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Preparation and comparison of various formulations of solid lipid nanoparticles (SLNs) containing the essential oil of *Zataria multiflora*

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ABSTRACT

Purpose: The aim of this study was to formulate a new delivery system by the incorporation of *Zataria multiflora*. essential oil into solid lipid nanoparticles (SLN). **Research Method:** SLN formulations were prepared following the high-pressure homogenization after starring and ultra-trax homogneization techniques. In this experiment, three SLNs formulations were prepared using three types of lipids. Lipids included glycerol monostearate lipid, precirol and stearic acid lipid. The SLNs were characterized by Differential Scanning Calorimetery (DSC), Transmission Electron Microscopy (TEM) and particle size analysis. **Findings:** The results showed that particle size, polydispersity index and zeta potential of the above formulations were about 255, 220, 486 nm, 0.369, 0.251, 0.296,-37.8, -17.6 -27.2 mV respectively. The results obtained from transmission electron microscopy (TEM) revealed that in all 3 formulations, particle size less than 200 nm were spherical. Thermal analysis by DSC, confirmed the presence of solid particles in the prepared SLNs. Also, the essential oil encapsulation percentage of Formulations 1, 2 and 3 were 85.3, 91.3 and 95.2% respectively. Stability studies of particle size and zeta in four months revealed that SLNs containing essential oils had relatively good stability. **Research limitations:** Limitations of SLNs are: Lipid particle growth, Unpredictable gelation tendency. **Originality/Value:** Due to the chemical structures of essential oils, EOs can be easily degraded after exposure to humidity, heat, oxygen, light, owing to chemical and enzymatic reactions. To overcome the drawbacks of EOs, several researchers have suggested the encapsulation of these active ingredients into nanocarriers. The results of the present research revealed that SLNs composed of glycerin monostearate lipids, precirol and stearic acid, were good carriers for *Z. multiflora* essential oil.

INTRODUCTION

Plant essential oils include a wide range of secondary metabolites, which in most cases have anti-microbial, anti-oxidant and allopathic and bio-regulatory properties. Chemically, essential oils are complex compounds, where different types of chemicals are found, including hydrocarbons, alcohols, ketones, aldehydes, etc. (Plotto et al., 2003). In this regard, research has shown that herbal essential oils have several advantages, which include lower bioaccumulation and toxicity, rapid decomposition, and being very broad-spectrum when compared with synthesized fungicides and pesticides, not to mention that they possess new effective which are not open to deactivation by fungi. Despite the mentioned advantages, using the essential oil of herbs has some limitations, such as instability, evaporation, and decomposition against environmental and chemical conditions (light, oxygen, and humidity) (Donsi et al., 2011). In this regard, one of the methods to solve the problems caused by volatility and instability of essential oils is encapsulating them in the nano size. In this method, the active ingredient is encapsulated within a small case or shell at the nano size. This shell both protects the chemical from damage caused by external factors and helps in increasing solubility and penetration to tissue. Opening of this shell to release the desired compound is possible through altering external conditions, such as pH (Perez-de-Luque and Rubiales, 2009).

There are various methods for encapsulation of the essential oil compounds of medicinal plants, one of the major ones being a carrier system called solid lipid nanoparticles (SLNs). The SLNs are colloidal carrier systems which are similar to nano-emulsions (Ekambaram et al., 2011). SLNs replaced other common colloidal carriers like emulsions, liposomes, and micro-polymers. Their size varies between 50 and 1000 nm. SLNs can enhance the stability and solubility of essential oil in water (Shi et al., 2012). SLNs guard the essential oil against environmental factors such as oxygen, light, moisture and acidity, and also create more nano-sized carriers and easily increases levels of solubility and bioavailability of essential oil and improve the controlled release of the essential oil (Donsi et al., 2011).

Research has shown that SLNs can be utilized for encapsulation of essential oils; they can also increase the stability and solubility of essential oil in water (Shi et al., 2012). Researchers have used the SLNs for controlled and targeted release of drugs (Lai et al., 2006). Lai et al. (2006) reported reducing volatility and evaporation of *Artemisia Warborescens* essential oil using SLNs.

Zataria multiflora is a plant of Lamiaceae family that grows only in Iran, Afghanistan and Pakistan (Hosseinzadeh et al. 2000; Shaiq Ali et al. 2000). Thymol and carvacrol are the two important and major substances, such that up to 78% of the levels of these two compounds have been reported in the essential oil of *Z. multiflora* (Avaei et al., 2015; Nasseri et al., 2015). Thymol and carvacrol are two compounds that have potent antifungal and antibacterial activities (Bacchella et al., 2009; Burt, 2004; Nasseri et al., 2015).

