Innovare
Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 6, Issue 10, 2014

Original Article

QUANTIFICATION OF *NIGELLA SATIVA* OIL (NSO) FROM BIODEGRADABLE PLGA NANOPARTICLES USING FTIR SPECTROSCOPY

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Received: 01 Jul 2014 Revised and Accepted: 10 Sep 2014

ABSTRACT

Objective: Quantification of medicinal plant, *N. sativa* oil, in biodegradable nanoparticles fabricated from PLGA and chitosan was impossible due to the difficulty in the oil extraction-partition method of which the nanoparticle did not dissolve in any solvent.

Methods: FTIR method was developed to quantify the loading efficiency of NSO from the intact nanoparticles without the need to solubilise the nanoparticles and extract the NSO thereafter. Beer-Lambert law was applied in the quantification following selection of a few wave number combinations.

Results: The method exhibited linearity in the range NSO/PLGA=5-150% with R^2=0.9911, RSD=1.68%, LOD=0.89% and LOQ=2.68%. NSO-PLGA nanoparticles revealed complete encapsulation of NSO (loading efficiency=101.5±2.2%) while chitosan-NSO-PLGA nanoparticle showed lower loading efficiency (84.5±1.7%) due to presence of the hydrophilic polymer, i. e. chitosan. This method is fast and easy to apply and does not require sample processing. The method will help to accelerate and improve routine characterisation of NSO nanoparticles during development and optimisation stage.

Keywords: *Nigella sativa*, FTIR, Loading efficiency, PLGA, Chitosan, Nanoparticle.

INTRODUCTION

Nigella sativa, an annual herbaceous plant from Ranuculacea family, is an established ethno-medicine widely used for edible and medicinal purposes. The seeds and its oil/extracts have been reported to possess multiple medical effects including neuroregeneration. The medicinal benefits of *N. sativa* had been reviewed and documented to show antitumor, antidiabetic, antidepressant, anti-inflammatory, antioxidant, anticonvulsant, antinociceptive and anxiolytic properties [1, 2]. Its neuroregenerative property was demonstrated in rats pre-exposed to toluene whereby the *N. sativa* volatile oil appeared to regenerate the degenerative neurons of the frontal cortex and the brain. [3]. Further, Azzubaidi, Saxena [4].

Reported that the fixed oil of *N. sativa* conspicuously preserved the spatial cognition of rats when challenged with chronic cerebral hypoperfusion. In addition, it was found that the *N. sativa* oil (NSO), via its free radical scavenging mechanism, protected the rat's brain against oxidative stress caused by a carbamate insecticide, propoxur [5]. Neurodegenerative diseases are characterized by the loss of neuronal activities in the brain and other parts of central nervous system. It appears that the Alzheimer's and Parkinson's diseases are the most common of such diseases [6] affecting more than 24.3 million people globally [7]. It was predicted that, by 2040, neurodegenerative cases would increase up to 71% in the developed countries and 300% in the developing countries [7]. These facts demand searching for an effective and of low cost therapy. One of possible treatment approaches is to employ the well-documented, ethno-therapeutic *N. sativa* oil. It is envisaged that the encapsulation of the NSO into biodegradable polymer will enhance its therapeutic efficacy and widen its therapeutic spectrum to include treatment in the neurodegeneration.

Micro or nanoencapsulation is known to improve the efficacy of drugs (or therapeutic agents) by providing a sustained release character with potential of targeting to the desired site. Quantification of the encapsulated agent is crucial and is part of the routine analysis in the characterization of the micro/nanoparticles. Studies were investigating micro or nanoencapsulation of *N. sativa* oil (NSO) and especially its quantification post encapsulation are profoundly lacking. ALHaj, Shamsudin [8] fabricated *N. sativa* essential oil in the form of solid lipid nanoparticles and characterized the nanoparticles. However, quantification of the oil was not done making the actual loading and encapsulation efficiency of the oil in the nanoparticles remain unknown. In the present study, NSO was encapsulated in the biodegradable poly (D, L-Lactic-co-Glycolic Acid) (PLGA) [9], which is approved by Food and Drug Administration (FDA) for use in human, and the nontoxic biopolymer chitosan [10, 11]. By encapsulating this NSO, it is envisaged that the NSO nanoparticles can be delivered to the brain or central nervous system as neuroprotective and neuroregenerative therapy.

