ADMET & DMPK 5(4) (2017) 224-233; doi: ***http://dx.doi.org/10.5599/admet.5.4.426***

ADMET

Open Access : ISSN : 1848-7718

http://www.pub.iapchem.org/ojs/index.php/admet/index

Original scientific paper

Spectroscopic examination and release of microencapsulated oregano essential oil

Ioannis Partheniadis[1], Panagiota Karakasidou[1], Souzan Vergkizi[2], Ioannis Nikolakakis[1]*

[1] *Department of Pharmaceutical Technology, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece*

[2] *Department of Microbiology, School of Medicine, Faculty of Health Sciences, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece*

*Corresponding Author: E-mail: yannikos@pharm.auth.gr; Tel.: +302310997635; Fax: +302310997652

Received: August 29, 2017; Revised: September 28, 2017; Published: December 24, 2017

Abstract

Oregano essential oil (EO) of Greek origin with high carvacrol content (86.84 %) was encapsulated by spray drying using Arabic gum, modified starch and maltodextrin (75:12.5:12.5) as wall materials. The spray-dried product (EOSD) consisted of roundish particles with narrow size distribution. FT-IR and Raman spectroscopy identified the EO in EOSD, with Raman spectra showing more distinct peaks and a small shift of the peak at 1260 cm^{-1} (assigned to the stretching vibration of the bond of C-O of the phenol), implying only minor chemical interaction with the wall materials. Release of the EO from EOSD was described by the Hixson-Crowell equation (R^2=0.986) with apparent diffusion coefficient 8.3x10^{-10} m^2/s. These findings indicate that microencapsulation by spray drying did not affect the quality of the oregano EO and provided relatively fast and complete release.

Keywords

oregano essential oil; microencapsulation; spray drying; FT-IR; Raman spectroscopy; *in vitro* release

Introduction

Essential oils are complex mixtures of natural, aromatic and volatile compounds synthesized by aromatic plants. Selected oils have been shown to act on microbial cell surface causing disruption of the cell wall and the cytoplasmic membrane leading to lysis and leakage of intracellular compounds [1]. Because of the increasing problem of bacterial resistance to several antibiotics and their accepted safety profile, essential oils may be interesting candidates against microbial infections [2].

Oregano essential oil (EO) has been receiving attention because of its antimicrobial activity against both Gram negative and Gram positive bacteria and to its colicidal and colistatic properties due to the presence of carvacrol and thymol in its composition [3-5]. Since oregano EO contains volatile and easily oxidized active ingredients protection from environmental factors is important for retaining its activity in the marketed product. This problem may be tackled by spray drying, which is an industrially established continuous process that in combination with emulsification offers a method for EO microencapsulation [6-9]. For this purpose, an EO in water emulsion is initially prepared and the EO droplets are stabilized by a

mixture of gums and carbohydrates [10-11]. The emulsion is then spray-dried under appropriate conditions of temperature and feed rate, and a powder product consisting of single or agglomerated particles is obtained where the EO is encapsulated and thus protected from humidity and light that normally lead to oxidation of alcohols, reduced antimicrobial activity and even inactivation [6, 12-14]. Moreover, microencapsulation is expected to increase the solubility of the EO by facilitating wetting of the wall materials and re-emulsification of sprayed dried product (EOSD), a process that possibly occurs when in contact with gastric fluids [15].

The aim of this work was to prepare a spray-dried encapsulated product of oregano EO of Greek origin that had high carvacrol content, using a composition of Arabic gum/starch/maltodextrin [7] as wall materials. In particular, the purpose was to identify the presence of EO in the product and possible interactions of the EO with the wall materials using Fourier transform infrared (FTIR) and Raman spectroscopy and to elucidate the mechanism of *in vitro* release of the EO in aqueous medium by dialysis dissolution method. The essential oil of oregano was used instead of a methanol extract since it has been reported that the former has stronger and broader spectrum of antimicrobial activity [16]. Arabic gum was used as the major of the three encapsulating materials because it forms a low viscosity colloidal dispersion suitable for spray drying, stabilizes EO emulsions in water across a wide pH range as encountered in the gastrointestinal tract, has good retention of volatile compounds and is compatible with other encapsulants [12, 17]. Modified starch was used since besides protection from oxidation and loss of volatiles, it also has emulsification ability. Maltodextrin with high dextrose equivalent (DE 20-23) was added to protect from oxidation and also to facilitate spray drying and improve product wettability [7, 12, 18].

