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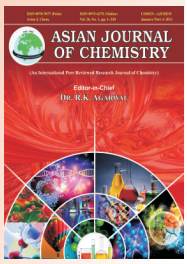

Effect of Viscosity on Formulation of Olive Oil Nanoemulsion Using Ultrasonicator

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Olive oil has been reported to have many medicinal properties and it forms an important constituent of human diet. The efficacy of olive oil for drug delivery can be improved by the formulation of nanoemulsion. The present work focuses on olive oil nanoemulsion formulation using non ionic surfactant Tween 20 and co surfactant ethanol. Olive oil nanoemulsions have been successfully prepared mechanically by high energy sonicator. The physical state of the nanoemulsion is represented using pseudo ternary phase diagram. Concentration of oil, surfactant and co surfactant is optimized to obtain nanoemulsion of small size. The obtained nanoemulsion is checked for its stability and other characteristics like pH, particle size and viscosity. Viscosity of the nanoemulsion increased with increasing concentration of Tween 20. Surface morphology is observed using transmission electron microscopy. This study paved the way for development of a novel nanoemulsion for various applications.

Keywords: Olive oil, Nanoemulsion, Sonicator, Non ionic surfactant.

INTRODUCTION

Nanoemulsions are one of the promising techniques in field of nanotechnology. It constitutes a group of dispersed particles which is used in biomedicine. Size of a nanoemulsion is mainly dependent on its non ionic surfactant phase structure which is optically translucent and thermodynamically stable with droplet size 50-200 nm[1]. Nanoemulsions (NEs) due to its remarkable properties like higher drug solubilization capacity, better thermodynamic stability, long self-life and rapid onset of action form a promising technology to achieve optimum targeted drug delivery[2].

Olive oil forms a major constituent of human diet and it confers many health benefits particularly reduced incidence of degenerative diseases like coronary heart disease, cancers of breast, skin *etc*.[3-5]. The minor component of olive oil are constituted by sterols (β-sitosterol), hydrocarbons (squalene), polyphenols more than 20 phenolic compounds, volatile compounds, terpenols and terpenic acids (maslinic acids) and water[6]. Recent findings suggest that olive oil have powerful antioxidants, both *in vitro* and *in vivo* that account for various potent health effects[7].

To the best of our knowledge, there is hardly any report available on formulation of olive oil nanoemulsion using high energy method. Therefore, the present study aims to look at the effect of viscosity on olive oil nanoemulsion prepared by Ultrasonicator and asses the stability of the optimal formulation obtained through size. The finding of this study will contribute to the possibility of this formulation to be used as one of the method to improve olive oil bioavailability for drug delivery.

EXPERIMENTAL

Olive oil and Tween 20 [polyoxyethylene (20) sorbitan monolaurate] were purchased from sigma - Aldrich (St Louis, Missouri, USA) and stored at room temperature under lab conditions. Ethanol was supplied by Merck. Deionized water (Milli-Q water; Millipore Corporation) was used for all experiments. All chemicals were of analytical grade.

Construction of pseudoternary phase diagram: The pseudo ternary phase diagrams were developed to optimize the concentration of olive oil, S_{mix} (surfactant-cosurfactant mixture) and distilled water. Tween 20 and ethanol were used as surfactant and co surfactant, respectively. Tween 20, a non ionic surfactant was known to be less affected by changes in pH[8]. Olive oil nanoemulsion was formulated using high energy sonicator (750w, 20 kHz, vibra-cell™, Sonics, USA). Nanoemulsion was formed after sonication of 1-3 h. Surfactant and cosurfactant were mixed in 1:1 ratio. For each phase diagram, oil and S_{mix} ratio were mixed properly in different weight ratios in separate glass vials. Eight different combinations of oil and S_{mix}, 1:1, 1: 1.5, 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7 were made, so that maximum ratios were covered, in order to define the

boundaries of phases specifically formed in each phase diagram. The shaded area (nanoemulsion area) in each phase diagram was plotted.

Optimization of nanoemulsion: In view of the application of olive oil nanoemulsion in drug delivery, certain oil-S_{mix}-water mixtures within the nanoemulsion region were prepared and final composition of nanoemulsion was optimized based on transparency, droplet size, zeta potential, and polydispersity index (PDI). Nanoemulsions were prepared by varying the stirring parameters and sonication time for optimization. Five different (1:3, 1:4, 1:5, 1:6, 1:7) formulations were selected based on stability by varying the percentage of oil and S_{mix}.