The main aim of this research is to encapsulate *Z. multiflora* essential oil (ZE) using solid lipid nanoparticles carrier systems (by 3 types of lipids; stearic acid, glycerol monostearate and precirol) and to determine their properties which can be used in food, medicine and agriculture industries. In addition to particle size analysis, thermal analysis studies are required to assess the prepared formulations completely. This test is essential for analyzing crystalline or thermal characteristics of SLNs formulations containing ZE.

MATERIALS AND METHODS

Preparation and characterization of essential oil

Thyme essential oil was purchased from the Barij Company (Barij Essential Oil Co., Iran). The compounds of the essential oil were then identified by gas chromatography. GC-MS spectrometry analysis was carried out in a Varian 3400 GC/MS (California, USA) system equipped with a DB-5 fused silica column (30m × 0.25mm i.d., film thickness 0.25 µm; J and W Scientific). The temperature of oven was raised from 50 to 240 °C at a rate of 4 °C min^{-1}, the transfer line temperature was 260 °C, the carrier gas was helium at a linear velocity of 31.5 cm s^{-1}, the split ratio was 1: 60, the ionization energy was 70 eV (Nasseri et al., 2015)

Preparation of solid lipid nanoparticles containing essential oil

Preparation of SLNs containing the essential oil was done through the ultrasonic method. In this method, lipid and aqueous phases are required. The lipid phase contains glyceryl monostearate, percirol, and stearic acid, the essential oil introduced into this same phase after achieving the final formulation, since the essential oils are lipid-soluble. The aqueous phase contains Tween 80 or Poloxamer 188 together with water. Having weighed the substances required for the formulation (lipid, water, and emulsifier) with certain percentages, the lipid and aqueous phases were individually placed inside Ben mari in two separate falcons. The Ben mari was adjusted at 80°C, the two falcons containing both phases were put in it to allow the lipid phase to melt and the two phases become isothermal. A beaker containing distilled water was also placed inside the Ben mari, to be later used as water bath. At this stage, first the essential oil was added to the lipid phase, and then, the two phases were taken out from the Ben Mari after 2 min, with the aqueous phase being rapidly and suddenly added to the lipid phase. If the lipid phase is slightly allowed to cool down within this interval, it returns to its solid-state. Thus, the stage of mixing the two phases should be done inside the Ben Mari. The beaker containing the mixture was put inside the water bath for its temperature not to decline. At this stage, a white emulsion was formed (at every volume load, as much as 20 mL of the sample was prepared), and homogenized with a homogenizer for 5 min. Following homogenization, the compound was transferred to a Probesonicator Device (3 cycles with each cycle for 30 s). The mixture was then subject to room temperature to fully cool down and become isothermal with the environment, such that the SLNs loaded with the ZE are formed (Golmohammadzadeh et al., 2012).

Particle Size and Zeta Potential Measurement

To investigate the particle size and zeta potential, particle size analyzer was used. The zeta potential is of importance in the stability of particle systems. This potential determines the range between adjacent particles with a similar charge. If the zeta potential is lower than a certain value, attraction forces overcome repulsion, resulting in aggregation of particles. The importance of measuring zeta potential is owing to the fact that its value plays an important role in the stability of SLNs against aggregation, attachment, and interference among lipids and charged compounds. To measure the size of the particle and zeta potential, 10 µl of fresh synthesized and the cooled sample was poured into a microtube with a volume of 1.5 ml, further diluted with 990 µl external phase, that is, deionized water (1 to 100) and each measurement was performed in triplicate. The instrument expresses particle size in terms of the number, volume, and intensity (Mader & Mehnert, 2005).

Investigation of the morphology of the SLNs using electron microscopy

To image and investigate the morphology of the SLNs, transmission electron microscopy (TEM) (located in the Central Laboratory of Mashhad Ferdowsi University) was employed. The sample was diluted with almost 50 times diluted water, then 20 µl of the sample was placed on grids with

coated carbon, and finally dried using a paper filter after 30 s. A total of 20 µl of Uranil acetate 2% in water was put on the grids, and then filtered with a paper filter after 30 s. Following drying, the sample was observed under the electron microscope (Jores et al., 2004).

Encapsulation efficacy (EE)

The encapsulation efficacy (EE%) was determined by measuring the concentration of the major component of ZE as an index (thymol) (Shah et al., 2007). To calculate the encapsulation efficiency, it must be dilute a specific amount of nanoparticles in a buffer. In this way, 500 µl of the SLN dispersion was transferred to the upper chamber of an ultrafilter then Amicon tubes were centrifuged. The filtrate was analyzed for encapsulated thymol at *gas chromatography* method after suitable dilution with chloroform: methanol (2:1 v/v).

Then, the percent of entrapment was determined using the following Equation 1.

$$EE\% = \frac{\text{actual Thymol concentration sample}}{\text{input Thymol concentration}} \times 100 \quad (1)$$

Differential scanning calorimetry (DSC)

Differential scanning calorimetry (DSC) is a very helpful technique in the pharmaceutical field to characterize drug delivery systems such as melting and recrystallization behavior of crystalline materials after solidification, therefore, used to evaluate the polymorphism, crystal or drying and interactions between the lipid and the drug. Samples of 5 mg were prepared in sealed pans. DSC was done at 25 to 250°C temperature range by the rate of 5°C/min under N2 flow (Mosallaei et al., 2013).

