

Characterization of *Nigella Sativa* L. Essential Oil-Loaded Solid Lipid Nanoparticles

^{1,4}Nagi A. ALHaj, ²Mariana. N. Shamsudin, ²Norfarrah. M. Alipiah, ²Hana F. Zamri,
³Ahmad Bustamam, ³Siddig Ibrahim and ⁴Rasedee Abdullah

¹Department of Medical Microbiology, Faculty of Medicine and Health Sciences,
Sana'a University, P.O. Box 13078, Sana'a, Yemen

²Department Marine Science and Aquaculture, Institute of Bioscience,
University Putra Malaysia, 43400, Serdang, Selangor, Malaysia

³Laboratory of Cancer Research MAKNA-UPM, Institute of Biosciences,
University Putra Malaysia, Serdang, Selangor DE, Malaysia

⁴Department of Microbiology and Pathology, Faculty of Veterinary Medicine,
University Putra Malaysia, Serdang 43400, Selangor, Malaysia

Abstract: Problem statement: Seeds of *Nigella sativa* L., commonly known as black seed, have been used in traditional medicine by many Asian, Middle Eastern and Far Eastern Countries to treat headache, coughs, abdominal pain, diarrhea, asthma, rheumatism and other diseases. The seeds of this plant are the most extensively studied, both phytochemically and pharmacologically. The aqueous and oil extracts of the seeds have been shown to possess especially nowadays in pharmaceutical antioxidant, anti-inflammatory, anticancer, analgesic, antimicrobial activities and medicinal and cosmetic applications, sanitary, cosmetic, agricultural and food industries. **Approach:** The aim of this study was to formulate a new delivery system for dermal and cosmetic application by the incorporation of *Nigella sativa* essential oil into solid lipid nanoparticles SLN. SLN formulations were prepared following the high-pressure homogenization after stirring and ultra-trax homognization techniques using hydrogenated palm oil Softisan 154 and *N. sativa* essential oil as lipid matrix, sorbitol and water as surfactants. The SLN formulation particle size was determined using Photon Correlation System (PCS). **Results:** The change of particle charge was studied by Zeta Potential (ZP) measurements, while the melting and re-crystallization behavior was studied using Differential Scanning Calorimetry (DSC). Data showed a high physical stability for both formulations at various storage temperatures during 3 months of storage. In particular, average diameter of *N. sativa* essential oil-loaded SLN did not vary during storage and increased slightly after freeze-drying the SLN dispersions. **Conclusion:** Therefore, obtained results showed that the studied SLN formulations are suitable carriers in pharmaceutical and cosmetic fields.

Key words: Solid lipid nanoparticles, *Nigella sativa*, palm oil, high pressure homogenization, supercritical fluid extraction

INTRODUCTION

Recent advances in nanoparticulate systems for improved drug delivery display a great potential for the administration of exigent active molecules. Solid Lipid Nanoparticles (SLNs) have emerged as an alternative to other novel delivery approaches due to various advantages such as feasibility of incorporation of lipophilic and hydrophilic drugs, improved physical stability, low cost compared to liposomes and ease of scale-up and manufacturing. Moreover, the potential of

SLNs in epidermal targeting, follicular delivery, controlled drug delivery, increased skin hydration due to greater occlusivity and photostability improvement of active pharmaceutical ingredients has been very well established (Shah *et al.*, 2007; Muller *et al.*, 2000; Mehnert and Mader, 2001). Solid lipid nanoparticles are colloidal carrier systems composed of a high melting point lipid/s as a solid core coated by surfactants. The term lipid in a broader sense includes triglycerides, partial glycerides, fatty acids, hard fats and waxes. A clear advantage of SLNs is the fact that

