

Influence of a nonionic surfactant on curcumin delivery of nanocellulose reinforced chitosan hydrogel

Article

Accepted Version

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To link to this article DOI: <http://dx.doi.org/10.1016/j.ijbiomac.2018.06.147>

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Available at <https://centaur.reading.ac.uk/102063/>

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4 **1 Influence of a nonionic surfactant on curcumin delivery of nanocellulose reinforced**
5 **2 chitosan hydrogel**
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7 **3**

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21 17

22
23 **18 Abstract**
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25
26 19 Nanocellulose reinforced chitosan hydrogel was synthesized using chemical crosslinking method
27
28 20 for the delivery of curcumin which is a poorly water-soluble drug. Curcumin extracted from the
29
30 21 dried rhizomes of *Curcuma longa* was incorporated to the hydrogel via in situ loading method. A
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32 22 nonionic surfactant (Tween 20) was incorporated into the hydrogel to improve the solubility of
33
34 23 curcumin. After the gas foaming process, hydrogel showed large interconnected pore structures.
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36 24 The release studies in gastric medium showed that the cumulative release of curcumin increased
37
38 25 from 0.21% ± 0.02% to 54.85% ± 0.77% with the increasing of Tween 20 concentration from
39
40 26 0% to 30% (w/v) after 7.5 h. However, the entrapment efficiency percentage decreased with the
41
42 27 addition of Tween 20. The gas foamed hydrogel showed higher initial burst release within the
43
44 28 first 120 minutes compared to hydrogel formed at atmospheric condition. The solubility of
45
46 29 curcumin would increase to 3.014 ± 0.041 mg/mL when the Tween 20 concentration increased
47
48 30 to 3.2% (w/v) in simulated gastric medium. UV-visible spectra revealed that the drug retained
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50 31 its chemical activity after in vitro release. From these findings, it is believed that the nonionic
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59 32 surfactant incorporated chitosan/nanocellulose hydrogel can provide a platform to overcome
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61 33 current problems associated with curcumin delivery.
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64 34 *Key words:* Nanocellulose, chitosan, hydrogel, curcumin, Tween 20
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72 73 37 **1. Introduction** 74 75

76 38 Curcumin is a lipophilic polyphenol compound which is derived from rhizomes of *Curcuma*
77
78 39 *longa*. *Curcumin* is usually a mixture of three curcuminoids (curcumin, demethoxycurcumin, and
79
80 40 bisdemethoxycurcumin) and volatile oil [1]. Among all former studies on antibacterial activity of
81
82 41 curcumin, the most promising result showed is its effectiveness in against *Helicobacter pylori*
83
84 42 [2]. *H. pylori* is a microaerophilic bacterium which lives in the sticky mucus that lines
85
86 43 the stomach and it has attracted great attention as a main cause of peptic ulcer disease.
87
88 44 International agency for research on cancer has defined *H. pylori* as a group I carcinogenic agent
89
90 45 of human gastric cancer [3]. Recent studies on the inhibition of *H. pylori* using curcumin by
91
92 46 Mahady, Pendland, Yun and Lu [4] showed that both of the curcumin and methanolic extract of
93
94 47 turmeric rhizome has inhibited the growth of 19 different strains of *H. pylori*. Furthermore, De,
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96 48 Kundu, Swarnakar, Ramamurthy, Chowdhury, Nair and Mukhopadhyay [3] mentioned that the
97
98 49 minimum inhibitory concentration of curcumin for 23% strains of *H. pylori* was about 10
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100 50 $\mu\text{g/mL}$ and for 58% strains, it was 15 $\mu\text{g/mL}$. Sometimes antibiotics are likely to be associated
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102 51 with adverse side effects on the host such as allergic reactions, hypersensitivity and immune-
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104 52 suppression. Therefore, there is an increasing demand to develop an alternative antimicrobial
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106 53 drugs using medicinal plants for the treatments of infectious diseases [5].
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115 54 Although curcumin exerts various biological effects, its limited aqueous solubility and rapid
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117 55 presystemic metabolism has restricted its bioavailability. Therefore, the advanced drug delivery
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119 56 systems like nanoparticles, liposomes, micellar formulations, cyclodextrin inclusion complexes,
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121 57 microemulsions and different hydrogel based delivery systems have been developed to
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123 58 circumvent their bioavailability issues [6-8]. Due to the variations on pH along the
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125 59 gastrointestinal tract, the stimuli responsive (e.g.: pH sensitive) hydrogels have been used to
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127 60 deliver various types of drugs to the different locations of the gastro intestinal tract [6-8]. In this
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129 61 study, nanocellulose reinforced chitosan hydrogel was used as a carrier for drug delivery of
130
131 62 curcumin. From our previous study [9-11], we observed an improvement of mechanical
132
133 63 properties of the biopolymer based composite and hydrogel with physical reinforcement of
134
135 64 nanocellulose into the biopolymer matrix. Further, it showed that the swelling characteristics of
136
137 65 the hydrogel has improved with the addition of low concentrations of nanocellulose. The system
138
139 66 has also exerted the highest swelling properties in acidic medium [10]. It is also observed that the
140
141 67 chitosan hydrogel reinforced with 0.5% CNC has successfully achieved the highest swelling
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143 68 properties and cumulative release of curcumin [8,10]. Therefore, in this study, 0.5% CNC-
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145 69 chitosan hydrogel formulation was selected for further improvement of drug delivery properties
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147 70 of the hydrogel. Previous results [8,10] also showed that the percentage of curcumin
148
149 71 encapsulation and the amount of curcumin release increased in the gas foam hydrogel (due to the
150
151 72 formation of large interconnected pore structures). However, poor solubility of curcumin has
152
153 73 caused less concentrations of released drug in simulated gastric medium [8]. Therefore, a
154
155 74 nonionic surfactant (Tween 20) has been incorporated into the hydrogel as a solubilizing agent
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157 75 for curcumin in this study.
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171 76 Surfactants can improve the solubility of some poorly soluble drugs. Tween surfactants contain
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173 77 hydrophilic ethylene glycol head and a hydrophobic alkyl chain [12]. The commonly used
174
175 78 Tween are Tween 20, 40, 60 and 80. They possess same hydrophilic group with different length
176
177 79 of alkyl chains. The alkyl chain length will influence the hydrophile–lipophile balance (HLB)
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180 80 value of the surfactant, which in turn directly influences the entrapment efficiency of the drug
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182 81 [13]. Low HLB value corresponds to high hydrophobicity. Higher HLB value indicates the
183
184 82 more water soluble the surfactant [14]. Tween 20 with HLB value of 16.7 is more hydrophilic in
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186 83 nature compared to other Tween surfactants [15]. Ratanajajaroen, Watthanaphanit, Tamura,
187
188 84 Tokura and Rujiravanit [16] reported that the addition of 2% (v/v) of Tween 20 has increased the
189
190 85 solubility of curcumin from 11 ng/mL to 0.767 mg/mL in acetate buffer (pH 5.5). In addition,
191
192 86 they obtained higher release rate of curcumin from chitin beads as the amount of Tween 20
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194 87 increased. O’Toole, Henderson, Soucy, Fasciotto, Hoblitzell, Keynton, Ehringer and Gobin [17]
195
196 88 observed that the curcumin can be released completely from the submicrometer spray-dried
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198 89 chitosan/Tween 20 particles in both 1% acetic acid and phosphate buffered saline solutions over
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200 90 a 2 h period. Furthermore, the research works by Petchsomrit, Sermkaew and Wiwattanapatapee
201
202 91 [18] also reported that the percentage of curcumin release increased with increasing the Tween
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204 92 80 concentration in oil entrapped alginate beads. Their results showed that the cumulative drug
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206 93 release increased up to 70% with the incorporation of 25% (w/v) of Tween 80.

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210 94 Previous cytotoxicity studies revealed that nonionic surfactant such as Tween has lower toxic
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212 95 effect than cationic, anionic and amphoteric ones [19]. Due to its less cytotoxicity properties, the
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214 96 nonionic surfactant was selected for this study. Besides that, surfactants have a profound effect
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216 97 on the release rate of the drug from the encapsulated matrix. Using proper concentration of
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218 98 surfactant and a suitable HLB value, the cumulative release and rate of release of the drug can be

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227 99 controlled. According to the previous studies, the surfactants with lower HLB values such as
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230 100 Tween 80 would cause lower release rates of hydrophobic drugs. This is because the surfactants
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232 101 having lower HLB values are more lipophilic and less water soluble. But with higher HLB
233
234 102 values, hydrophobic drug release rate will increase as these surfactants are more hydrophilic and
235
236 103 water soluble [20]. Tween 20 has a high HLB value of 16.7 and this will help to improve the
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238 104 release of hydrophobic drug to a desired level. Tween 80 had been successfully applied in many
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240 105 hydrophobic drug delivery systems [21-25]. However, the investigations on polysorbate-chitosan
241
242 106 association by Picone and Cunha [26] has shown that the longer hydrophobic polysorbate tail
243
244 107 length of Tween 80 has made it difficult to form homogeneous association with chitosan. Their
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246 108 further investigations had found that the shorter hydrophobic tail length of Tween 20 was more
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248 109 appropriated to form mixed surfactant–chitosan polymer systems. So, Tween 20 which is a less
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250 110 cytotoxic, nonionic, with shorter hydrophobic tail length surfactant was used to improve the drug
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252 111 release of curcumin from nanocellulose reinforced chitosan hydrogel matrix in this study.