RESULTS

The compounds in the essential oil

Having investigated the spectra of GC and GC/MS, the indices of inhibition was calculated and the mass spectra of the compounds with standard compounds were compared. Twenty-one different compounds were identified in the essential oil of *Z. multiflora*, with the major compounds being thymol (35.31%), carvacrol (33.9%), parasimin (9.89%), gamatripnen (5.88%), and alphapinen (4.22%) (Table 1).

Characterizations

The physical stability of SLNs depends on their particle size and it is the most important parameters. The particle size is affected by formulation compound, methods of production, and environmental conditions (Bunjes, 2005). Zeta potential (ZP) refers to the surface charge of the particles. ZP (±) indicates the degree of repulsion between particles. ZP prevents aggregation of the particles. Therefore, ZP predicts the stability of the solid lipid nanoparticles dispersions. The result of particle size distribution, zeta potential, and polydispersity index of three ZE- SLNs formulations are given in Table 2.

Table 1. Composition of *Z. multiflora* L. Essential Oil

	Component	Retention Time R	Area %
1	Alpha.-Thujene	930	0.36
2	Alpha.-Pinene	939	4.22
3	Camphene	954	0.1
4	Beta-Pinene	979	0.34
5	Beta-Myrcene	990	1.01
6	Alpha-Phellandrene	1002	0.19
7	Alpha-Terpinene	1017	1.26
8	Cymene<p->	1024	9.89
9	1,8-Cineole	1023	0.37
10	Gamma.-Terpinene	1059	5.88
11	Linalool	1096	0.88
12	Terpinene-4-ol	1177	0.36
13	Terpineneol<gama>	1199	0.29
14	Thymyl Methyl Ether	1235	0.41
15	Carvacrol Methyl Ether	1244	0.73
16	Thymoquinone	1248	0.36
17	Thymol	1289	35.3
18	Carvacrol	1299	33.9
19	Trans-Caryophyllene	1439	1.12
20	Aromadendrene	1439	0.36
21	Ledene	1475	0.22
		---	98.71

Table 2. Particle size distribution, zeta potential, and polydispersity index of ZE- SLN formulations

Formulation	Z-Potential (mv)	PDI	(nm)Z-average
1	-37.8 ± 0.5	0.369±0.08	255±6
2	-17.6 ±0.9	0.251±0.06	220±12
3	-27.2 ± 0.3	0.296±0.05	486± 9

Formulation 1: SLN_1: 70% Glyceryl mono stearate +30% percirol+ Toween 80+ Poloxamer 188+ Water
Formulation 2: SLN_2: 30% Glyceryl mono stearate +70% percirol+ Poloxamer 188+ Water
Formulation 3: SLN_3: Stearic Acid+ Poloxamer 188+ Water

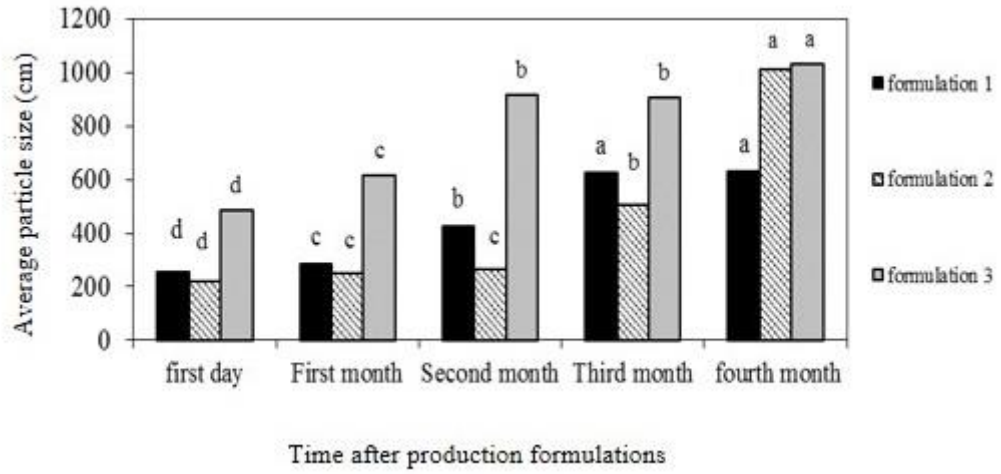

Fig. 1. *Z*-average (nm) of ZE-SLN prepared by probe sonication technique through 4 weeks of storage at 4°C
(Values followed by the same letter are equivalent according to Fisher's protected LSD at the 0.05 probability level)

The stable average particle size of the three formulations of SLNs containing ZE was measured for four months (Fig. 1).