Experimental

Materials

Oregano essential oil (EO), obtained from *Oreganum vulgare* (*Heracleoticum)* was a gift from Ecopharm Hellas, Kilkis, Greece Batch 0614 and had in its composition: 86.84 % carvacrol, 2.82 % thymol, 0.96 % γ-Terpinene, 3.46 % p-Cymene, 1.82 % β-Caryophyllene and 4.11 % other terpenes and phenols (Manufacturer's data). The encapsulating materials: Arabic gum (AG, Spraygum AB) was purchased from Nexira, France. Maltodextrin (MD, Glucidex 21) and modified starch (MS, Clearam CH20 20, food grade acetylate di-starch adipate (E1422), waxy maize basis) were from Roquette Italia, both gifts from Interallis Chemicals, Sindos, Greece.

Preparation of encapsulated oregano EO

A 30 % w/w concentration of dispersed phase of sprayed emulsion used for encapsulation was selected after preliminary trials since its viscosity allowed feeding and passing through the nozzle of the spray drier with minimal losses to the walls of the drying chamber at the applied conditions. The emulsion consisted of 3 % w/w oregano EO as the internal dispersed phase, 27 % w/w encapsulating materials (AG 75 %, MS 12.5 %, MD 12.5 %) and 70 % deionised water as the external phase [7]. For the preparation of the emulsion, AG and MD were hydrated overnight in deionised water at 4-5 °C and then heated to 82 °C. MS was added to this and the mixture homogenized at 15000 rpm for 20 min using an Ultra Turrax (IKA, Germany). The mixture was cooled to about 5 °C and oregano EO was added, followed by further homogenization for 5 min. The prepared emulsion was spray-dried using a mini spray dryer (B-191, Büchi, Switzerland) operated under the following conditions: feed rate 5 mL min^{-1}, inlet air temperature 180 °C, outlet temperature 117 °C, aspiration rate 100 % and airflow 600 mL/min. The nominal content of oregano EO in the final spray-

dried product was 10 %. Three replicate batches were prepared and used for the determination of physicochemical properties and drug release. A spray-dried product without EO was also prepared for comparison purposes.

Particle size and moisture content

The spray-dried product consisted of reasonably well formed particles with a narrow normal size distribution and particle diameters in the range D10=3.9 μm to D90=16.9 with mean value 8.1 μm as previously reported [19]. The moisture content was measured by heating at 105 °C to constant weight and was found to be 3.4±0.2 expressed as % of weight loss on a dry basis (Table 1).

Table 1. Technological properties of spray-dried oregano EO product (mean ± standard error, n=3)

Property	Value
Refractive Index of unprocessed EO	$1.51387\pm5.2\times10^{-5}$
Refractive indexof EO after spray drying	$1.51566\pm8.5\times10^{-5}$
Particle size (μm)	
D10	4.7
D50	11.0
D90	26.0
Moisture content (%)	3.4 ± 0.2

Refractive index

The refractive index of the unprocessed EO and after its extraction from EOSD was measured using a refractometer (Bellingham and Stanley, Kent, England), illuminated with a sodium D1 (yellow) lamp at 589.6 nm and 20 °C. The units read on the scale of the instrument were converted to refractive index from the manufacturer's conversion table.

FT-IR and Raman spectroscopy

FT-IR Spectra were obtained using a Shimadzu FT-IR-Prestige-21 spectrometer (Shimadzu Corporation) attached to a horizontal Golden Gate MKII single-reflection ATR system (Specac, Kent, UK) equipped with a Diamond/ZnSe crystal (45° angle to infrared beam, 1.66 μm at 1000 cm^{-1} depth of penetration, 2.4 refractive index and 525 cm^{-1} long wavelength cut-off). A few drops of oregano EO or a small amount of the EOSD powder product were placed on the diamond disk and 64 scans were collected over the range of 4000–400 cm^{-1} at resolution 4 cm^{-1} using appropriate software (Shimadzu IRsolution 1.3). For the spray-dried powder product a sapphire anvil was used to restrain the powder in the path of the beam.

