# Characterization of Spray Dried Chitosan-Tripolyphosphate Nanoparticles Containing Curcuminoid

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## Abstract

This research was conducted to prepare nanoencapsulated curcuminoid powder by using spray drying chitosan -tripolyphosphate (CS-TPP) nanoemulsion to improve curcuminoid solubility for extending its use in the food industry. Curcuminoid was extracted by sonication of turmeric powder in 80% (v/v) ethanol for 15 minutes, then dried by using vacuum evaporator and vacuum suction. The type of emulsifier and acid solution used to dissolve chitosan with the ratio of CS:TPP for curcuminoid encapsulation by using spray drying were evaluated. The results showed that nanoemulsion with the smallest particle size of 381.60 nm was obtained by homogenization of curcuminoid oleoresin in 0.1% (w/v) chitosan dissolved in 5% citric acid with 1% lecithin powder as an emulsifier with a CS:TPP ratio of 0.125:1. The encapsulation efficiency was 67.25%. After spray drying, the curcuminoid nanoparticles were observed by TEM and had spherical morphology (300-400 nm) and good water solubility. The contents of curcumin, demethoxycurcumin and bisdemethoxycurcumin in the nanoparticles were 1.3734, 1.0803 and0.3954 mg/g DW respectively. After heating at 80°C for 3 hours, only 32.13% of curcumin content in the nanoencapsulated curcuminoid solution was lost. An MTT assay was used to compare the cell viability of the curcuminoid nanoparticles and the curcuminoid oleoresin. The results indicated that the curcuminoid nanoparticles are nontoxic to cells.

***Keywords*****:** *cucuminoid nanoparticles, nanoemulsion, ionotropic gelation, spray drying*

**บทคัดย่อ**

งานวิจัยนี้มีวัตถุประสงค์เพื่อห่อหุ้มสารเคอร์คิวมินอยด์ในระดับนาโนโดยวิธีเตรียมให้อยู่ในรูปนาโนอิมัลชันด้วยไคโตซาน (CS) และไตรพอลิฟอสเฟต (TPP) จากนั้นทำให้อยู่ในรูปผงแห้งด้วยเทคนิคการทำแห้งแบบพ่นฝอย เพื่อปรับปรุงสมบัติการละลายของเคอร์คิวมินอยด์ ซึ่งจะเป็นการเพิ่มการนำไปใช้ประโยชน์ในอุตสาหกรรมอาหาร ในการทดลองนี้เคอร์คิวมินอยด์จะถูกสกัดจากขมิ้นชันผงโดยใช้เอทานอลความเข้มข้นร้อย 80 (ปริมาตร/ปริมาตร) เป็นตัวทำละลาย สกัดด้วยเครื่องโซนิเคเตอร์เป็นเวลา 15 นาที จากนั้นกำจัดเอทานอลออกด้วยเครื่องกลั่นระเหยแบบหมุนภายใต้สุญญากาศ และการดูดภายใต้สุญญากาศ โดยในการวิจัยจะศึกษาชนิดของอิมัลซิไฟเออร์ และสารละลายกรดที่ใช้ในการละลายไคโตซาน รวมทั้งอัตราส่วนของ CS และ TPP ที่เหมาะสมในการเตรียมเคอร์คิวมินอยด์นาโนอิมัลชันก่อนการทำแห้งด้วยเครื่องทำแห้งแบบพ่นฝอย ผลการทดลองแสดงให้เห็นว่าสภาวะที่เหมาะสมคือ การโฮโมจิไนซ์เคอร์คิวมินอยด์โอลิโอเรซินในสารละลายไคโตซานความเข้มข้นร้อยละ 0.1 (น้ำหนัก/ปริมาตร) ที่เตรียมโดยการละลายไคโตซานในสารละลายกรดซิตริกความเข้มข้นร้อยละ 5 (น้ำหนัก/ปริมาตร) และใช้เลซิทินผงปริมาณร้อยละ 1 เป็นอิมัลซิไฟเออร์ จากนั้นห่อหุ้มด้วย CS และ TPP ในอัตราส่วน 0.125:1 วิธีนี้จะได้เคอร์คิวมินอยด์นาโนอิมัลชันที่มีขนาดเล็กสุดเท่ากับ 381.60 นาโนเมตร และมีประสิทธิภาพการห่อหุ้มร้อยละ 67.25 ซึ่งหลังจากนำไปทำแห้งด้วยเครื่องทำแห้งแบบพ่นฝอย จะได้อนุภาคเคอร์คิวมินอยด์ที่มีสมบัติการละลายดี เมื่อนำมาส่องดูด้วยกล้องจุลทรรศน์อิเล็กตรอนแบบส่องผ่านจะเห็นลักษณะของอนุภาคเป็นทรงกลมขนาด 300 ถึง 400 นาโนเมตร และจากการวิเคราะห์องค์ประกอบของเคอร์คิวมินอยด์ผงที่ถูกห่อหุ้มระดับนาโน พบว่าประกอบด้วย เคอร์คิวมิน ดีเมทอกซีเคอร์คิวมิน และบิสดีเมทอกซีเคอร์คิวมิน ในปริมาณร้อยละ 1.3734, 1.0803 และ 0.3954 มิลลิกรัม/กรัมน้ำหนักแห้ง ตามลำดับ ผลการทดสอบความเสถียรต่อความร้อนของสารละลายเคอร์คิวมินอยด์ที่ถูกห่อหุ้มระดับนาโนที่อุณหภูมิ 80 องศาเซลเซียส เป็นเวลา 3 ชั่วโมง พบว่าปริมาณเคอร์คิวมินสูญเสียไปร้อยละ 32.13 การศึกษาความเป็นพิษต่อเซลล์ด้วยวิธี MTT แสดงให้เห็นว่าเคอร์คิวมินอยด์ผงที่ถูกห่อหุ้มระดับนาโนไม่มีความเป็นพิษต่อเซลล์

