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# Preparation and Characterization of Curcumin Nanoemulsion in Olive Oil-Tween 80 System using Wet Ball Milling Method

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Nanoemulsion has been developed as a drug delivery system which increases bioavailability and effectiveness of curcumin. Many methods and formulations have been applied in order to fabricate the most efficient nanoemulsion system to deliver curcumin. Wet ball milling is a simple technique to grind solids like powder to produce nano emulsion. On the other hand, a combination of olive oil and curcumin in Mediterranian Diet shows a promising anticancer activity. This research aims to elaborate the preparation and characterization of curcumin nanoemulsion in olive oil-Tween 80 system with wet ball milling method. It is expected that the procedure yields a combination of curcumin and olive oil in nanoemulsion system with simple preparation. This research uses curcumin as active compound, olive oil as solvent for curcumin, Tween 80 as stabilizer, and water as dispersing agent. Particle size and polydispersity index are determined using Dynamic Light Scattering technique. The results show that the best milling time is 8 hours to produce nanoemulsion that has diameter of 303 nm and polidispersity index of 0.29. Nanoemulsion system is stable for 60 days storage at 4 °C and 25 °C. The maximum curcumin mass that can be loaded in the system while maintaining particle size in nanoemulsion range is 300 mg.

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## 1 Introduction

Curcumin is a well-known active compound with various health benefits. Curcumin shows antibacterial, antifungal, antiviral[1], anti-diabetic[2], anti-cancer[3] and anti-inflammatory activity[4]. The application of this versatile chemical is hampered by its low solubility, hence lower its bioavailability in the biological system. In order to overcome this problem, curcumin is developed in nanoemulsion system. Several studies proved the increase of curcumin bioavailability following formulation of curcumin in nanoemulsion system[5–7].

Further study also examines the effect of olive oil and curcumin on cancer cell. Individually, as well as in combination, curcumin and olive oil show anticancer activity[8]. Study by Esposito et.al[9] reveals that olive oil and curcumin in Mediterranian diet combination gives a significant effect on the growth of tumour. In addition, Kamel et.al[10] reported that nanoformulation of curcumin and olive oil in photodynamic therapy of breast cancer enhances active compound cell penetration, hence improved its cytotoxicity. Thus, it is necessary to explore the potency of curcumin-olive oil combination in treating cancer.

Formulation of curcumin-olive oil to produce nanoemulsion has also been developed. Kamel et.al[10] employs high shear hot homogenization to produce curcumin nanoemulsion using Tween 80 and lecithin as surfactant to stabilize the dispersion of curcumin-olive oil droplet in water. Meanwhile, Sharma et.al[11] and Franklyne et.al[12] use ultrasound to produce curcumin-olive oil nanoemulsion. Hernandez et.al[13] prepares curcumin-olive oil nanoemulsion using high intensity technique including ultrasound and microfluidization. These methods demand high energy in its application. On the other hand, wet ball milling is a simple technique using milling beads stirred with magnetic stirrer at low energy to grind curcumin powder and disperse it in a surfactant-oil system[14].

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Studies regarding curcumin solubility show that formulation of curcumin in a combination with casein and glutathione increases curcumin water solubility to 40-350 times compared to the native curcumin [15]. Similarly, combining curcumin with TPGS (D-$\alpha$ Tocopherol Polyethylene Glycol 1000 Succinate) successfully rises curcumin solubility from $0.6\,\mu g\,mL^{-1}$ in pure water to $260\,\mu g\,mL^{-1}$ in TPGS formulation. Furthermore, milling technique is proved to be able to improve hydrophobic chemical solubility in polar media [16,17]. This research aims to elaborate the preparation and characterization of curcumin nanoemulsion in olive oil-Tween 80 system prepared using wet ball milling method. It is expected that the procedure yields a combination of curcumin and olive oil in nanoemulsion system with simple preparation. This research uses curcumin as active compound, olive oil as solvent for curcumin, Tween 80 as stabilizer, and water as dispersing agent. Particle size and polydispersity index are examined to determine the optimum milling time as well as the stability of nanoemulsion stored at low temperature and room temperature for 60 days.

## 2 Methodology

### 2.1 Materials

Materials used in this research are curcumin powder (pharmaceutical grade, Health-Ingredients, China) which is used without further purification, Tween 80 (pharmaceutical grade), olive oil (food grade), distilled water, yttrium-stabilized zirconium beads size 0.5 mm (Hosokawa Alpine, Japan) and ethanol p.a.