While *N. sativa* components have been commonly analysed by GC [12, 13] and HPLC [14], these methods require extraction of the oil from the nanoparticles followed by laborious time-consuming procedures. In fact, the fabricated nanoparticles were difficult to dissolve in the common and powerful solvents like dichloromethane, chloroform, acetone or acetonitrile; and especially for chitosan-PLGA nanoparticles, it appeared impossible to completely solubilize the nanoparticles in any solvent. This made the whole steps of oil extraction from the particles inefficient. An alternative quantification method based on Fourier-transform infrared spectroscopy (FTIR) was hereby employed instead of traditional extraction-partition method of quantification. FTIR is an easy and fast quantitative method with satisfactory results obtained by various studies. In pharmaceutical sciences, FTIR has been widely applied for various testing including identification of drug identity, purity and crystalline structures and investigation of possible interactions between active medicaments and excipients [15]. However, application of FTIR in quantitative studies of pharmaceuticals is less frequent than of food science. For instance, Attenuated Total Reflectance FTIR (ATR-FTIR) spectroscopy has been applied quantitatively to determine numerous materials including solid non-fat content in raw milk [16], α-tocopherol in refined bleached and deodorized palm olein [17], fatty acid contents

in a micro-encapsulated fish-oil supplement [18], and sugar and organic acid contents in apricot fruit slurries [19]. Therefore, in this study, we describe application of FTIR as a rapid quantification method for NSO encapsulated in PLGA and PLGA-chitosan nanoparticles using simple Beer-Lambert law computed by the software without the need for extraction or sample processing.

MATERIALS AND METHODS

Materials

PLGA5002A [lactic to glycolic acid ratio is 50:50, intrinsic viscosity is 0.2 dl/g and is acid terminated] was obtained from PURAC (Biochem, Netherland). Polyvinyl alcohol (PVA; MW ~115 kDa, 88% hydrolysed) was purchased from BDH Laboratory Supplies (Poole, England). Dichloromethane (DCM), ethyl acetate (EA) and low molecular weight chitosan (CS) of 50-190 kDa were obtained from Sigma-Aldrich (Germany). Cold-pressed *Nigella sativa* oil (NSO), was obtained from Hemani (Karachi, Pakistan).

Transmission FTIR measurements of the raw materials

In order to identify the peaks with minimal interference by other materials, FTIR spectra of each raw material used in the nanoparticle fabrication was acquired. Briefly, PVA and chitosan powders were individually mixed with KBr then the discs were prepared by compression. After that, FTIR spectra were recorded by using the Perkin-Elmer FTIR spectrometer (Perkin Elmer Corp., Norwalk, CT, USA) in the range of 4000–400 cm^{-1} and 4 cm^{-1} resolution. The ambient air was used as background. 10 µl of 5% w/v PLGA in DCM was loaded on a blank KBr disc and dried by the aid of hair-dryer. Similarly, 10 µl of 5% w/v NSO in DCM was loaded on another blank KBr disc and the DCM was evaporated leaving NSO in the KBr disc. PLGA-KBr and NSO-KBr discs were scanned at the same wavelength..

Preparation of NSO-PLGA standard mixtures for generation of calibration curve

5% w/v of PLGA in DCM and 5% w/v of NSO in DCM were prepared as stock solutions. DCM was chosen as it is highly volatile and hence can be easily evaporated at room temperature. The standard mixtures were then prepared by mixing PLGA and NSO stock solutions to have a series of NSO: PLGA ratio from 0.05 to 1.5. These mixtures were subsequently used to generate a calibration curve. To record FTIR spectra of the standard mixtures, blank discs were prepared from KBr alone and then 10 µl of each standard mixture was loaded on the disc and dried by the aid of hair-dryer to ensure complete evaporation of the DCM. Following solvent evaporation, a thin film of NSO-PLGA remained on the KBr disc which was ready to be scanned as described above.

