PREPARATION AND CHARACTERIZATION OF CURCUMIN (O/W) NANOEMULSIONS USING CANOLA OIL AND TWEEN 80/SPAN 20

PEMBUATAN DAN KARAKTERISASI NANOEMULSI KURKUMIN (MINYAK/AIR) MENGGUNAKAN MINYAK KANOLA DAN TWEEN 80/SPAN 20

Donny Ramadhan*, Sjaikhurrizal El Muttaqien, Nuralih,
Swasmi Purwajanti, Etik Mardliyati

Center for Pharmaceutical and Medical Technology, Agency for the Assessment and Application of Technology (BPPT)
LAPTIAB I, PUSPIPTEK, Serpong, Tangerang Selatan, 15314, Indonesia
*Corresponding author: donny.ramadhan@bppt.go.id

ABSTRACT

Curcumin, a polyphenolic compound derived from turmeric (Curcuma longa) has been reported to have some therapeutic properties such as anti-tumor, anti-oxidant, anti-inflammatory, anti-microbial and hepatoprotective activity. However, it has poor water solubility resulting in the low bioavailability for oral administration route. In this work, we reported the preparation of curcumin oil-in-water (o/w) nanoemulsions with improved solubility and stability of curcumin using food grade canola oil and tween 80/span 20 as the oil phase and emulsifiers, respectively. It is found that oil droplet particle size was 51.03 ± 0.03 nm. This formulation could enhance the solubility of curcumin up to 8.663 ± 1.211 mg/ml. Moreover, it is also demonstrated that this formulation has an impressive stability up to 200 times dilution. These results suggested that this formulation is promising to be used in oral administration.

Keywords: Curcumin, Nanoemulsion, Particle size, Solubility, Stability

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ABSTRAK

Kurkumin adalah senyawa aktif golongan polifenol dari rimpang kunyit (*Curcuma longa*) yang telah dilaporkan memiliki beberapa aktifitas terapeutik, seperti anti-tumor, anti-oksidan, anti-inflamasi, anti-mikroba, dan hepatoprotektif. Akan tetapi, kurkumin memiliki kelarutan dalam air yang rendah sehingga ketersediaan hayati kurkumin melalui rute oral juga sangat rendah. Dalam riset ini, telah dilakukan penyiapan nanoemulsi (minyak/air) kurkumin menggunakan minyak kanola sebagai fase minyak dan kombinasi tween 80/span 20 sebagai emulsifier. Nanoemulsi yang dihasilkan dapat meningkatkan kelarutan dari kurkumin serta memiliki stabilitas yang cukup baik. Ukuran partikel dari droplet nanoemulsi berkisar 51,03 ± 0,03 nm dengan peningkatan kelarutan kurkumin mencapai 8,663 ± 1,211 mg/ml. Stabilitas nanoemulsi juga dapat terjaga hingga 200 kali proses pengenceran dengan air. Hasil yang diperoleh menunjukan potensi dari nanoemulsi dengan minyak kanola sebagai fase minyak dan tween 80/span 20 sebagai emulsifier untuk dapat digunakan sebagai sistem penghantar dari kurkumin untuk pemberian oral.

Kata kunci: Kurkumin, Nanoemulsi, Ukuran partikel, Kelarutan, Stabilitas

INTRODUCTION

Curcumin (diferuloylmethane) is a natural polyphenolic compound extracted from the rhizomes of turmeric (*Curcuma longa*) and other *Curcuma* spp. (Anand et al., 2007). It has been reported that curcumin possesses some pharmacological activities such as anti-tumor, anti-oxidant, anti-inflammatory, anti-microbial and hepatoprotective effects (Bahramsoltani et al., 2017). Numerous studies in clinical trials have shown that curcumin is safe and well tolerated in high daily doses of 12 g (Lin et al., 2014). However, its application as bioactive ingredient is still restrained by its limitations (Xu et al., 2018). For instance, curcumin has poor water solubility (11 ng/ml in aqueous buffer at pH 5) which becomes a main obstacle for its formulation. As consequence of this limited water solubility, curcumin has low oral bioavailability (only 1% in rats) that thereby reduces its health-promotion effect (Ahmed et al., 2012; Hu et al., 2012).