Investigation of the morphology of the SLNs using electron microscopy

The results showed that the particle size was lower than 200 nm with almost spherical particle shape (Fig. 2, 3, and 4). Spherical SLNs made of them to have the highest ability for controlled-release and protection against encapsulated essential oil.

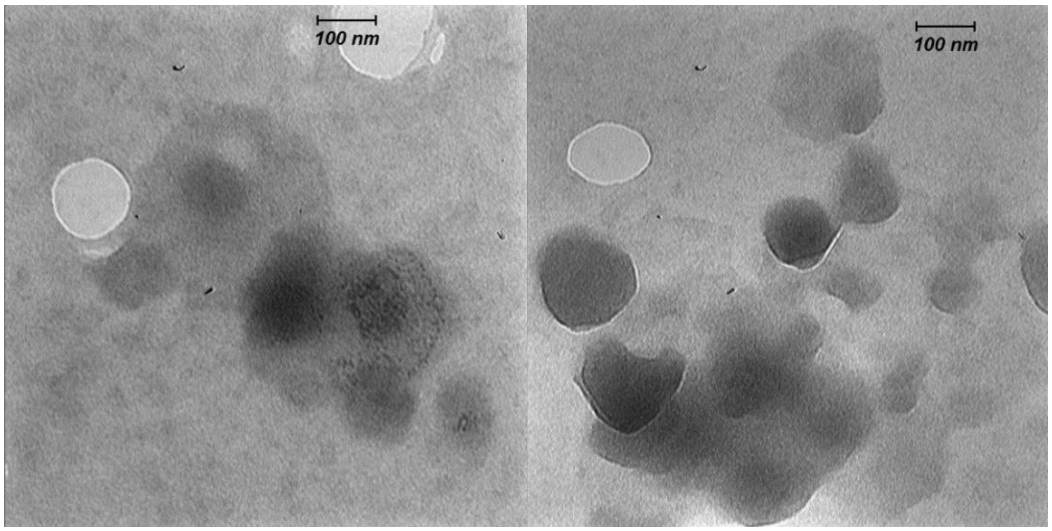

Fig. 2. Electron microscopy image of TEM from SLNs loaded with the ZE (Formulation 1)

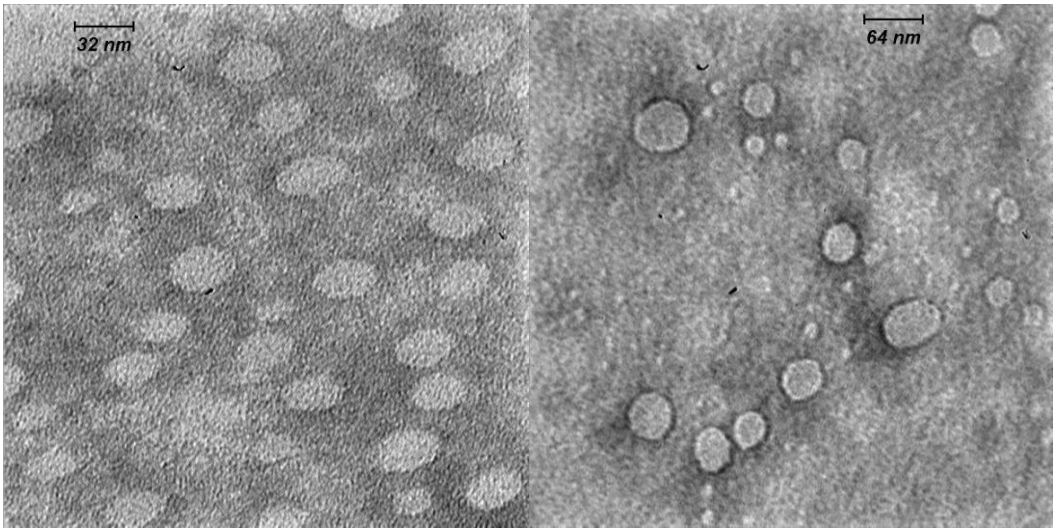

Fig. 3. Electron microscopy image of TEM from SLNs loaded with the ZE (Formulation 2)