Fabrication of NSO-loaded nanoparticles

The nanoparticles were prepared by a modified multiple emulsion solvent-diffusion method. Two formulations were fabricated: NSO loaded PLGA (NSO-PLGA) nanoparticle and NSO loaded PLGA nanoparticle modified with chitosan (CS-NSO-PLGA nanoparticle). The contents of the two formulations were listed in Table 1. Briefly, the oil phase was prepared by dissolving the PLGA in DCM prior to addition of EA and NSO. Then, two different aqueous phases were prepared namely; 1% w/v PVA alone and 1% w/v PVA with additional 1% w/v chitosan acetate. The oil phase was mixed with the aqueous phase and homogenized at 20500 rpm for 3 min using IKA® T10 basic homogenizer (IKA Werke GmbH and Co., Germany). The resulting emulsion was added to a continuously stirred dispersion medium (Table 1) and stirring was continued for 2 h under vacuum to accelerate evaporation of the organic solvents. Hardened nanoparticles were collected by centrifugation and washed three times with deionized water, lyophilized for 24 h and kept at 4°C in an air-tight container supplied with silica gel.

Table 1: Contents of the two nanoparticle formulations

	NSO-PLGA nanoparticle	CS-NSO-PLGA nanoparticle
Oil phase	PLGA: 30 mg	PLGA: 30 mg
	NSO: 30 mg	NSO: 30 mg
	DCM: 0.5 ml	DCM: 0.5 ml
	EA: 2.5 ml	EA: 2.5 ml
Aqueous phase	2% w/v PVA: 4.5 ml	2% w/v PVA: 4.5 ml
	H_2O: 4.5 ml	2% w/v CS: 4.5 ml
Dispersion medium	H_2O: 36 ml	1% w/v CS: 36 ml

FTIR spectra processing and method development

Quantification of NSO in the particles was determined by FTIR using Beer-Lambert law and the principle of constant ratio method described by Reig, Adelantado [20]. The computation was done by Essential FTIR software v3.10.016 (Operant LLC).

The constant ratio method is based on the fact that the absorption bands in an infrared spectrum of a material have a constant relationship that is related only to the material itself. When the concentration or thickness of the material varies, the ratio between spectral peaks of the different concentration or thickness of the same material remains constant. Consequently, when two different materials are mixed together, the ratio between the resulting peaks is related to the ratio of the mixture regardless of the thickness of the sample. However, in order to obtain the best results experimentally, selection of peaks with minimal interference needs to be meticulously done. Based on this principle, the amount of standard mixtures or nanoparticles that were added to or mixed with KBr had no effect on the ratio between the selected peaks. Even though NSO is in liquid form, this procedure did not require the special liquid cuvette with the fixed path length. Instead, the common solid holder with KBr disc was utilised and found to be reliable.

To set a quantitative method based on Beer-Lambert law using the software, the analyte or the material to be analysed was defined first as "NSO/PLGA". Then the spectra of the standard mixtures was loaded and the peaks and baselines were selected for quantification. To validate the data, the predicted values were plotted against the actual values of the standard mixtures. As suggested by the software developer, to avoid minor shifts in the peaks due to instrument, peaks in a specific range were selected instead of peaks at a specific wavelength number.

In this study, the interference between PLGA and NSO peaks was minimised by selecting the absorption peaks for each material that appeared to be minimally interfered by the other materials. This was performed by comparing the spectra of all the raw materials as shown in Fig. 1. Furthermore, the interference between PLGA and NSO was minimised by selecting the ratio A_{NSO}/A_{PLGA} and assigned it to be the "quantitative response" for this method. Selected peaks are shown in Tables 2 and Fig. 2.

