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Research Article

Curcumin nanosuspension: Screening for antioxidant and antibacterial activities and study of protein adsorption

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Abstract

Curcumin is considered to be a powerful bioactive agent, which is extensively known for its medicinal use. Despite its being excellent therapeutic agent and natural antioxidant, curcumin shows poor solubility and bioavailability. Therefore, the development of formulation of curcumin nanoparticles to improve its aqueous-phase solubility and absorption is beneficial. In the present study, we investigated the formulation of a curcumin nanosuspension (CN) at curcumin concentration of 0.025-0.100 mg/mL with/without surfactant by sonication method and examined its antioxidant activity. Surface modification of CN was carried out by coating with 1% sodium docecyl sulfate (SDS) as surfactant to obtain the modified CN with SDS (CNS). The particle size and morphology of CN and CNS were studied by scanning electron microscopy (SEM). An antioxidant activity was measured using a 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The CN without SDS at curcumin concentration of 0.025 mg/ml exhibited the highest antioxidant activity compared with other formulations. An antimicrobial effect of CN with/without SDS was tested by agar-well diffusion method. It was found that the highst antimicrobial activity against *Bacillus Cereus* was observed for the CNS at the curcumin concentration of 0.050 mg/mL with an inhibition zone of 15.7 ± 1.5 mm. Protein adsorption was investigated using model protein bovine serum albumin (BSA). At curcumin concentration of 0.100 mg/mL, BSA adsorption of CNS was approximately 2 times greater than the unmodified at the same conditions after 24 h which suggests that this formulation of CN increases its bioavailability and may serve as potential bioactive agent for combination therapy.

Keywords: curcumin nanosuspension**,** antioxidant activity**,** antimicrobial activity, protein adsorption

Introduction

Curcumin has shown potent antioxidant, anti-inflammatory and inhibitory effects on the initial stages of carcinogenesis (Sharma et al., 2005; Amalraj et al., 2017). It has shown anti-proliferative effect on a variety of cancers as an inhibitor of the transcription factor NF-κB and downstream gene products (Wilken et al., 2011). However, a poor solubility of curcumin in water at physiological pH and low bioavailability limited the absorption of curcumin after administration (Jäger et al., 2014; Siviero et al., 2015). To overcome the problems of poor solubility and low bioavailability, recently several nanotechnology approaches have been reported for the encapsulation of curcumin into nanoparticles such as liposomes, polymeric nanoparticles,

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micelles, nanogels, cyclodextrins, dendrimers, silvers and solid lipids as one of the useful alternatives (Ghalandarlaki et al., 2014). Although these biocompatible nanocarriers hold a promise to enhance the bioavailability and deliver therapeutic concentrations of curcumin the safety and toxicity issues cannot be ignored. In this study, curcumin nanosuspensions (CN) were developed by sonication method. The prepared CN were further modified by surface coating with sodium docecyl sulfate (SDS) and examined its antioxidant activity, antimicrobial effect and protein adsorption profile.

Materials and methods

Materials

Curcumin was obtained from local market in Bangkok. Sodium docecyl sulfate (SDS) and lyophilized bovine serum albumin (BSA) with molecular weight of 69 kDa (grade A9418) were purchased from Sigma-Aldrich. All reagents were of analytical grade and used as received.

Methods

Synthesis of curcumin nanosuspension (CN)

CN was prepared by a method previously described (Pandit et al., 2015). Briefly, a solution of curcumin was dissolved in dichloromethane (5 mg/ml). The stock solution was added to water (50 ml) in drop-wise manner under ultrasonication condition with an ultrasonic frequency of 50 kHz to prepare the CN at a curcumin concentration of 0.025-0.100 mg/ml and sonicated for 30 min. The prepared particles was lyophilized to obtain the powder CN. For modification of CN, the curcumin stock solution were resuspended with 1% SDS using the same method previously described to obtain the curcumin nanosuspension with SDS (CNS).

Particle characterization

Particle size and particle morphology was measured by scanning electronic microscopy (SEM) (JEM-1200EX, Japan). The freeze dried samples were placed on sample holder and coated with gold. The sample was observed under SEM at the electron acceleration of 60 kV.

Color measurement

The colors of CN and CNS at the concentration of 0.025, 0.05 and 0.10 mg/ml were measured as Hunter L (lightness), a (±, redness/greenness), and b (±, yellowness/blueness) values using colorimeter (Hunter Lab Colorflex 4510, USA).