***คำสำคัญ:*** *อนุภาคนาโนเคอร์คิวมินอยด์ นาโนอิมัลชัน ไอโอโนโทรปิคเจเลชัน การทำแห้งแบบพ่นฝอย*

## 1. Introduction

Curcuminoids are natural bioactive agents found in turmeric (*Curcuma longa* L.) roots. Curcuminoids consist primarily of three phenolic compounds: curcumin, demethoxycurcumin and bisdemethoxycurcumin. The most abundant component, curcumin, has been shown to pose antioxidant activity (Jayaprakasha et al., 2006), antimicrobial activity against oral bacteria (Mohammed & Habil, 2015), and can be used as a therapeutic agent for a variety of inflammatory conditions and cancer (Jurenka, 2009). However, the low solubility of curcumin causes a practical problem that restricts its usage as a food supplement. To overcome this problem, many encapsulation techniques have been reported such as encapsulation in liposomes (Wang et al., 2008), production of solid lipid microparticles using bovine serum albumin (Gupta et al., 2009) or complexing with cyclodextrin (Yallapu et al., 2010).

Chitosan, a functional linear polysaccharide is produced from N-deacetylation of chitin, has been considered as a versatile polymer for encapsulation and has delivery potentials for various nutrients. Chitosan micro/nano particles can be easily prepared by ionotropic gelation process using TPP which is a non-toxic crosslinker. The process is based on the interaction between the negative groups of TPP and the positively charged amino group of chitosan (Elgadir et al., 2015). The CS-TPP nanoparticles were loaded with tea catechins (Hu et al., 2008), quercetin (Zhang et al., 2008), and rutin (Konecsni et al., 2012) were successfully prepared via the ionic gelation method by controlling the critical fabricating parameters including CS molecular mass, CS concentration, and CS-TPP mass ratio. Parize and Stulzer (2012) revealed that chitosan microparticles containing curcumin cross-linked with TPP were efficiently prepared by a one step process using a spray drying technique. The microparticles were in an amorphous and dispersed state at the molecular level that promoted an increase in its solubility in aqueous solutions. The spray drying technique is quite suitable for the encapsulation of oils and oleoresins for several important reasons. It is an economical, flexible, and continuous operation which produces dry, stable food additives and flavors (Gharsallaoui et al., 2007). Microencapsulation of turmeric oleoresin using gum Arabic and maltodextrin as well as other materials by spray drying was also reported by Delfiya et al. (2015). In this paper, we describe the improvements in solubility and stability of curcuminoids by spray drying of CS-TPP curcuminoid nanoemulsion that were prepared via an emulsification-ionotropic gelation process.