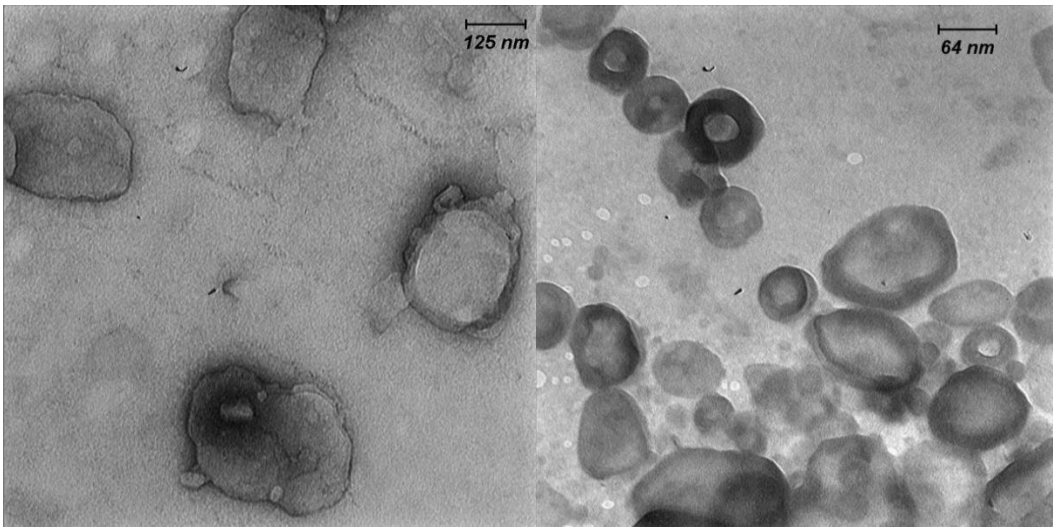

Fig. 4. Electron microscopy image of TEM from SLNs loaded with the ZE (Formulation 3)

Thermal analysis of ZE-SLN

Thermal analysis of lipid was conducted by differential scanning calorimetry (DSC).
One of the advantages of SLNs is that their crystalline stability can be easily measured in the carrier using DSC. Thermal analysis of bulk lipids used in SLNs, ZE and Formulations 1, 2 and 3 were carried out by DSC (Fig. 5, 6, 7, and 8).

Encapsulation efficacy (EE)

According to GC/Mass assay results, encapsulation percentage of Formulations 1, 2 and 3 were 85.3, 91.3 and 95.2% respectively. Therefore, a high amount of essential oil could be incorporated in nanoparticle dispersion. It is because of the high lipophilicity of ZE and its excellent compatibility with Glyceryl monostearat, Glyceryl palmito stearate (Percirol) and stearic acid.