Antioxidant activity test using DPPH assay

The radical scavenging activity of CN and CNS was determined using DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay. Briefly, 150 µl of 0.3 mM DPPH methanolic solution was added to an aliquot of CN or CNS in 3 ml of methanol. The mixture was protected from light and left to stand for 15 min. The absorbance was observed at 516 nm using a spectrophotometer (Optima SP-3000 plus, Japan). The absorbance of DPPH solution without sample was used as control. % % Antioxidant activity was calculated using the formula (1):

$$\% \text{ Antioxidant activity} = \frac{(\text{Control Absorbance} - \text{Sample Absorbance})}{\text{Control Absorbance}} \times 100 \qquad (1)$$

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Determination of antibacterial activity using disk-diffusion assay

Agar Disk-Diffusion method was carried out to test an antibacterial activity of CN and CNS against *Escherichia coli* and *Bacillus Cereus*. The lyophilized samples of CN and CNS were dissolved in sterile water and impregnated on to a small disc of sterile filter paper (6 mm diameter) and placed on nutrient agar plates containing a standardized inoculum of each test microorganism. The petri dishes were incubated at 37°C for 24 h and then the diameters of inhibition growth zones were measured.

Protein adsorption study

0.1 g lyophilized samples of CN and CNS were added in 10 ml BSA (0.1 mg/ml) and incubated at 37°C for 2 h to 72 h. The obtained supernatants were collected at each time intervals. The protein adsorption was determined indirectly by analyzing the non-adsorbed portion with UV-VIS spectrometry at wavelength of 562 nm using Lowry assay. % BSA adsorption was calculated from the amount of protein left in the supernatant (*m* BSA supernatant) and the total amount of protein added initially (*m* BSA int) using the formula (2):

$$\% \text{ BSA adsorption} = \frac{m\,\text{BSA int} - m\,\text{BSA supernatant}}{m\,\text{BSA int}} \times 100 \qquad (2)$$

Statistical analysis

All data were presented as means ± S.D. and all experiments were done in triplicate. Data were analyzed by analysis of variance (ANOVA) using sigma-plot stat. The differences between mean values were compared using Duncan's multiple-range test as $p \leq 0.05$.

Results and discussion

Particle characterization

According to poor solubility of curcumin in water at neutral pH values, its low bioavailability is a major problem for the application of curcumin as effective therapeutic agent (Anand et al., 2007). In this study CN was prepared by sonication technique with different curcumin formulation unmodified CN and modified CN using 1% SDS in distilled water as surfactant (CNS) to improve the solubility and prevent particle aggregation. The formulation and pH of all curcumin samples are shown in Table 1. As pH was found at 7-8, it would be advantages to employ these curcumin formulation to investigate their physicochemical properties and bioactivites. Unlike curcumin which is water insoluble, the CN and CNS formed a very fine aqueous dispersion and there was no particle aggregation. The particle size and the particle morphology of CN and CNS at a curcumin concentration of 0.1 mg/ml was observed using SEM (Figure 1). The diameter size of most particles ranged from 200 to 300 nm and exhibited a rice seed-like morphology which was was similar to polymorphism of curcumin prepared by ultrasound and stabilizers from previous study (Thorat et al., 2014). As shown in Figure 1, the unmodified CN and modified CNS were comparable in size and particle morphology. There was not a statistically significant difference between the size of CN and CNS at curcumin concentration of 0.1 mg/ml. Therefore, the use of 1% SDS as surfactant for CN modification did not play a role in the size and morphology of CN.

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Table 1. pH valules of various formulations of unmodified curcumin nanosuspension (CN) and modified curcumin nanosuspension with SDS (CNS).

Sample	Curcumin concentration (mg/ml)	Surfactant	pH
CN-1	0.025	-	7.7 ± 0.1
CN-2	0.050	-	7.6 ± 0.1
CN-3	0.100	-	7.6 ± 0.1
CNS-1	0.025	1% SDS	8.0 ± 0.1
CNS-2	0.050	1% SDS	7.9 ± 0.1
CNS-3	0.100	1% SDS	7.9 ± 0.1

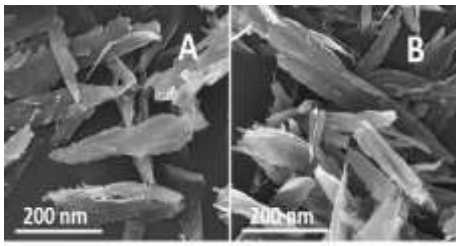

Figure 1. SEM images of CN (A) and CNS (B) at curcumin concentration of 0.1 mg/ml.