### 2.2 Procedures

Briefly, 1 mg curcumin is mixed together with $60\,\mu L$ of Tween 80, $200\,\mu L$ of olive oil, and 5 mL of distilled water. The 2.5 mL of milling beads are then added to the mixture and mixture is then milled for 24 hours with the aid of magnetic stirring bar set at 100 rpm speed. Sample is taken $300\,\mu L$ periodically to determine the optimum milling time based on the particle size and polydispersity index. Sample is then stored in 4 °C and 25 °C for 60 days and the particle size and polydispersity index are re-examined to measure system stability. Furthermore, set of nanoemulsion curcumin are prepared with the increasing of curcumin mass loaded, vary from 100 mg to 500 mg to examine the effect of curcumin mass on particle size.

### 2.3 Instruments

Particle size and polydispersity index examination is conducted using DelsaTM Nano C Particle Analyzer (Beckman Coulter, USA) based on Dynamic Light Scattering technique. The particle size of curcumin nanoemulsion is analyzed at 25 °C; refractive index of 1.3328; viscosity of 0.8878 Pa s; dielectric constant of 78.3; Sample is measured in a disposable cell. Volume of sample taken is $50\,\mu L$ and diluted with $2450\,\mu L$ of distilled water.

## 3 Results and discussion

Figure 1 displays particle size as a function of milling time. Based on that, the optimum milling time to produce the smallest particle size is 8 hours. As the milling time increases, particle size decreases and reach minimum at 8 hours (303 nm). Prolonged milling time (24 hours) does not induce further particle size reduction, instead, particle enlargement is observed. Similarly, particle size homogeneity, indicated by the index of polydispersity, decreases at the first 4 hours and remain constant at 8 hours, signifying that the particle size distribution is narrowing. However, longer milling time does not further reduce particle heterogeneity. Polydispersity index at 24 hours is higher than at 8 hours indicating that the particle size is uneven that could be due to aggregation of the particles. Our results show a different pattern with Sholihat et.al [14] which shows a decreasing pattern of particle size in 24 hours milling time. This could be attributed to the different oil being used in the previous research. Prolonged milling provides heat and agitation which contribute to structure change in the nanoemulsion components inducing agglomeration. Moreover, prolonged agitation creates particle with large interfacial area, high free energy and less thermodynamic stability [17]. Ketoconazole, as an example, is one of the chemicals prone to agglomeration due to prolonged milling time [18].

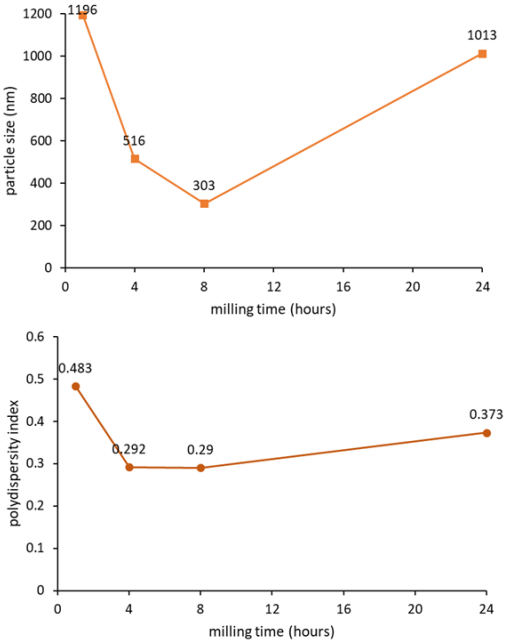


**Figure 1** Particle size (top) and polydispersity index (bottom) as a function of milling time.

Our results show higher particle size compared to the previous reported research. Sholihat et.al [14] stated that by using wet ball milling method, the particle size of nanoemulsion made was 127-330 nm. Using sonication, Franklyne et.al [12] produces less than 150 nm particle size. Similarly, Hernandez et.al [13] are able to make less than 240 nm particle size using sonication and microfluidization. Meanwhile, Sharma et.al [11] produce particle size of curcumin nanoemulsion using olive oil in a range of 595 nm using sonication method. Kamel et.al [10] reported particle size ranging from 101-1248 nm in their experiment using high shear hot homogenization method in which olive oil also being used in their nanoemulsion. Those results show that nanoemulsion particle size is various based on the applied method. Moreover, the for-