To improve water solubility and bioavailability of curcumin, recently, many researchers have employed various types of nano-based drug delivery carriers as therapeutic cargo, such as nanoemulsion (Hani & Shivakumar, 2014; Helson, 2013). Oil-in-water (o/w) nanoemulsions are colloidal dispersion system consisting of small lipid droplets in the range 20-200 nm that are dispersed in aqueous medium (Shah et al., 2010). Attributed to its small droplet size, nanoemulsions offer several benefits compared to those of conventional emulsions including its stability against droplet aggregation and gravitational separation, clearer optical properties and novel rheological properties (Jaiswal et al., 2015). To fabricate nanoemulsions, high-energy emulsification methods such as high-pressure valve homogenizer, microfluidizer, ultrasonic homogenizer, and high-pressure homogenizer can be employed to efficiently reduce its droplet size. The small droplet size of nanoemulsions may increase the bioavailability of encapsulated lipophilic components in the gastrointestinal tract (Ahmed et al., 2012).

To further improve the stability of nanoemulsions, the optimization of emulsifier type and its concentration are needed. Emulsifiers could reduce the surface tension, therefore reducing the energy needed for the droplet disruption (Abbas et al., 2014). Moreover, the adsorption of emulsifiers can stabilize droplets to the freshly formed interface, concomitantly, preventing the re-coalescence of droplet (McClements & Jafari, 2018). Moreover, the preparation of food grade nanoemulsions using small-molecule surfactant (e.g., Tweens, Spans), phospholipids (e.g., lecithins), amphiphilic proteins (e.g., whey proteins) and amphiphilic polysaccharides (e.g., modified starches, gums) are preferable due to their low cost and better efficiency.

In food and pharmaceutical industries, canola oil consumption has increased in recent years due to the perception of its health promoting properties and lower cost compared to olive oil (Lauretti & Praticò, 2017; Mehmood, 2015). Also, canola oil has low level of saturated fatty acids (7%), and it contains a lot of polyunsaturated fatty acids, including oleic acid (61%), linoleic acid (21%) and α-linolenic acid (11%) (Lin et al., 2013). Several *in vivo* and *in vitro* studies demonstrated that canola oil exhibited antioxidant activities due to its carotenoids and phenolic compounds. Moreover, some studies also demonstrated that canola oil has several beneficial effects such as anti-hypertensive, anti-inflammatory, anti-mutagenic, and anti-microbial (Loganes et al., 2016).

In this study, nanoemulsion formulation of curcumin was prepared using canola oil as an oil phase and tween 80/span 20 as the emulsifiers. The emulsification was performed by using high-intensity ultrasonic waves. The physical character of the nanoemulsions and its stability were assessed to evaluate the potency of nanoemulsions for increasing curcumin oral bioavailability.

MATERIALS AND METHODS

Materials

The food grade canola oil was obtained from Nutrifood Indonesia (Bogor, Indonesia). Tween 80 and Span 20 were purchased from Merck (France) and Sigma-Aldrich (St. Louis, USA), respectively. Curcumin powder was supplied from Sigma Aldrich (St. Louis, USA).

Solubility Study of Curcumin

The solubility of curcumin in the oil phase and emulsifiers was investigated by adding excess curcumin (200 mg) into 1 ml of each medium in the microtubes. The microtubes containing the mixture were kept in the shaking incubator at 250 rpm and 25^{o}C for 2 days to reach equilibrium, and followed by centrifugation at 5,000 rpm for 10 minutes. Then, they were kept overnight at room temperature and the supernatant samples were filtered by 0.45 µm filtration to remove the excess drug. The concentration of curcumin in each medium was measured by UV-Vis spectrophotometer after the appropriate dilution by methanol at a wavelength of 426 nm.

Preparation and Characterization of Curcumin O/W Nanoemulsions

Curcumin o/w nanoemulsions were prepared by adding excess curcumin (0.67% w/w) into food grade canola oil (6.33% w/w), tween 80 (19.28% w/w) and span 20 (3.72% w/w) in a test tube, then they were kept at 50^{o}C and mixed using a vortex mixer. The mixtures then were added by ultrapure water (70.00% w/w), followed by heating at 50^{o}C and mixed. The mixtures were sonicated using ultrasonic homogenizer in a container filled with ice cubes at amplitude of 35% for 30 minutes (pulse in and pulse off were set for 7.0 sec and 3.0 sec, respectively) (Figure 1). The diameter distribution of curcumin o/w nanoemulsions was measured by laser scattering particle size distribution analyzer.

