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Beta casein-micelle as a nano vehicle for solubility enhancement of curcumin; food industry application

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ABSTRACT

Curcumin is a potent anticancer and antioxidant natural polyphenol poorly soluble in aqueous solutions. Beta-casein (B-CN), an amphiphilic self-assembling protein that can form micellar nanostructures, could be used as a carrier system for hydrophobic therapeutic agents such as curcumin. In this study, camel B-CN was used for curcumin encapsulation. Critical micelle concentration of camel B-CN was determined at 25, 30 and 37 °C using pyrene fluorescence and the solubility of curcumin was evaluated according to the solvent-evaporation technique. Presence of camel B-CN increased the solubility of curcumin at least 2500 fold. Analysis of fluorescence emission of curcumin showed that hydrophobic interactions are predominant in its formulation with B-CN. Additionally, the cytotoxicity of curcumin to human leukemia cell line K-562 was enhanced in the presence of B-CN micelles giving inhibitory concentration (IC_{50}) values of 26.5 and 17.7 μmol/L for free and encapsulated curcumin, respectively. Antioxidant activity of curcumin encapsulated in B-CN was higher than that of both free B-CN and curcumin.

1. Introduction

Two main approaches are commonly used when inventing a new drug carrier. Either, the bulk material is dispersed in fine structures or molecules capable of self-assembly are used. Physicochemical properties of proteins including emulsification, foaming and gelation make them a good matrix in development of new nutraceutical applications and as drug delivery vehicles (Branco & Schneider, 2009; Chaudhry et al., 2008; Nezhadi, Choong, Lotfipour, & Dass, 2009; Torchilin, 2001).

Curcumin (1,7-bis(4-hydroxy-3-methoxyphenyl)1,6-heptadiene -3,5-dione) is a polyphenol natural product derived from *Curcuma longa* (Chattopadhyay, Biswas, Bandyopadhay & Banerjee, 2004; Niranjan & Prakash, 2008) with antiproliferative (Kunnumakkara, Arnand, & Aggarwal, 2008; Shishodia, Chaturvedi, & Aggarwal, 2007; Schaaf et al., 2009), antiangiogenetic (Bhandarkar & Arbiser, 2007; Rafiee, Otterson, & Binion, 2008) and antioxidant properties (Ak & Gulcin, 2008), and it is of special importance among multi-target components in cancer chemotherapy (Goel, Jhurani, & Aggarwal, 2008). Curcumin is poorly soluble in water what is limiting its bioavailability (Singh, 2007), thus impending its conversion from cooking to clinical applications (Goel, Kunnumakkara, & Aggarwal, 2008).

To increase curcumin bioavailability and incorporating it in functional foods, several carriers including hydrophobically modified starch (Yu & Huang, 2010), cyclodextrins (Baglole, Boland, & Wagner, 2005), polyvinyl alcohol/polyvinyl alcohol hydrogel (Shah, Mishra, Kumar, Priyadarsini, & Bajaj, 2008), polymeric nanoparticles (Bisht et al., 2007; Thangapazham, Puri, Tele, Blumenthal, & Maheshwari, 2008) and bovine whole casein micelles (Sahu, Kasoju, & Bora, 2008) have been investigated.

Worldwide efforts are taking place to discover and consume new sources of food proteins. Nowadays camel milk has become quite attractive from scientific and industrial points of view. However, the literature is lacking any basic and applied reports

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about some camel dairy proteins for example beta casein (B-CN), which is one of the most important proteins in the case of microstructure formation. Our laboratory has focused for some years on investigation of camel caseins' structure and elucidation of their physico-chemical properties in order to fill one of these gaps in knowledge. B-CN is an amphiphilic self-assembling protein, which forms small oblate micelles (as demonstrated by cryo-electron microscopy and dynamic light scattering studies) with a diameter of approximately 13 nm in aqueous solutions. Their structure reminds amphiphilic diblock copolymers forming micellar aggregates (Dauphas et al., 2005; Mikheeva, Grinberg, Grinberg, Khokhlov, & de Kruif, 2003; Portnaya et al., 2008). Camel B-CN (accession number Q9TVD0) has 217 amino acids and a pI of 5.7. This protein share some main characteristics with bovine B-CN in terms of number of hydrophobic residues, surface hydrophobicity plots and number of serine and threonine, which are potential phosphate group acceptors. Moreover, these proteins show 84.5% similarity and 67.2% identity (Barzegar et al., 2008).

