medium, and faster drug release, these drug delivery systems are more suitable for stomach delivery of curcumin.

The higher drug release by TW/CNC-SGF and TW/CNC-PBS nanoparticles, which immersed in 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 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 affinity towards aqueous medium and thereby improve the solubility of the drug in SGF and PBS medium. In contrast, for METH/CNC-SGF and METH/CNC-PBS conditions, the interactions 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,

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

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

467 *Drug activity*

468 The drugs are often inevitable and therefore, the chemical reactivity related to biological activity
469 of the drug is most important parameter to be concerned when selecting a drug delivery carrier.
470 For some drug delivery systems, drugs deteriorate due to the destructive interactions with the
471 carrier molecules. In order to prevent this, the carrier should be prone to destructive interactions
472 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 nm, which can be assigned to the low-energy π- π* excitation of the chromophore, that formed due to the enolization of the diketone group and conjugation between the π-electron clouds of the two vinylguaiacol (Zsila et al. 2004). In this study, curcumin is in contact with nonionic surfactant, cellulose, simulated gastric fluid and phosphate buffer saline solution. Fig. 9b represents the UV-Vis spectra of curcumin, which is released from nanocellulose (drug loaded in surfactant medium) into simulated gastric medium and phosphate buffered saline solution. It is 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 spectra of curcumin, which is released from nanocellulose (drug loaded in methanolic medium) into simulated gastric medium and phosphate buffered saline solution. Since the released amount of drug from these nanoparticles is very low, the absorption peak around 427 nm is slightly small compared to the absorption maximum of Fig. 9b. However, in this situation the absorption maximum (427 nm) also remain unchanged (without shifting upward or downward direction of 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 any denaturation reaction with drug containing media or with carrier molecules (nanocellulose).