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256 112 The objective of this study was to improve the bioavailability of less water-soluble curcumin by
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258 113 incorporating a nonionic surfactant (Tween 20) to nanocellulose reinforced chitosan hydrogel.
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260 114 Curcumin extracted from dried rhizomes of *Curcuma longa* and Tween 20 was incorporated to
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262 115 the hydrogel via in situ loading method. From our previous study, a large extent of chitosan
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264 116 matrix swelling was found to occur in acidic medium [10]. Therefore, the drug molecules are
265
266 117 expected to diffuse extensively through the swollen gel into the exterior medium at gastric pH
267
268 118 levels. Nonionic surfactant will further facilitate dissolution of the drug by partitioning the drug
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270 119 into the aqueous phase of gastric fluid. Based on the results of this study, it is expected that the
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272 120 nanocellulose reinforced chitosan hydrogel/Tween 20 drug delivery system will provide a
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283 121 platform to overcome poor bioavailability of curcumin to yield its broad range of therapeutic
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285 122 benefits.
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288 123 **2. Materials and Methods**

289 290 291 124 *2.1. Materials*

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294 125 Chitosan with medium molecular weight (viscosity 200–500 cP, 0.5% acetic acid at 20 °C),
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296 126 acetic acid glacial grade AR, sodium chloride, hydrochloric acid, methanol and sulfuric acid
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298 127 were purchased from Friendemann Schmidt Chemicals (Parkwood, Australia). The drug
299
300 128 curcumin was provided by HIMEDIA laboratories Pvt Ltd. (Mumbai, India). Glutaraldehyde
301
302 129 25% (for crosslinking of chitosan) was obtained from Thermo Fisher Scientific Inc. (Victoria,
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304 130 Australia). Microcrystalline cellulose, Tween 20 and phosphate-buffered saline were supplied by
305
306 131 R&M chemicals (Essex, UK).
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310 132 *2.2. Research Methodology*

311 312 133 *2.2.1. Extraction of curcumin from turmeric*

313
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315 134 Curcumin was extracted from rhizomes of *Curcuma longa* following the method proposed
316
317 135 elsewhere [5]. Dried rhizomes were crushed in a mortar and pestle. Crushed rhizomes were
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319 136 soaked in methanol for 3 days and then filtered with Whatman filter paper (pore size 0.2 µm).
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321 137 After that, filtrate was poured into a petri plate and the solvent was evaporated under vacuum
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323 138 condition to obtain semi-dry oily mass.
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327 139 *2.2.2. Preparation of curcumin loaded chitosan/nanocellulose/Tween20 hydrogel*

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330 140 Cellulose nanocrystals (CNC) were prepared from microcrystalline cellulose by sulfuric acid
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332 141 hydrolysis method reported in our previous study [10]. Chitosan was dissolved in 5% (v/v)
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339 142 aqueous acetic acid solution at room temperature and left overnight in the shaker with the
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Formulation	Chitosan (w/v)%	Nanocellulose (w/v)%	Tween 20 (w/v)%	Glutaraldehyde (v/v)%	Curcumin (mg per 2.5 g of hydrogel disc)
CH/CNC/ TW-0%	2	0.5	0	0.2	1.5
CH/CNC/ TW-5%	2	0.5	5	0.2	1.5
CH/CNC/ TW-10%	2	0.5	10	0.2	1.5
CH/CNC/ TW-15%	2	0.5	15	0.2	1.5
CH/CNC/ TW-20%	2	0.5	20	0.2	1.5
CH/CNC/ TW-25%	2	0.5	25	0.2	1.5
CH/CNC/ TW-30%	2	0.5	30	0.2	1.5

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143 rotation rate of 250 rpm and then filtered through the filter paper to remove any insoluble
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367 144 matters. To prepare curcumin loaded chitosan/nanocellulose/Tween 20 hydrogel, nanocellulose,
368
369 145 extracted curcumin and Tween 20 were added to chitosan solution and stirred (250 rpm) for one
370
371 146 hour. After that glutaraldehyde was added and stirred (350 rpm) for 1 min at room temperature.
372
373 147 The mixture was then poured into the mold and the hydrogel was allowed to solidify at room
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375 148 temperature for 24 h.
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377

378 149 Table 1.
379
380

381 150 Table 1: Composition of curcumin entrapped chitosan/nanocellulose/Tween 20 hydrogel
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152 *2.2.3. CO₂ gas foaming of curcumin entrapped chitosan/nanocellulose/Tween 20 hydrogels*

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395 153 After mixing the CNC, drug, Tween 20 and crosslinker with chitosan solution as described
396
397 154 above, the mixture was poured into the mold and placed inside the gas foaming apparatus. Then
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399 155 the apparatus was pressurized with CO₂ to predetermined pressure (50 bar). The pressure was
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401 156 maintained to allow for CO₂ saturation and chitosan crosslinking for 48 h. The system was then
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403 157 depressurized at 1 bar/min to result in the generation of numerous gas bubbles which induce gas
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405 158 foaming.
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418 162 *2.3. Characterization*
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420 163 *2.3.1. Characterization of curcumin*
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422
423 164 UV-visible spectra of both curcumin (HIMEDIA Co.) and curcumin extracted from turmeric
424
425 165 were obtained by scanning the drug solutions within the range of 350-800 nm using UV-visible
426
427 166 spectrophotometer (Shimadzu, Kyoto, Japan). FTIR studies on curcumin purchased from
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429 167 HIMEDIA Co. and curcumin extracted from turmeric, were carried out using PerkinElmer
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431 168 spectrum 400 FTIR spectrometer over the range 3000–500 cm⁻¹.
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438 170 *2.3.2. Characterization of the curcumin entrapped chitosan/nanocellulose/Tween 20 hydrogels*
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441 171 *2.3.2.1. FTIR analysis*
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451 172 FTIR studies of raw materials and various composition of hydrogel composites were carried out
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453 173 by using PerkinElmer spectrum 400 FTIR spectrometer over the range 3000–500 cm⁻¹.
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456 174 *2.3.2.2. Morphology Studies* 457 458

459 175 The morphology of the hydrogels was examined using field emission scanning electron
460 176 microscope (FE-SEM, SU8220). Hydrogels were freeze dried using freeze dryer to remove water
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462 without disturbing the morphology. The hydrogels were then coated with platinum in order to
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464 prevent the charging effects at an accelerating voltage of 5 kV.
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471 180 *2.4. Drug delivery studies* 472 473

474 181 *2.4.1. Estimation procedure of curcumin by UV-Vis spectrophotometer* 475 476

477 182 The absorption maxima (λ_{\max}) for curcumin was determined by scanning the drug solution within
478
479 183 the range of 350-800 nm using UV-Vis spectrophotometer. It was found that the drug exhibited
480
481 184 λ_{\max} at 427 nm in both of the distilled water and simulated gastric medium as shown in Fig. 1a
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483 185 and Fig. 2a, respectively. The concentration of curcumin extracted from turmeric was determined
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485 186 by the standard calibration curve (λ_{\max} at 427 nm) prepared using standard solutions of curcumin
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487 187 (HIMEDIA Co).
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491 188 The concentration of curcumin present in distilled water was determined from the calibration
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493 189 curve (Fig. 1b) prepared from standard solutions of curcumin (HIMEDIA Co.), dissolved in
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495 190 methanol and diluted by distilled water.
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497

498 191 **Fig. 1.** a) UV–Vis absorbance spectrum of curcumin in distilled water and b) calibration curve of
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500 192 curcumin.
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507 193 The concentration of curcumin present in simulated gastric fluid was determined from the
508
509 194 calibration curve (Fig. 2b) prepared from standard solutions of curcumin (HIMEDIA Co.),
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511
512 195 dissolved in methanol and diluted by simulated gastric fluid [27, 28].
513

514 196 **Fig. 2.** a) UV–Vis absorbance spectrum of curcumin in simulated gastric medium and b)
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516
517 197 calibration curve of curcumin.
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519 198 *2.4.2. Drug entrapment efficiency*

522 199 Disc shape hydrogel (1.80 cm diameter and 1.20 cm height) was immersed in 30 mL ethanol for
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524 200 24 h. Then, the solution was filtered and diluted suitably to measure the absorbance, from which
525
526
527 201 the concentration of drug was calculated using the standard calibration data. Each formulation
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529 202 was analyzed in triplicate and average values were taken. The drug entrapment efficiency was
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531 203 calculated based on the ratio of actual amount of drug present in the hydrogel to the initial
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533 204 amount of drug contained in the hydrogel by using Equation (1).
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$$535$$
$$536 205 \text{Entrapment efficiency} = \frac{\text{Actual amount of curcumin in hydrogel}}{\text{Initial amount of curcumin contained in the hydrogel}} \quad (1)$$
$$537$$
$$538$$

539 206 *2.4.3. Drug release*

542 207 In vitro drug release from hydrogel networks with different Tween 20 concentrations was
543
544 208 investigated in simulated gastric fluid (simulated gastric fluid was prepared by dissolving 2 g
545
546 209 NaCl in 7.0 mL HCl and water up to 1000 mL) at 37 °C [29]. In order to study the release, 3 mL
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548
549 210 aliquot was withdrawn at predetermined time intervals and returned it back to the solution after
550
551 211 the analysis. The concentration of released curcumin was determined by the calibration curve
552
553 212 (Fig. 2b) prepared using the curcumin (HIMEDIA Co.) in simulated gastric fluid. The
554
555 213 experiments were performed triplicates and average values were taken.
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563 214 *2.4.4. Drug activity*
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566 215 In drug delivery systems, the chemical and biological activity of drug after release into the body
567
568 216 is the most critical parameter. The drug activity of curcumin before loading and after release
569
570 217 could be studied by using UV-Vis spectrophotometer [30]. UV- visible spectra of pure drug and
571
572 218 the drug released from the hydrogel formed at atmospheric condition were obtained by scanning
573
574 219 the solutions within the range of 350-800 nm using UV-visible spectrophotometer. Drug activity
575
576 220 was determined through comparison between the spectra (the absorption maxima (λ_{\max})) of pure
577
578 221 drug and released drug.
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580

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582 222 *2.5. Curcumin solubility studies*
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585 223 To determine a saturated concentration of curcumin in simulated gastric fluid, an excess amount
586
587 224 of curcumin (extracted from turmeric) was added in to 30 mL of simulated gastric fluid with
588
589 225 different concentrations of Tween 20 (0.8%, 1.6%, 2.4%, 3.2%, 4%, 5.6% (w/v)). Then, the
590
591 226 mixtures were stirred (350 rpm) using magnetic stirrer at 37 °C for 12h. Samples were covered to
592
593 227 avoid exposition to the light. After that, the solutions were centrifuged at 10,000 rpm for 10 min,
594
595 228 supernatant was decanted and the dissolved curcumin was determined using the standard
596
597 229 calibration curve prepared using the curcumin (HIMEDIA Co.) in simulated gastric fluid (Fig.
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599 230 2b).
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602 231 **3. Results and discussion**
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605 232 *3.1 Characterization of curcumin*
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619 233 As shown in Fig. 3, the UV-visible spectra of curcumin (HIMEDIA Co.) and curcumin extracted
620
621 234 from turmeric were obtained by scanning the drug solutions within the range of 350-800 nm
622
623
624 235 using UV-visible spectrophotometer.