Corresponding Author: Nagi A. ALHaj, Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Sana'a University, P.O. Box 13078, Sana'a, Yemen Tel: +967-1-218889 H/S +967-714-222-561

the lipid matrix is made from physiological lipids which decreases the danger of acute and chronic toxicity. Highly purified natural solid lipids such as stearine fractions of fruit kernel are low cost alternative to the commercial lipids used for SLN production. This specialty material is derived from indigenous source, available in abundance and supplemented with essential bio-actives. They are completely biodegradable. High saturated fatty acid-oleic acid-saturated fatty acid (SOS) content and exceptional high oxidation resistance of these fractions (Gunstone, 2004) would be beneficial for drug encapsulation efficiency and the drug stability upon the encapsulation, respectively. The main aim of this investigation was to develop SLNs from indigenous solid lipids by using a simple method such as micro-emulsion technique. Furthermore, the aim was to characterize these SLNs and evaluate its potential in the topical delivery system using a lipophilic drug model. *Nigella sativa* is an annual herb of the Ranunculaceae family, which grows in countries bordering the Mediterranean Sea, Pakistan and India. This widely distributed plant is native to Arab countries and other parts of the Mediterranean region. For thousands of years, this plant has been used in many Asian, Middle Eastern and Far Eastern Countries as a spice and food preservative as well as a protective and health remedy in traditional folk medicine for the treatment of numerous disorders. The seed of this plant is commonly known as black seed and is referred to by the prophet Mohammed as having healing powers. The seeds are commonly eaten alone or in combination with honey and in many food preparations. The oil prepared by compressing the seeds of *N. sativa* is used for cooking. Black seed is also identified as the curative black cumin in the Holy Bible and is described as the Melanthon of Hippocrates and Discroides and as the Gith of Pliny. *N. sativa* plant is one of the most extensively studied, both phytochemically and pharmacologically. The extracts of *N. sativa* seeds have been used by patients to suppress coughs disintegrate renal calculi, retard the carcinogenic process (Worthen *et al.*, 1998; Hosseinzadeh *et al.*, 2007) treat abdominal pain, diarrhea, flatulence and polio (Enomoto *et al.*, 2001), exert choleric and uricosuric activities, anti-inflammatory (Chakravarty, 1993; Houghton *et al.*, 1995) and antioxidant effects (Mansour *et al.*, 2002; Mansour, 2000). Besides, the essential oil was shown to have antihelminthic (Agarwal *et al.*, 1979), antinematodal (Akhtar and Riffat, 1991), antischistosomal (Mahmoud *et al.*, 2002), antimicrobial (Aboul-Ela *et al.*, 1996; Hanafy and Hatem, 1991; Aboul-Ela, 2002) and antiviral (Salem and Hossain, 2000) effects. The pharmacological properties appear to

be involved in the beneficial effects of *N. sativa* oil on headache, flatulence, blood homeostasis abnormalities, rheumatism and related inflammatory diseases (Boulos, 1983). Moreover, the seeds are believed to have carminative, stimulatory and diaphoretic properties and are used in the treatment of bronchial asthma and eczema (Boulos, 1983). In view of this, exploring the potential of SLNs in improving the topical delivery of *N. sativa* oil seems worthwhile wherein; indigenous natural solid lipids were explored to offer their inherent merits to improve treatment efficacy and patient compliance. An alternative, lab-scale technique of micro-emulsion template was utilized for production of SLNs in the preparation of topical dosage form.

MATERIALS AND METHODS

***Nigella sativa* seeds:** The seeds were purchased from a local herbal shop in Yemen. Seeds were stored in dark at 4°C for 20 days. Immediately prior to the extraction process, the seeds were ground in a blender to produce a powder with an approximate size of 150 μ m.

Supercritical Fluid Extraction (SFE): A SFE system mode (Thar, Germany) at the Food Science and Biotechnology Laboratory UPM was used for the extraction. In this study, extraction was performed by filling extraction vessel with 150.0 g of the completely crushed seeds. The plant was then extracted with SFE under 400 atm pressure and 40°C temperature for 15 min static followed by 20 min dynamic. A duraflo manual variable restrictor (Suprex) was used in the SFE system to collect the extracted analytes as shown in the (Fig. 1 and 2). The SFE flow rate through the duraflo restrictor was approximately 25 g min⁻¹ (compressed). The extracted analyses were collected in a volumetric flask. The final volume of the extract was adjusted to 35 mL at the end of the extraction. In order to have better collection efficiency, the 35 mL volumetric flask was placed in an ice bath during the dynamic extraction stage.