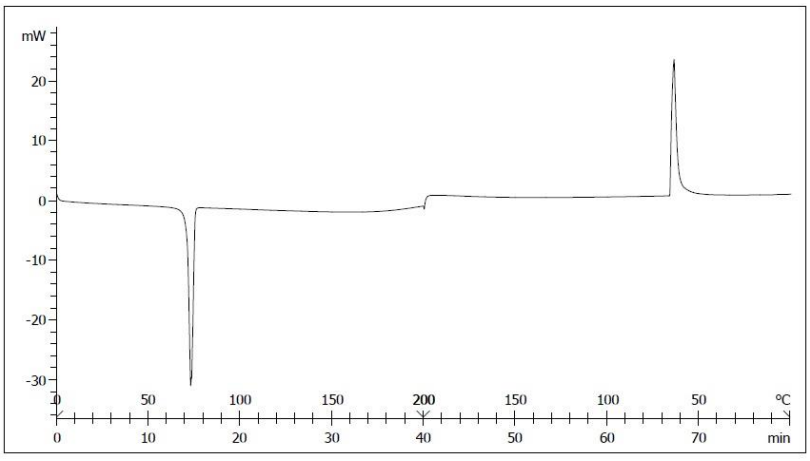

Fig. 5. DSC of *Zataria multiflora* essential oil

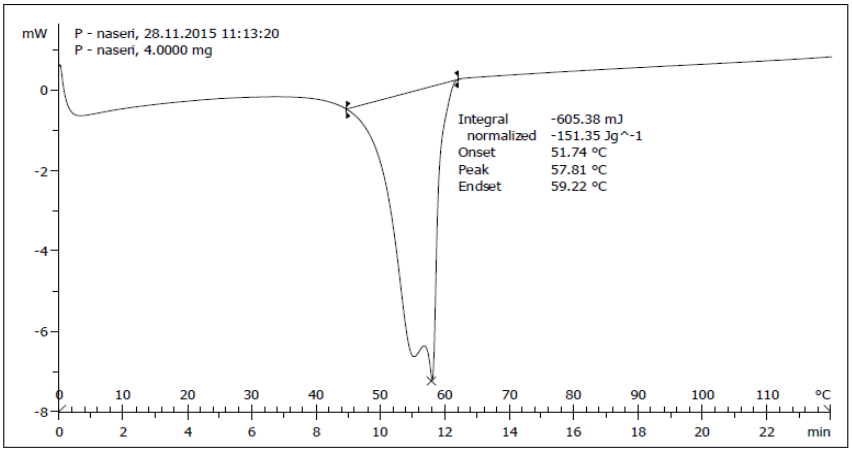

Fig. 6. DSC of Formulation1

1

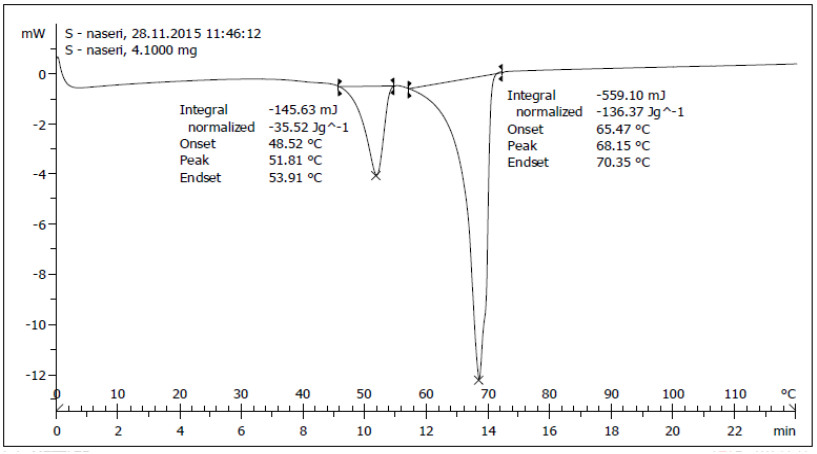

Fig. 7. DSC of Formulation 2

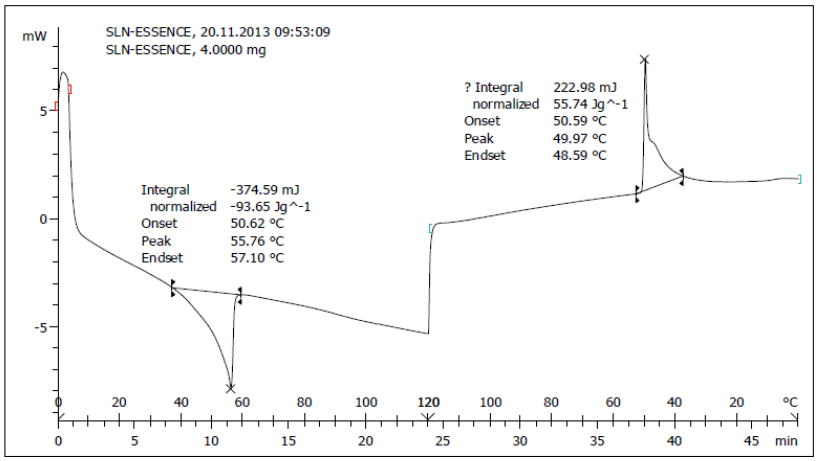

Fig. 8. DSC of Formulation 3

DISCUSSION

Among the advantages of SLNs, is slow release of the active ingredient, blocking characteristic and film-forming property (Wissing & Muller, 2002). The formulation of these carriers is derived from nanoemulsions; but the difference is that in SLNs, dispersed phase, which is the lipid phase, is solid at room and body temperature (Domb, 1993). The particle size of SLNs was measured using particle size measurement device (PSA) and the photo taken with an electron microscope which confirmed the tiny size of these particles. The difference between the results of PSA and electron microscope on stearic acid formulation is probably because of the particular structure of this acid, which slightly differentiates light, thereby making some differences while measuring the size of the particles (Pizzol et al., 2014).

Thermal analysis was carried out using DSC. DSC test charts on glycerol monostearate and precirol substances, shows a similar melting point of references of about 56 to 57 °C (Fig. 6 and 7). Also, a small peak at about 82°C can be observed for ZE peak (Fig. 5), which is probably due to oxidative degradation of the essential oil in this area. In particular, Formulation 1 shows that components of SLNs are uniformly mixed and form a regular lipid network similar to the bulk lipid. In fact, this peak is located at the glycerol monostearate and precirol peaks (Fig. 6 and 7). ZE peak is about 82°C (Fig. 5), in which there is no other peak in this range, there can be the possibility that the essential oil is completely trapped in the SLNs network and possibly is somehow protected against oxidative damage. Also, the prepared nanoparticles may have a matrix type network or nucleus-focused network, because DSC diagram of SLNs containing essential oils generates no additional peak. The absence of peak oil in the DSC diagram and more than 85% essential oil retention in the SLNs, support each other.

