Thai Journal of Pharmaceutical Sciences (TJPS)

Journal homepage: http://www.tjps.pharm.chula.ac.th

# Low-Energy Emulsification of Edible Nanoemulsions

Jaemsak K and Warisnoicharoen W*

*Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Road, Pathumwan, Bangkok, Thailand*

**Keywords:** Nanoemulsion(s); Extra virgin olive oil; Surfactant; Cosurfactant; Emulsification

**Objectives:** Nanoemulsions are attractive for use in many food applications. The composition in the formulation should have an ability to form stable nanoemulsions and must be in food grade. The aim of this study was to characterize edible nanoemulsions incorporating extra virgin olive oil prepared by a low energy approach.
**Methods:** Oil-in-water nanoemulsions were prepared at different concentrations of extra virgin olive oil, polyoxyethylene sorbitan monolaurate or Tween® 20 (surfactant) and glyceryl monocaprylate or Imwitor® 308 (cosurfactant). Briefly, after being weighed, the mixture was heated up to 70-75 °C for 10 min before cooling down to the ambient temperature with stirring. The freshly prepared samples were characterized for the size and size distribution using dynamic light scattering technique. The linear regression analysis was used to analyse the correlation between concentration of surfactants and particle sizes in order to forecast the droplet size at infinite dilution. Moreover, nanoemulsions were examined for refractive index and viscosity.
**Results:** The refractive indices (RI) of nanoemulsions were approximately 1.3-1.4 while the higher values of RI were found with increasing oil and surfactant concentrations. Nanoemulsions were found to be slightly more viscous than the dispersed aqueous phase. For the particle size measurement, nanoemulsions formed in conjunction with a cosurfactant clearly showed the smaller particle size than the systems without it. Increasing amount of oil led to larger particle sizes. Most of the nanoemulsions presented the polydispersity index of less than 0.7 indicating the rather narrow size distribution of the preparations. The results indicated the requirement of the cosurfactant and sufficient amount of surfactant for the formation of nano-size of emulsion droplets. The calculated particle size at infinite dilution of nanoemulsions indicated the droplet sizes of less than 100 nm when assumed that the particles are spherical or near-spherical in shape and monodisperse.
**Conclusion:** The edible oil-in-water nanoemulsions with sufficiently small particles could be formed using a low-energy emulsification method. The compositions, namely oil, surfactant and cosurfactant, were controllable in order to optimize the formation of such nanoemulsions.

* Corresponding author: Department of Food and Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Phyathai Road, Pathumwan, Bangkok, Thailand; Tel. +66(022188293) ; Fax. +66(022188291)
E-mail: wwarangk@gmail.com

## Introduction

In recent years, nanotechnology-based products have been developed to employ in many applications including food and pharmaceuticals. Nanoemulsions (NEs) contain droplet sizes in nanoscale typically less than 100 nm. They are considered to be more stable to gravitational separation and aggregation than conventional emulsions.[1] NEs formation can be obtained by either high-energy strategies such as high pressure homogenization or low-energy approaches such as spontaneous emulsification. Although preparation of NEs by low-energy methods is economical and reasonably suitable for small scale production, the formation of NEs is largely dependent on compositional selection.[1] In order to prepare NEs for food application, it is of necessity that ingredients used in the formulation including surfactant and cosurfactant are in food registration. Triglyceride oils such as olive oil, soybean oil are mostly employed in the NEs formulation for food use. Lately, extra virgin olive oil (EVOO) is attractive not only from its health benefit but also antimicrobial activity.[2] The latter has been arisen from high phenolic compounds in the olive oil. This study aimed to investigate the formation of oil-in-water NEs containing extra virgin olive oil as an oil phase using low-energy approach. NEs were emulsified by polyoxyethylene sorbitan esters and co-emulsified by glyceryl monocaprylate. NEs were determined for their physicochemical properties including size and size distribution, refractive indices and viscosity.

## Materials and Methods

### Materials

Polyoxyethylene sorbitan monolaurate or Tween® 20 (Acros, Belgium) was a non-ionic surfactant and glyceryl monocaprylate or Imwitor® 308 (Sasol, Germany) was a co-surfactant for NEs formation. An oil phase in NEs was extra virgin olive oil (Bertolli®, Italy) purchased from a local supermarket. Ultrapure water was used for all NEs preparation.