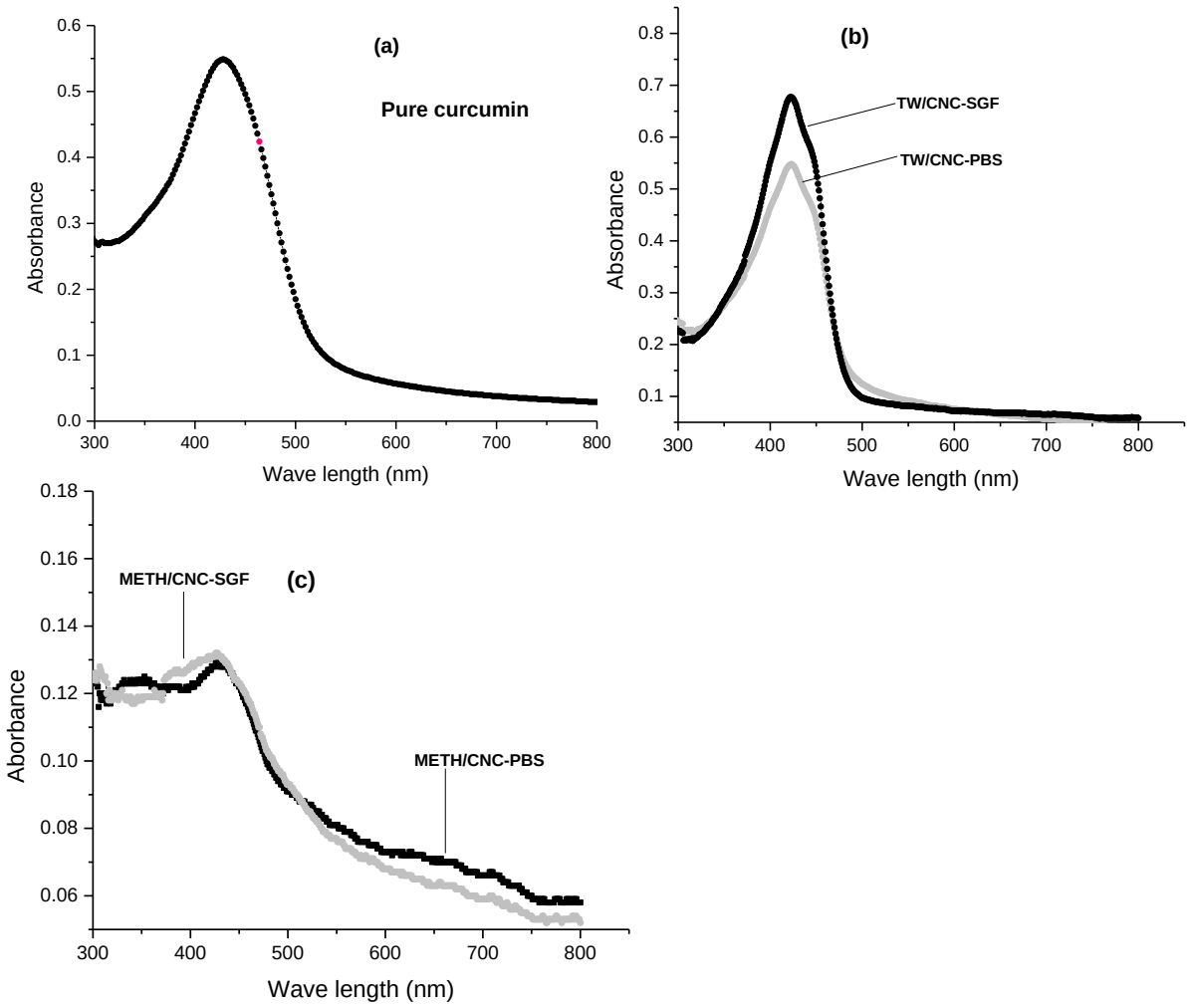

Fig. 9 UV-Vis spectra of: **a** pure curcumin; **c**urcumin release from TW/CNC into PBS and SGF media; **c** curcumin release from METH/CNC into PBS and SGF media

Solubility studies

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 water with the presence of nonionic surfactantat 37° C. As shown in Fig. 10 the dissolution rate of curcumin increased with increasing thesurfactant concentration in dissolution medium and the 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 facilitating the dispersion of drug by micelle solubilization in the bulk medium. The amount of surfactant needed depends on the critical micelle concentration and the solubilization capacity for curcumin in surfactant micelles.It may be the reason for the fact that the amount of dissolved curcumin did not increase above 4% of surfactant in distilled water. It reached a minimum surface tension at 4% of surfactantwith no significant change at higher concentrations. Similar 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 mg/mL in 20% aqueous solution of Tween 20. From our previous study, we achieved the solubility of curcumin with an upper limit of 3.014 ± 0.041 mg/mL in the presence of 3.2% (w/v) Tween 20 in simulated gastric medium. This may be due to the higher stability of curcumin in acidic medium compared to neutral or alkaline medium. Previous studies have also confirmed 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).

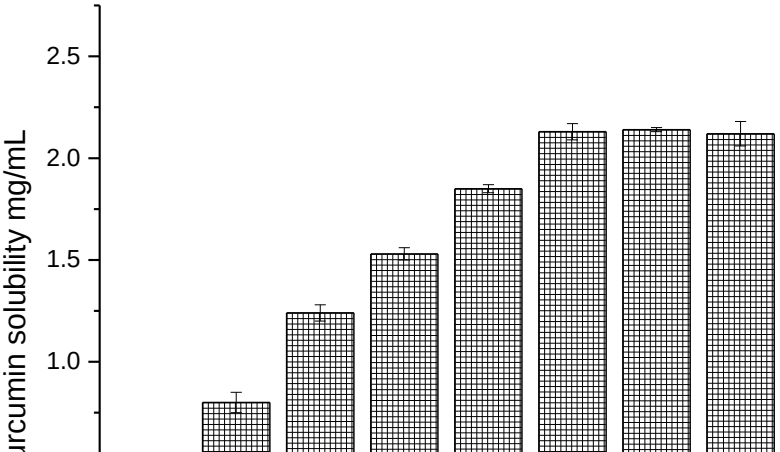

Fig. 10 Solubility of curcumin in distilled water with different concentrations of nonionic surfactant

Conclusion

Cellulose nanocrystals were synthesized using microcrystalline cellulose via sulphuric acid 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 nanoparticles was verified through the FTIR characterization. The drug loading capacity of nanocellulose increased from 0.1mg/g to 7.73 mg/g with increasing the surfactant concentration from 0% to 4%. This may be due to the formation of micelles facilitating the incorporation of 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 nanoparticles drug loaded in surfactant medium (TW/CNC) compared to the nanoparticles drug 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 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 retained its structural integrity after release to the SGF and PBS media, which is a critical

requirement for preserving drug activity. In conclusion, the enhancement of bioavailability of curcumin using curcumin/nonionic surfactant-incorporated cellulose nanoparticles would represent an important step forward in nanopharmaceutical field.

Acknowledgments

The authors would like to acknowledge the financial support from the Ministry of Education of Malaysia FRGS-FP053-2015A and PRGS-PR005-2017A and University of Malaya PG160-2016A, ST017-2018, GPF 033A-2018, RU018I-2016 and International Funding AUA Scholars IF025-2018 for the success of this project.