The aim of this study was to examine the potential of camel B-CN micelles as a carrier system to improve the solubility and hence, the bio efficacy of curcumin.

2. Material and methods

2.1. Chemicals

Camel milk was generously provided by Faculty of Veterinary, University of Tehran, Iran. Curcumin (purity $<$ 90%) was purchased from Merck (Hohenbrunn, Germany). ABTS [2,2′-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)], pyrene and 8-anilino-1-naphthalenesulfonic acid (ANS) were obtained from Sigma (Munich, Germany). All other chemicals were of analytical grade.

2.2. Beta-casein purification

Camel B-CN was purified according to the modified methods of Rasmussen, Due, and Petersen (1995) and Egito et al. (2002) as previously described (Barzegar et al., 2008). The purity of B-CN was assessed using SDS-PAGE and estimated to be more than 95%.

2.3. Preparation of B-CN and curcumin stock solutions

Camel B-CN (M_r 24,650 Da) was dissolved in 5 mmol/L phosphate buffer (pH 7.0) containing 80 mmol/L NaCl and the solution was passed through filter membrane to avoid formation of large protein aggregates, according to Portnaya et al. (2008) and the concentration was determined using an extinction coefficient of 0.302 at 280 nm. Curcumin stock solution was first dissolved in ethanol and then diluted to 370 μmol/L in double distilled water. In all the experiments, the final concentration of ethanol did not exceed 10 ml/L.

2.4. Fluorescence spectroscopy

Pyrene (benzo,d,e,f, phenanthrene) is one of the fluorescent probes that are widely used in determination of critical micelle concentration (CMC, a minimum concentration at which micelles are starting to form) of surfactants and polymers since the relative intensity of the first and third emission peaks (which are denoted as I_1 and I_3 at 373 and 393 nm, respectively) is a good marker of the environment polarity (Liu & Guo, 2008). Pyrene (1.5 μmol/L) was excited at 337 nm and the emission spectra were recorded between 350 and 600 nm using Cary-Eclipse spectrofluorimeter. Both emission and excitation slits were set to 2.5 nm. Plots of I_3/I_1 versus log B-CN concentration were drawn using Sigma plot 11 software and the concentration at which a sudden increase in I_3/I_1 had been occurred (turning point) was considered as CMC value, in other word the lowest concentration at which the micelles are starting to form.

Curcumin fluorescence spectra (excitation 420 nm) were measured in the range of 450–700 nm, in the presence of different concentrations of B-CN. Emission and excitation width slits were set to 5 and 2.5 nm, respectively (Mandeville, Froehlich, & Tajmir-Riahi, 2009).

Intrinsic fluorescence (IF) of B-CN was recorded from 315 to 600 nm (excitation at 280 nm) at different temperatures and in the presence of different curcumin concentrations. In order to estimate binding parameters accurately, casein concentration was below the CMC values in these experiments.

ANS (8-Anilinonaphthalene-1-sulfonate) binding to camel B-CN at different temperatures was assessed after 10 min incubation of 5 μmol/L B-CN with 200 μmol/L ANS. Fluorescence spectra (excitation at 390 nm) were recorded between 400 and 600 nm on Cary-Eclipse spectrofluorimeter. The bandwidths for both excitation and emission were 5.0 nm (Barzegar et al., 2008).

2.5. UV–Vis spectroscopy

UV–Vis absorption spectra of curcumin alone (10 μmol/L) or in the presence of various concentrations of B-CN were recorded on Shimadzu-3100 double beam spectrophotometer (Japan).

2.6. Aqueous and micellar solubilities of curcumin

Value of aqueous solubility of curcumin has been determined according to Tønnesen, Másson, and Loftsson (2002). The micellar solubility was calculated in a 10 g/L B-CN solution by the solvent-evaporation method (Letchford, Liggins, & Burt, 2007). Briefly, curcumin and B-CN were dissolved in 150 ml/L ethanol, and then the solvent was removed at 40 °C under vacuum using a Rotavapor. Micelles were formed by the addition of phosphate buffer (5 mmol/L, pH 7.0) and 5 min vortexing. This solution was incubated overnight in a water bath (37 °C) and the undissolved curcumin was removed by centrifugation (10,000 rpm, 10 min). The supernatant was diluted in methanol and the absorption was measured at 428 nm. The solubility was calculated using the extinction coefficient of curcumin in methanol (48,000 cm^{-1} M^{-1}) (Bong, 2000).