## 2. Objectives

To investigate the preparation of curcuminoid nanoparticles by ionotropic gelation method using chitosan and tripolyphosphate followed by spray drying technique including characterization of their properties for food application.

## 3. Materials and methods

### 3.1. Raw Materials

Turmeric powder from the rhizome of *C. longa* L. was supplied by New Concept Product Co., Ltd, Thailand. Tri-polyphosphate (TPP) was sourced from Sigma, citric acid, purchased from Duksan Pure Chemical Co., Ltd. (Gyeonggi-do, Korea), chitosan powder from shrimp-shell chitin, with a degree of deacetylation of 90% was purchased from TS Agritech, Thailand. Lecithin powder was purchased from Lipoid GmbH, Germany. All other reagents were of analytical grade.

### 3.2 Preparation of Curcuminoid Oleoresin

Curcuminoid was extracted by sonication of turmeric powder in 80% (v/v) ethanol for 15 minutes then dried by using vacuum evaporator and vacuum suction until curcuminoid oleoresin was obtained.

### 3.3 Preparation of Curcuminoid Nanoemulsion

The curcuminoid emulsion was prepared in two steps. First, chitosan solution was prepared following the method of Parize and Stulzer (2012). Chitosan (2.0 g) was briefly dissolved in 100 ml of 5% acid solutions (citric acid or acetic acid) containing different kinds of emulsifiers (1% Tween 80 or lecithin powder), the solutions were stirred continuously for 12 hours. Dispersions containing 25 mg of curcuminoid oleoresin were homogenized in chitosan solution at 10,000 rpm for 5 minutes, and 40 ml of 2% (w/v) TPP

was added to these solutions. After the addition of TPP, the solutions were stirred continuously for 2 hours, and kept at room temperature before measuring the color (L*, a*, b* value) at 0, 3, 6 and 9 days to evaluate the stability of curcuminoid in different acid solutions and emulsifiers.

Suitable acid solution and emulsifier options were chosen to prepare curcuminoid nanoemulsions with different mass ratios of CS:TPP as 0.125:1, 0.5:1 and 1.25:1 respectively. These emulsions were adjusted to pH 5.5 using 2 N NaOH following the method of Konecsni et al. (2012), then ultrasonicated at 50 W for 3 minutes before the particle sizes were measured by Dynamic Light Scattering (Zetasizer Nano ZS90, Malvern Instruments) and the encapsulation efficiency (%EE) was detected using the method of Gomez-Estaca et al. (2015). The nanoemulsion sample was briefly diluted in 80% ethanol in the ratio of 1:1, then vortexed for 5 minutes followed by centrifugation (15,000 rpm, 5 minutes). The amount of curcuminoid in the supernatant was determined from its absorption at 427 nm. The percentage of curcuminoid incorporation was then calculated using a calibration curve ranging from 20-70 ppm of the original curcuminoid solution which was initially dissolved in 80% ethanol as per the following equation:

$$\% \text{ EE} = \frac{\text{Total curcuminiod amount} - \text{Free curcuminoid}}{\text{Total curcuminoid amount}} \times 100$$

3.4 Spray Drying of Curcuminoid Nanoemulsion

The curcuminoid nanoemulsion was dried using a Eyela Spray Dryer SD 1000 (Eyela, Japan). The sample was atomized under drying air at 160 ± 1°C, air outlet temperature of 95±1°C, feed rate of 480 ml/h, flow rate of 700 $m^3$/min and pressure of 2.5 bar. The drying process varied between 30-50 minutes. The product was collected in the form of fine powder and was kept in aluminum foil bag.