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

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Figure 1

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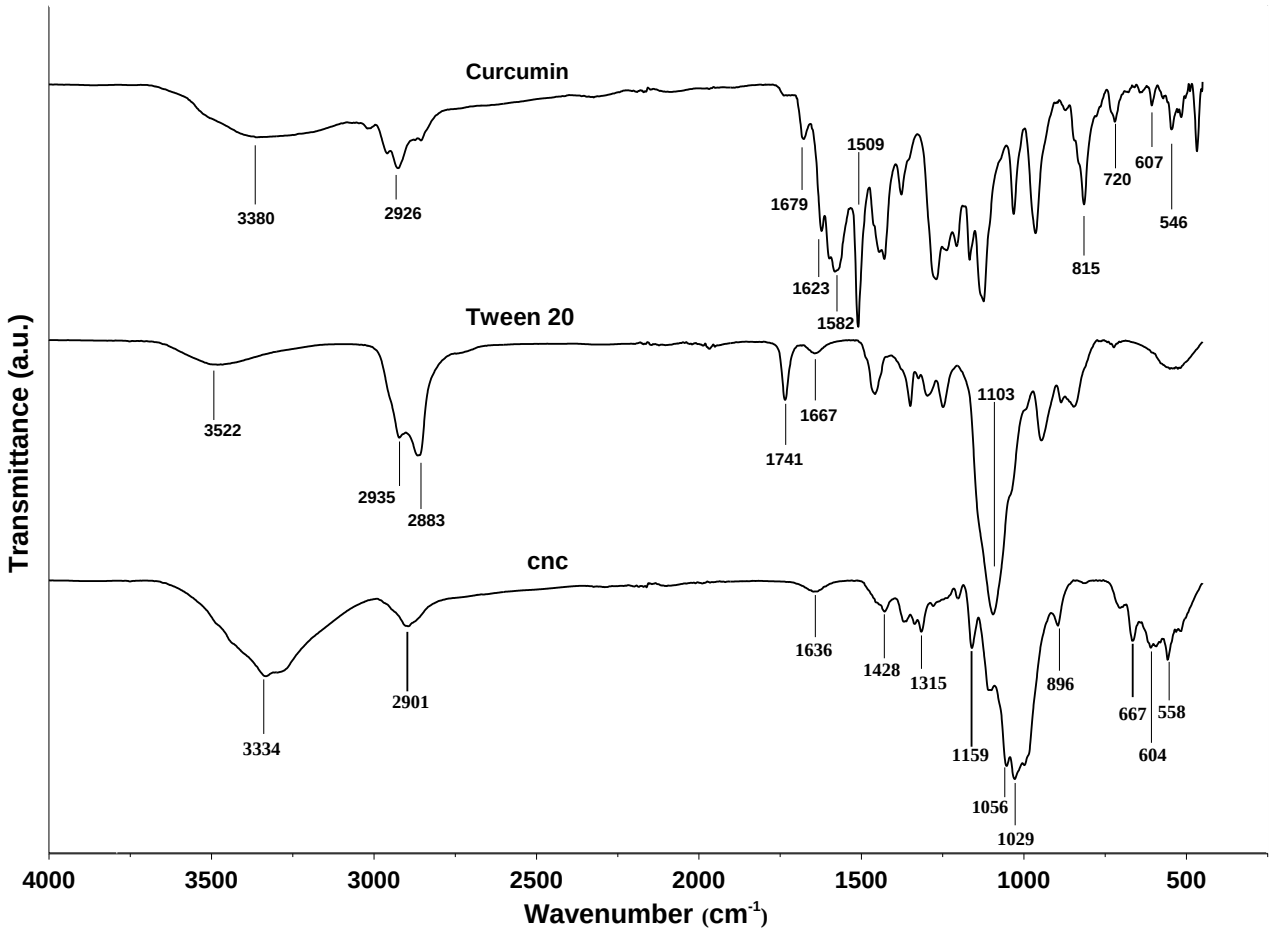

Fig. 1 FTIR spectra of curcumin, nonionic surfactant and nanocellulose (CNC)