The predictive values were calculated by the software based on Beer-Lambert law. Beer-Lambert law gives the linear function between the absorbance and the concentration which can be further interpreted in this study based on the following equation:

$$\left(\frac{A_{NSO} - A_{NSO.baseline}}{A_{PLGA} - A_{PLGA.baseline}}\right) = Slope.\frac{NSO}{PLGA} + constant \quad \text{.....Eqn. 1}$$

Repeatability test represented as relative standard deviation (RSD) was performed by carrying out 6 repeated FTIR scans for the standard mixture NSO: PLGA 1:1. The predicted quantitative response, A_{NSO}/A_{PLGA}, was calculated for each scan to derive the RSD.

Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on the equations 2 & 3, respectively:

$$LOD = 3.3\frac{\sigma}{S}$$ ..... Eqn. 2

$$LOD = 10\frac{\sigma}{S}$$ ..... Eqn. 3

Where σ and S are the standard deviation of the response and the slope, respectively.

Following generation of standard curves, the R^2 and the RSD values were compared between the selected peaks (Table 2) in order to choose the best calibration curve to be applied for quantification of NSO from nanoparticles. The best curve is characterised by the R^2 closest to 1 and the lowest RSD.

Table 2: Selected peaks from FTIR spectra of the NSO-PLGA standard mixtures in order to construct the best standard curve LOD and LOQ are represented by %w/w NSO/PLGA.

NSO peak (cm^{-1})			PLGA peak (cm^{-1})			R^2	RSD	LOD	LOQ	Figure
Region	**peak**	**baseline**	**Region**	**peak**	**baseline**					
2846-2861	~2854	2455-2550	1065-1113	~1092	2455-2550	0.9830	3.68%	4.05	12.27	Fig. 4A
2846-2861	~2854	2455-2550	1718-1793	~1749	2455-2550	0.9311	6.51%	8.74	26.48	Fig. 4B
2846-2861	~2854	2455-2550	1250-1295	~1272	2455-2550	0.9816	5.23%	2.40	7.26	Fig. 4C
2888-2954	~2927	2455-2550	1065-1113	~1092	2455-2550	0.9895	1.90%	1.29	3.91	Fig. 4D
2888-2954	~2927	2455-2550	1065-1113	~1092	800-819	0.9911	1.68%	0.89	2.68	Fig. 4E

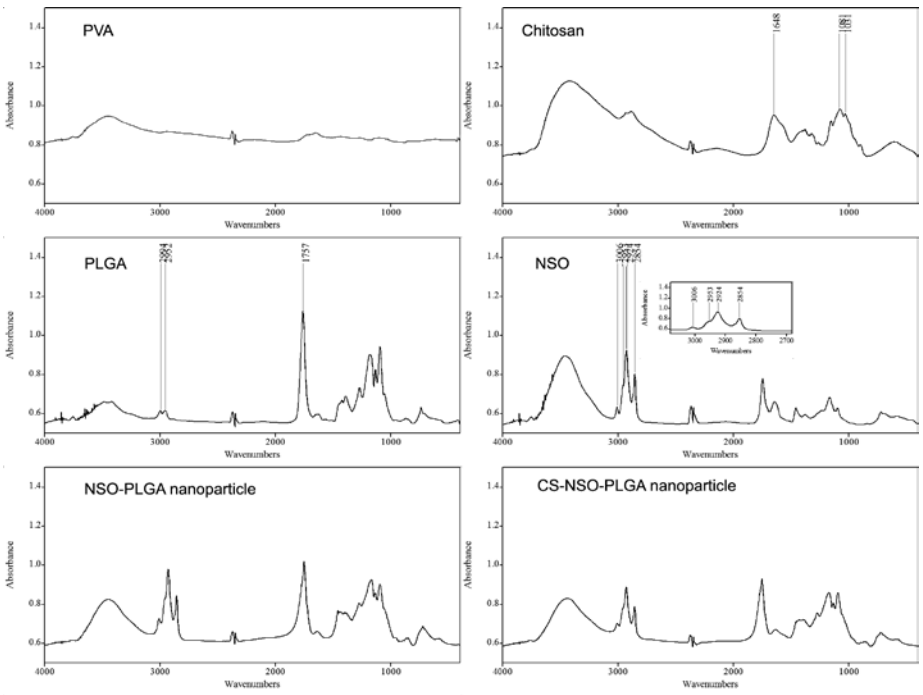

Fig. 1: FTIR spectra of the raw materials used in nanoparticle fabrication, i. e. PLGA, NSO, PVA and Chitosan.