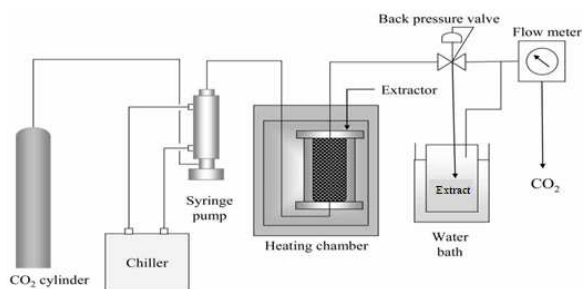


Fig. 1: Schematic diagram of one stage SFE apparatus (SFE 1)

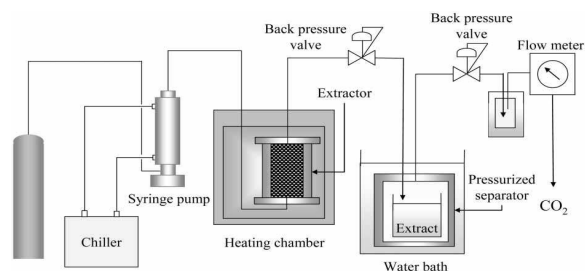


Fig. 2: Schematic diagram of SFE apparatus using high pressure separator (SFE 2)

Lipid materials and affilation of study: Our study was conducted in the University Putra Malaysia (UPM). Hydrogenated palm oil Softisan 154, Sorbitol, (Caesar and Loretz, Germany) and polysorbate 80 (tween 80) (Sigma Aldrich, Germany) were used as procured from their manufacturers without further purification. Bidistilled water was used for nanoparticle preparation.

Formulation of the lipid matrix: The lipid matrix used in SLN formulation corresponded to 30% w/w of Hydrogenated palm oil Softisan 154 and *N. sativa* essential oil as lipid matrix. The lipids were weighed with an electronic balance (Sartorius, Germany), melted together at 10°C higher than melting point on a hot plate (RCT, IKA, Germany) and stirred to fully yellow color.

Formulation of Solid Lipid Nanoparticles (SLN): SLN were formulated to contain 20% w/w of lipid matrix (30% w/w of Softisan 154 and *N. sativa* essential oil as lipid matrix), 1.0% w/w of polysorbate 80, 4.5% w/w of sorbitol and enough bidistilled water to make 100% w/w. The hot homogenisation technique was adopted. Lipid matrix was melted at 10°C higher than the melting point and the water containing polysorbate 80 and sorbitol at the same temperature was added to the molten lipid matrix with gentle stirring with a magnetic stirrer. The mixture was further dispersed with a mixer (Ultra-Turrax, Ika Staufen, Germany) at 13,000 rpm for 5 min to produce the hot primary emulsion. The hot primary emulsion at 70°C was immediately passed through a heated high pressure homogeniser (EmulsiFlex-C5, Avestin, Canada) at a pressure of 500 bars for 5 cycles to produce the nanoparticles, which were collected in a hot container and allowed to recrystallize at room temperature.

Particle size analysis: The mean hydrodynamic (peak) and size distribution (width) of the SLN were assessed at 25°C by Photon Correlation System (PCS) with Zetasizer 4 (Malvern, UK). Prior to measurement, the samples

were diluted with demineralized water to PIDS obscurity of not less than 40%. Size distribution by volume was calculated by applying an optical module created by the instrument software. Measurements were performed in triplicate. Particle size measurements were done 24 h, 1 week, 1 and 3 months after SLN preparation.

Zeta potential measurement: Zeta potential of a colloidal sample determines whether the particles within a liquid will tend to flocculate (stick together) or not. It is the potential within the hydrodynamic shear or slipping plane of the electric double layer of a charged particle. The zeta potentials of the formulated SLN were determined after 3 months of preparation in a Zetasizer 4 (Malvern Instruments, Malvern, UK). Each sample was diluted with bidistilled water (pH 7.0) and the electrophoretic mobility determined at 25°C and dispersant dielectric constant of 78.5. The obtained electrophoretic mobility values were used to calculate the zeta potentials using the software DTS Version 5.0 (Malvern, UK).