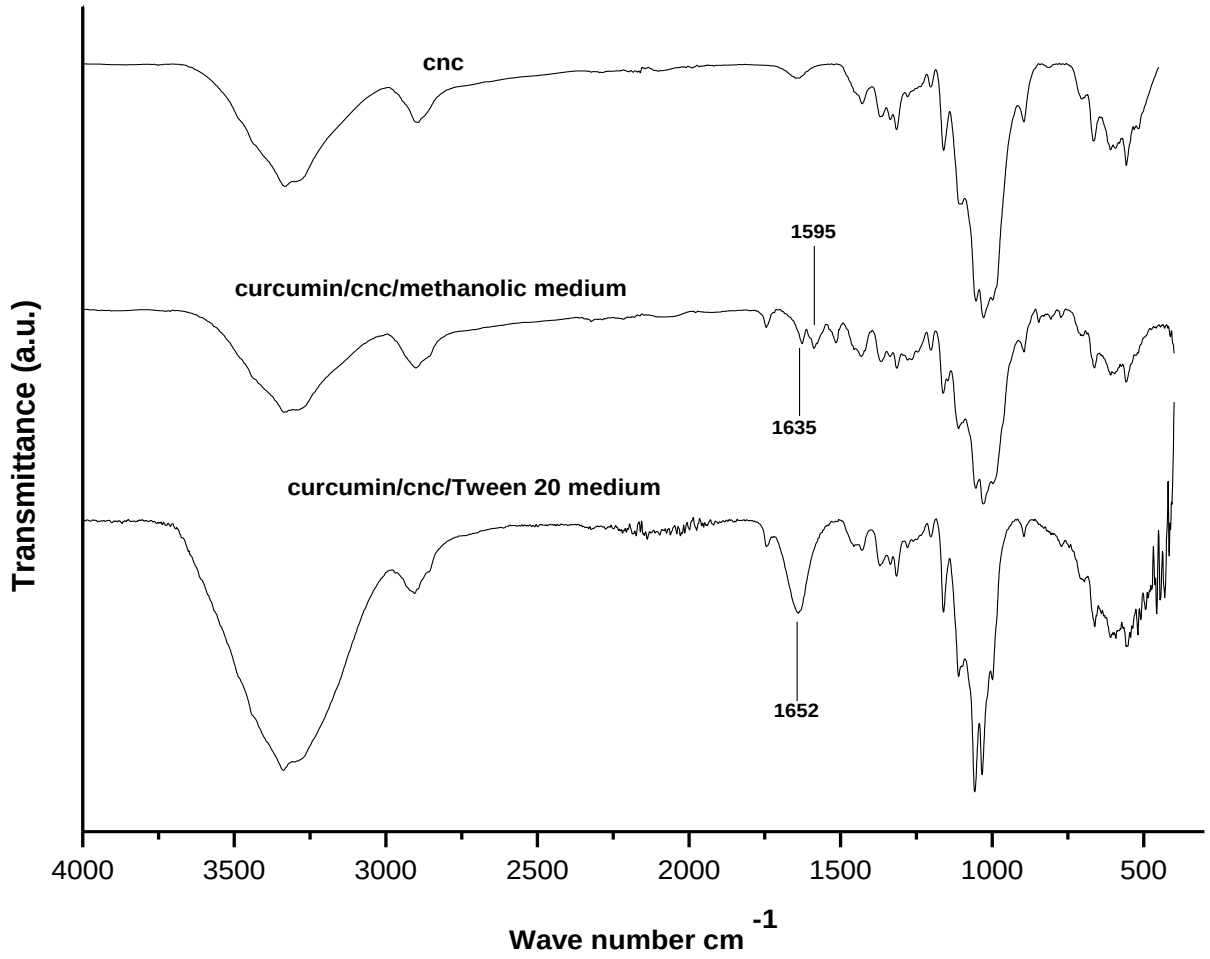

Fig. 2 FTIR spectra of cellulose nanoparticles, curcumin loaded cellulose nanoparticles in methanolic medium and curcumin loaded cellulose nanoparticles in surfactant medium