625
626 236 **Fig. 3.** UV-visible spectra of curcumin extracted from turmeric powder and curcumin
627
628 237 (HIMEDIA Co.).

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630
631 238 The UV- visible spectra of curcumin represent the transition between the electronic energy
632
633 239 levels. The maximum absorption wavelength of a compound is a measure of the difference
634
635 240 between energy levels of the orbitals concerned. An isolated double bond/lone pair produces
636
637 241 strong absorption maximum around 190 nm, whereas the presence of conjugation decreases the
638
639 242 energy separation between orbitals and give rise to the absorption at longer wavelengths. In
640
641 243 organic solvents enolization of diketone of curcumin conjugates between the π -electron clouds of
642
643 244 the two vinylguaiacol parts leading to a common conjugated chromophore, resulted in decrease
644
645 245 in energy. As a result of low-energy π - π^* excitation of that chromophore, curcumin in organic
646
647 246 solvents (primarily in methanol or ethanol) typically absorbs around \sim 420 nm and appears in
648
649 247 yellow color [31].

650
651
652 248 The FTIR spectra of curcumin (HIMEDIA Co.) and curcumin derived from turmeric powder are
653
654 249 shown in Fig. 4. The differences in the 3100-3600 cm^{-1} range may be attributed to the OH
655
656 250 stretching of the methanol molecules adsorbed in the curcumin derived from turmeric powder
657
658 251 [32]. The appearance of strong peak at 1582 cm^{-1} and no peak at 1317 cm^{-1} in the derived
659
660 252 curcumin as well as various displacements of the peaks may be due to different interactions
661
662 253 between functional groups of curcumin. As shown in Fig. 5, the chemical composition of the
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675 254 extracted curcumin is a mixture of curcuminoids (curcumin, demethoxycurcumin and bis-
676
677 255 demethoxycurcumin).
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679
680 256 **Fig. 4.** FTIR spectra of curcumin (HIMEDIA Co.) and curcumin derived from turmeric.
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682
683 257 **Fig. 5.** Curcuminoids present in turmeric powder.
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685
686 258 The IR spectrum of curcumin derived from turmeric is more similar to the IR spectrum of
687
688 259 crystalline curcumin derived from turmeric powder (extracted using actone/ethanol/methanol),
689
690 260 which was reported in previous studies [32, 33]. A broad peak at 3380 cm^{-1} indicates the
691
692 261 presence of $-\text{OH}$ group. In the highest frequency region, phenolic vibrations of the curcumin has
693
694 262 theoretical frequency at 3595 cm^{-1} , but in practice this band could be shifted downwards due to
695
696 263 the intramolecular and intermolecular hydrogen bonds [33, 34]. The appearance of bands with
697
698 264 low intensity in the region from $2700\text{-}3000\text{ cm}^{-1}$ can be attributed to the C-H stretches [33]. The
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700 265 highest frequency bands observed within $2700\text{-}3000\text{ cm}^{-1}$ region are assigned to the aromatic C
701
702 266 C-H stretches, while the lower frequency bands are attributed to the methyl group motions [34].
703
704 267 The peak at 1679 cm^{-1} appeared due to the C=O vibrations [33]. The band at 1623 cm^{-1} can be
705
706 268 assigned to the $\nu(\text{C=C})$ of the benzene ring [35]. The strong peak at 1582 cm^{-1} has a
707
708 269 predominantly mixed $\nu(\text{C=C})$ and $\nu(\text{C=O})$ characteristic. The most prominent band in the IR
709
710 270 spectrum is at 1509 cm^{-1} . This can be attributed to highly mixed vibrations ($\nu\text{C=O}$, $\delta\text{CC}^{10}\text{C}$,
711
712 271 $\delta\text{CC=O}$) [33]. The peaks at 1430 cm^{-1} appeared due to the asymmetric angular deformation
713
714 272 vibrations of methyl groups [34]. The observed bands at 1377 cm^{-1} and 1207 cm^{-1} can be
715
716 273 attributed to $\nu(\text{C-O})$ and $\delta(\text{C=C-H})$ of interring chain, respectively. One band and one shoulder at
717
718 274 $1270/1238\text{ cm}^{-1}$ and peak at 1167 cm^{-1} are attributed to the in-plane deformation vibrations of
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720 275 (CCH) of phenyl rings and skeletal in-plane deformations, respectively. A prominent band at
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731 276 1124 cm^{-1} is assigned also to the (C-O-C) vibrations [33]. The peak at 1031 cm^{-1} appeared due to
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733 277 C-O stretching coupling with the adjacent C-C stretching vibrations [32]. The bands at 964 cm^{-1}
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735 278 and 815 cm^{-1} assigned to the $\nu(\text{C-O})$ vibrations. The IR bands at 815 cm^{-1} and 720 cm^{-1} belongs
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737
738 279 to the $\nu(\text{C-H})$ out of plane vibration of the aromatic ring [34]. In the range of 700-500 cm^{-1} , we
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740 280 could see deformation vibrations of both benzene rings and the out of plane vibrations of both
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742 281 OH groups, which are at 607 cm^{-1} and 546 cm^{-1} [33].
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751 752 753 285 *3.2. Characterization of curcumin entrapped chitosan/nanocellulose/Tween 20 hydrogels*

754 755 756 286 *3.2.1. FTIR analysis*

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758
759 287 Fig. 6 displays the FTIR spectra of curcumin (extracted), Tween 20, and curcumin/Tween 20
760
761 288 incorporated chitosan based hydrogel. From the spectrum of curcumin/Tween 20 incorporated
762
763 289 chitosan hydrogel, it can be seen that the bands corresponded to the functional groups of Tween
764
765 290 20 are more prominent together with the bands assigned to the functional groups of the hydrogel.
766
767 291 The sharper peaks related to the functional groups of curcumin are super imposed by the broader
768
769 292 peaks of Tween 20 and chitosan hydrogel [35] (in the spectrum of curcumin/Tween 20
770
771 293 incorporated hydrogel). Also, the drug concentration loaded to the hydrogel is very low when
772
773 294 compared to Tween 20 concentration. Therefore, the peaks related to Tween 20 are more
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775 295 prominent in the spectrum of curcumin/Tween 20 incorporated hydrogel. However, there are no
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777 296 new peaks appeared in the spectrum of curcumin/Tween 20 incorporated hydrogel.
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787 **Fig. 6.** FTIR spectra of curcumin (extracted), Tween 20, nanocellulose reinforced chitosan
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789 hydrogel and curcumin/Tween 20 incorporated hydrogel.
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792 **3.2.2. Morphology studies**
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795 The morphology of 0.5% CNC-chitosan hydrogels formed at atmospheric condition and high-
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797 pressure conditions (50 bar) was examined using field emission scanning electron microscope.
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799 The micrographs of the cross section of hydrogels are shown in Fig.7. The micrographs clearly
800
801 show the porous network of the gels. As shown in Fig. 7a, hydrogel formed at atmospheric
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803 condition showed closed pore structures with around 100-300 μm pore size. After the gas
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805 foaming, the pore size of the hydrogel significantly increased (more than 10-fold higher
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807 compared to the hydrogel formed at atmospheric condition) with the formation of interconnected
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809 pore network structures (Fig. 7b).
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813 **Fig. 7.** Micrographs of a) hydrogel formed at atmospheric condition and b) gas foamed hydrogel
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816 Skin layer formation and poor pore interconnectivity are common issues in porous fabrication
817
818 techniques. However, these can be overcome by fabrication of polymer matrices using gas
819
820 foaming method [37]. The porous structure is generated when the discontinuous dispersed gas
821
822 phase is removed from the continuous phase of polymer. These polymeric foams have low
823
824 kinetic stability due to the significant difference between the densities of the gas and liquid. The
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826 liquid phase tends to move down while the gas tends to move upwards, which leads to the
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828 formation of interconnected porous structure with highly porous top surface [38].
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832 **3.3 Drug delivery studies**
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835 **3.3.1. Entrapment efficiency**
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843 318 The drug entrapment efficiency of the hydrogel with different concentrations of surfactant was
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845 319 studied and the results are shown in Fig. 8. To form curcumin loaded hydrogel, 1.5 mg of
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847 320 curcumin was entrapped in 2.50 g of hydrogel (disc diameter 1.8 cm and height 1.2 cm). The
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849 321 hydrogel with 0% (w/v) surfactant revealed the highest drug entrapment efficiency value of
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851 322 92.09% \pm 0.15%, whereas hydrogel containing 30% (w/v) Tween 20 demonstrated the least
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853 323 value of 70.21% \pm 0.26%. The percentage entrapment efficiency decreased significantly from
854
855 324 92.09% \pm 0.15% to 77.92% \pm 0.70% with the addition of 5% (w/v) surfactant to the hydrogel.
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857 325 After that, it slightly decreased from 77.92% \pm 0.70% to 70.21% \pm 0.26% with increasing the
858
859 326 surfactant concentration from 5% to 30% (w/v).
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862