Determination of Curcumin Loading Capacity

For measuring curcumin loading capacity of the nanoemulsions, the insoluble curcumin o/w nanoemulsions was taken out by centrifugation at 5,000 rpm (25°C) for 10 minutes, and the supernatant samples were filtered through 0.45 µm filter. The drug content of the supernatant samples was measured by UV-Vis spectrophotometer after the appropriate dilution by methanol at a wavelength of 426 nm.

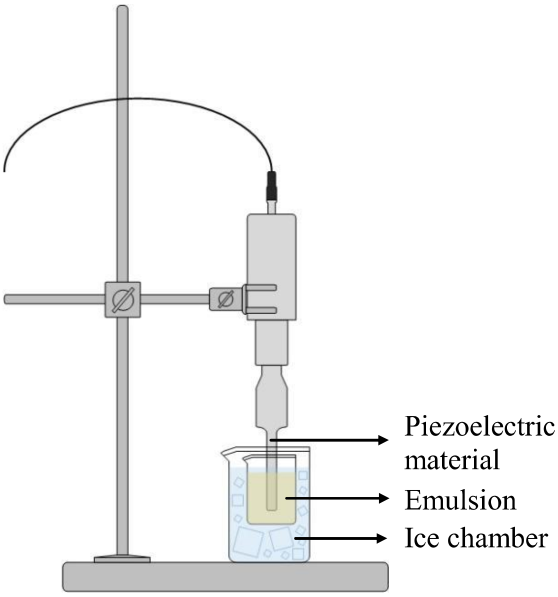


Figure 1. Schematic representation of ultrasonication process of emulsion

Stability of Curcumin O/W Nanoemulsions

The stability of curcumin o/w nanoemulsions was evaluated after 50, 100, 200 times dilution with ultrapure water. The diameter distribution of these diluted curcumin o/w nanoemulsions was measured by particle size analyzer.

Data Analysis

All of the experiments in this study were performed 3 times, and the data were presented as the average ± standard deviation (SD).

RESULT AND DISCUSSION

Preparation of Nanoemulsions

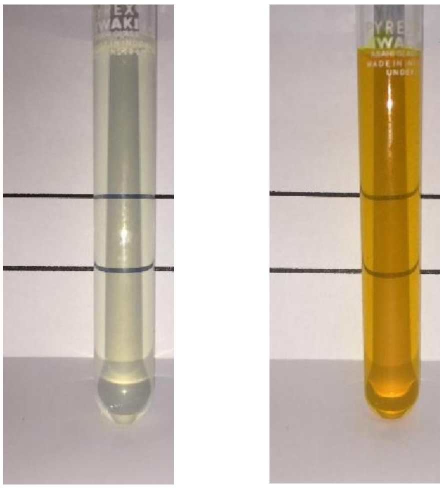
The solubility study of curcumin in the mediums (canola oil, tween 80 and span 20) was required as a preliminary study to estimate the loading capacity of curcumin in o/w nanoemulsions. The result was shown in Table 1.

The o/w nanoemulsions were constructed for the encapsulation of curcumin. The weight ratio of curcumin/canola oil/tween 80/span 20/water for the formula was 0.67/6.33/19.28/3.72/70.00, respectively. Ultrasonic homogenization was used for preparing curcumin o/w nanoemulsions. A piezoelectric transducer of ultrasonic homogenizer produces high-intensity ultrasonic waves that creates cavitation effects to break oil droplets containing dissolved curcumin. The ultrasonicator probe contains piezoelectric quartz crystals which oscillate to produce the ultrasonic wave when a high-intensity electrical field is implemented. The ultrasonic wave generates cavitation force and mechanical vibration that induce the formation, growth and collapse of vapor cavities in the liquid. The vapor cavities collapse generates high pressure and sheer gradients in the liquid around the sonicator probe, causing the disruption and droplets breakdown (Je Lee et al., 2018).

Nanoemulsions are thermodynamically stable transparent or translucent multiphase colloidal dispersions of water and oil, stabilized by an interfacial film of emulsifier molecules. Some literatures mentioned that the droplet size of nanoemulsions is less than 200 nm, underlying the clear appearance of this dispersion system (Walker et al., 2015). Consistent with this theory, the appearance of our nanoemulsions, without and with curcumin was transparent (Figure 2), that could indicate the formation of nano-sized droplet in the emulsion system.