Differential Scanning Calorimetry (DSC): The degree of crystallinity of the lipid nanoparticles was determined on a calorimeter (DSC 28°C) connected to a disc station of a Mettler DSC 822e (Mettler Toledo, Greifensee, Switzerland). Approximately 5 mg of SLN was weighed into an aluminum pan and sealed hermetically and the thermal behavior determined in the range of 10-125°C at a heating rate of 5°C min⁻¹. Baselines were determined using an empty pan and all the thermo grams were baseline-corrected. Transition temperatures were determined from the endothermic peak minima while transition enthalpies were obtained by integration of the endothermic transitions using linear baselines.

RESULTS

An adequate characterization of the solid lipid particles is a necessity for the control of the quality of the product. PCS is the most powerful techniques for routine measurements of particle size. The zeta potential distribution of SLN was showed in Table 1. The mean zeta potential was -15.4 mV (n = 5). Therefore, this method had gained a relative good stability and dispersion quality. The zeta potential of SLN (3) varied in dependence of the emulsifier concentration from K14.8 mV, prior to any addition of nonionic emulsifier, to about 0 mV at an emulsifier lipid ratio of 1-2. Due to the decrease in particle size and zeta potential with increasing surfactant concentration it is supposed that the surfactant is present at least partly close to the particle interface.

Table 1: Particle size and zeta potential with Polydispersity Index (PI) of SLN

SLN	Particle size (nm)	PI	Zeta potential (mV)
SLN (1)	142.70	0.27	-14.4
SLN (2)	107.30	0.21	-18.7
SLN (3)	95.50	0.22	-14.8
SLN (4)	66.27	0.18	-16.9
SLN (5)	128.50	0.20	-12.2

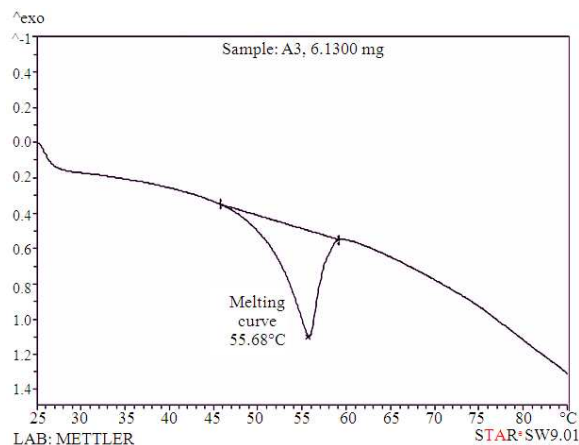


Fig. 3: Differential scanning calorimetry scans of SLN

The cooling curves obtained 1 day after production showed that the formulation re-crystallized in different polymorphic forms. SLN cooling curve shows a main peak at 55.68°C which can be attributed to the beta modification (Fig. 3).

DISCUSSION

Extraction of essential oil components using Super-Critical Fluids (SCF), has received much attention in the past several years, especially in food, pharmaceutical and cosmetic industries. The seeds, rich in essential oil, are consumed widely as condiment. In the indigenous system of medicines, seeds are regarded as stimulants and carminatives and found to be useful in diarrhoea and dyspepsia. The particle size distribution expressed by the polydispersity colloidal triacylglycerol emulsions for parenteral nutrition (Westesen and Wehler, 1993). The particle size decrease with increasing o/w surfactant concentration was accompanied by a decrease of the polydispersity index from about 0.4 down to below 0.2. Different emulsifier concentrations enabled not only a control of the particle size and in consequence specific surface area, but allowed the preparation of surface charge modified particles in addition. The measurement of the zeta potential allows predictions about the storage stability of colloidal dispersion. In general, particle