863 327 **Fig. 8.** Entrapment efficiency of curcumin for gas foamed hydrogel and hydrogel formed at
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865 328 atmospheric condition.
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868 329 Similar results were obtained by Petchsomrit, Sermkaew and Wiwattanapatapee [18] for oil
869
870 330 entrapped alginate bead formulation of curcumin. Tween 80 was used as the surfactant for
871
872 331 their study and the entrapment efficiency of curcumin was found to decrease from 73.69% \pm
873
874 332 2.04% to 40.28% \pm 0.23% with increasing the Tween 80 content from 0% to 30%. From the
875
876 333 study of variability of (poly lactic-co-glycolic acid) PLGA nanoparticles quality of protein
877
878 334 loaded PLGA nanoparticles by Plackett–Burman design, Rahman, Zidan, Habib and Khan
879
880 335 [39] described that the decreasing of the entrapment efficiency with increasing the surfactant is
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882 336 due to the fact that the higher concentration of the emulsifier increases the partition of the drug
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884 337 from internal to external phase due to the increased solubility of the drug in the external phase. In
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886 338 addition, the alkyl chain length influences the hydrophile–lipophile balance (HLB) value of the
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888 339 surfactant, which in turn directly affects the drug entrapment efficiency [13]. Non-ionic
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890 340 surfactants have hydrophilic and lipophilic properties and are characterized by its' hydrophile–
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899 341 lipophile balance values. Low HLB value corresponds to high hydrophobicity. The higher HLB
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901 342 value the more water soluble the surfactant [40]. The lower the HLB of the surfactant the higher
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903 343 will be the drug entrapment efficiency as in the case of niosomes prepared using Span 60 (HLB
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905 344 = 4.7), compared with Span 40 with a higher HLB of 6.7 [41-43]. The HLB value of Tween 20
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907
908 345 is 16.7. The higher HLB value represents higher hydrophilic property. According to Dinarvand,
909
910 346 Moghadam, Sheikhi and Atyabi [44], the higher internal aqueous volume may increase the
911
912 347 volume of water droplets in the hydrophobic phase. This will promote more contact and
913
914 348 exchange (drug loss) to the external water phase. Polymer phase acts as a diffusion barrier
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916 349 against movement of drug molecules between the internal and external aqueous phases; the
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918 350 thickness of this layer decreases when the internal aqueous volume is increased. This may lead to
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920
921 351 the reduction of drug entrapment efficiency.

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923 352 In this study, we have compared the drug delivery behavior of the gas foamed hydrogel with the
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925 353 hydrogel prepared at atmospheric condition. As can be seen from the Fig. 8, the entrapment
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927 354 efficiency of curcumin in gas foamed hydrogel is slightly lower than that of the hydrogel formed
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929 355 at atmospheric condition. This result is different compared to our previous study [8], which has
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931 356 reported that the gas foamed hydrogel has higher drug entrapment efficiency compared to
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933 357 hydrogel formed in atmospheric condition. This may be caused by the different drug loading
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935 358 methods that have been applied in these two different studies. In previous study [8], the drug
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937 359 was encapsulated by post-loading method. However, in this study we are applying in situ
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939 360 loading method for the comparative study between the hydrogels prepared under gas foamed
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941 361 and atmospheric condition. In situ loading method is used in this study to incorporate both of
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943 362 the surfactant and the drug to the hydrogel matrix. This in situ loading method does not
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945 363 perform the same as the previous post-loading method [44]. From the studies on the effects of
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955 364 different drug loading methods on drug delivery, Wong and Dodou [45] reported that the drug is
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957 365 embedded within polymeric network when in situ loaded, instead of being deposited in
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959 366 microporous spaces of the hydrogel when post loaded method was applied. As can be seen
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961 367 from Fig. 7, the hydrogel prepared at atmospheric condition composed of small pore
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963 368 structures when compared to the gas foamed hydrogel. The decrease of pore size of the
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965 369 hydrogel simply correlated with the higher hydrogel density, which contributing to more
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967 370 embedded drug in the hydrogel matrix and higher encapsulation efficiency. Therefore, in this
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969 371 study, the hydrogel prepared at atmospheric condition showed higher encapsulation efficiency
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971 372 compared to gas foamed hydrogel. Petchsomrit, Sermkaew and Wiwattanapatapee [6] also
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973 373 reported that the higher hydrogel density contributed to the increase of curcumin content on in
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975 374 situ drug loaded, alginate-based composite sponge.

978
979 375 From the comparison between post loaded formulations from our previous study [8] and in situ
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981 376 loaded formulations used in this study, it is found that the in situ loading method provides better
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983 377 drug entrapment efficiency within hydrogel network for all the hydrogel prepared under gas
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985 378 foamed and atmospheric condition due to the specific interactions between polymer and drug
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987 379 molecules [45].

989 380 *3.3.2. Drug release*

992 381 *3.3.2.1. Drug release from the hydrogel formed at atmospheric condition*

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994 382 The oral bioavailability of a drug relies upon on its solubility and/or dissolution rate, and
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996 383 dissolution can be the rate determining step for the onset of drug action. Hence there are
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998 384 numerous approaches available and reported in literature to enhance dissolution and drug
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1000 385 bioavailability of poorly water-soluble drugs [7-10, 46-47]. The use of surfactant to improve the
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1011 386 solubility of hydrophobic/lipophilic drugs is a common practice in the industry and it has been
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1013 387 extensively studied by many researchers [46-47]. In this study, curcumin and Tween 20 were
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1015 388 incorporated into hydrogel matrix via in-situ loading method to allow hydrogel network
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1018 389 formulation and drug encapsulation are accomplished simultaneously.
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1020
1021 390 **Fig. 9.** Curcumin release from the hydrogels containing different concentrations of Tween 20.
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1023 391 The hydrogel discs (1.2 cm height and 1.8 cm diameter) containing 1.5 mg of drug with different
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1025 392 concentrations of Tween 20 (5%, 10%, 15%, 20%, 25% and 30% (w/v)) were immersed in
1026
1027 393 simulated gastric fluid and drug release was monitored over a period of 7.5 h. Since the solubility
1028
1029 394 of curcumin is very low [46], a large volume of the releasing medium was used to maintain a
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1031 395 good sink condition [47]. As can be seen in Fig. 9, the cumulative release of curcumin increased
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1033 396 from 0.21% \pm 0.02% to 54.85% \pm 0.77% with increasing the Tween 20 concentration from 0%
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1035 397 to 30% (w/v), after 7.5 h immersion. Release studies in the presence of Tween 20 show a burst
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1037 398 release profile for curcumin, up to 20% of curcumin released in the first 60 min of the
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1039 399 experiment. As shown in Fig. 9, with the increase of Tween 20 concentration from 5-30% (w/v),
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1041 400 at a fixed drug concentration, the amount of drug release increased from 31.82% \pm 0.75% to
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1043 401 54.85% \pm 0.77%. This is almost 1.7-fold increase after 7.5 h. During the time of monitoring, the
1044
1045 402 formulations containing lower amount of Tween 20 produced an incomplete solubilization of
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1047 403 curcumin, whereas complete solubilization showed at high concentration of Tween 20.
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1049 404 Therefore, larger amount of surfactant produced a higher drug release. Ratanajajaroen and
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1051 405 Ohshima [7] showed that the solubility of curcumin increased from 11 mg/mL to 0.767 mg/mL
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1053 406 with Tween 20, at a concentration of 2% (v/v) in acetate buffer (pH 5.5). In addition, they found
1054
1055 407 that the drug release rate increased from chitin beads as the Tween 20 concentration increased.
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1057 408 O'Toole, Henderson, Soucy, Fasciotto, Hoblitzell, Keynton, Ehringer and Gobin [17]
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409 investigated that the curcumin saturation point increased linearly with Tween 20 concentration
410 throughout the range used in their experiments with an upper limit of 294 μM curcumin with
411 0.05% (w/v) Tween 20. Further, they reported that curcumin can be released completely from the
412 submicrometer spray-dried chitosan/Tween 20 particles in both 1% acetic acid and phosphate
413 buffered saline solutions over a 2 h period.

414 Moreover, in terms of therapeutic applications for abdominal disorders, the doses of curcumin up
415 to the healing levels should also be concerned. Considering the strong association of *H. pylori*
416 and gastric cancer the authors of the study by Mahady, Pendland, Yun and Lu [4] showed that
417 both curcumin and methanolic extract of turmeric rhizome inhibited the growth of 19 different
418 strains of *H. pylori*. They reported that curcumin inhibited the growth of all the *H. pylori* strains
419 by 100% at a concentration of 12.5 $\mu\text{g/mL}$ with a minimum inhibitory concentration from 6.25-
420 12.5 $\mu\text{g/mL}$. De, Kundu, Swarnakar, Ramamurthy, Chowdhury, Nair and Mukhopadhyay [3]
421 investigated that the minimum inhibitory concentration of curcumin for *H. pylori* strains ranged
422 from 5 $\mu\text{g/mL}$ to 50 $\mu\text{g/mL}$, and the majority of the strains (81%) showed a minimum inhibitory
423 concentration of either 10 $\mu\text{g/mL}$ (23%) or 15 $\mu\text{g/mL}$ (58%). In addition, curcumin is able to
424 suppress the proliferation and survival of cancer cells by directly or indirectly binding to various
425 cellular molecular targets [48]. Liu, Xiang, Wu and Wang [49] reported that curcumin inhibited
426 the growth of gastric cancer cells in a concentration and a time-dependent manner. Their studies
427 showed that as compared to untreated cancer cells, the cell proliferation was significantly
428 inhibited in the curcumin treated samples after 48 h of treatment with 50 μM curcumin. In our
429 study, the maximum concentration of curcumin released in the simulated gastric fluid was 3.98
430 $\mu\text{g/mL}$ after 7.5 h. Even so, we found that the drug release of the hydrogel increased (≥ 10
431 $\mu\text{g/mL}$) with increasing the initial concentration of the drug which incorporated to the hydrogel

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1123 432 (for same Tween 20 concentration). However, with increasing the initial amount of drug, the
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1125 433 percentage release of drug dramatically decreased during the period of monitoring. Therefore,
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1127 434 1.5 mg of drug per hydrogel disc (1.2 cm height and 1.8 cm diameter) was used for the
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1129 435 encapsulation in this study. However, the initial drug loading amount to the hydrogel can be
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1131 436 varied in order to get the drug release to the extent desired (to reach the therapeutic levels) or to
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1133 437 obtain the minimum inhibitory concentration. As a summary from the results obtained from drug
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1135 438 release studies, the nanocellulose reinforced chitosan/Tween 20 hydrogel can be suggested as a
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1137 439 promising candidate for carrying curcumin for the absorption of stomach and upper intestinal
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1139 440 tract.