2.7. In vitro cytotoxicity study

Human leukemia cell line K562 was grown at 37 °C in the presence of 5% CO_2 to the logarithmic phase in RPMI 1640 (Gibco, Invitrogen) supplemented with 100 ml/L fetal bovine serum (FBS), 100 unit/ml penicillin and 100 μg/ml streptomycin (Biosera).

Cells were seeded in a 96-well plate (2×10^4 cell/well) and treated with different amounts of free curcumin (diluted in the medium), and in the case of B-CN–curcumin complex, curcumin was diluted with B-CN solution with a final concentration of B-CN of 32 μmol/L in each well (four times higher than the CMC value at 37 °C, when almost all the B-CN molecules are in micelles form). The cytotoxicity of free and micelle-entrapped curcumin was determined using an MTT [(3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Briefly, 10 μl of MTT stock solution (5 mg/ml in PBS) was added to each well and incubated for further 4 h. The resulting formazone crystals, formed by living cells, were dissolved in 50 μl of a solution containing 500 ml/L dimethylformamide (DMF) and 100 g/L SDS (Bharti, Donato, Singh, & Aggarwal, 2003) and the absorbance was read against a blank at 570 nm. Viability was calculated according to the Eq. (1) and data were analyzed as three independent experiments with SPSS 16 software using formula:

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$$\%\ \text{viability} = \frac{OD_{570}\ \text{of treated cells}}{OD_{570}\ \text{of control cells}} \times 100 \quad (1)$$

In these experiments two kinds of control were used, the first without both B-CN and curcumin, and the second containing B-CN only at a concentration of 32 μmol/L.

2.8. Quantification of total antioxidant activity (ABTS assay)

Total antioxidant activity (TAA) was measured by the Trolox equivalent antioxidant capacity test (TEAC) (Miller & RiceEvans, 1996). The ABTS radical was generated by reacting a 20 mmol/L ABTS solution with 70 mmol/L potassium persulfate stored in the dark for 12 h. The $ABTS^{\bullet+}$ solution was diluted to give an absorbance of 0.750 ± 0.025 at 734 nm in phosphate buffered saline (PBS) (pH 7.4). PBS was prepared by mixing 5 mmol/L NaH_2PO_4, 5 mmol/L Na_2HPO_4 and 153.84 mmol/L NaCl in 1 L of distilled water.

Upon addition of 10 μl of antioxidant sample or Trolox standard to 1.0 ml of the diluted $ABTS^{\bullet+}$ solution, the absorbance at 734 nm was recorded periodically for 6 min. The scavenging capability of test compounds was calculated using Eq. (2):

$$ABTS^{\bullet+}\ \text{scavenging}(\%) = \left(1 - \frac{A_s}{A_c}\right) \times 100 \quad (2)$$

where A_s and A_c are the absorbance of test and control solutions, respectively. PBS was used as blank.

3. Results and discussion

3.1. Fluorescence spectroscopy

CMC values for B-CN decreased with temperature increasing from 25 to 37 °C (Fig. 1A). At 37 °C CMC was reached at 8 μmol/L, hence the micellization of camel B-CN is temperature dependent.