aggregation is less likely to occur for charged particles (high zeta potential) due to electric repulsion (Enomoto *et al.*, 2001). The DSC thermal behavior of the untreated S154 was chosen as reference for the examination of the crystallinity of colloidal SLN matrices. Untreated S154 showed a single endothermic peak upon heating with a minimum at 55.68°C. DSC thermograms of SLN displayed one endothermic minimum upon heating as well. The crystallinity of the different lipid matrices was only determined by the triglyceride content and lecithin was present in a non-crystalline state. In conclusion, analysis of the melting enthalpy of SLN with regard to the triglyceride content within the lipid matrices revealed no enthalpy decrease in comparison to the untreated bulk. In contrast to the untreated bulk, the melting points of colloidal systems were distinctly decreased by about 3-8°C. According to (Hou *et al.*, 2003), the melting point decrease of colloidal systems can be assigned to the colloidal dimensions of the particles in particular to their large surface to volume ratio and not to re-crystallization of the lipid matrices in a metastable polymorph possessing a lower melting point. Nevertheless, the melting point reduction of the different formulations has no apparent relation to the particle size. SLN cooling curve shows a main peak at 55.68°C which can be attributed to the beta modification. But if the SLN cooling curve of the peak higher than 62°C suggested the presence of alpha modifications (Zur Muhlen *et al.*, 1998). Solid lipid nanoparticles do not, as proposed, “combine the advantages of other colloidal drug carriers and avoid the disadvantages of them”. They cannot simply be regarded as nanoemulsions with a solid core. Clear advantages of SLN include the composition (physiological compounds), the rapid and effective production process including the possibility of large scale production, the avoidance of organic solvents and the possibility to produce high concentrated lipid suspensions. Disadvantages include low drug-loading capacities, the presence of alternative colloidal structures (micelles, liposomes, mixed micelles, drug nanocrystals), the complexity of the physical state of the lipid (transformation between different modifications, possibility of supercooled melts) which cause stability problems during storage or administration (gelation, particle size increase, drug expulsion). Sample dilution or water removal might significantly change the equilibria between the different colloidal species and the physical state of the lipid. The appropriate characterization of the complex surfactant/lipid dispersions requires several analytical methods in addition to the determination of the particle size. The lipid matrix of solid lipid nanodispersions is

more mobile compared to polylactide-co-glycolide based nanoparticles and therefore, controlled release due to restricted diffusion of the drug within the lipid matrix is questionable because of the drug mobility and the short way length. In natural product extraction and isolation, Supercritical Fluid Extraction (SFE), especially that employing SFE, has become the method of choice. Sophisticated modern technologies allow precise regulation of changes in temperature and pressure and thus manipulation of solvating property of the SCF, which helps the extraction of natural products of a wide range of polarities. According to these advantages of supercritical fluid extraction we recommended to use as clean tool for essential oil extraction.

CONCLUSION

The results of this study show that it is possible to formulate solid lipid nanoparticles with good properties with mixed lipid consisting of 3:1 mixture with black seed oil and Phospholipon 80 G. The mixed lipid used in this study yielded SLN of lower crystallinity. It is thus envisaged that use of mixed lipids, which do not form highly crystalline matrix, would overcome the problem of partial or total drug expulsion encountered with use of high purity lipids in the formulation of solid lipid nanoparticles and nanostructured lipid carriers. In terms of nanostructured lipid carriers, use of mixed lipids that result in a matrix of low crystallinity may be better than a solid lipid and liquid lipid mixed together from the viewpoint of handling, possible sustenance of the incorporated drug and