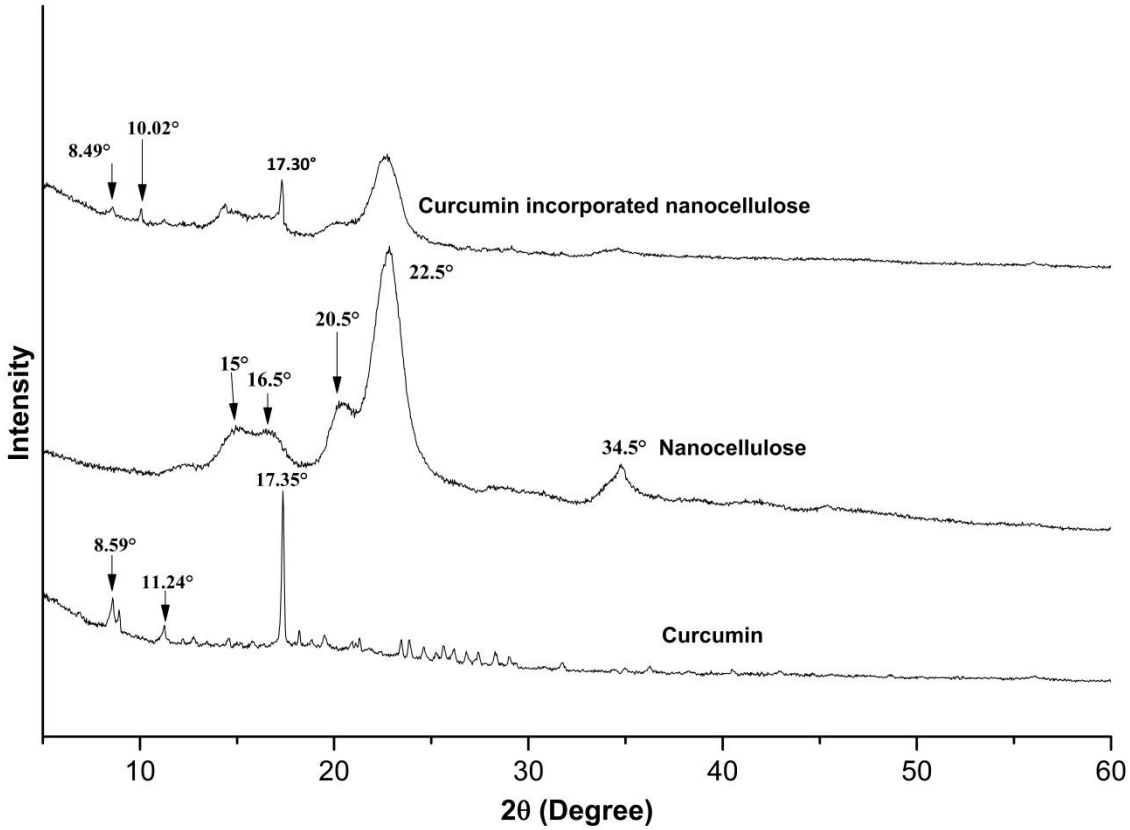

Fig. 3 X-ray diffraction (XRD) patterns of curcumin, nanocellulose and curcumin incorporated nanocelulose

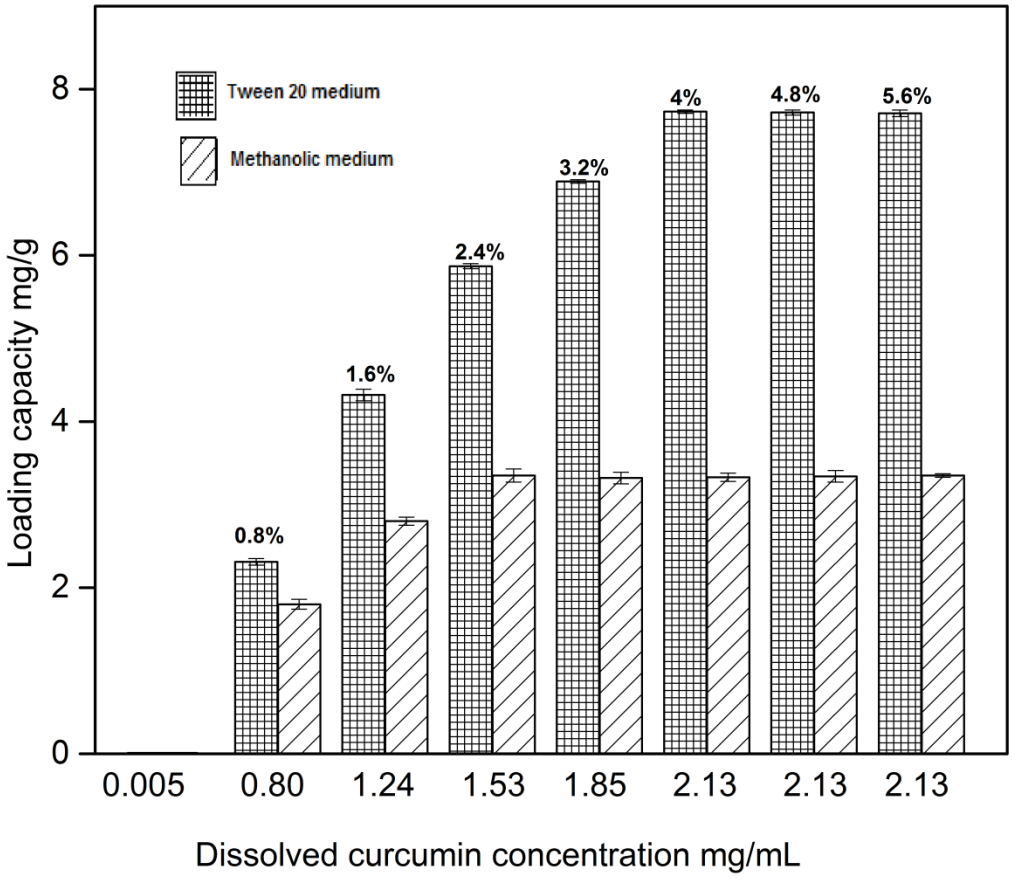

Fig. 4 Drug loading capacity of nanocellulose in surfactant and methanolic medium