## Methods

**Preparation of nanoemulsions:** Oil-in-water nanoemulsions were formulated at varied concentrations of extra virgin olive oil (EVOO), Tween® 20 (surfactant) and Imwitor® 308 (cosurfactant). NEs formulation in the absence of a cosurfactant was also prepared. For the formulation containing a cosurfactant, a weight ratio of surfactant to cosurfactant at 2:1 was used. NEs were prepared by weighing desired amounts of the ingredients and adding water to adjust the final weight. The mixture was heated up to 70-75 °C for approximately 10 min. Then the samples were cooled down to the ambient temperature with stirring throughout. The samples were kept in airtight containers. For the experiment on characterization of NEs, the samples were freshly prepared and used within 24 hr.

**Determination of refractive index:** The value of refractive index (RI) was used as a parameter for a light scattering experiment. The RI measurement was performed by RM40 refractometer (Mettler Toledo, Switzerland) with a built-in solid state thermostat. A minimal sample volume of 0.4 mL of a test sample was dropped on the measuring cell and the value was read at a controlled temperature of 25 °C.

**Determination of viscosity:** The NEs prepared at different concentrations of surfactant were studied for viscosity change. The viscosity of NEs was determined using SV-10 sine-wave vibro viscometer (A&D, Japan) at 28 °C. The NEs samples were diluted to 5% w/w surfactant before test.

**Particle size measurement of nanoemulsions:** Droplet sizes of NEs were measured by dynamic light scattering (DLS) using non-invasive back scatter technology. The experiment was performed at 25 °C on Zetasizer Nano ZS (Malvern Instruments, UK) equipped with a helium-neon laser operating at 633 nm. The NEs samples were diluted to surfactant concentrations within a range of 0.2-1% w/w so as to avoid the interparticulate interaction and multiple scattering. Prior to size measurement, all sample solutions were clarified by ultrafiltration through a cellulose acetate Millipore membrane (Merck, UK). The DLS result was analyzed by a method of Cumulants in which the intensity correlation function was related to a diffusion coefficient and eventually converted to a hydrodynamic size.[3] The width of size distribution was referred as a polydispersity index (PDI). The data obtained was represented as an average of three determinations.

**Statistical analysis:** The relationship between droplet sizes and surfactant concentrations were analysed using a linear regression and the square of Pearson's correlation coefficient ($r^2$) was observed. A linear regression equation obtained was used to calculate the particle size of NEs at an infinite dilution.

## Results

### Refractive index and viscosity

The values of refractive indices of NEs at varying concentrations of Tween® 20 (surfactant), Imwitor® 308 (cosurfactant) and extra virgin olive oil (EVOO) are shown in Table 1. The NEs formulations were encoded depending on the compositions. The refractive indices of NEs fell in the range of 1.3-1.4 while the higher values of RI were found with an increment of oil concentration. Increasing amount of surfactant and cosurfactant also caused a higher number of RI (Table 1). For the viscosity study, the dilute NEs containing 1% w/w EVOO stabilized by Tween® 20 and Imwitor® 308 were measured and the result was tabulated in Table 1. NEs were found to be slightly more viscous than the dispersed aqueous phase (1.08 mPa·s).

**Table 1**. Values of refractive index and viscosity of nanoemulsions. The surfactant (SAA), cosurfactant (CoSAA) and oil are Tween® 20, Imwitor® 308 and extra virgin olive oil (EVOO), respectively.

| Weight ratio of SAA: CoSAA: EVOO | Sample coding | Refractive index[a] | Viscosity (mPa·s) |
|---|---|---|---|
| 5:0:1 | T5 | 1.340 ± 0.001 | *nd* |
| 10:0:1 | T10 | 1.346 ± 0.001 | *nd* |
| 20:0:1 | T20 | 1.360 ± 0.001 | *nd* |
| 30:0:1 | T30 | 1.375 ± 0.001 | *nd* |
| 5:2.5:1 | T5i1 | 1.343 ± 0.001 | 1.24 |
| 10:5:1 | T10i1 | 1.352 ± 0.002 | 1.27 |
| 20:10:1 | T20i1 | 1.373 ± 0.001 | 1.32 |
| 30:15:1 | T30i1 | 1.393 ± 0.001 | 1.41 |
| 5:2.5:2 | T5i2 | 1.343 ± 0.001 | *nd* |
| 10:5:2 | T10i2 | 1.354 ± 0.001 | *nd* |
| 20:10:2 | T20i2 | 1.375 ± 0.001 | *nd* |
| 30:15:2 | T30i2 | 1.395 ± 0.001 | *nd* |

[a] mean ± S.D. (*n*=3).
*nd* = not determined.