3.5 Characterization of Curcuminoid Nanoparticles

*3.5.1. Morphology and Size Measurement*

The morphology and the mean particle size of the curcuminoid nanoparticles were investigated via a transmission electron microscope (TEM, JEOL:JEM 1010, Korea). To prepare samples, 0.025 g powder was dissolved in 10 ml of water then diluted 10-fold and applied to the carbon-coat copper grids, with excess solution removed with filter paper. The image was analyzed at 30 kV.

*3.5.2. HPLC analysis of curcuminoid nanoparticles*

The curcuminoid nanoparticles were dissolved in distilled water and its components were analyzed using high performance liquid chromatography (Column: EC150/4.6 NUCLEODUR 100-5 C18ec, 250 L× 4.6 mm, 5 µm). The mobile phase consisted of acetonitrile:5% acetic acid (50:50). The eluent flowed isocratically at a flow rate of 1 ml/min. The Photo Diode Array detector was adjusted at 427 nm with the injection volume of 20 µl. Standard curcumin, demethoxycurcumin and bisdemethoxycurcumin were dissolved in methanol. The sample solutions were diluted with methanol and filtered through 0.45 µm membrane filter before injecting into HPLC.

*3.5.3. Solubility Test*

To evaluate the solubility of curcuminoid nanoparticles, 100 mg of the sample was dissolved in 10 ml of distilled water, vortexed for 1 minute and then centrifuged at 4,500 rpm for 10 minutes. The remaining precipitate after centrifugation was dried in a hot air oven at 105°C until constant weight was obtained.

*3.5.4. Thermal Stability Test*

The stability of nanoencapsulated curcuminoid solutions were investigated by dissolving 0.085 g of sample powder in 10 ml of distilled water then vortexed for 1 minute. The solutions were heated at 80°C for 0, 15, 30, 45, 60, 120 and 180 minutes. After thermal treatment, the samples were diluted with 80%

ethanol and curcumin content were determined at 427 nm using a calibration curve of curcumin ranging from 0.5-1.0 µg/ml.

*3.5.5. Cytotoxicity Test*

*In vitro* cytotoxicity of curcuminoid oleoresin and curcuminoid nanoparticles was evaluated by MTT test using the mouse monocyte RAW 264.7 cell line as previously published by Anuchapreeda et al., (2012).

*3.5.6 Statistical Analysis*

Results are expressed as mean standard deviation from triplicate measurements. Statistical analysis was performed using SPSS software (SPSS Ver. 22). A one-way analysis of variance (ANOVA) with a Duncan New Multiple Range Test was used to determine statistical significance ($p \leq 0.05$).

**4. Results and discussions**

4.1 Optimization of process condition for preparing curcuminoid nanoparticles

In order to prepare the nanoencapsulated curcuminoids using CS and TPP by using spray drying technique, nanocurcuminoid emulsion has to be prepared. In this study, different acid solutions (citric acid and acetic acid) for dissolving chitosan and different emulsifiers (Tween 80 and lecithin powder) were investigated by measuring the color stability of curcuminoids during storage time at room temperature as shown in Figure 1.