Characterization of nanoemulsion

Droplet size and polydispersity index determination: The droplet size of nanoemulsion was determined by dynamic light scattering with Malvern particle size distribution (Zetasizer ver. 6.20) based on laser light scattering phenomenon, which analyzes the fluctuations in light scattering. Light scattering was monitored at 25ºC at a 90º angle. Properly diluted samples of nanoemulsions were used for droplet size analysis. Average droplet size and polydispersity index were determined. All the quantitative determinations were done in triplicate. Droplet size was expressed as nm and polydispersity index determines size distribution. The higher the polydispersity index the wider is the droplet size distributed.

Viscosity: The viscosity measurements of the nanoemulsion were made using Brookfield viscometer DV-II+ Pro (Brookfield Engineering Laboratories, Inc., USA) coupled with S-00 spindle and shear rate is adjusted 60 rpm at 25 ± 2 ºC.

pH: The nanoemulsion pH was checked by pH meter (ELICO instruments; model LI 120) at 20 ± 5 ºC.

Stability of nanoemulsion: Stability of formulated nanoemulsion was carried out by centrifuging at 6000 rpm for 10 min. Optimized nanoemulsion was also stored at room temperature (26 ºC) and refrigerator temperature (4 ºC) and observed for phase separation, creaming and cracking.

Transmission electron microscopy: Surface morphology of nanoemulsion was studied with the help of field emission transmission electron microscope (Joel FE-TEM, model No. JEM-2100F) capable of point to point resolution. A drop of nanoemulsion was deposited on a copper grid and observed after drying.

Statistical analysis: Values were expressed as mean ± standard error (SE). All the experiments were performed in triplicate.

RESULTS AND DISCUSSION

Construction of pseudo ternary phase diagram: Pseudo ternary phase diagrams were constructed by varying oil: surfactant concentration (Fig. 1). The shaded areas of phase diagrams show the nanoemulsion regions, whereas the nonshaded area displays the multiphase turbid regions. In Fig. 1, nanoemulsion regions are plotted to avoid overcrowding of phases in the diagram. Mustafa *et al.*[2] reported the effect of shorter chain length and less viscosity of the cosurfactant on the fluidity of the interfacial film and interdroplet interactions which improves the nanoemulsion region. Small droplet size and reduced turbidity was observed with increase in surfactant concentration and decrease in olive oil concentration (Fig. 2).

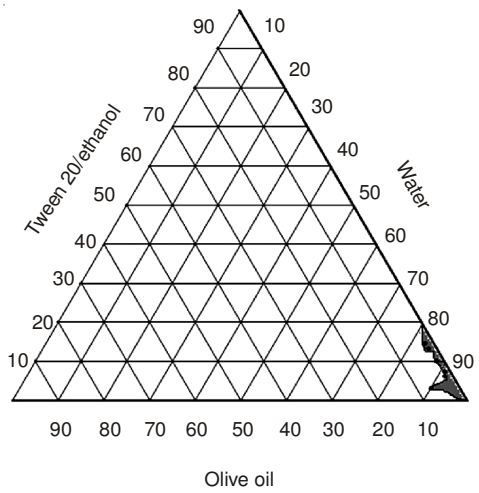

Fig. 1. Pseudo ternary phase diagram of olive oil, Tween 20/ethanol and water

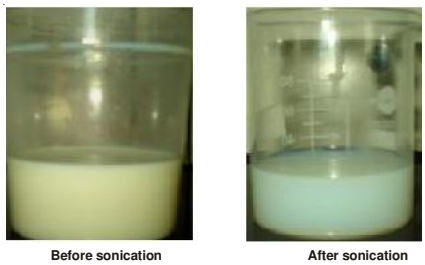

Fig. 2. Diagram showing change in appearance of 1:7 nanoemulsion before and after sonication

Optimization of nanoemulsion: The optimization of nanoemulsions with S_{mix} of 1:1 was carried out on the basis of droplet size and polydispersity index. The formulation of oil: surfactant/co surfactant ratio (1:7) was selected as optimized formulation as it displayed optimum characteristics such as least globule size (76.2 nm) and polydispersity (0.220).

Characterization of nanoemulsion

Droplet size and polydispersity index determination: Apart from the pseudo ternary phase diagram, droplet size determination was also performed as it could provide supportive evidence. It was clearly evident that an increase in the concentration of Tween 20 resulted in a decrease in droplet size (Fig. 3). Table-1 shows the droplet size distribution of five selected formulations. Thus, at the lowest concentration of surfactant droplet size was 248.4 nm, whereas with increase in concentration of Tween 20, it was reduced to 76.2 nm.

