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Preparation of Neem Seed Oil Nanoemulsion

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Abstract- Presently, there is a growing interest in natural, especially herbal, bioactive agents. Neem (*Azadirachtaindica*) has been used in traditional medicine over centuries and it showed different biological activities which makes it a good target for antibacterial studies. On the other hand, nanoemulsions serve as delivery agents for lipophilic bioactive compounds such as drug in the pharmaceutical industry. In this study an attempt was made to prepare water based nanoemulsions from Neem seed oil using Tween 20 as a surfactant. Ultrasonic waves were used for applying shear to micro-scale droplets and altering them to nano-scale. Nanoemulsion with Neem oil and surfactant ratio of 1:4.5 which was sonicated for 45 minutes contained 50.21 nm droplets.

***Keywords*:** Neem, Ultrasonic, Nanoemulsion, Tween 20.

1. Introduction

Neem is an ever green Indian tree (Fig. 1) which is planted in almost 30 countries. It usually grows in southeast countries like Pakistan, Bangladesh and Iran. It has been found in south and southeast of Iran (Kausik et al., 2002). Although Neem extracts have shown many bioactive properties such as antibacterial, antimalarial, antifungal, antifeedant, they usually have been used as pesticides (Rajendran, 2012). These bioactive properties have been found in almost every part of the tree, especially seeds (Kausik et al., 2002). Different solvents have been tested for preparation of Neem extracts from different parts, such as acetone and ethanol (Liauw et al., 2008; Boursier et al., 2011).

Nanoemulsions are dispersions of nano-scale droplets formed by shear-induced rupturing. Nanoemulsionsare defined as O/W (oil in water) or W/O (water in oil) emulsion producing a transparent product that has a droplet size from 20-200nm and does not have the propensity to coalesce. Nanoemulsions have many interesting physical properties that are different from or are more extreme than those of micro-scale emulsions. Nanoemulsions appear visibly different from micro-scale emulsions since the dropletscan be much smaller than optical wavelengths of the visible spectrum. So nanoemulsions can appear nearly transparent in the visible spectrum and exhibit very little scattering (Mason et al., 2006).

Fig. 1. Picture of a Neem tree (Kausik et al., 2002)

In this study, we made an effort to make nanoemulsions from Neem seed oil. We used Tween 20 as a nonionic surfactant because non-ionic surfactants are known to be less affected by pH (Jones et al., 1989) [Mason et al., 2006; Rajasekaran et al., 2008]. For altering microemulsions to nanoemulsions, ultrasonic agitation of microemulsions is one of the methods which can be used (Chiang et al., 2012). The premixed emulsion has been made in advance using regular mixing methods. In the ultrasonic method, a vibrating solid surface agitates the premixed emulsion at ultrasonic frequencies causing extreme shear and cavitations that breaks up droplets and makes nanoemulsions (Nakabayashi et al., 2011).

With nano-sizing the extracts, the surface area of particles increase and as a result the number of functional groups on the surface increase thus when extracts are attacked by bacteria more hydroxyl and carboxyl groups and tricyclicdihydrofuran ring are in contact with bacteria and so can better prevent their growth.

2. Materials and Methods

Fresh grown leaves and seeds of Neem tree was provided from Bandar Abbas, one of the southern cities of Iran. Picture of Neem seeds used in this experiment are shown in Fig. 2.

Fig. 2 . Neem seed kernels.

Acetone which was used for extracting Neem leaf and Neem seed oil was of analytical grade and was purchased from Merk Company. Tween 20, with IUPAC name Polyoxyethylene (20) sorbitanmonolaurate and molecular formula of $C_{58}H_{114}O_{26}$, produced in Daejung Company, Korea, was supplied by Behnojen Company. Distillated water (Mahdarou; Iran) was used for all experiments.

2.1 Preparation of Neem seed oil

First, Neem's seeds were washed three times thoroughly till no dust or other impurities left. Neem seeds were heated in the temperature of 50 °C for one hour so they would dry out so the kernels can be separated from wooden parts of seeds. The kernels were ground using a grinder (32002, Moulinex, France). Pictures of ground Neem kernels are shown in Fig. 3.

Fig. 3. Grounded Neem seed kernels.