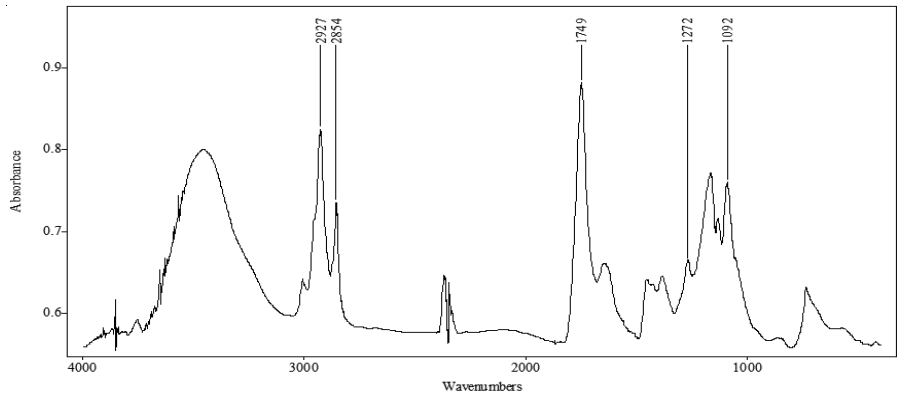

Fig. 2: Selected peaks from FTIR spectrum of NSO-PLGA mixture at 1:1 ratio.

Quantification of NSO from nanoparticles

Briefly, the lyophilized nanoparticles, NSO-PLGA and CS-NSO-PLGA, were directly mixed with KBr and their spectra were recorded at the same conditions as aforementioned. The spectrum of each nanoparticle formulation was loaded into the software being set with the selected method of analysis. The actual amount of NSO in each nanoparticle formulation was obtained by applying the Eqn. 1 using the best standard curve. Finally, the 'loading efficiency' of the NSO into the nanoparticles was expressed as the ratio of the actual amount of NSO to the theoretical loading. The latter was NSO/PLGA=1.

Detection of PLGA degradation

Degradation of PLGA is anticipated over entire fabrication process which may compromise its IR absorbance as nanoparticles. To detect its degradation, the dispersion medium (Table 1) at the end of fabrication process was subjected to UV scan in the range of 200-300 nm using Hitachi spectrophotometer model U-1900 (Hitachi, Tokyo, Japan) controlled by Hitachi UV Solutions Application software. Similarly, PLGA was dissolved in methanol and subjected to the UV scan at the same wavelength. In addition, equal volume of 1 M sodium hydroxide was added to the PLGA in methanol and left undisturbed at the room temperature for 30 min to induce PLGA degradation. After that, the degraded PLGA was subjected to UV scan as mentioned.

RESULTS AND DISCUSSION

FTIR spectra of the raw materials

FTIR spectra of the raw materials are shown in Fig. 1. PVA did not exhibit any important absorption peak in the range of interest (400-3000 cm^{-1}). Chitosan exhibited the characteristic band of NH_2 at 1648 cm^{-1} (scissioning vibration), carbonyl at 1564 cm^{-1} (asymmetric stretching vibration), and C–O of the pyranose ring at 1081 cm^{-1} and 1031 cm^{-1} [21, 22]. PLGA exhibited characteristic bands of the ester group at 1757 cm^{-1}, and the axial stretching of sp^2 and sp^3 carbons at 2952 cm^{-1} and 2994 cm^{-1} [23]. The prominent characteristic peaks for NSO were at 2854 cm^{-1} (C-H in -CH_2), 2924 cm^{-1} (C-H in -CH_2), 2955 cm^{-1} (C-H in -CH_3) and 3006 cm^{-1} (C-H in HC=CH) [18, 24] due to predominance of carbon chains in the fatty acids. In general, fatty acids compose more than 98% of NSO [13, 25, 26].