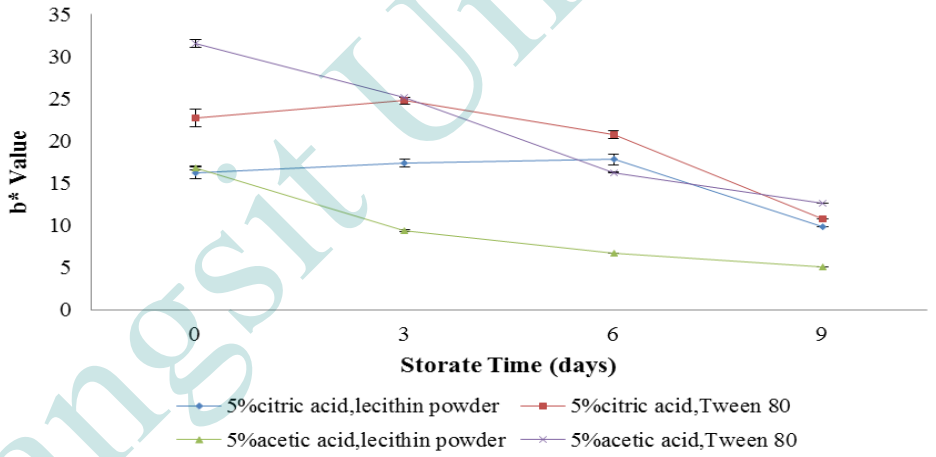


**Figure 1** Effect of acid solutions and emulsifiers on color stability of curcuminoid nanoemulsion

The yellow color of curcuminoid nanoemulsion in chitosan was dissolved in citric acid and using lecithin powder as an emulsifier which remain unchanged during the storage period of 6 days and no precipitation was observed. Joshi et al. (2010) also reported that the presence of citric acid, tartaric acid and ascorbic acid in the solution enhanced the curcumin aqueous stability relatively by 3-folds at pH 7.4.

The particle size and encapsulation efficiency of curcuminoid nanoemulsion in 5% citric acid were then investigated as a function of CS:TPP mass ratios. In general, nanoemulsions are obtained when the size of an emulsion globule reaches approximately 20-500 nm. A small droplet size can resist physical destabilization caused by gravitational separation, flocculation and /or coalescence (Tadros et al., 2004). As shown in Table 1 there were a direct correlation between particle sizes and the CS:TPP ratios.

**Table 1** Particle size and encapsulation efficiency of curcuminoid nanoemulsion using different CS:TPP mass ratios

| CS:TPP mass ratios | Polydispersity index | Particle size (nm) | Zeta potential (MV) | Encapsulation efficiency (%) |
|---|---|---|---|---|
| 0.125:1 | 0.253±0.00$^{c}$ | 381.60±15.04$^{c}$ | -25.60±0.10$^{a}$ | 67.25±2.31$^{b}$ |
| 0.5:1 | 0.461±0.01$^{b}$ | 766.70±5.74b | -37.80±0.04$^{c}$ | 75.88±0.15$^{a}$ |
| 1.25:1 | 0.887±0.09$^{a}$ | 1285.00±170.09$^{a}$ | -32.40±0.08$^{b}$ | 71.95±3.33$^{a}$ |

$^{a-c}$: Mean in the same column with different superscripts are significantly different ($p< 0.05$)

An increased in the CS:TPP mass ratios caused a resultant increase in particle sizes, since at high mass ratios (when TPP is more limiting) fewer inter-particle cross links may be formed. Similar results were also reported for selenite-loaded CS:TPP nanoparticles (Luo et al., 2010). According to polydispersity index (PDI), the higher value of PDI (>0.5) of nanoemulsions can be correlated with less stability on storage (Sari et al., 2013). In addition, zeta potential which indicates the characteristic of the globule surface, its sufficient value is required (±30 mV) to ensure a high energy barrier against coalescence of the dispersed globule (Rachmawati et al., 2016).

Therefore, the CS:TPP mass ratio of 0.125:1 was chosen for curcuminoid nanoencapsulation because it corresponded to the higher stability of nanoemulsion. At this CS:TPP mass ratio, the loading efficiency of curcumimoid nanoemulsion was quite high as 67.25%. The loading efficiency of curcumin in sunflower oil/ethanol microemulsion was reported as 48% (Chin et al., 2014), whereas it was 89.89% when curcumin nanoemulsion was performed using self-nanoemulsification method (Rachmawati et al., 2016) . Encapsulation efficiency of 75.5% was obtained when curcumin was encapsulated in gelatin microspheres by using emulsion crosslinking method (Cao et al., 2009).

4.2 Characterization of curcuminoid nanoparticles

The morphology of curcuminoid nanoparticles using CS:TPP ratio of 0.125:1 after spray drying observed by TEM showed a spherical shape with a thin wall and uniform particle size as shown in Figure 2 and the data from HPLC chromatogram (Figure 3) indicated that the nanoparticles composed of curcumin, demethoxycurcumin and bisdemethoxycurcumin as 1.3734, 1.0803 and 0.3954 mg/g DW respectively. Several researches on curcumin nanoencapsulation by natural polymers also reported the spherical in shape and homogeneously distributed of the curcumin nanoparticles that had much better physical stability and solubility (Parize & Stulzer, 2012; Sari et al., 2013; Zamarioli et al., 2015).