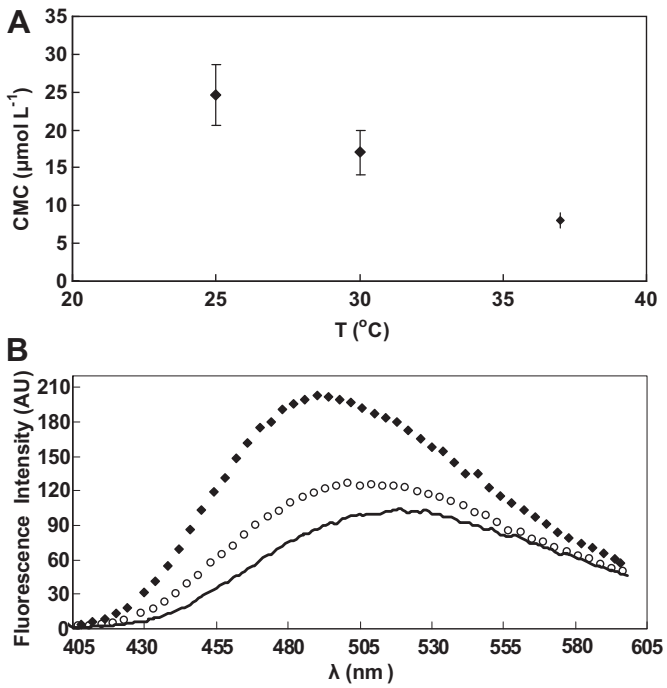

Fig. 1. (A) Critical association concentration (CMC) values of camel B-CN were calculated using pyrene fluorescence at 25, 30 and 37 °C; (B) ANS fluorescence of B-CN (5 μmol/L) at 25 °C (solid line), 30 °C (○) and 37 °C (◆).

Analysis of surface hydrophobicity (as shown in Fig. 1B) using ANS fluorescence indicated that as the temperature increases, ANS intensity is enhanced and an obvious blue shift occurred in the spectra measured at 30 °C and 37 °C. It means that more hydrophobic patches are exposed during heating. The main reason for this phenomenon is that the entropy increases along with temperature, and hydrophobic interactions are the only entropy driven forces mainly responsible for self-association of proteins. The same results have also been reported for bovine B-CN and the obtained CMC values of camel B-CN at 37 °C is nearly similar to 8.3 μmol/L reported by Portnaya et al. (2008) using isothermal titration microcalorimetry (ITC). It is worth mentioning that the experimental conditions in terms of pH and ionic strength (5 mmol/L phosphate buffer, pH 7) were similar. O'Connell, Grinberg, & de Kruif (2003) measured this parameter via dynamic light scattering (DLS) under different conditions i.e. in water at pH 6.5, and reported value of 0.5 mg/ml (21 μmol/L) at 40 °C. It is clear that the slight differences in CMC value of these proteins are mainly due to variability of experimental conditions including the instruments used, ionic strength and to some extent pH.

In contrast to other GRAS carriers such as whole casein micelle and hydrophobically modified starch (with CMC values of 1 mg/ml and 5 mg/ml, respectively), B-CN micelle has a lower CMC value and consequently represents an obvious advantage among other biopolymers that have been proposed so far as curcumin delivery vectors.

Fluorescence intensity of a protein is mainly due to Trp residues. Camel B-CN contains 5 Tyr and 10 Phe residues, which are mainly located in the hydrophobic part of its primary structure, which is devoid of Trp (comparing to bovine B-CN which contains 1 Trp, 4 Tyr and 8 Phe residues). Interaction between curcumin and B-CN at different temperatures resulted in a quenching of the intrinsic fluorescence at 280 nm (Fig. 2). Quenching of the protein fluorescence upon interaction with curcumin does not induce any shift in the spectra neither to blue nor to red. The binding constants and the number of binding sites were comparatively calculated assuming the static quenching by Eq. (3) (Hu, Liu, Wang, Xiao, & Qu, 2004; Muresan, van der Bent, & de Wolf, 2001; Zolghadri et al., 2009).

$$\text{Log}\frac{\Delta F}{F} = \text{Log}\ K_b + n\ \text{Log}[\text{curcumin}] \quad (3)$$

where ΔF represents the difference between fluorescence intensity in the presence or absence of curcumin (because curcumin presence leads to a decrease in fluorescence intensity), K_b and n represent the intercept and the slope of straight line, respectively. These experiments were carried out at least three times and the calculated values are mean ± SD of the triplicates.

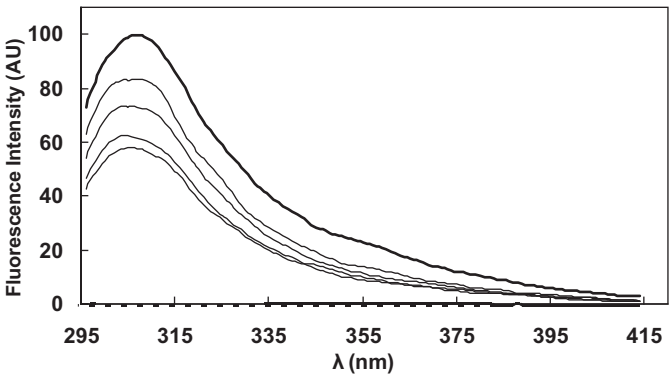

Fig. 2. Intrinsic fluorescence (excitation at 280 nm) of camel B-CN (5 μmol/L) in the presence of different concentration of curcumin (0, 4, 8, 12 and 16 μmol/L from top to bottom) at 25 °C. Baseline is shown as a dashed line.