The DSC diagram for Formulation 2 shows that in addition to the peak formation and the presence of crystals of total lipids, this peak is slightly shifted to the left, which is probably due to the presence of surfactant and the SLN nature and small size of particles (Fig. 7). In addition, little change in the peak shape may be due to different polymorphic forms of crystalline lipid mixtures, which causes disruption to the network and create crystals with less regularity (Wissing & Muller, 2002). The DSC results of stearic acid-lipid also showed a peak at about 72 °C, which was optimum according to the stearic acid melting point of 70 °C (8). In the DSC diagram for Formulation 3, it can be seen that the peak is slightly inclined to the left when converting to SLNs, which is because that lipid is nanosized and surrounded by a layer of surfactant. As a result, the lipid nature changes and little change in its DSC diagram are seen (Fig. 8). Furthermore, some changes are made in the polymorphism of the created crystalline, which probably disturbed lipid crystalline regularity at the time of recrystallization and crystals with less regularity is formed (Wissing & Muller, 2002). The encapsulation percentage of essential oil in Formulations 1, 2 and 3 were, 85.3, 91.3 and 95.2% respectively. Due to the lipid nature of the essential oil, high lipophilic property and its high solubility, it is expected that oil particles will be well accumulated and higher encapsulation percentage will be achieved in the lipid phase of nanoparticles. One of the reasons for selection of precirol as SLNs containing the essential oil in this study is that it is a diglyceride with two different fatty acids (C_{18} and C_{16}), which creates an irregular network. Moreover, loading can be well predicted when it is used (Hamadani et al., 2003). The existence of an irregular lipid network causes controlled release of active ingredient and increases the essential oil encapsulation (Wang et al., 2007). Wissing et al. (2004) investigated the stability of lipid nanoparticles made of precirol in terms of the particle size and the polydispersity index. It became clear that SLNs were stable for up to 3 years. The particle size and zeta stability study in this research confirms the fact that SLNs containing the essential oil were stable, although Formulation 1 was more stable than the other two formulations in terms of size and zeta potential. The average particle size in Formulation 1 was about 255 (the first day) to 630 nm (fourth month), which had better size than the other

formulations (Fig. 1). According to the results of this study, SLNs composed of stearic acid lipids, glycerol monostearate lipids and precirol are appropriate carriers for the ZE. On the other hand, considering the antimicrobial effects of the ZE (Nasseri et al., 2016) and its application in the pharmaceutical, food industry and agriculture industries, the formulation of the essential oil using nanotechnology can facilitate its use and increase its efficiency. Based on this, Moghimipour et al. (2013) also formulated ZE using SLNs, but in their study, the particle size (650 nm) and the encapsulation percentage (up to 38.66%) were larger and less than those of the present study, respectively. However, in the present experiment, particle size was smaller (from 255 to 486 nm) and the encapsulation percentage ranges from 85 to 95% using different lipids and convenient preparation method. However, further studies are recommended on the preparation of SLNs containing ZE.

CONCLUSION

In this study, ZM-SLNs were prepared by high-pressure homogenization. Essential oil of Zataria multiflora L was encapsulated into the nanoparticles. Particles prepared under proper formulation conditions with diameters of < 220 nm. The highest percentage of encapsulation was obtained in Formula 3. Physicochemical characterization of ZM_SLNs revealed that efficiently SLNs encapsulated the essential oil. The results of the present research showed that SLNs were good carriers for *Z. multiflora* essential oil.

CONFLICT OF INTEREST

The authors have no conflict of interest to report.

REFERENCES

Avaei, A., Mohamadi Sani, A., & Mahmoodzadeh Vaziri, B. (2015). Chemical composition and antimicrobial effect of the essential oil of *Zataria multiflora*Boiss endemic in Khorasan-Iran.*Asian*Pacific. *Journal of Tropical Disease*, *5*(3), 181-185. http://dx.doi.org/10.1016/S2222-1808(14)60649-6

Bacchella, R., Testoni, A., & Lo Scalzo, A. (2009). Influence of genetic and environmental factors on chemical profile and antioxidant potential of 84 commercial Strawberry (*Fragaria* × *ananassa* Duchesne). *Electronical Journal of environmental, Agricultural and Food Chemistry*, *8*(4), 230-242. http://dx.doi.org/10.1016/j.jhazmat.2015.10.026.

Bunjes, H. (2005). Characterization of solid lipid nano- and microparticles. in: lipospheres in drug targets and delivery. Edited by Nastruzzi C, pp: 41-66. Florida: CRC press.

Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods. *International Journal of Food Microbiology*, *94*, 223-253. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.03.022.

Domb, A.J. (1993). Lipospheres for controlled delivery of substances. In: microencapsulation: methods and industrial applications. Ed. Benita, S. 2th ed, 158, pp.188-836. USA: CRC Press.

Donsi, F., Annunziata, M., Sessa, M., & Ferrari, G. (2011). Nanoencapsulation of essential oils to enhance their antimicrobial activity in foods. *Food Science and Technology*, 1908-1914. http://dx.doi.org/10.1016/j.lwt.2011.03.003.

Ekambaram, P., Abdul Hasan Sathali, A., & Priyanka, K. (2011). Solid lipid nanoparticles: a review. *Scientific Reviews & Chemical Communications Journal*, *2*(1):80-102. http://dx.doi.org/10.20959/wjpr20165-6066.