### Size measurement and analysis

The hydrodynamic diameters of NEs and the corresponding PDI were determined by dynamic light scattering (DLS). The DLS results are illustrated in Figure 1A-1F. NEs formation in the presence of Imwitor® 308 clearly showed the

smaller particle size than the systems without the cosurfactant. NEs prepared using Imwitor® 308 and incorporated EVOO at 1% w/w had the droplet sizes in a range of 50-150 nm. Upon increasing the percentage of oil from 1% to 2% w/w, particle sizes of NEs were slightly different from those containing the lower amount of oil except for NEs formulated using 5% w/w surfactant. Most of the PDI values of NEs did not exceed 0.7 suggesting the rather narrow size distribution of NEs.[3] The data from DLS results were further analysed using a linear regression equation; Y = *a* + bX, where Y, X and b is droplet sizes, surfactant concentration, and slope, respectively. The y-intercept (*a*) was considered as a size of NEs at infinite dilution.

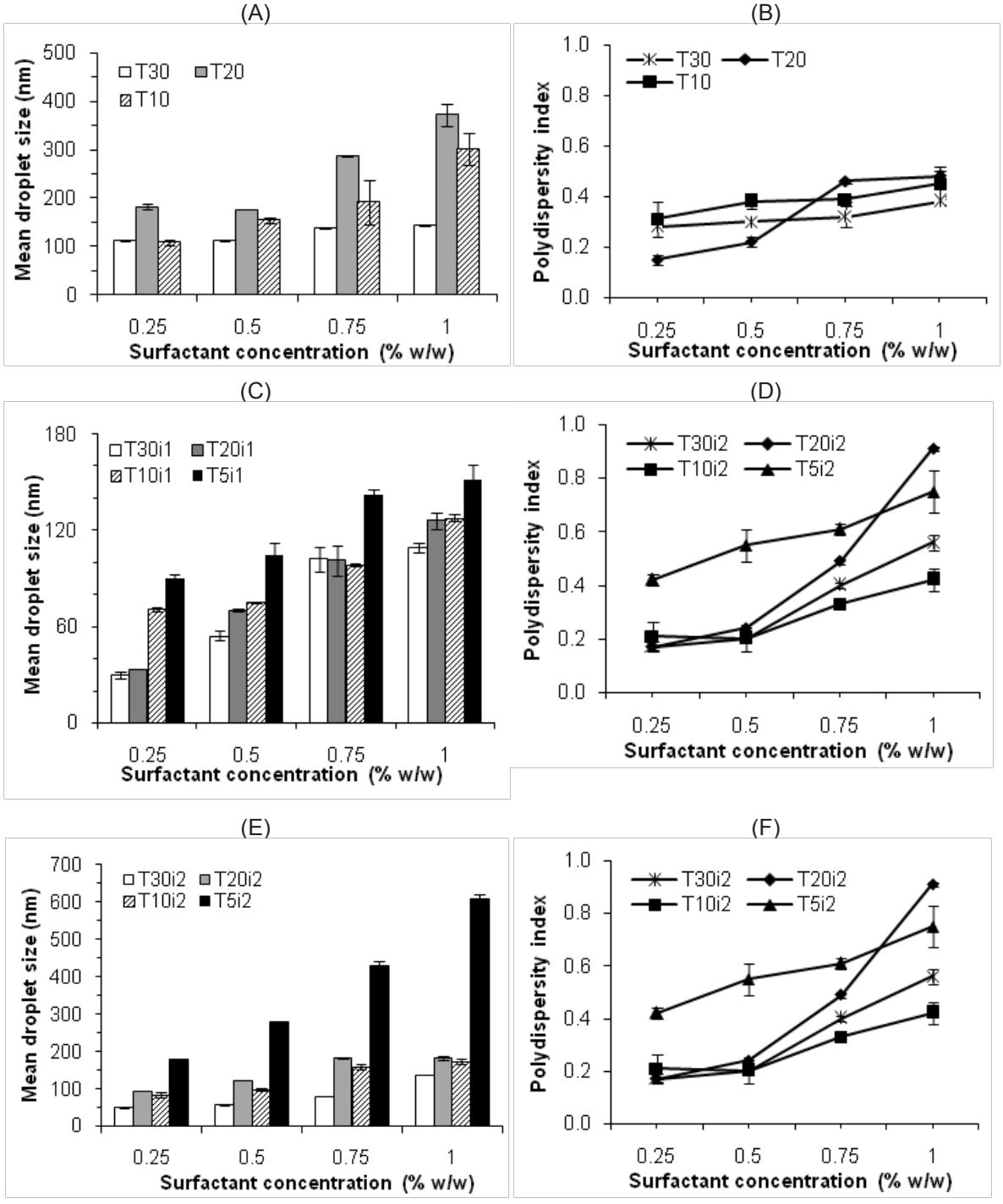


**Figure 1.** Hydrodynamic diameter and polydispersity index of nanoemulsion droplet after dilution to different surfactant concentrations.

**Table 2.** Droplet size of nanoemulsions (*a*) derived from a linear regression equation. The $r^2$ is a coefficient of determination.

| Sample | Slope | $r^2$ | *a* (nm) | Sample | Slope | $r^2$ | *a* (nm) |
|---|---|---|---|---|---|---|---|
| T10 | 61.86 | 0.9338 | 34.23 | T30i1 | 28.68 | 0.9364 | 2.02 |
| T20 | 68.32 | 0.8856 | 83.37 | T5i2 | 142.93 | 0.9848 | 15.70 |
| T30 | 12.46 | 0.8644 | 95.20 | T10i2 | 32.46 | 0.9187 | 47.76 |
| T5i1 | 22.43 | 0.9474 | 66.03 | T20i2 | 32.14 | 0.9020 | 64.78 |
| T10i1 | 19.47 | 0.9190 | 44.37 | T30i2 | 28.78 | 0.8663 | 8.98 |
| T20i1 | 30.85 | 0.9929 | 5.52 | | | | |

## Discussion

There is of great potential in food application for oil-in-water NEs. The emulsification using low energy approach depended upon the compositions of the system to spontaneously form emulsion droplet in nano-scale (<200 nm). In this study, oil-in-water NEs were prepared using food-grade surfactant, Tween® 20, and cosurfactant, Imwitor® 308. The long chain triglyceride, extra virgin olive oil, was incorporated in the NEs. The results indicated the requirement of any cosurfactant especially at low amount of surfactant in order to form NEs with smaller droplet sizes. The cosurfactant can help lower an interfacial tension and increase the fluidity of the interfacial layer, thus allowing greater penetration of the oil.