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Stern-Volmer constants were calculated according to Eq. (4):

$$\frac{F_0}{F} = 1 + K_{sv}[\text{Curcumin}] \quad (4)$$

where F_0 and F are fluorescence intensities in the presence and absence of quenching agent, respectively. The plots of F_0/F are shown in Fig. 3B.

The obtained values of K_{sv} (Table 1) correlate well with temperature increase Moreover, increasing the temperature from 25 to 37 °C enhanced the binding constant about 46 times, due to hydrophobic interactions (Fig. 3A and Table 1). The thermodynamic parameters are good indicators of main force contributing in the interaction hence, these parameters were calculated at different temperatures via van't Hoff Eq. (5):

$$\text{Ln } K_b = -\frac{\Delta H}{RT} + \frac{\Delta S}{R} \quad (5)$$

where ΔS and ΔH describe the change in entropy and enthalpy of the binding, respectively, K_b is the binding constant related to each temperature, R is gas constant and T is the absolute temperature.

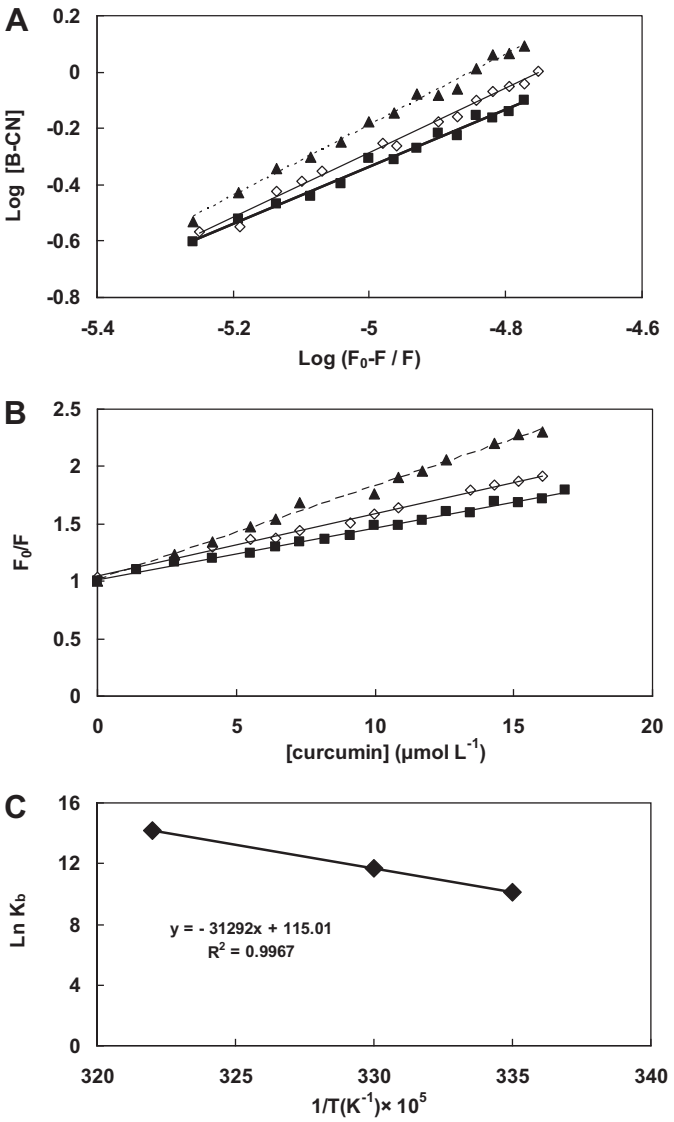

Fig. 3. (A) Binding curves of the interaction at 25 °C (■), 30 °C (◇) and 37 °C (▲) calculated according to Eq. (3); (B) Stern- Volmer plots of the interaction between curcumin and B-CN at 25 °C (■), 30 °C (◇) and 37 °C (▲); (C) van't Hoff plot.