**Table 1** Particle size and polydispersity of curcumin nanoemulsion during storage

| | Milling time (hours) | | | |
|---|---|---|---|---|
| | 1 | 4 | 8 | 24 |
| **Day 1** | | | | |
| Particle size (nm) | 1196 | 515 | 303 | 1012 |
| Polydispersity index | 0.483 | 0.292 | 0.291 | 0.373 |
| **Day 60 at 4**°C | | | | |
| Particle size (nm) | 834 | 390 | 268 | 508 |
| Polydispersity index | 0.346 | 0.333 | 0.290 | 0.217 |
| **Day 60 at 25**°C | | | | |
| Particle size (nm) | 834 | 484 | 270 | 608 |
| Polydispersity index | 0.346 | 0.329 | 0.290 | 0.369 |

mulation of nanoemulsion in each experiment is different. Hence, a conclusion of the efficiency and effectivity of each method in producing nanoemulsion is difficult to be drawn unless the comparison is made side by side with identical formulation.

To further examine nanoemulsion stability, sample is then stored at low (4 °C) and room (25 °C) temperature. Particle size and polydispersity index are measured at day 1 and day 60 of storage. The results are summarized in Table 1.

During storage, there is particle size and polydispersity index fluctuation observed. At milling time of 1- and 24-hours, particle size changes considerably both in low and room temperature storage. At 4- and 8-hours milling time, particle size changes, but not drastically unlike in 1- and 24-hours milling time. Furthermore, 8-hours milling time shows the smallest particle size changes during storage. This indicates that 8-hours milling time produce a stable nanoemulsion and agglomeration is avoided. After 60 days of storage, in 1- and 24-hours milling time sample, the particle size distribution observed is wider, marked with higher polydispersity index, which substantiates the heterogeneity in particle size. Since particle size in uneven, Ostwald ripening might occur in which small and big particle fuse together forming agglomerate. In contrast, after 60 days of storage in 8-hours milling time sample, polydispersity index remains constant which designates that there is no significant change in particle size distribution. Thus, it confirms that 8-hours milling time is the optimum time to produce smallest particle size with the highest uniformity and stability during 60 days of storage.

In order to evaluate the maximum curcumin mass that can be added in the system while maintaining particle size in nanoemulsion particle size range, we add more curcumin from 100 to 500 mg. The results are shown in Figure 2.

Based on the measurement results obtained, it can be seen that 300 mg of curcumin is the maximum curcumin mass can be loaded in the system while maintaining particle size in the nanoemulsion range. The particle size and polydispersity index are below 1000 nm and 0.5, respectively. This substantiates that the system is in nanoemulsion particle size range and have homogeneous particle size distribution.

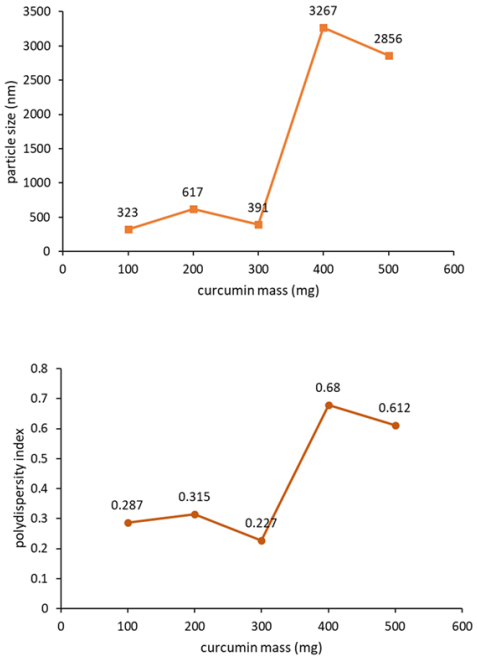


**Figure 2** Particle size (top) and polydispersity index (bottom) as a function of curcumin mass.

## 4 Conclusions

Our experiment describes the preparation and characterization of curcumin nanoemulsion using wet ball milling. The experiment successfully produces nanoemulsion and identifies the optimum milling time at 8-hours, which produce curcumin nanoemulsion with smallest particle size and polydispersity index. The characterization of nanoemulsion produced in optimum milling time shows that the particle size produced is 303 nm with polydispersity index of 0.29 nm. The system is stable in storage both at 4 °C and 25 °C for 60 days. The maximum curcumin mass can be added in the system while maintaining particle size in nanoemulsion range is 300 mg.

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