1141 441 *3.3.2.2. Drug release from gas foamed hydrogel*

1142
1143 442 Fig. 7b has clearly illustrates that nanocellulose reinforced chitosan hydrogel fabricated using
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1145 443 carbon dioxide gas foaming process possess large-scale macroporous with wide interconnected
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1147 444 pores and large accessible surface area. Due to the large pore size of hydrogels, a rapid initial
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1149 445 burst release of drug was typically observed as observed in previous study [8].

1150
1151 446 **Fig. 10.** Curcumin release from gas foamed hydrogel and hydrogels formed at atmospheric
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1153 447 condition with different concentrations of Tween 20.

1154
1155 448 As shown in Fig. 10, the concentration of released drug from gas foamed hydrogel was greater
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1157 449 when compared to the hydrogel formed at atmospheric condition within the first 120 minutes.
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1159 450 The increased pore size and pore interconnectivity of gas foamed hydrogel act as a capillary
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1161 451 system causing a rapid diffusion of drug solution through the hydrogel matrix [50]. At 60
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1163 452 minutes, For the hydrogel containing 5% (w/v) Tween 20, the cumulative release of drug at
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1165 453 60 minutes was $8.51\% \pm 0.61\%$ and $7.95\% \pm 0.39\%$ for gas foamed hydrogel and hydrogel

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1178
1179 454 formed at atmospheric condition respectively. While, the cumulative release of drug at 60
1180
1181 455 minutes was $21.58\% \pm 0.32\%$ and $20.87\% \pm 0.46\%$ respectively for hydrogel containing 30%
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1183 456 (w/v) Tween 20 prepared under gas foam and at atmospheric condition. After the burst
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1185
1186 457 release, the rate of drug release of both types of hydrogels become almost similar.

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1188
1189 458 From our previous study [8], the gas foamed hydrogel showed higher drug release when
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1191 459 compared to the hydrogel formed at atmospheric condition. However, gas foamed hydrogel
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1193 460 did not show any improvement of the drug release over the hydrogel formed at atmospheric
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1195 461 condition in this study. Kawase, Michibayashi, Nakashima, Kurikawa, Yagi and Mizoguchi
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1197 462 [51] reported that the permeation rates of the drug from post-loaded formulations are
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1199 463 generally more rapid compared to the in situ loaded ones. This may be due to drug being
1200
1201 464 deposited in microporous spaces of the hydrogel when post-loaded instead of being embedded
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1203 465 within polymeric network when loaded in situ. In our previous study by applying post-loading
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1205 466 method, gas foamed hydrogel with large and interconnected pore structures has allowed the
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1207 467 drug deposited in the pore spaces of the hydrogel to be easily transported [52]. Therefore, the
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1209 468 prepared gas foamed hydrogel with post-loading method has attributed to higher drug release
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1211 469 compared to the hydrogel prepared at atmospheric condition in the previous study [8].
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1215 470 In the current research work, we have applied in situ loading method for the drug loading. The
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1217 471 gas foamed hydrogel with larger interconnected pore structures would cause less impact on
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1219 472 the drug release from the in situ loaded formulations [44, 45, 52]. Therefore, the gas foamed
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1221 473 hydrogel did not show any improvement of the drug release over the hydrogel formed at
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1223 474 atmospheric condition in this study.

1224 1225 1226 475 *3.4. Drug activity* 1227 1228 1229 1230 1231 1232

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1234
1235 476 The chemical reactivity and biological activity of the drug are the most critical parameters in
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1237 477 drug delivery systems after drug release process [30]. Curcumin has three reactive functional
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1239 478 groups which associated with its different biological activities: one diketone moiety, and two
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1241
1242 479 phenolic groups. The presence of C=O groups as hydrogen acceptors and C-4 as a hydrogen
1243
1244 480 donor are the important chemical reactions associated with its biological activities [53-54].
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1246
1247 481 **Fig. 11.** UV-visible spectra of (a) pure drug and (b) released drug.
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1250 482 As shown in Fig. 11a and 11b, both of the UV-visible spectra indicate absorption peak around
1251
1252 483 427 nm. This can be assigned to the low-energy π - π^* excitation of the chromophore which
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1254 484 formed due to the enolization of the diketone group and conjugation between the π -electron
1255
1256 485 clouds of the two vinylguaiacol [31]. The presence of the absorption peak around 427 nm in both
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1258 486 of the spectra revealed that the reactive functional groups which associated with the biological
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1260 487 activity of curcumin retained without any deterioration due to any denaturation reaction with the
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1262 488 carrier molecules.
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1264 1265 489 *3.5. Solubility study* 1266

1267
1268 490 From our previous study [8,10], we observed lower drug release profiles of the hydrogel due to
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1270 491 poor solubility of curcumin in simulated gastric fluid. To improve curcumin's solubility, Tween
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1272 492 20 was selected as potential solubilizing agent due to its biocompatibility and previous
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1274 493 successful application in curcumin drug delivery systems [16, 17, 55].
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1276
1277 494 **Fig. 12.** Solubility of curcumin in simulated gastric fluid with different concentrations of Tween
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1279 495 20.
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1291 496 The ability of Tween 20 to solubilize curcumin in simulated gastric fluid was evaluated by
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1293 497 measuring the curcumin concentration in curcumin-saturated simulated gastric fluid with various
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1295 498 amounts of Tween 20. As shown in Fig. 12, the solubility of the drug gradually increased with
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1297 499 increasing the concentration of nonionic surfactant (Tween 20), with an upper limit of $3.014 \pm$
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1299 500 0.041 mg/mL in the presence of 3.2% (w/v) Tween 20. Similar results were obtained from the
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1301 501 studies on solubility of curcumin in aqueous polysorbate micelle reported by Inchai, Ezure,
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1303 502 Hongwiset and Yotsawimonwat [56]. Their studies had showed that the solubility of curcumin
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1305 503 increased up to 2.7 mg/mL in 20% aqueous solution of Tween 20. O'Toole, Henderson, Soucy,
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1307 504 Fasciotto, Hoblitzell, Keynton, Ehringer and Gobin [17] showed that the saturation point of
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1309 505 curcumin increased linearly with increasing the Tween 20 concentration in 1% acetic acid
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1311 506 medium. Their findings revealed that the solubility of curcumin increased to an upper limit of
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1313 507 294 μ M (~ 0.108 mg/L) with 0.05% (w/v) Tween 20.

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1315 508 With higher pH, curcumin degrades rapidly (on the timescale of minutes). However, the
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1317 509 solubility of curcumin decreases rapidly with decreasing of pH [56-57]. In our study, the
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1319 510 solubility of curcumin in simulated gastric medium was ~ 6 μ g/mL (without addition of the
1320
1321 511 surfactant). It showed a slight decrease when compared to the results obtained by Hung, Chen,
1322
1323 512 Lee, Sun, Lee and Huang [58] which was 25 μ M (~ 9.2 μ g/mL) in pH 7 buffer solution. This
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1325 513 may be due to the low pH of the simulated gastric fluid.