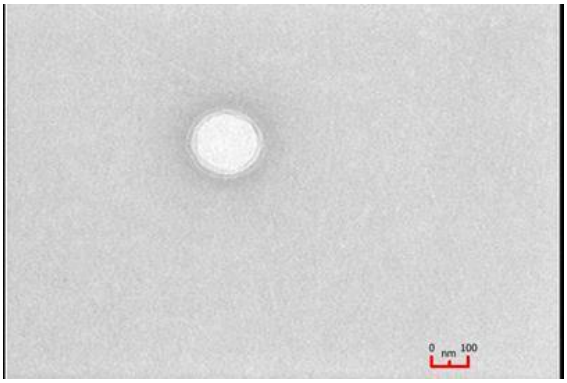


**Figure 2** The morphology of curcuminoid nanoparticle using CS:TPP ratio of 0.125:1

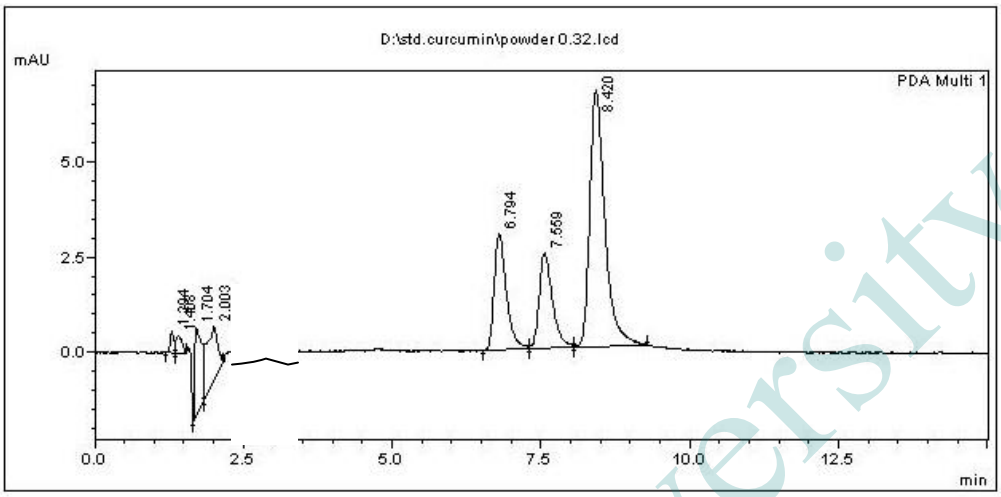


**Figure 3** Chromatography profile of curcumin (C), demethoxycurcumin (DMC) and bisdemethoxycurcumin (BDMC) in curcuminoid nanoparticles

To compare the solubility of curcuminoid nanoparticles and original curcuminoid, the two samples which contained equal amounts of curcumin (analyzing by HPLC) were weighed to dissolve in 10 ml of distilled water (Figure 4). The maximum solubility of curcuminoid nanoparticles was found to be 8.5 mg/ml. The solubility of curcuminoid nanoparticles was then investigated for thermal stability at 80°C. Results showed that a gradual decrease in curcumin content was observed as the heating time increased. The possible changes could be caused by the shifting of double bonds, polymerization or degradation of curcumin to lower molecular weight compounds indicating a vulnerability of the "diketone bridge" of curcumin to heat (Kumavat et al., 2013). Therefore, 32.13% curcumin was lost after a 3 hour of incubation at 80°C as shown in Figure 5. Similar result was reported by Delfiya et al. (2015) that oleoresin microcapsules (prepared by spray drying technique using gum arabic and maltodextrin as wall materials) in water solution was stable after heating at the temperature of 100°C for 15 minutes whereas the solvent extracted and commercial turmeric oleoresin were not stable.