Effect of addition of curcumin extract on color of the nanosuspension

The color interference of antioxidant could be a limitation of antioxidant applications in commercial food products and supplements. Therefore, the effect of addition of curcumin extract on color of the nanosuspension was evaluated as Hunter *L, a, b* values. The Hunter *L, a, b* values of CN and CNS are shown in Table 2. The colors of CN and CNS at the curcumin concentration of 0.025, 0.050 and 0.100 mg/ml were measured as lightness (*L* value), redness (*a* value), and yellowness (*b* value). *L* and *a* values of the samples did not change at curcumin concentration of 0.025 to 0.100 mg/ml. Interestingly, the *b* value increased in a concentration-dependent manner for both CN and CNS corresponding to the yellow pigment found in curcumin. The *b* value of all CN preparations increased signifiacntly as the concentration of curcumin was increased. While in the case of CNS, all CNS preparations showed an increase in the *b* value with increase in concentration of curcumin similar to that of CN. There was a statistically significant difference among each formulations (p <0.05). To study the effect of surfactant, the *b* value of CN was compared to that of CNS at the curcumin concentration of 0.025, 0.050 and 0.100 mg/ml, respectively. At the same concentration of CN and CNS, the *b* value significantly (p <0.05) increased at any of the curcumin concentrations studied. Hence, the color of curcumin nanosuspension was significantly affected by curcumin concentration and surfactant.

Table 2. Hunter *L, a, b* value of curcumin nanosuspension.

Sample	Color values		
	L	***a***	***b***
CN-1	57.52 ± 0.06	-2.04 ± 0.01	12.63 ± 0.04
CN-2	55.07 ± 0.10	-0.05 ± 0.01	22.16 ± 0.04
CN-3	51.54 ± 0.03	2.20 ± 0.01	25.05 ± 0.04
CNS-1	58.00 ± 0.02	-9.77 ± 0.01	35.54 ± 0.01
CNS-2	56.81 ± 0.04	-10.65 ± 0.03	53.72 ± 0.06
CNS-3	54.21 ± 0.06	-7.30 ± 0.01	69.28 ± 0.31

Antioxidant activity test using DPPH assay

To access the antioxidant properties of curcumin, the DPPH assay is a rapid, simple and widely used for investigating the free radical scavenging activity of a compound (Kakran et al., 2012). As shown in Figure 2, the radical scavenging activity of CN and CNS was found in the ranges approximately 65-80% at curcumin concentration of 0.025-0.100 mg/ml. The CN exhibited concentration-independent radical scavenging activity. At curcumin concentration of 0.025 mg/ml, CN-1 exhibited highest antioxidant activity compared with other concentrations and

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there was a statistically significant difference between that of CN-1 and CN-3 (p <0.05). Moreover, a statistically significant difference was indicated between the antioxidant activity of CN-1 and CNS-1 (p <0.05), however, no significant difference was observed between the antioxidant activity of CN-2 and CNS-2 as well as that of CN-3 and CNS-3. Therefore, adding SDS as a surfactant did not improve the curcumin antioxidant activity. The previous study showed that the antioxidant activity of curcumin probable attribute to its phenolic hydroxyl groups (Malik and Mukherjee, 2014).

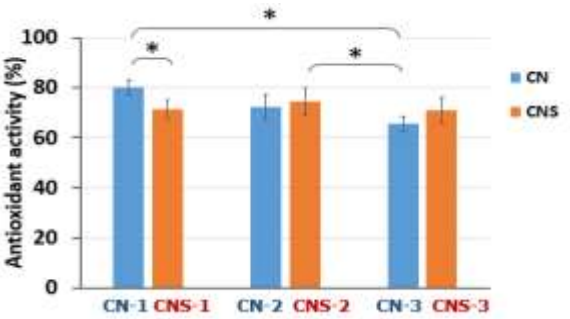

Figure 2. DPPH radical scavenging activity of curcumin nanosuspension.
(* represent significant differences at p <0.05)