Soxhlet extractor was used for preparation of Neem seed oil. Grinded seed kernels were packed tightly in filtration paper so substance would get out and the packed material was placed in middle part of the extractor. Then acetone was used as the solvent with the ratio of 1:5 (W/V). The solvent was heated at 50 °C for eight hours so no oil was left in the Neem kernels. Thereupon, the mixture of oil and solvent was kept in room temperature for 48 hours so the solvent evaporates completely and eventually what was left was Neem seeds oil. Picture of Neem seed oil is shown in Fig. 4.

Fig. 4. *Neem seed oil.*

2.2 Preparation of Nanoemulsion Neem extract

Neem oil, Tween 20 and distillated water were used in the preparation of water-based (O/W) emulsion. The emulsions contained Neem oil and surfactant with ratios of 1:1.5, 1:3.0 and 1:4.5, respectively. Each emulsion with micro-scale droplets was sonicated for 30 minutes and 45 minutes using high-energy sonication in a sonicator (UP400S, Hielscher, Germany) so nanoemulsions were prepared.The nanoemulsions particles size were determined using DLS device (Nanophox, Sympatech Germany).

3. Results

3.1 Droplet sizes

Droplet sizes of various nanoemulsions are shown in Table 1. The droplet size decreased with increase in surfactant concentration and time of sonication. The smallest droplet size of the nanoemulsion consisting of 1 part oil (3.5%) and 4.5 parts surfactant (15.75%) with 45 minutes sonication was 50.21 nm.

As it is shown in Table 1, increase in oil and surfactant ratio and sonication time of nanoemulsions cause decrease in droplet sizes and also distribution of particle sizes of emulsions decrease. As it is indicated in Table 1, the effect of ultrasonic waves is greater than oil and surfactant ratio. By comparing nanoemulsion with the same oil and surfactant ratio it is noted that maximum droplet size of nanoemulsionsonicated for 45 minutes is less than 100 nm while droplets in nanoemulsionsonicated for 30 minutes are almost two times bigger. However, on the other hand, increase in oil and surfactant ratio has greater effect on nanoemulsion which was sonicated for 30 minutes. Once the oil and surfactant ratio increases three times, droplets' sizes for nanoemulsionsonicated for 30 minutes decrease with ratio of 2.5 and for nanoemulsionsonicated for 45 minutes decrease with ratio of 1.8.

Table. 1. Three different ratios of oil and surfactant used for nanoemulsion with two different sonication times

Oil and surfactant ratio	Time of sonication (min)	Average droplet size (nm)	Standard deviation	VMD/SMD
1:1.50	30	169.09	6.54	1.002
1:1.50	45	89.48	0.93	1.003
1:3.00	30	127.29	2.37	1.003
1:3.00	45	70.08	0.74	1.001
1:4.50	30	67.85	0.74	1.001
1:4.50	45	50.21	1.20	1.000

Decrease in droplet size cause increase in surface area of droplets and as mentioned before, the number of active groups should be increased hence the antibacterial activity of nanoemulsion expected to be enhanced. The antibacterial tests were performed to investigate the effect on nano-sizing on antibacterial activity of emulsions.

VMD given in Table 1 is the average diameter based on unit volume area of a particle and SMD the average diameter based on the unit surface of a particle. Proximity of these two parameters indicates how close the shapes of droplets are to spherical form. As it is shown in Table 1, all nanoemulsions are near to spherical form.

Fig.5 shows cumulative distribution of particle size diagram. Their density distribution diagram is shown in Fig. 6. Droplet size of nanoemulsions can be read from cumulative distribution diagrams of particles size.