REFERENCES

- Aboul-Ela, M.A., N.S. El-Shaer and N.B. Ghanem, 1996. Antimicrobial evaluation and chromatographic analysis of some essential and fixed oils. *Pharmazie*, 51: 993-994. PMID: 8985990
- Aboul-Ela, E.I., 2002. Cytogenetic studies on *Nigella sativa* seeds extract and thymoquinone on mouse cells infected with schistosomiasis using karyotyping. *Mutat. Res. Genet. Technol. Environ.*, 516: 11-17. DOI: 10.1016/S1383-5718(01)00333
- Agarwal, R., M.D. Kharya and R. Shrivastava, 1979. Antimicrobial and anthelmintic activities of the essential oil of *Nigella sativa* Linn. *Indian J. Exp. Biol.*, 17: 1264-1265. PMID: 549848
- Akhtar, M.S. and S. Riffat, 1991. Field trial of *Saussurea lappa* roots against nematodes and *Nigella sativa* seeds against cestodes in children. *J. Pak. Med. Assoc.*, 41: 185-187. PMID: 1942479
- Boulos, L., 1983. *Medicinal Plants of North Africa*. 1st Edn., Reference Pubns, Algonac, MI., ISBN: 10: 0917256166, pp: 286.
- Chakravarty, N., 1993. Inhibition of histamine release from mast cells by nigellone. *Ann. Allerg.*, 70: 237-242. PMID: 7680846
- Enomoto, S., R. Asano, Y. Iwahori, T. Narui, Y. Okada, A.N. Singab and T. Okuyama, 2001. Hematological studies on black cumin oil from the seeds of *Nigella sativa* L. *Biol. Pharm. Bull.*, 24: 307-310. DOI: 10.1248/bpb.24.307
- Gunstone, F.D., 2004. *The Chemistry of Oils and Fats: Oils and Fats: Sources and Constituents*. Blackwell Publishing, UK., ISBN: 13: 9780849323737, pp: 288.
- Hanafy, M.S. and M.E. Hatem, 1991. Studies on the antimicrobial activity of *Nigella sativa* seed (black cumin). *J. Ethnopharmacol.*, 34: 275-278. PMID: 1795532
- Hosseinzadeh, H., S. Parvardeh, M.N. Asl, H.R. Sadeghnia and T. Ziaee, 2007. Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. *Phytomedicine*, 14: 621-627. DOI: 10.1016/j.phymed.2006.12.005
- Houghton, P.J., R. Zarka, B. De Las Heras and R.S. Hoult, 1995. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. *Planta Med.*, 61: 33-36. PMID: 7700988
- Hou, D.Z., C.S. Xie, K.J. Huang and C.H. Zhu, 2003. The production and characteristics of Solid Lipid Nanoparticles (SLNs). *Biomaterials*, 24: 1781-1785. DOI: 10.1016/S0142-9612(02)00578-1
- Mansour, M.A., 2000. Protective effects of thymoquinone and desferrioxamine against hepatotoxicity of carbon tetrachloride in mice. *Life Sci.*, 66: 2583-2591. DOI: 10.1016/S0024-3205(00)00592-0
- Mansour, M.A., M.N. Nagi, A.S. El-Khatib and A.M. Al-Bekairi, 2002. Effects of thymoquinone on antioxidant enzyme activities, lipid peroxidation and DT-diaphorase in different tissues of mice: A possible mechanism of action *Cell. Biochem. Funct.*, 20: 143-151. PMID: 11979510
- Mahmoud, M.R., H.S. El-Abhar and S. Saleh, 2002. The effect of *Nigella sativa* oil against the liver damage induced by *Schistosoma mansoni* infection in mice. *J. Ethnopharmacol.*, 79: 1-11. <http://cat.inist.fr/?aModele=afficheN&cpsid=13673981>

- Mehnert, W. and K. Mader, 2001. Solid lipid nanoparticles production, characterization and applications. *Adv. Drug Deliv. Rev.*, 47: 165-196. PMID: 11311991
- Muller, H., K. Mader and S. Gohla, 2000. Solid lipid nanoparticles (SLN) for controlled drug delivery-a review of the state of the art. *Eur. J. Pharm. Biopharm.*, 50: 161-177. DOI: 10.1016/S0939-6411(00)00087-4
- Salem, M.L. and M.S. Hossain, 2000. Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. *Int. J. Immunopharmacol.*, 22: 729-740. DOI: 10.1016/S0192-0561(00)00036-9
- Shah, K.A., A.A. Date, M.D. Joshi and V.B. Patravale, 2007. Solid Lipid Nanoparticles (SLN) of tretinoin: Potential in topical delivery. *Int. J. Pharm.*, 345: 163-171. DOI: 10.1016/j.ijpharm.2007.05.061
- Worthen, D.R., O.A. Ghosheh and P.A. Crooks, 1998. The *in vitro* anti-tumor activity of some crude and purified components of black seed, *Nigella sativa* L. *Anticancer Res.*, 18: 1527-1532. <http://cat.inist.fr/?aModele=afficheN&cpsidt=2325194>
- Westesen, K. and T. Wehler, 1993. Characterization of a submicron-sized oil-in-water emulsion. *Colloids Surf. A Physicochem. Eng. Aspects*, 78: 115-123. <http://cat.inist.fr/?aModele=afficheN&cpsidt=4034331>
- Zur Muhlen, A., C. Schwarz and W. Mehnert, 1998. Solid Lipid Nanoparticles (SLN) for controlled drug delivery-Drug release and release mechanism. *Eur. J. Pharm. Biopharm.*, 45: 149-155. PMID: 9704911