Determination of antibacterial activity using disc diffusion assay

The antibacterial activities of CN and CNS were evaluated against two pathogenic bacteria (*Escherichia coli* and *Bacillus Cereus*) using disc diffusion assay. It was found that CN and CNS showed the intermediate inhibiting effect on *B. Cereus* with the inhibition zone ranging from 11-16 mm (Table 3 and Figure 3). It was found that the ratio of clear zone diameter over paper filter's diameter was in the range of 1.8-2.6. However, there was no a statistically significant difference in the mean values among these formulations (p > 0.05). In the present study the concentration of curcuimn (0.025-0.100 mg/ml) and the addition of SDS were not a major factor for the antibacterial activity of curcumin nanosuspension against *B. Cereus.* However, the concentration-dependent behavior of the curcumin extract could promote the inhibitory effect on *E. Coli*, *S. aureus*, *B. Cereus* and other pathogenic bacteria at minimum inhibitory concentration (MIC) value of 4 to 16 g/L which was higher than that of CN and CNS used in this study (Moghadamtous et al., 2014).

Table 3. Antibacterial activities of curcumin nanosuspension against *B. Cereus*.

Sample	Clear zone (mm)	Ratio of clear zone diameter over paper disc's diameter
CN-1	12.0 ± 2.7	2.00
CN-2	11.0 ± 1.0	1.83
CN-3	13.5 ± 5.8	2.25
CNS-1	14.0 ± 0.2	2.33
CNS-2	15.7 ± 1.5	2.62
CNS-3	13.2 ± 3.6	2.20

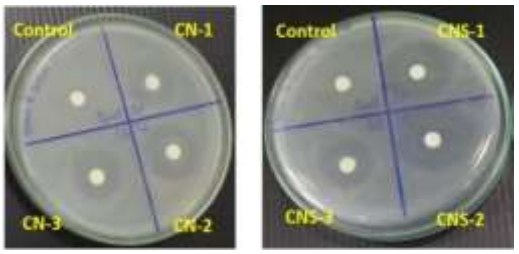

Figure 3. Clear zone of curcumin nanosuspension against *B. Cereus*.

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Protein adsorption study

Protein adsorption was investigated using bovine serum albumin (BSA) as a model protein. Albumin is the most abundant plasma protein in human body. As shown in Figure 4, the adsorption of BSA on CN and CNS increased in a dose-dependent manner. The initial uptake of BSA on both particles was slightly fast up to 24 h and followed by steady state after 24-72 h. After 24 h, BSA adsorption of CNS was approximately 2 times greater than the unmodified CN at the curcumin concentration of 0.1 mg/ml which suggested that the surfacemodification of CN with SDS enhanced the protein adsorption. The mechanism of protein adsorption resulted from hydrophobic interaction, electrostatic interaction and H-bond (Shubhra et al., 2014). The increased BSA adsorption of CN in the presence and absence of SDS could result from different interaction patterns between protein and CN.

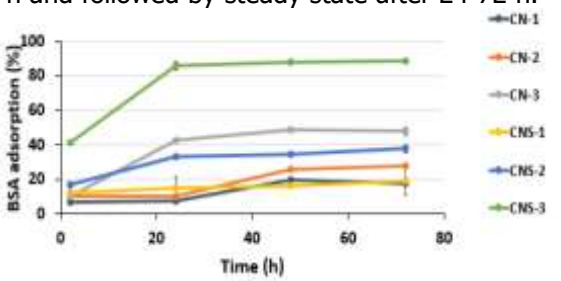

Figure 4. BSA adsorption of curcumin nanosuspension.

Conclusion

In summary, CN and CNS was successfully prepared by one-step sonication method. The size in diameter of CN and CNS was about 200-300 nm at curcumin concentration of 0.100 mg/ml. The addition of SDS did not change the particle size and morphology. These results suggest that the size of particle was not affected by adding SDS. There was no evidence that the size of particle can be affected by curcumin concentration in this study. However, the further studies on the effect of curcumin concentration on the particle size of CN and CNS are nedded. The unmodified CN and modified CNS with SDS were tested for their in vitro antioxidant and antibacterial activities. Both CN and CNS exhibited the radical scavenging activity ranging from 65 to 80% approximately and promoted the moderate antibacterial activity against *B. Cereus*. Compared with unmodified CN, the modified CNS showed a higher BSA adsorption capacity at the curcumin concentration of 0.05 and 0.10 mg/ml. The effective surface modification of CN with SDS could increase its bioavailability and this formulation may serve as potential bioactive agent for combination therapy.

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