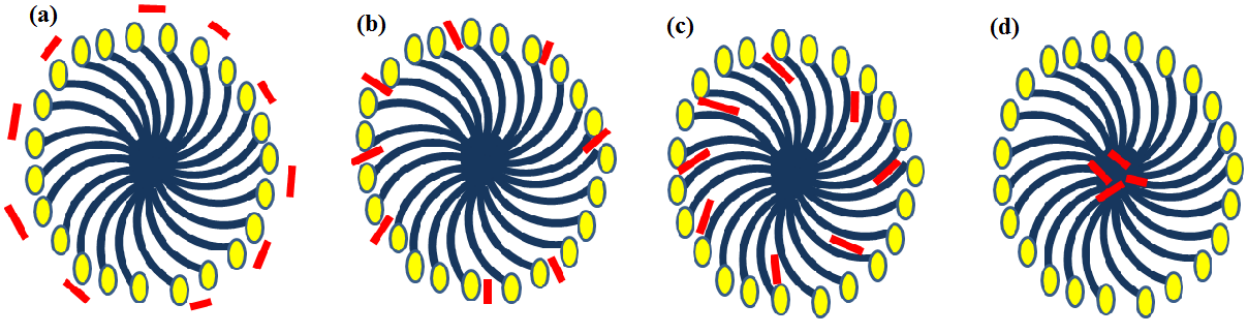

Fig. 5 Possible locations of drug in surfactant micelles, based on the drug hydrophobicity. The red bold lines represent the drug molecules, yellow circles display the surfactant heads and blue curved lines represents the hydrophobic tails of surfactants