Standard curve construction

Quantification of NSO was based on Beer-Lambert law and constant ratio method [20]. Absorbance peaks were selected with minimal interference from the other components by comparing the spectra of the raw materials (Fig. 1). Chitosan was present in the final nanoparticle formulation but in low amount wherein its peaks were not really visible in the FTIR spectrum of CS-NSO-PLGA nanoparticle (Fig. 1). Consequently, peak selection was made mainly by comparing the FTIR spectra of PLGA and NSO and their mixture as shown in Fig. 1-3. The two distinct peaks at about 2924 cm^{-1} and 2854 cm^{-1} were selected for NSO. For PLGA, peak at 1757 cm^{-1}, 1272 cm^{-1} and 1094 cm^{-1} were chosen. Different mathematical combinations of the peaks' heights and the baseline regions were tested in order to obtain the best standard curve (Fig. 4 and Table 2).

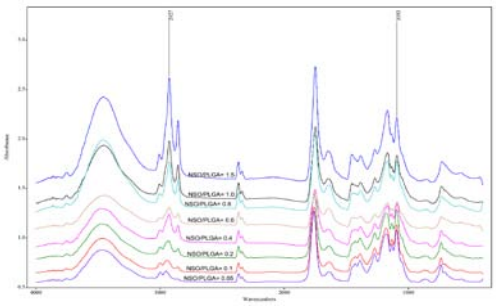

Fig. 3: FTIR spectra for the standard NSO-PLGA mixtures. It is obvious that the ratio A_{2927}/A_{1093} increases when the ratio NSO/PLGA increases.

Based on Table 2, the best standard curve was obtained at the absorbance 2924 cm^{-1} with the baseline correction at 2455-2550 cm^{-1} for NSO, and the absorbance 1094 cm^{-1} with baseline correction at 800-819 cm^{-1} for PLGA (Fig. 4E). At these range of wavelength, the R^2 and the RSD values are the closest to 1 (0.99) and the lowest (1.68%), respectively. Even though chitosan has its own IR spectrum but the prominent peaks appeared to be shared with PLGA and NSO at 2924 cm^{-1} and with PLGA alone at 1094 cm^{-1} (Fig. 1). Due to this profile, it was concluded that interference from chitosan was minimum.

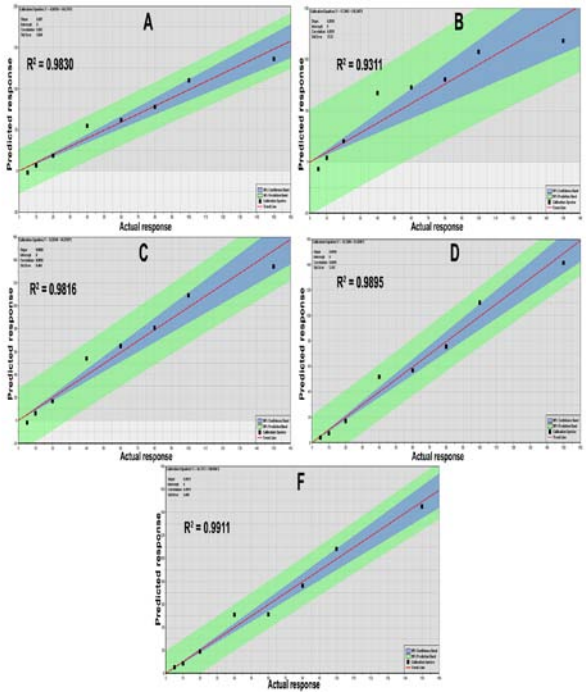

Fig. 4: Standard curves obtained from FTIR spectra based on Beer-Lambert law. Each curve represents a set of selected peaks explained in Table 2.