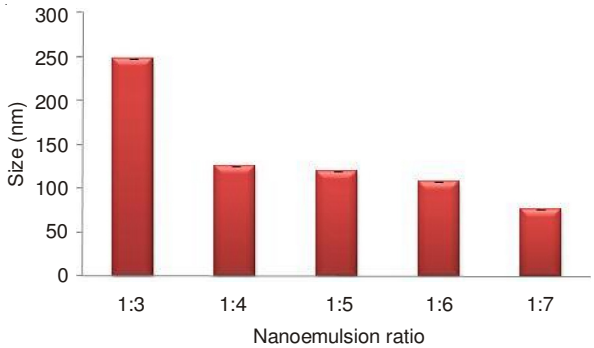

Fig. 3. Graphical representation of nanoemulsion ratio *versus* size

TABLE-1
REPRESENTS NANOEMULSION SIZE WITH INCREASE IN RATIO OF SURFACTANT

Nanoemulsion ratio	Size (nm)
1:3	248.40
1:4	124.80
1:5	119.10
1:6	107.00
1:7	76.20

Polydispersity index indicates the uniformity of droplet size within the formulation. High polydispersity index results in low uniformity of the droplet size in the formulation[9]. This was indicative of nanoemulsion approaching a monodispersed stable system that could effectively deliver the drug effectively owing to larger surface area. The polydispersity of droplet size was observed to be for small size nanoemulsion.

Stability of nanoemulsion: Five selected nanoemulsion formulations were observed to be stable with no phase separation, creaming or cracking. The steric effect results in nanoemulsion stability[10]. The present study showed that the formulated nanoemulsions survived the stability test. pH of formulated nanoemulsion was observed to be in acidic range since human body maintains acidic pH.

Viscosity: Viscosity of the formulated nanoemulsions was observed to increase with increase in surfactant concentration (Fig. 4). This may be due to increased hydration by water molecules around the hydrophilic portion of the surfactants[11]. Viscosity of the selected formulation is shown in Table-2.

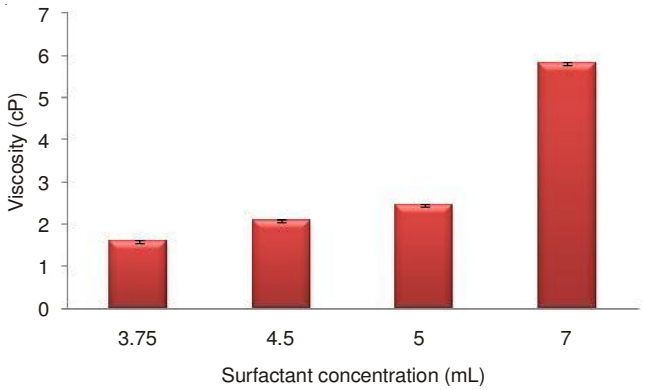

Fig. 4. Graphical representation of increase in viscosity with surfactant concentration

TABLE-2
SHOWS VISCOSITY OF FORMULATED NANOEMULSIONS

Nanoemulsion ratio (surfactant concentration, mL)	Viscosity (cP)
1:3 (3.75)	1.6
1:4 (5.0)	2.45
1:5 (3.75)	1.65
1:6 (4.5)	2.07
1:7 (7.0)	5.82

Transmission electron microscopy: Transmission electron microscopy showed that the formulated nanoemulsion droplets were almost spherical in shape (Fig. 5). Some droplet sizes were measured and the droplets in nanometer range varied from nm.

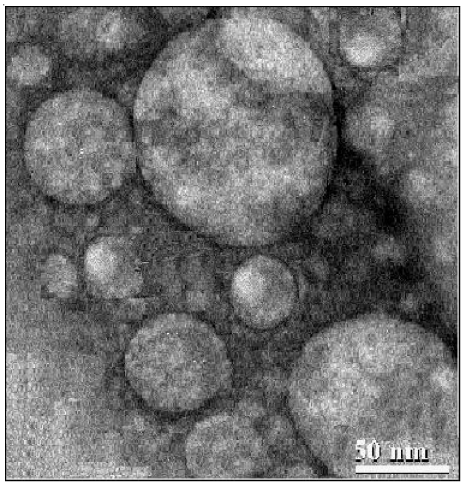

Fig. 5. Transmission electron microscopic image of nanoemulsion

In the present study, olive oil nanoemulsion of size 76.2 nm was formulated using Tween 20 as a surfactant and ethanol as co surfactant. The presence of various antiinflammatory compounds in olive oil improves its application in pharmaceutics as antiinflammatory agent. However further work is going on in authors laboratory to evaluate its antiinflammatory efficacy.

Conclusion

Olive oil nanoemulsion was successfully formulated using sonicator. The nanoemulsion was observed to be stable for more than 3 months. Stability and various medicinal properties of olive oil nanoemulsion pave way for its development as a potent product in area of drug delivery.

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