1330 1331 514 **4. Conclusions**

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1333 515 In this study, curcumin was extracted from dried rhizomes of *curcuma longa* using methanol for
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1335 516 preparation of curcumin loaded chitosan/nanocellulose/Tween20 hydrogel. As a result of low-
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1337 517 energy π - π^* excitation of the chromophore, both curcumin (HIMEDIA Co.) and curcumin
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1347 518 (extracted) showed typical absorption peaks around 420 nm in the UV-visible spectrum. The
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1349 519 drug release of the hydrogel increased from $0.21\% \pm 0.02\%$ to $54.85\% \pm 0.77\%$ with increasing
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1351 520 of Tween 20 concentration from 0% to 30% (w/v) after 7.5 h immersion. FESEM micrographs
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1353 521 had proven that the pore size of the hydrogel increased more than tenfold after the gas foaming
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1355 522 process (at 50 bar). Gas foamed hydrogel showed a high burst release of the drug compared to
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1357 523 the hydrogel formed at atmospheric condition. The entrapment efficiency of the hydrogels
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1359 524 decreased with increasing the Tween 20 concentration. Solubility studies showed that the
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1361 525 saturation point of curcumin increased linearly with increasing the concentration of nonionic
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1363 526 surfactant (Tween 20). The maximum limit of 3.014 ± 0.041 mg/mL was achieved with the
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1365 527 introduction of 3.2% (w/v) of Tween 20. Furthermore, curcumin retained its structural integrity
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1367 528 after release to the gastric medium, which is a critical requirement for preserving drug activity.
1368
1369 529 The solubility of a drug is a fundamental parameter in terms of promoting any effect impacting
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1371 530 the therapeutic effect of the drug. Thus, the Tween 20 incorporated chitosan/nanocellulose
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1373 531 hydrogel has provided promising platform as a carrier for curcumin with improved solubility
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1375 532 characteristics for the absorption from stomach and upper intestinal tract. Since biocompatibility
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1377 533 of the hydrogel system is a critical concern for researchers in drug delivery field. Therefore,
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1379 534 investigations on the cytotoxicity and biocompatibility of the Tween 20 incorporated
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1381 535 chitosan/nanocellulose hydrogel for in vivo drug delivery applications will be conducted in
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1383 536 future works.
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1388 537 **Acknowledgments:** The authors would like to acknowledge the financial support from the
1389 538 Ministry of Education Malaysia: FP053-2015A and PR005-2017A; and University Malaya
1390 539 research grant: PG160-2016A, RU005D-2016, and ST012-2017 and RU018I-2016 and
1391 540 International Funding AUA Scholars IF025-2018 for the success of this project.
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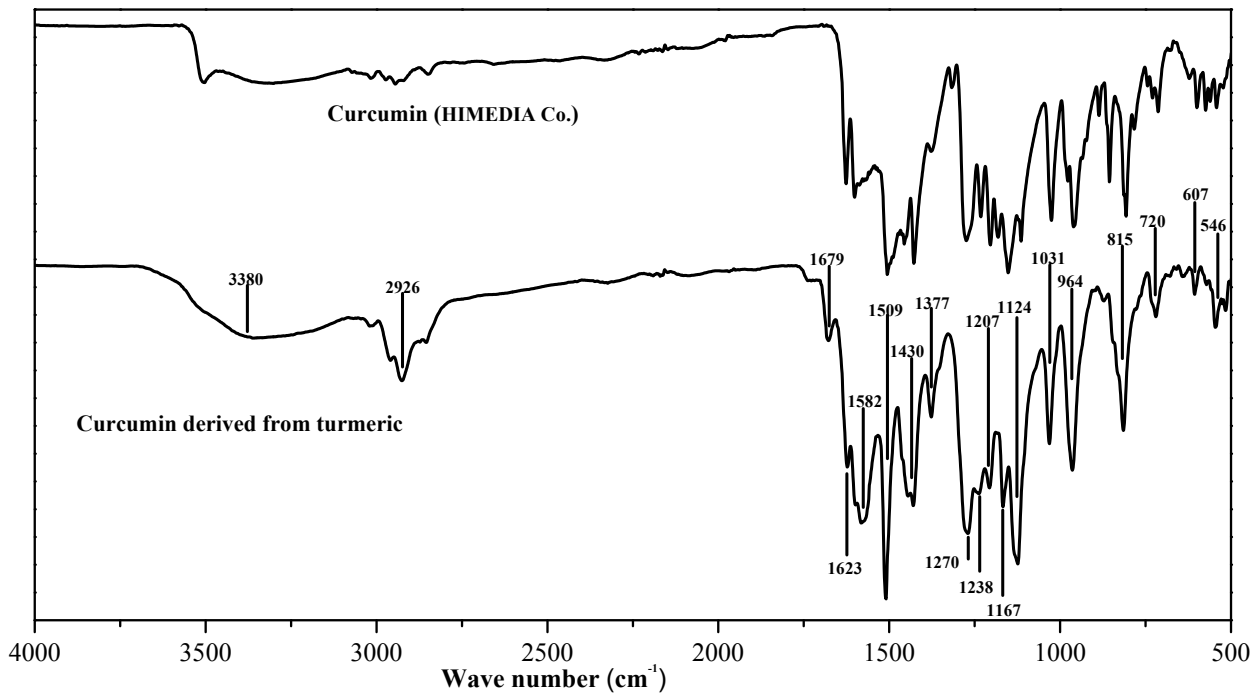


Fig. 1. FTIR spectra of curcumin (HIMEDIA Co.) and curcumin derived from turmeric.

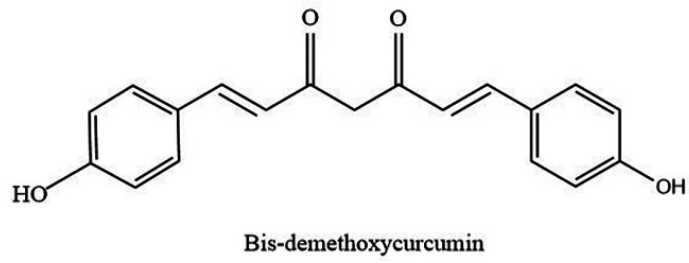
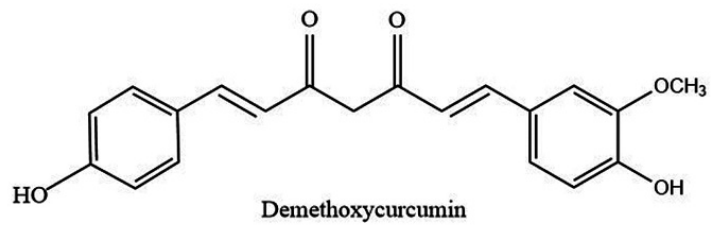
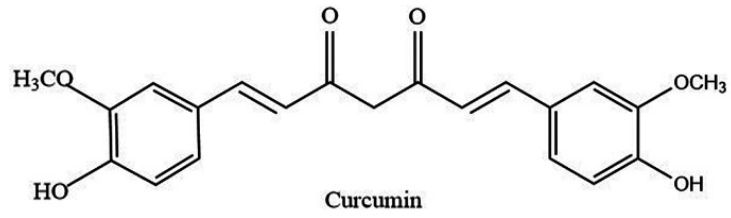


Fig. 2. Curcuminoids present in turmeric powder.

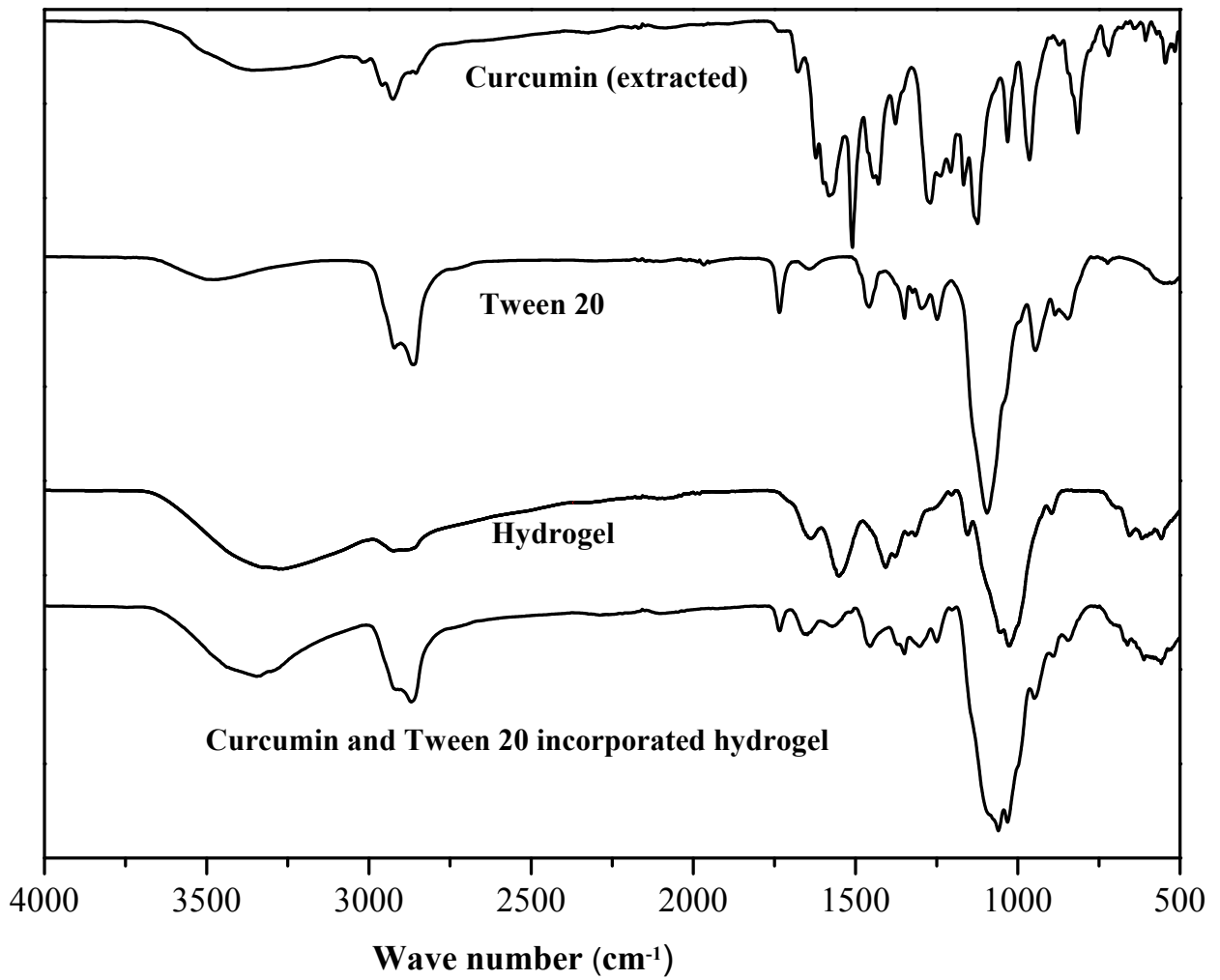


Fig. 3. FTIR spectra of curcumin (extracted), Tween 20, nanocellulose reinforced chitosan hydrogel and curcumin/Tween 20 incorporated hydrogel.