[4] A two-fold increase in oil amount resulted in larger NEs droplets. An increase in oil molecules inside the core of the droplet brings about more surfactant molecules needed to form an interfacial film around the droplet. Hence, only the NEs forming with higher surfactant concentrations (> 5 % w/w) presented the nano-scale droplet size. The calculated particle size at infinite dilution of dilute NEs form a regression analysis indicated that the particle diameters of NEs were less than 100 nm. A decrease in regression line with increasing surfactant concentrations indicated the presence of some extent of attractive interaction among particles.[5] However, the size analysis of DLS results relies on the assumption that particles are spherical or near-spherical in shape and monodisperse.[3,5]

## Conclusion

The food-grade oil-in-water NEs with sufficiently small particles could be formed using a low-energy emulsification method. The compositions, namely oil, surfactant and cosurfacant, were controllable in order to optimize the formation of such NEs.

## References


1. McClements DJ, Rao J. Food-grade nanoemulsions: formulation, fabrication, properties, performance, biological fate, and potential toxicity. Crit Rev Food Sci Nutr.2011;51:285-330.
2. Medina E, De Castro A, Romeo C, Brenes M. Comparison of the concentrations of phenolic compounds in olive oils and other plant oils: correlation with antimicrobial activity. J Agric Food Chem. 2006;54:4954-61.
3. Malvern Instruments. Inform white paper: Dynamic light scattering common terms defined. Worcestershire: Malvern Instrument; 2011, pp 1-6.
4. Wooster TJ, Labbett D, Sanguansri P, Andrews H. Impact of microemulsion inspired approaches on the formation and destabilisation mechanisms of triglyceride nanoemulsions. Soft Matter. 2016;12:1425-35.
5. Warisnoicharoen W, Lansley AB, Lawrence MJ. Light scattering investigations on dilute nonionic oil-in-water microemulsions. AAPS PharmSci. 2000;2(2):E12.