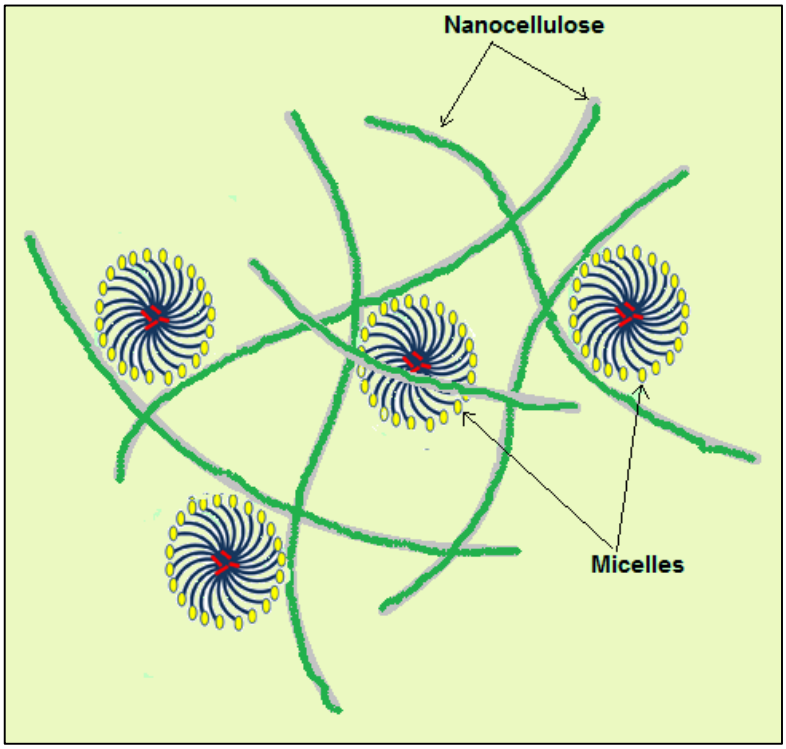

Fig. 6 Possible interactions of surfactant micelles with nanocellulose

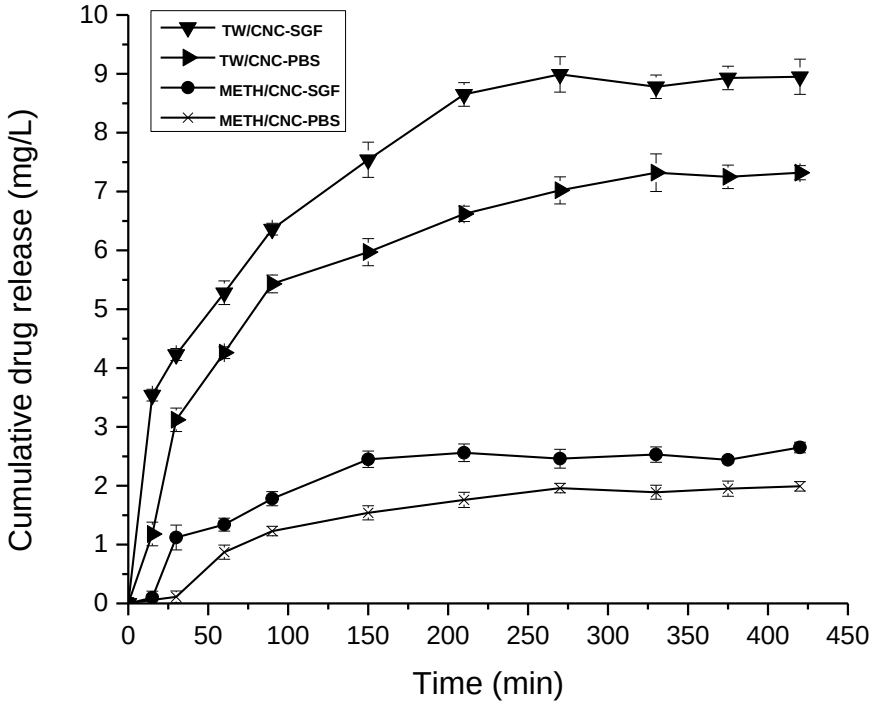

Fig. 7 Drug release from cellulose nanoparticles (TW/CNC and METH/CNC) to SGF and PBS media

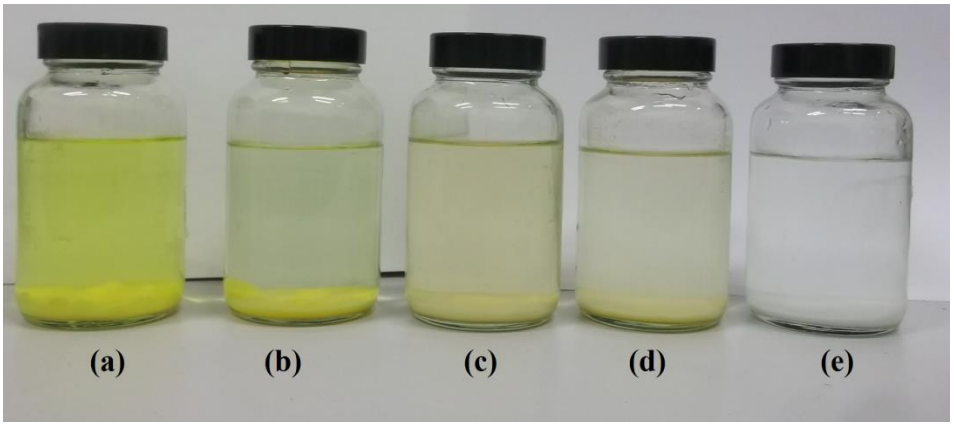

Fig. 8 Drug release from: **a** TW/CNC into SGF medium; **b** METH/CNC into SGF medium; **c** TW/CNC into PBS medium; **d** METH/CNC into PBS medium and **e** control