Table 1
Binding parameters of the interactions between curcumin and camel B-CN at different temperatures.[a]

Temperature (K)	[b]ΔG (kJ mol^{-1})	[c]$K_{binding}$ (mol/L)$^{-1}$	[d]$10^{-4} \times$ Ksv (mol/L)$^{-1}$	[e]*n*
298	−339.6	$(1.8 \pm 0.4) \times 10^4$	4.8 ± 1	0.9 ± 0.02
303	−345.2	$(2.2 \pm 0.7) \times 10^5$	6.33 ± 2	1.1 ± 0.05
310	−353.1	$(8.3 \pm 0.7) \times 10^5$	7.16 ± 1	1.3 ± 0.05

[a] Data are expressed as mean ± SD of triplicate values.
[b] Values of Gibbs free energy.
[c] Binding constants calculated according to Eq. (3).
[d] Stern- Volmer constant calculated according to Eq. (4).
[e] Number of binding site.

Then, ΔG values were calculated according to Gibbs equation (6) (Jang, Liu, Chen, & Zou, 2009):

$$\Delta G = \Delta H^0 - T\Delta S^0 \quad (6)$$

Using Fig. 3C, the obtained ΔS^0 is positive (1.124 ± 0.2 kJ mol^{-1}K^{-1}) indicating that interactions are entropy driven, the characteristics emphasize that hydrophobic interactions are mainly involved in this process. The negative value of ΔH^0 (−4.505 ± 0.8 kJ mol^{-1}) shows that the interaction is exothermic. As shown in Table 1, ΔG values at three temperatures are negative. This parameter indicates that the reaction proceeds spontaneously.

Estimation of binding parameters including binding constants, ΔG, ΔH and Stern-Volmer constants is one of the basic routes toward our understanding of stability of the complex. In addition, these factors are good indicators of micelle and drug compatibility, hydrophobicity and charge at core micelle, and affect thermodynamic and kinetic stability of the complex and consequently will be of crucial importance from usage point of view (Yamamoto, Yokoyama, Opanasopit, Hayama, Kawano, & Maitini, 2007).

3.2. Change in fluorescence and in absorption spectra of curcumin in the presence of B-CN

Curcumin is a fluorophore, which can be excited at 420 nm and like other fluorophores such as ANS its emission is affected by the polarity of the medium (Wang et al., 1997). Consequently, free curcumin in a polar solvent has a broad low intensity emission spectrum but after transition in a less polar environment (e.g. adhesion to hydrophobic domains of proteins such as bovine serum albumin (BSA) and inclusion into hydrophobic cores) its emission spectrum exhibits a sharp peak. Titration of free curcumin with different concentrations of B-CN (Fig. 4A) shows a shift of λ_{max} toward lower wavelengths (blue shift). This change in λ_{max} is accompanied with an increase of fluorescence intensities, suggesting the hydrophobic character of interactions between curcumin and camel B-CN. In contrast, the polarity of local environments does not influence UV–Vis absorption spectrum of curcumin. As shown in Fig. 4B, the absorption spectra of free curcumin and curcumin–B-CN complexes are significantly different during intensity measurements, which indicate an increase in its solubility (assuming no significant effect of B-CN on the extinction coefficient of curcumin). The extinction coefficient of curcumin was calculated in the presence of B-CN according to the method of Moosavi-Movahedi et al. (2006). This value is actually by far smaller than that of free curcumin (data not shown). However, the spectra show negligible change in maximum wavelength. This observation is consistent with previous results indicating a very small solvent polarity effect on the absorption of curcumin (Baglole et al., 2005; Shah et al., 2008).