Tabel 1. Curcumin solubility in the mediums

Group	Medium	Curcumin Solubility (mg/ml)
Oil	Canola Oil	0.433 ± 0.005
Emulsifier	Tween 80	9.095 ± 0.213
	Span 20	2.122 ± 0.266



(A) **(B)**

Figure 2. Visual appearance of nanoemulsion without curcumin (A) and with curcumin (B)

Characterization of Curcumin O/W Nanoemulsions

Various factors affect the particle size of nanoemulsions including emulsion composition (type and concentration of emulsifier, oil and water phase) and the power intensity of sonication (Roohinejad et al., 2018). By optimizing such parameters, the particle size and its stability can be controlled. It has been reported that the particle size distribution of the emulsion should be smaller than 80 nm to render a transparent appearance of the nanoemelsion (McClements & Rao, 2011). Moreover, the particle size distribution should be less than 20-25 nm to ensure high optical transparency of a nanoemulsion (Walker et al., 2015). In this study, the particle size distribution of the optimized curcumin o/w nanoemulsion droplet was shown in Figure 3 with average size of the droplet around 51 nm. This relatively small droplet size was attributed to the ultrasonication process. The *d10*, *d25*, and *d90* of the nanoemulsion were 45.5, 47.6, and 56.7 nm, respectively, suggesting the low polydispersity index (PDI) of this nanoemulsion.

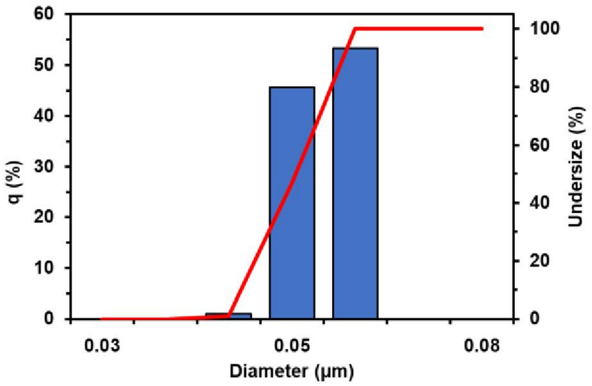


Figure 3. Particle size distribution of curcumin nanoemulsion droplet measured by static light scattering

The solubility of curcumin in nanoemulsion was assessed. From this evaluation, the solubility of curcumin in nanoemulsion was improved significantly into 8.663 ± 1.211 mg/ml, compared to the solubility of curcumin in plain aqueous buffer (11 ng/ml). When the solubility of drug is improved, the permeation rate of curcumin o/w nanoemulsion could be increased, and in turn, may promote the gastrointestinal absorption (Hu et al., 2012).

The stability of curcumin o/w nanoemulsions were evaluated by the gradual dilution using ultrapure water and its visual appearance as well as its droplet size was probed. Theoretically, the dilution of nanomeulsion may decrease the emulsifiers concentration in the emulsion system, allowing the coalescence between larger droplets.

Table 2. The droplet size of the formulation before and after dilution

Dilution (times)	Droplet Size (nm)
Not diluted	51.03 ± 0.03
50	51.62 ± 0.42
100	52.30 ± 0.16
200	53.06 ± 0.47

Besides the contribution of emulsifier to stabilize the droplets against coalescence, the nanoemulsion stability in this research may be caused by the ultrasonication technique used in the emulsification process. It was found consistent with stability study of nanoemulsion performed by Saharan and Coworker; the nanoemulsion prepared by ultrasonication exhibited no visible phase separation and change of droplet size, while those fabricated through mechanical agitation displayed low physical stability (Agrawal et al., 2017). The nanoemulsion stability even improved by increasing sonication time and intensity as more energy was dissipated into the emulsion, causing no separation (Carpenter & Saharan, 2017).

CONCLUSION

Curcumin o/w nanoemulsions were successfully prepared using food grade canola oil as the oil phase and tween 80/span 20 as the emulsifiers by ultrasonic homogenization method. This nanoemulsions formulation could improve the solubility of curcumin as well as display impressive stability against gradual dilutions. These results suggested that our curcumin o/w nanoemulsions is promisable to be used in oral administration.

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REFERENCES

Abbas, S., Bashari, M., Akhtar, W., Li, W. W., & Zhang, X. (2014). Process optimization of ultrasound-assisted curcumin nanoemulsions stabilized by OSA-modified starch. *Ultrasonics Sonochemistry*, *21*(4), 1265–1274.

Agrawal, N., Maddikeri, G. L., & Pandit, A. B. (2017). Sustained release formulations of citronella oil nanoemulsion using cavitational techniques. *Ultrasonics Sonochemistry*, *36*, 367–374.