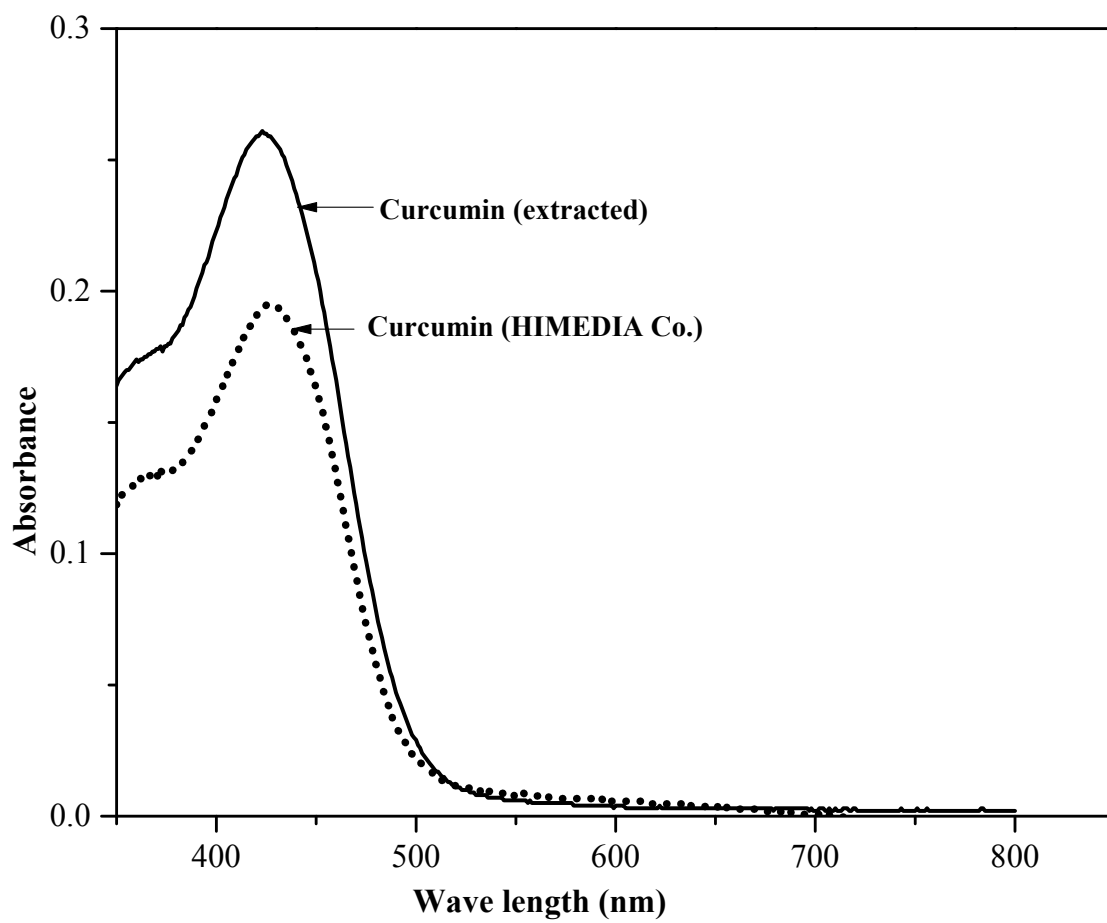


Fig. 4. UV-visible spectra of curcumin extracted from turmeric powder and curcumin (HIMEDIA Co.).

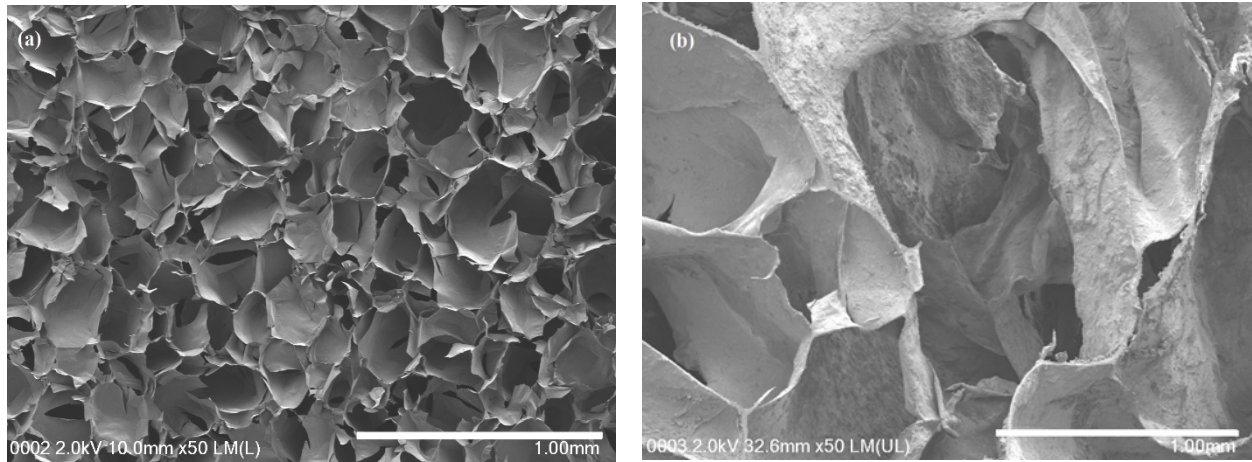


Fig. 5. Micrographs of a) hydrogel formed at atmospheric condition and b) gas foamed hydrogel.

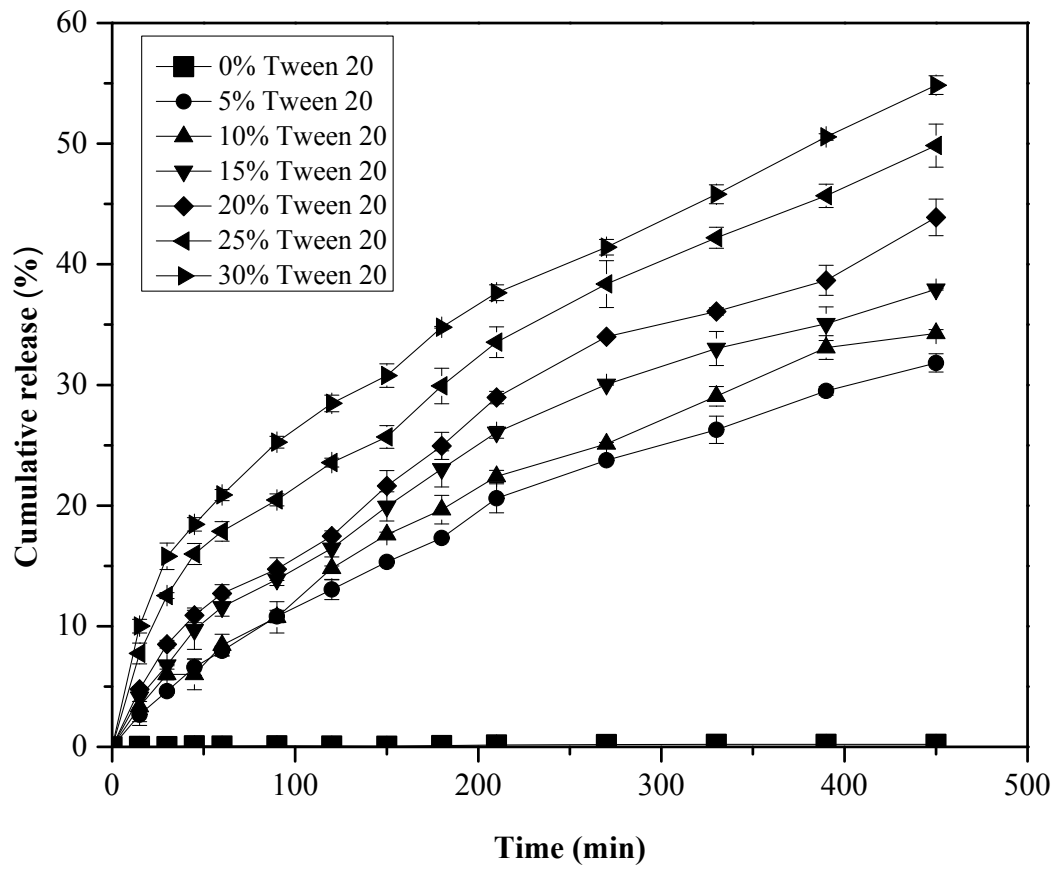


Fig. 6. Curcumin release from the hydrogels containing different concentrations of Tween 20.

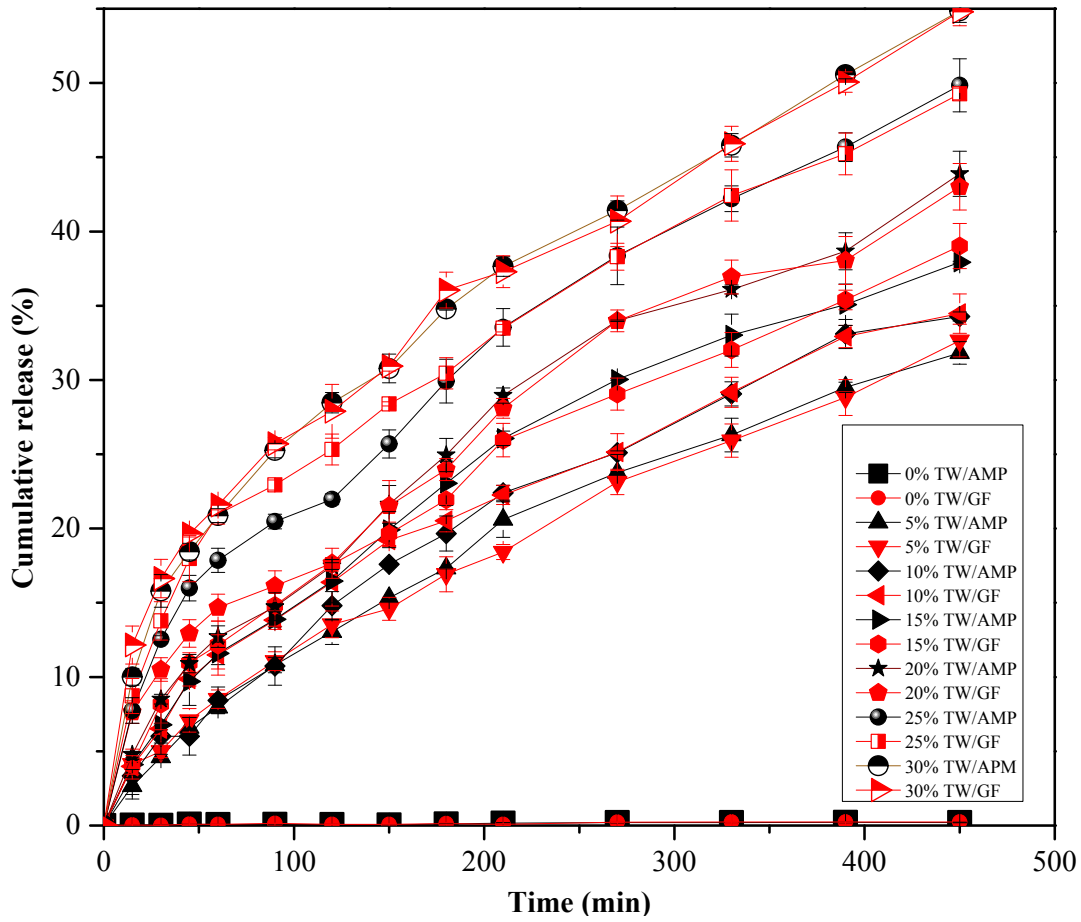


Fig. 7. Curcumin release from the hydrogels formed at atmospheric condition (AMP) and high-pressure condition (GF) with different concentrations of Tween 20 (TW).