Es'haghi Gorjim M., Noori, N., Nabizadeh Nodehi, R., Jahed Khaniki, G., Noushin Rastkari & Alimohammadi, M. (2014). The evaluation of Zataria multiflora boiss. essential oil effect on biogenic

amines formation and microbiological profile in Gouda cheese. *Letters in Applied Microbiology*, 59, 621-630. http://dx.doi.org/10.1111/lam.12319.

Fathi, M., Mozafari, M. R., & Mohebbi, M. (2012). Nanoencapsulation of food ingredients using lipid based delivery systems. *Trends Food Science & Technology. 23*, 13-27. http://dx.doi.org/10.1016/j.tifs.2011.08.003.

Golmohammadzadeh, Sh., Mokhtari, M. & Jaafari, M.R. (2012). Preparation, characterization and evaluation of moisturizing and UV protecting effects of topical solid lipid nanoparticles. Brazil Journal of Pharmaceutics Science, 48(4), 683-690. http://dx.doi.org/10.1590/S1984-82502012000400012.

Jores, K., Mehnerta, W., Drechslerb, M., Bunjesc, H., Johannd, C. & Mäder, K. (2004). Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. *Journal of Controlled Release*, *9*, 217-227. http://dx.doi.org/10.1016/j.jconrel.2003.11.012.

Lai, F., Wissing, S. A., Müller, R. H., & Fadda, A. A. (2006). *Artemisia arborescens* L. essential oil–loaded solid lipid nanoparticles for potential agricultural application: preparation and characterization. *American Association of Pharmaceutical Scientists*, *7*(1), 10-18. http://dx.doi.org/10.1208/pt070.

Mader, K., & Mehnert, W. (2005). Solid lipid nanoparticles-concepts, procedures and physicochemical aspects. In: Lipospheres in drug targets and delivery. Ed. Nastruzzi, C. Florida: CRC press, 1-22.

Moghimipour, E., Ramezani, Z., & Handali, S. (2013). Solid lipid nanoparticles as a delivery system for *Zataria multiflora* essential oil: formulation and characterization. *Current Drug Delivery*, *10*, 151-157. http://dx.doi.org/10.2174/1567201811310020001.

Nasseri, M., Arouiee, H.,Golmohammadzadeh, S.,Jaafari, M.R., & Neamati, H. (2015.) Antifungal effects of *Zataria multiflora* essential oil on the inhibitory growth of some postharvest pathogenic fungi. ***Notulae Scientia Biologicae****, 7*(4), 412-416. http://dx.doi.org/10.15835/nsb749650.

Pérez-de-Luque, A., & Rubiales, D. (2009). Nanotechnology for parasitic plant control. *Pest Management Science, 65*, 540-545. http://dx.doi.org/10.1002/ps.1732.

Pizzol, C.D., Filippin-Monteiro, F.B., Restrepo, J.A.S., Pittella, F., Silva, A.H., Souza, P.A., Campos, A.M., & Creczynski-Pasa, T.B. (2014). Influence of surfactant and lipid type on the physicochemical properties and biocompatibility of solid lipid nanoparticles. *International Journal of Environmental Research and Public Health*, *11*, 8581-8596. http://dx.doi.org/10.3390/ijerph110808581.

Plotto, A., Roberts, R.G., & Roberts, D.D. (2003). Evaluation of plant essential oils as natural postharvest disease control of Tomato (*Lycopersicon esculentum*). *Acta Horticulture,* 628: 737 - 745.*doi* doi.org/10.17660/ActaHortic.2003.628.93.

Schäfer-Korting, M., & Mehnert, W. (2005). Delivery of Lipophilic Compounds with LipidNanoparticles-Applications in Dermatics and for Transdermal Therapy. In: Lipospheres in drug targets and delivery: approaches, methods, and applications. Ed. Nastruzzi, C., 1st ed. pp. 128-139. London: CRC Press.

Shi, F., Zhao, J., Liu, Y., Wang, Z., Zhang, Y. & Feng, N. (2012). Preparation and characterization of solid lipid nanoparticles loaded with frankincense and myrrh oil. *International Journal of Nanomedicine*, *7*, 2033–2043. http://dx.doi.org/10.2147/IJN.S30085.

Wang, L., Liu, L., Kerry, J. F., & Kerry, J. P. (2007). Assessment of film-forming potential andproperties of protein and polysaccharide-based biopolymer films. *International Journal of Food Science and Technology*, *42*, 1128-1138. http://dx.doi.org/10.1111/j.1365-2621.2006.01440.

Wissing, S.A., & Muller, R.H. (2002). The influence of the crystallinity of lipid57- nanoparticles on their occlusive properties. *International Journal Pharmaceutical*, *242*, 377-379. http://dx.doi.org/10.1016/S0378-5173(02)00220-X.

Wissing, S.A., Kayser, O., & Muller, R.H. (2004). Solid lipid nanoparticles for parenteral drug delivery. *Advanced Drug Delivery* Reviews, *56*(9), 1257-1272. http://dx.doi.org/10.1016/j.addr.2003.12.002.