Ahmed, K., Li, Y., McClements, D. J., & Xiao, H. (2012). Nanoemulsion-and emulsion-based delivery systems for curcumin: Encapsulation and release properties. *Food Chemistry*, *132*(2), 799–807.

Anand, P., Kunnumakkara, A. B., Newman, R. A., & Aggarwal, B. B. (2007). Bioavailability of curcumin: problems and promises. *Molecular Pharmaceutics*, *4*(6), 807–818.

Bahramsoltani, R., Rahimi, R., & Farzaei, M. H. (2017). Pharmacokinetic interactions of curcuminoids with conventional drugs: A review. *Journal of Ethnopharmacology*, *209*, 1–12.

Carpenter, J., & Saharan, V. K. (2017). Ultrasonic assisted formation and stability of mustard oil in water nanoemulsion: Effect of process parameters and their optimization. *Ultrasonics Sonochemistry*, *35*, 422–430.

Hani, U., & Shivakumar, H. G. (2014). Solubility enhancement and delivery systems of curcumin a herbal medicine: a review. *Current Drug Delivery*, *11*(6), 792–804.

Helson, L. (2013). Curcumin (diferuloylmethane) delivery methods: a review. *Biofactors*, *39*(1), 21–26.

Hu, L., Jia, Y., Niu, F., Jia, Z., Yang, X., & Jiao, K. (2012). Preparation and enhancement of oral bioavailability of curcumin using microemulsions vehicle. *Journal of Agricultural and Food Chemistry*, *60*(29), 7137–7141.

Jaiswal, M., Dudhe, R., & Sharma, P. K. (2015). Nanoemulsion: an advanced mode of drug delivery system. *3 Biotech*, *5*(2), 123–127.

Je Lee, S., Yuan, Q., Teo, A., Goh, K. K. T., & Wong, M. (2018). Nanoemulsions. In *Emulsion‐ based Systems for Delivery of Food Active Compounds* (pp. 181–230). John Wiley & Sons, Ltd.

Lauretti, E., & Praticò, D. (2017). Effect of canola oil consumption on memory, synapse and neuropathology in the triple transgenic mouse model of Alzheimer’s disease. *Scientific Reports*, *7*(1), 1–9.

Lin, C.-C., Lin, H.-Y., Chi, M.-H., Shen, C.-M., Chen, H.-W., Yang, W.-J., & Lee, M.-H. (2014). Preparation of curcumin microemulsions with food-grade soybean oil/lecithin and their cytotoxicity on the HepG2 cell line.

Food Chemistry, *154*, 282–290.

Lin, L., Allemekinders, H., Dansby, A., Campbell, L., Durance-Tod, S., Berger, A., & Jones, P. J. H. (2013). Evidence of health benefits of canola oil. *Nutrition Reviews*, *71*(6), 370–385.

Loganes, C., Ballali, S., & Minto, C. (2016). Functional properties of canola oil: a review of literature. *The Open Agriculture Journal*, *10*(1).

McClements, D. J., & Jafari, S. M. (2018). Improving emulsion formation, stability and performance using mixed emulsifiers: A review. *Advances in Colloid and Interface Science*, *251*, 55–79.

McClements, D. J., & Rao, J. (2011). Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. *Critical Reviews in Food Science and Nutrition*, *51*(4), 285–330.

Mehmood, T. (2015). Optimization of the canola oil based vitamin E nanoemulsions stabilized by food grade mixed surfactants using response surface methodology. *Food Chemistry*, *183*, 1–7.

Roohinejad, S., Greiner, R., Oey, I., & Wen, J. (2018). *Emulsion-based systems for delivery of food active compounds: formation, application, health and safety*. John Wiley & Sons.

Shah, P., Bhalodia, D., & Shelat, P. (2010). Nanoemulsion: a pharmaceutical review. *Systematic Reviews in Pharmacy*, *1*(1).

Walker, R., Decker, E. A., & McClements, D. J. (2015). Development of food-grade nanoemulsions and emulsions for delivery of omega-3 fatty acids: opportunities and obstacles in the food industry. *Food & Function*, *6*(1), 41–54.

Xu, X.-Y., Meng, X., Li, S., Gan, R.-Y., Li, Y., & Li, H.-B. (2018). Bioactivity, health benefits, and related molecular mechanisms of curcumin: current progress, challenges, and perspectives. *Nutrients*, *10*(10).