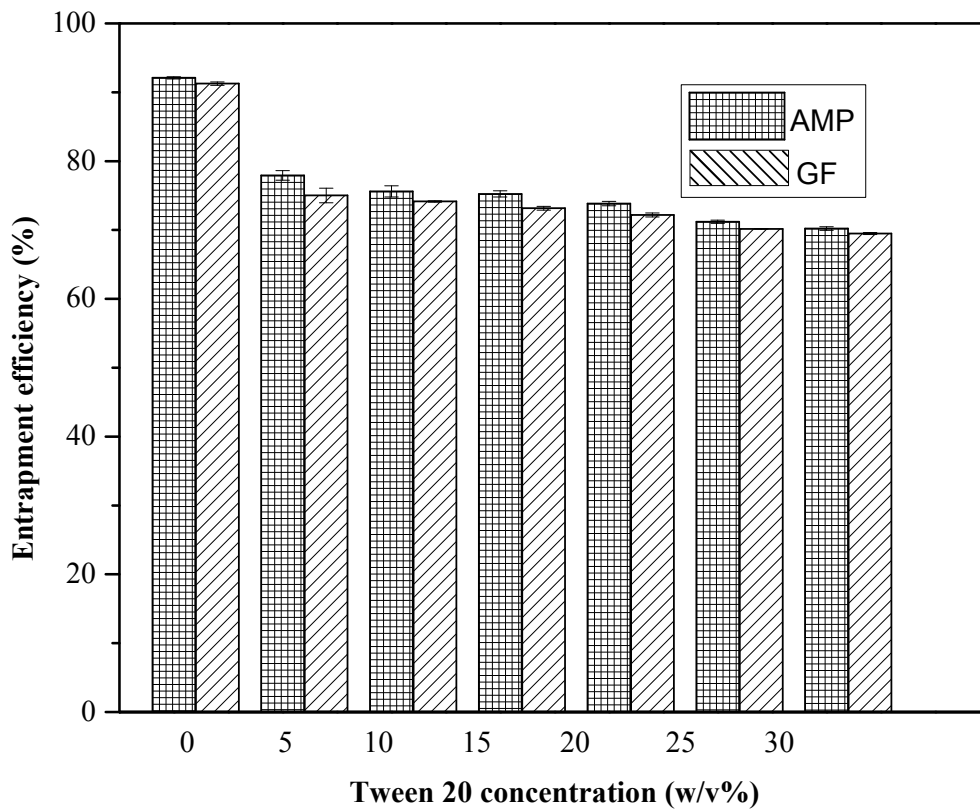


Fig. 8. Entrapment efficiency of curcumin in gas foamed hydrogel (GF) and hydrogel formed at atmospheric condition (AMP).

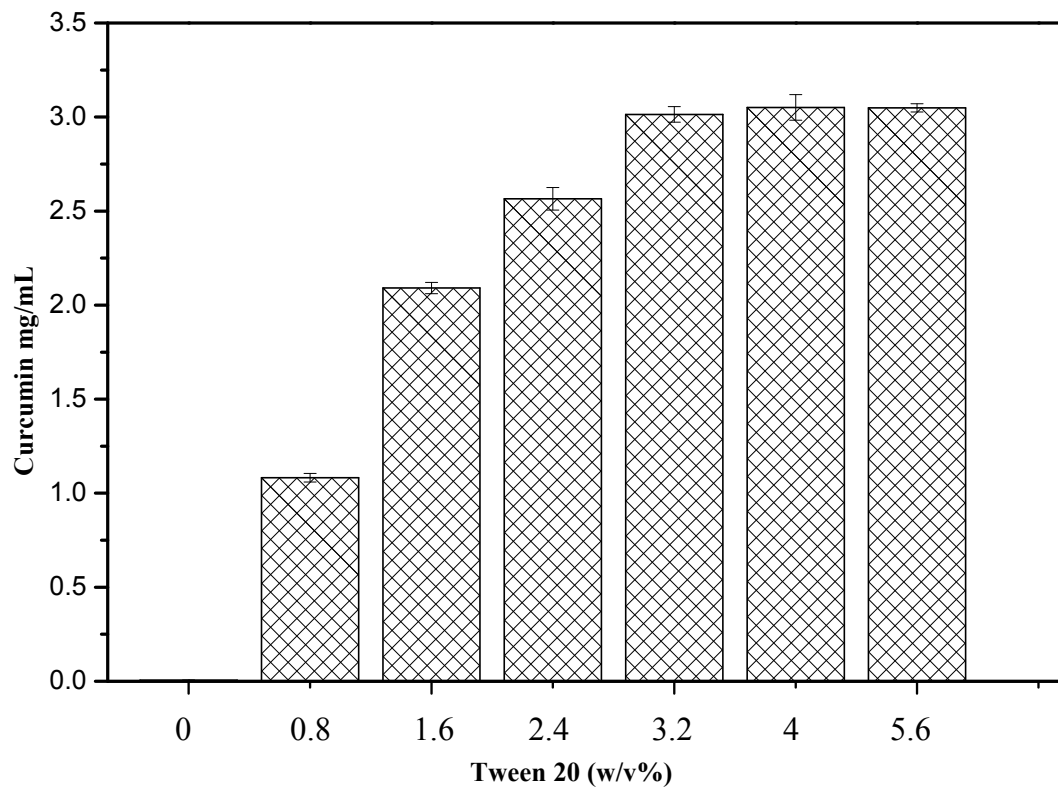


Fig. 9. Solubility of curcumin in simulated gastric fluid with different concentrations of Tween 20.

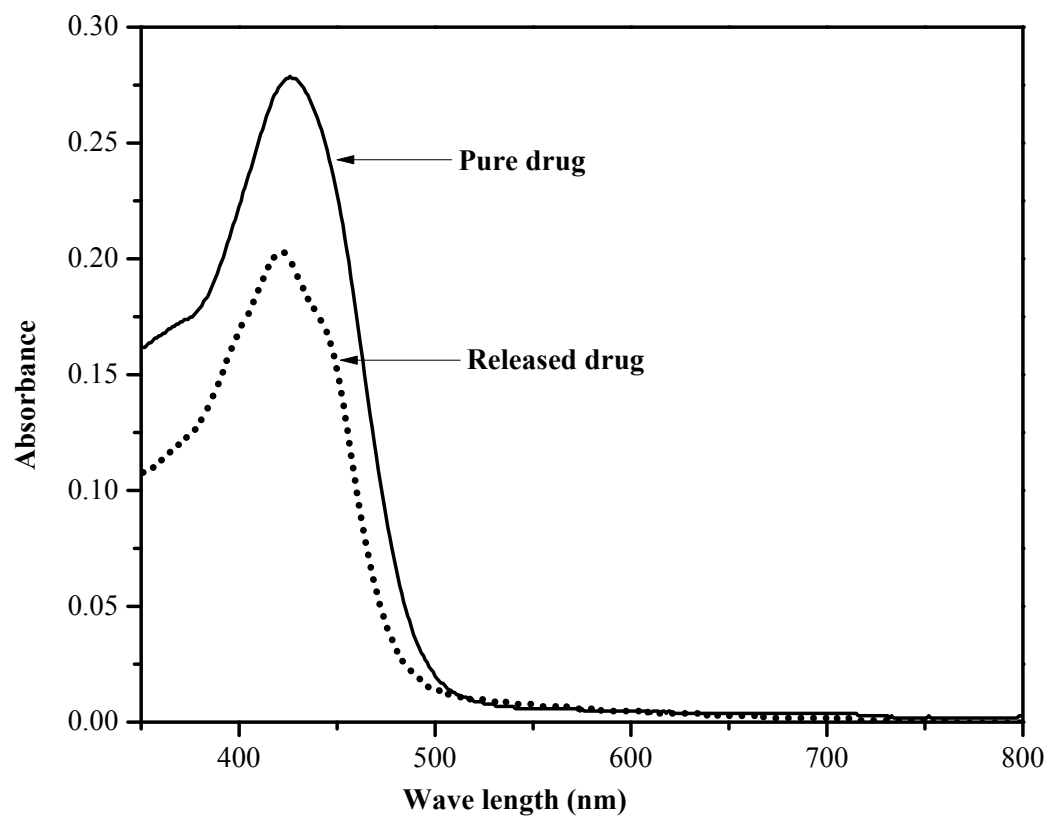


Fig. 10. UV-visible spectra of (a) pure drug and (b) released drug.

Table 1.

Composition of curcumin entrapped chitosan/nanocellulose/Tween 20 hydrogel formulations.

Formulation	Chitosan (w/v)%	Nanocellulose (w/v)%	Tween 20 (w/v)%	Glutaraldehyde (v/v)%	Curcumin (mg per 2.5 g of hydrogel disc)
CH/CNC/ TW-0%	2	0.5	0	0.2	1.5
CH/CNC/ TW-5%	2	0.5	5	0.2	1.5
CH/CNC/ TW-10%	2	0.5	10	0.2	1.5
CH/CNC/ TW-15%	2	0.5	15	0.2	1.5
CH/CNC/ TW-20%	2	0.5	20	0.2	1.5
CH/CNC/ TW-25%	2	0.5	25	0.2	1.5
CH/CNC/ TW-30%	2	0.5	30	0.2	1.5