Quantification of NSO in NSO-PLGA and CS-NSO-PLGA nanoparticles

FTIR spectra for the fabricated nanoparticles (Fig. 1) were used to quantify the loading efficiency of NSO. Using the abovementioned best standard curve (Fig. 4F), NSO loading efficiency was estimated to be 101.5±2.2% and 84.5±1.7% for NSO-PLGA and CS-NSO-PLGA, respectively. The lower loading efficiency of CS-NSO-PLGA nanoparticle may be related to the presence of chitosan which makes the nanoparticle relatively more hydrophilic making it unfavourable to encapsulate lipophilic materials. In contrast, the loading efficiency of 100% seems to be acceptable based on virtual inspection of any residual NSO in the dispersion media at the end of fabrication process. The 1.5% more may be contributed by the instrument's noise and could be also due to degradation of PLGA during fabrication process. The latter may compromise absorbance peaks attributed to the PLGA.

PLGA degradation

It was anticipated that during the fabrication process, PLGA was exposed to aqueous environment that may hydrolyse it to its water soluble monomers, namely, lactic acid and glycolic acid which can escape to the dispersion medium. The hydrolysis was accelerated for low molecular weight PLGA, as employed in this study (intrinsic viscosity = 0.2 dl/g), coupled with the presence of free terminal carboxyl groups; both factors predispose the PLGA to be rapidly degraded. UV scan at 200-300 nm range was conducted which inevitably detected present of monomers in the dispersion medium and in the induced degradation sample (Fig. 5). UV absorbance at

214 nm have been used to detect degradation of PLGA to lactic acid and glycolic acid [27, 28]. T.h.is finding indicates that the FTIR spectra of the nanoparticles were actually 'depleted' of peaks attributed to PLGA itself. Hence, this explains the loading efficiency of NSO that was higher than 100%.

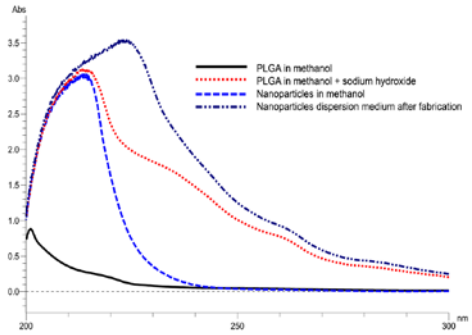

Fig. 5: UV spectra for dispersion medium of NSO-PLGA nanoparticle formulation post fabrication showing that portion of PLGA degraded during the fabrication.

CONCLUSION

Quantification of NSO in nanoparticles by means of FTIR is rapid and easy to apply. This method does not require sample processing especially when the nanoparticle are modified with chitosan and become difficult to dissolve in common solvents. The method will help to accelerate and improve the characterisation of NSO nanoparticles during development and optimization stage.

CONFLICT OF INTERESTS

Declared None

ACKNOWLEDGMENTS

This work was funded by Ministry of Science, Technology & Innovation of Malaysia (MOSTI) (Grant ID: 02-01-08-SF0101).