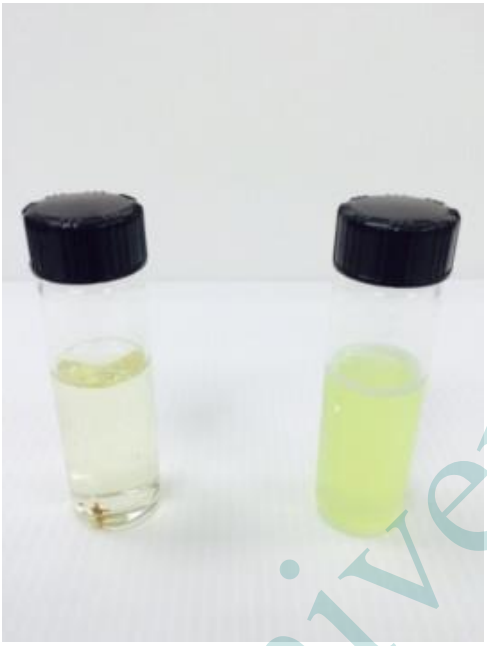

**Figure 4** Solubility of curcuminoid (left) and curcuminoid nanoparticles (right) in water

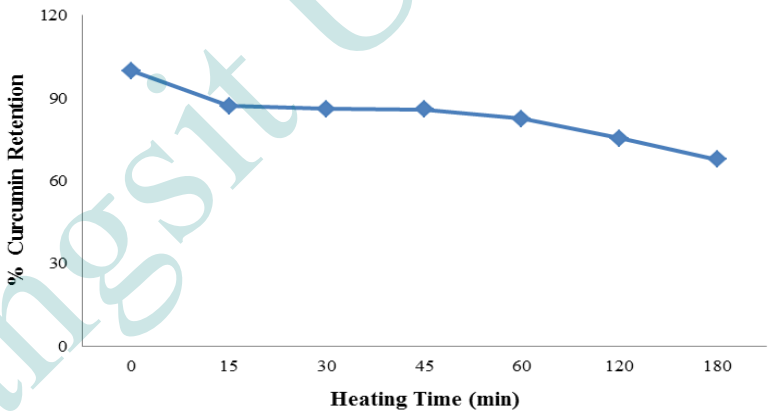


**Figure 5** Thermal stability of nanoencapsulated curcuminoid solution at 80 °C

Considering the result of cytotoxicity of curcuminoid nanoparticles, the MTT assayed indicated that more than 80% of normal cells could survive compared to the curcuminoid oleoresin (Figure 6). Therefore, the curcuminoid nanoparticles should be considered non-toxic and could be used as a food ingredient.

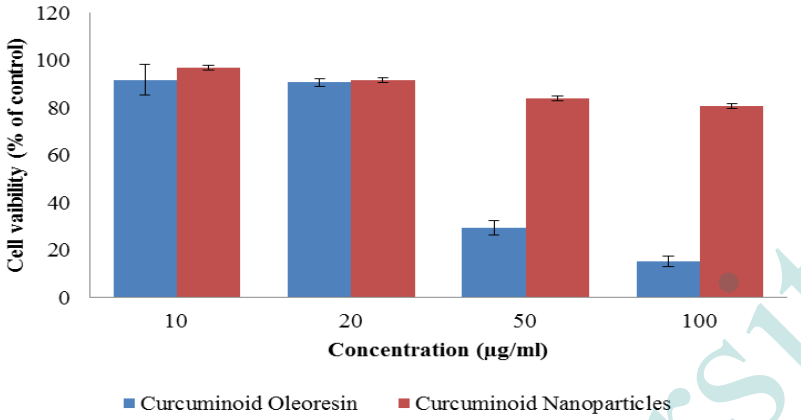


**Figure 6** Cytotoxic effect of curcuminoid nanoparticles and curcuminoid oleoresin on mouse monocyte RAW 264.7 cell line

## 5. Conclusion

In this study, nanoencapsulation of curcuminoid by ionotropic gelation technique using chitosan dissolved in 5% (w/v) citric acid: TPP ratio of 0.125:1 was used to prepare the nanoemulsuion before spray drying. The nanoparticle form of curcuminoid exhibited good water solubility and formed a translucent solution while dissolving in water. This method proved to be simple and efficient and resulted in low toxicity curcuminoid nanoparticles with improved solubility and thermal stability. These encouraging results prompt further studies of the applications and advantages of the curcuminoid nanoparticles in food products.

## 6. Acknowledgements

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