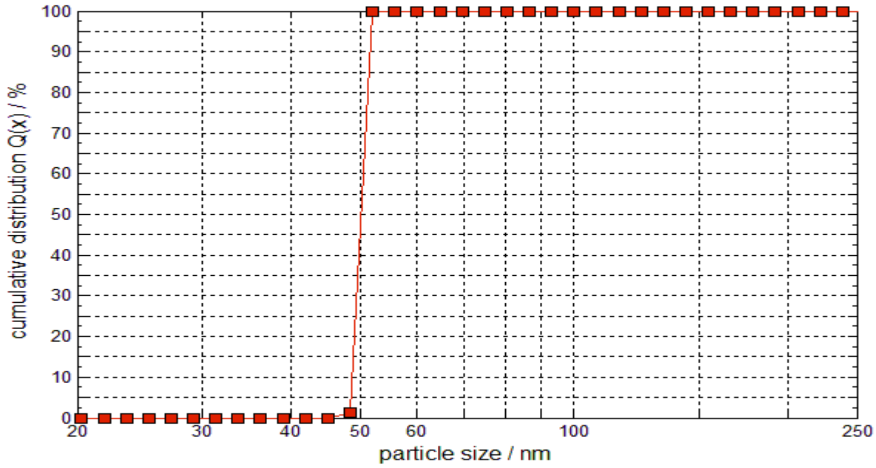

Fig. 5. Cumulative distribution diagram of nanoemulsionsonicated for 45 min with oil and surfactant ratio of 1:4.5

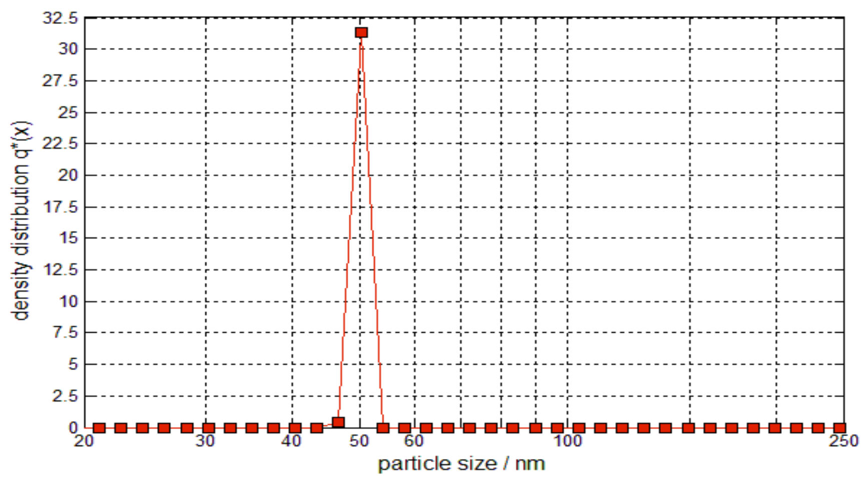

Fig. 6. Density distribution diagram of nanoemulsionsonicated for 45 min with oil and surfactant ratio of 1:4.5.

As can be seen in Fig.6 the density distribution diagram has one narrow peak which verifies the monodispersity of this nanoemulsion.

3.2 Transparency

The transparency of the nanoemulsions was compared with micro-emulsion with the same formulation and as it is shown in the right picture nanoemulsion has excellent transparency. This transparency makes nanoemulsions great materials for antibacterial finishing of white fabrics. Fig. 7 shows the difference between the transparency of Neem oil emulsion and nanoemulsion with the oil and surfactant ratio of 1:4.5.

Fig. 7. Left Neem oil emulsion, right Neem oil nanoemulsion.

4. Conclusion

The nanoemulsion formulation containing Neem oil, Tween 20 and distillated water was successfully optimized by the high-energy method. A smallest droplet size of 67.85 nm was obtained. Neem oil nanoemulsion may be a good alternative to other antibacterial agents in textile finishing.

References

Kausik B., Chattopadhyay I., Banerjee K. R., Bandyopadhyay U. (2002). J.Current Science, 82 1336-1345.

Rajendran R. (2012), Journal of Engineered Fibers and Fabrics, 7, 136-141.

Liauw M. Y., Natan F. A., Widiyanti P., Ikasari D., Indraswati N., Soetaredjo F.E. (2008), Journal of Engineering and Applied Sciences, 3, 49-54.

Boursier C. M., Bosco D., Coulibaly A., Negre M. (2011). Journal of Crop Protection, 30, 318-322.

Mason T. G., Wilking J. N., Meleson K., Chang C. B., Graves S. M. (2006), J. Phys.: Condense, 18, 635–666.

Rajasekaran C., Meignanam E., Vijayakumar V., Kalaivani T., Ramya S., Premkumar N., Siva R., Jayakumararaj R. (2008), Ethnobotanical Leaflets, 12, 1213-1217.

Chiang P., Li B. (2012), J. Ultrasonic Sonochem, 19, 192–197.

Nakabayashi K., Amemiya F., Fuchigami T., Machida K., Takeda S., Tamamitsub K., Atobe M. (2011), J. Chem. Commune, 47, 5765–5767.