REFERENCES

1. Paarakh PM, *Nigella sativa Linn. A comprehensive review.* Indian J Nat Prod Resour 2010;1(4):409-29.
2. Ramadan MF. *Nutritional value, functional properties and nutraceutical applications of black cumin (Nigella sativa L.): an overview.* Int J Food Sci Technol 2007;42(10):1208-18.
3. Kanter M. *Protective effects of nigella sativa on the neuronal injury in frontal cortex and brain stem after chronic toluene exposure.* Neurochemical Res 2008;33(11): 2241-9.
4. Azzubaidi MS, *et al*. Protective effect of treatment with black cumin oil on spatial cognitive functions of rats that suffered global cerebrovascular hypoperfusion. Acta Neurobiol Exp (Wars) 2012;72(2):154-65.
5. Mohamadin AM, *et al*. *Protective* effects of Nigella sativa oil on propoxur-induced toxicity and oxidative stress in rat brain regions. Pestic Biochem Physiol 2010;98(1):128-34.
6. Sahni JK, *et al*. Neurotherapeutic applications of nanoparticles in Alzheimer's disease. J Controlled Rel 2011;152(2):208-31.
7. Ferri CP, *et al*. Global prevalence of dementia: a Delphi consensus study. Lancet 2006;366(9503):2112-7.
8. ALHaj NA, *et al*. Characterization of Nigella sativa L. essential oil-loaded solid lipid nanoparticles. Am J Pharmacol Toxicol 2010;5(1):52.
9. Bala I, S Hariharan, M Kumar. *PLGA* nanoparticles in drug delivery: the state of the art. Crit Rev Ther Drug Carrier Syst 2004;21(5):387-422.
10. Rao SB, CP Sharma. Use of chitosan as a biomaterial: studies on its safety and hemostatic potential. J Biol Materials Res 1997;34(1):21-8.
11. Baldrick P. The safety of chitosan as a pharmaceutical excipient. Regul Toxicol Pharmacol 2010;56(3):290-9.
12. Nickavar B, *et al*. Chemical composition of the fixed and volatile oils of Nigella sativa L. from Iran Zeitschrift Fur Naturforschung C 2003;58(9/10):629-31.
13. Cheikh-Rouhou S, *et al*. Nigella sativa L Chemical composition and physicochemical characteristics of lipid fraction. Food Chem 2007;101(2):673-81.
14. Ghosheh OA, AA Houdi, PA Crooks. High performance liquid chromatographic analysis of the pharmacologically active quinones and related compounds in the oil of the black seed *(Nigella sativa L.).* J Pharm Biol Anal 1999;19(5):757-62.
15. Kalinkova GN. Infrared spectroscopy in pharmacy. Vibrational Spectroscopy 1999;19(2):307-20.
16. Bassbasi M, *et al*. FTIR-ATR determination of solid non fat (SNF) in raw milk using PLS and SVM chemometric methods. Food Chem 2014;146(0):250-4.
17. Che Man YB, W Ammawath, MES Mirghani. Determining α-tocopherol in refined bleached and deodorized palm olein by Fourier transform infrared spectroscopy. Food Chem 2005;90(1–2):323-7.
18. Vongsvivut J, *et al*. Quantitative determination of fatty acid compositions in micro-encapsulated fish-oil supplements using Fourier transform infrared (FTIR) spectroscopy. Food Chem 2012;135(2):603-9.
19. Bureau S, *et al*. Application of ATR-FTIR for a rapid and simultaneous determination of sugars and organic acids in apricot fruit. Food Chem 2009;115(3):1133-40.
20. Reig FB, JVG Adelantado, MCM Moya Moreno. FTIR quantitative analysis of calcium carbonate (calcite) and silica (quartz) mixtures using the constant ratio method. Application to geological samples. Talanta 2002;58(4):811-21.
21. Liu W, *et al*. An investigation on the physicochemical properties of chitosan/DNA polyelectrolyte complexes. Biomaterials 2005;26(15):2705-11.
22. Banerjee T, *et al*. Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. Int J Pharm 2002;243(1–2):93-105.
23. Da Silva-Junior AA. *et al*. Thermal behavior and stability of biodegradable spray-dried microparticles containing triamcinolone. Int J Pharm 2009;368(1–2):45-55.
24. Guillén MD, N Cabo. Characterization of edible oils and lard by fourier transform infrared spectroscopy. Relationships between composition and frequency of concrete bands in the fingerprint region. J Am Oil Chem Soc 1997;74(10):1281-6.
25. Kökdil G, H Yılmaz. Analysis of the fixed oils of the genus Nigella L. (Ranunculaceae) in Turkey. Biochem Systematics Ecology 2005;33(12):1203-9.
26. Mohamed BA. Some characteristics of nigella (Nigella sativa L.) seed cultivated in Egypt and its lipid profile. Food Chem 2003;83(1):63-8.
27. Man C, *et al*. Poly (lactic acid)/titanium dioxide composites: Preparation and performance under ultraviolet irradiation. Polymer Degradation Stability 2012;97(6):856-62.
28. De Villiers MM, DE Wurster, K Narsai. Stability of lactic acid and glycolic acid in aqueous systems subjected to acid hydrolysis and thermal decomposition. J Soc Cosmetic Chemists 1997;48(4):165-74.