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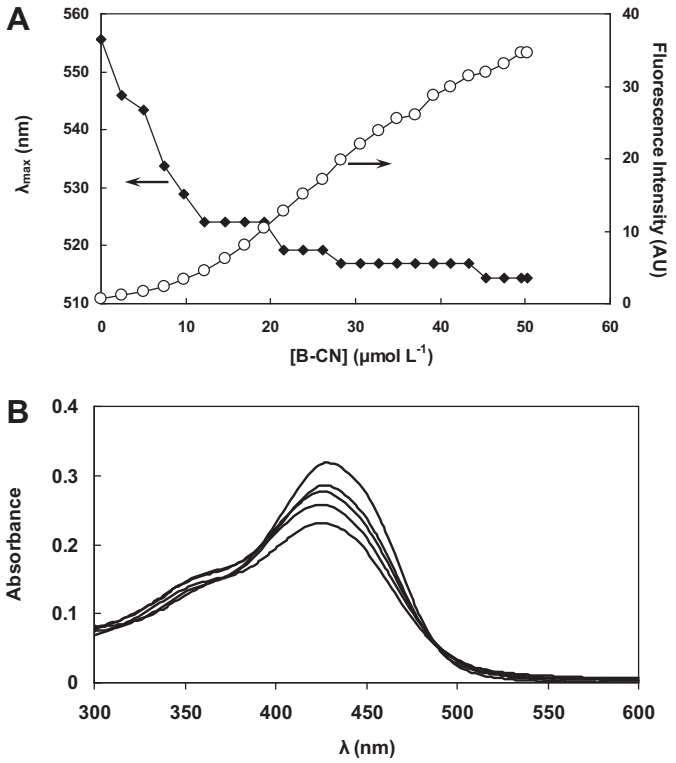

Fig. 4. (A) Measures of the change of fluorescence emission intensity (○) and maximum wavelength (◆) of curcumin (10 μmol/L) in the presence of different concentrations of B-CN; (B) Absorption spectra of curcumin (10 μmol/L) in the presence of increasing concentrations of B-CN (0, 10, 15, 20, 40 μmol/L from bottom to top).

3.3. Solubility of curcumin in camel B-CN micelles

The solubilization of curcumin in aqueous solution of B-CN (10 g/L) was investigated. This solution was centrifuged to remove any precipitate and the absorbance of supernatant was read in methanol solution at 428 nm. As observed in this study, aqueous and micellar solubilities of curcumin were 2.99×10^{-8} and 7.7×10^{-5} mol/L, respectively. Therefore, camel B-CN enhances markedly the solubility of curcumin (at least 2500 fold). It is worth mentioning that other synthesized chemical polymers including di block copolymer, hydrophobically modified starch and HP-γ-cyclodextrin were reported to enhance solubility about 131,000, 1670 and 4700 times, respectively (Baglole et al., 2005; Letchford et al., 2007; Yu & Huang, 2010). However, the significant lower CMC value of B-CN as compared with synthesized chemical copolymers and modified cyclodextrins makes B-CN the most efficient GRAS protein emulsifier for encapsulation of curcumin or possibly also other hydrophobic health beneficial compounds.

3.4. Evaluation of the cytotoxic effect of curcumin–B-CN complex on K-562 leukemia cell line

K562 leukemia cells were exposed to a number of equivalent concentrations of free and encapsulated curcumin and their viability was quantified using MTT assay. Either free or encapsulated curcumin showed a dose-dependent cytotoxicity effect (Fig. 5). However, B-CN alone has no harmful effect on the cells (control, gray bar) as compared with untreated cells (control, white bar). The calculated IC_{50} values of free and B-CN encapsulated curcumin were 26.5 and 17.7 μmol/L, respectively. Among other carrier systems, which have been reported till now, three systems

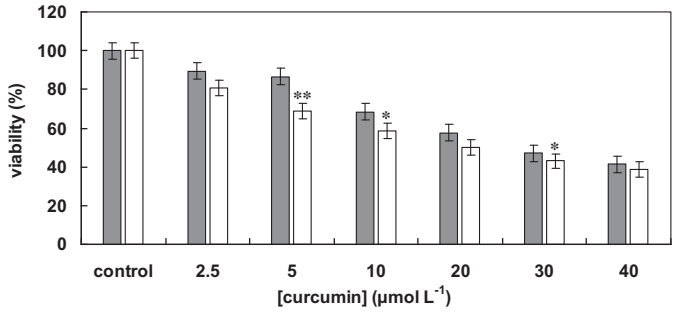

Fig. 5. Cytotoxicity of equal concentrations of free curcumin (gray bars) and curcumin–B-CN complex (white bars) on K562 human leukemia cells. Results are averages ± SD of three independent experiments ($n = 10$). * and ** are representative of $P_{value} < 0.05$ and $P_{value} < 0.01$, respectively. Controls are cells without curcumin and B-CN (control white bar) and cells in the presence of B-CN only (control grey bar).