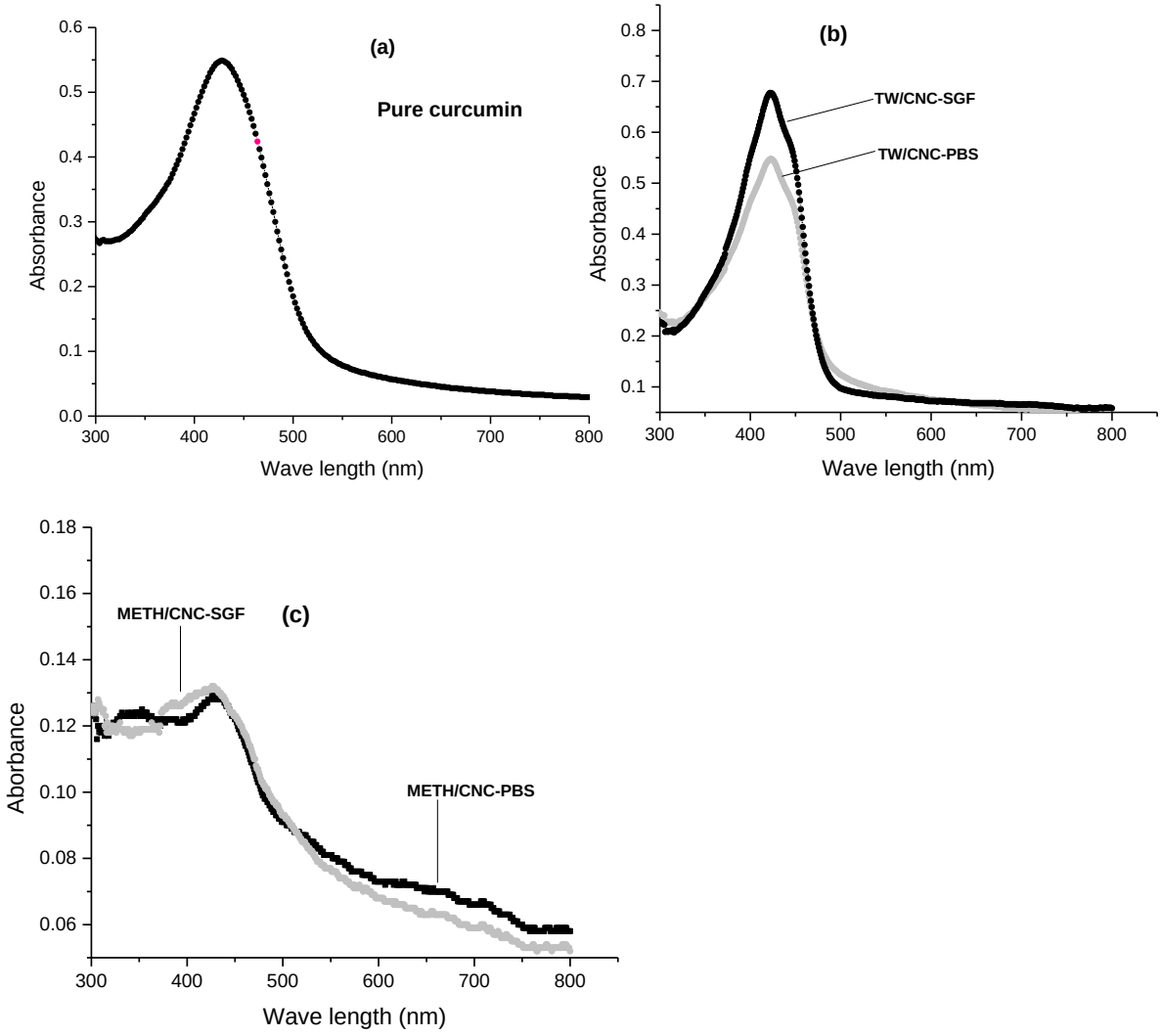

Fig. 9 UV-Vis spectra of: **a** pure curcumin; **b** curcumin release from TW/CNC into PBS and SGF media; **c** curcumin release from METH/CNC into PBS and SGF media

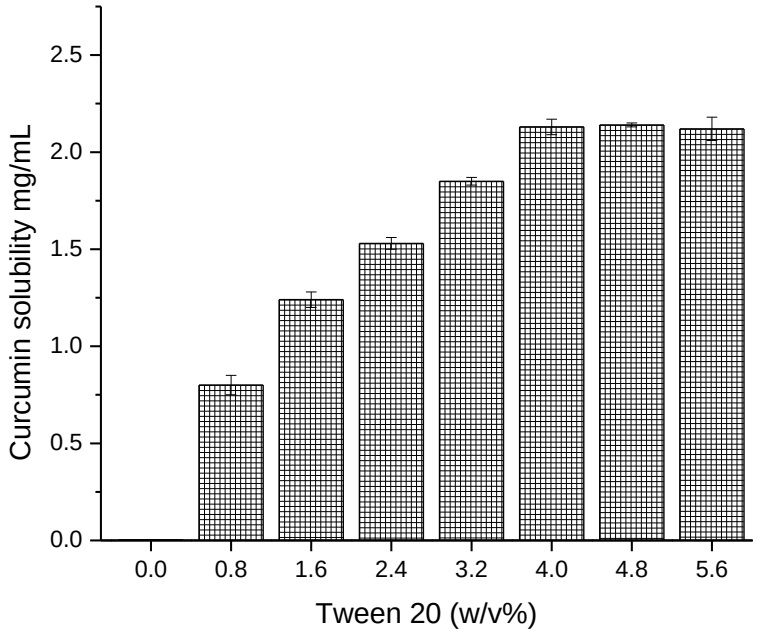

Fig. 10 Solubility of curcumin in distilled water with different concentrations of nonionic surfactant

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

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