including hydrophobically modified starch, bovine whole casein micelle and polymeric nanoparticle have been shown to increase the cytotoxicity but this property was tested on different cell lines (HepG2, Hela and human pancreatic cell lines, respectively) other than K-562 leukemia cells. It seems that modified starch is more potent than the other carriers since this complex could enhance cytotoxicity of curcumin up to 5 times at some concentrations (Yu & Huang, 2010), while whole casein micelles, our own reported complex and nano curcumin decrease the IC_{50} values about 2, 9 and 5 μmol/L, respectively. Regardless of what type of vehicle is used, various tumor cell lines show different preference toward curcumin uptake. Therefore, the conclusion should be taken cautiously. In addition, studies carried out on liposomal loaded curcumin have displayed *in vitro* efficacy on EL4 lymphoma and the authors demonstrated only just more uptake. However, they have not reported any more information about accurate evaluation of IC_{50} value (Kunwar, Barik, Pandey, & Priyadarsini, 2006).

3.5. Effect of solvents and interactions on the antioxidant activity of curcumin

Total antioxidant activity was analyzed by using TEAC test. As shown in Fig. 6, the antioxidant activity of 20 μmol/L B-CN is significantly higher than that of 120 μmol/L curcumin dissolved in 10% ethanol ($P_{value} < 0.01$). Antioxidant activity of proteins is mainly due to the presence of some amino acids including Trp, Tyr, Phe, and His, which have a hydrogen donor group such as indole, phenyl and imidazole, or others like Met, Cys, and Pro, considered as endogenous protein antioxidants (Levine, Mosoni, Berlett, & Stadtman; 1996; Zhang, Wang, & Xu, 2008). Antioxidant activity of curcumin (120 μmol/L), dissolved in 96% ethanol, is obviously higher than that of the same concentration of curcumin in 100 ml/L

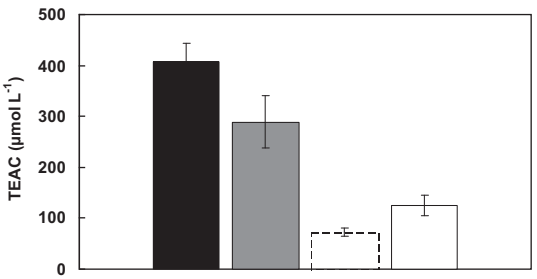

Fig. 6. Antioxidant activity of B-CN (20 μmol/L) (white bar), curcumin (120 μmol/L) in 100 ml/L ethanol (dashed line), curcumin (120 μmol/L) in 96% ethanol (grey bar), curcumin (120 μmol/L in 100 ml/L ethanol) in B-CN solution (20 μmol/L) (black bar) at 25 °C.

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hydro-ethanolic solution ($P_{value} < 0.001$). However, the mixture of B-CN (20 μmol/L) and curcumin (120 μmol/L in 100 ml/L ethanol) shows the highest antioxidant activity, and this effect is beyond a simple additive effect.

Regeneration mechanism and a combination of different antioxidant mechanisms for radical scavenging would account for this observation. Other factors such as pH, solubility, interactions (hydrogen bonds and hydrophobic interactions), emulsifier type (in terms of physical structure and charge) and physical location of an antioxidant might influence antioxidant property and affect the hydrogen donating capacity of phenolic compounds (Sørensen et al., 2008). A few studies about antioxidant activity in the presence of emulsifiers have been reported. Based upon our results, contributions of hydrophobic interactions and demonstrated more solubility in B-CN result in the consequent increase in antioxidant activity. Such an enhanced antioxidant activity has been noticed in other surveys as well (Altunkaya, Becker, Gökmen, & Skibsted, 2009; Capitani, Carvalho, Botelho, Carrapeiro, & Castro, 2009; Sørensen et al., 2008).

4. Conclusion

The present study demonstrates that camel B-CN micelles interact with curcumin mainly via hydrophobic interactions, what increases the solubility of curcumin and its bioavailability and antioxidant activity. These observations provide another candidate carrier for curcumin with GRAS status potentially useful in food industries and nutripharma. Curcumin loaded B-CN micelles can be an excellent nano vehicles (carriers) for this compound in the formulation of functional health food formulations.

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