Raman spectra of samples placed in standard vials were recorded using a bench top Raman spectrophotomer (Agility, dual band 785/1064 nm model, BaySpec, CA, USA) and supporting software (Agile 20/20). The laser excitation line was 1064 nm selected due to strong fluorescence of the sample at lower wavelengths, the resolution 12 cm^{-1}, the exposure time 2 s, the power of incident laser beam 350 mW and the recorded spectra were the average of 100 runs.

In vitro release of oregano EO

A dialysis method was used to test the release of encapsulated EO from EOSD. 100-mg sample corresponding to 10 mg nominal EO but in fact to about 7-8 mg due to losses during spray drying [7], was

placed in a dialysis cellulose tubing (molecular weight cut-off 12500, Sigma-Aldrich), closed at the two ends and immersed in 200 mL phosphate buffer (PBS, pH 6.8, 37 °C), under magnetic stirring. Since the cut-off M_W was greater than that previously used for similar systems [20, 21], no interference of the membrane pores was expected in the measured values of EO. For the construction of the standard reference curve, the absorption peaks of the oregano EO were first determined from spectra of oregano EO obtained using a UV-VIS spectrophotometer (Pharma Spec UV-1700 Shimandzu, Japan). A major peak appeared at wavelength 273 nm (Figure 1) and was used for the determination of released EO. In fact, this was very close to the peak obtained from the pure carvacrol (Sigma-Aldrich) (Figure 1) indicating its predominance in the composition of EO (86.84 % according to the manufacturer's data). The reference curve of EO in PBS was then constructed using standard solutions of unprocessed oregano EO prepared by diluting stock EO solutions in methanol with phosphate buffer (PBS) pH 6.8, to give final concentrations in the range 0.01125 – 0.1125 mg/mL. The standard reference curve was C = (Abs - 0.0192) / 11.525), where C is in mg mL^{-1} and was used for the determination of EO in the aliquots taken at timely intervals from the dissolution medium.

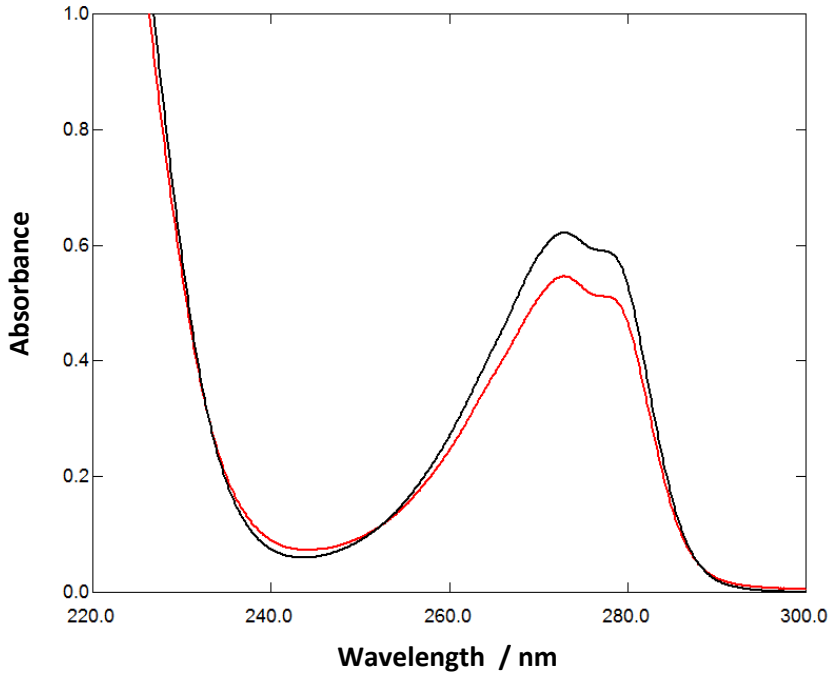

Figure 1. UV-VIS spectra of unprocessed oregano EO (black line) and carvacrol (red line)

Results

The values of the refractive indices of the unprocessed and extracted EO are shown on Table 1. It can be seen that they were only slightly different indicating that the oregano EO essentially retained its composition after spray drying.

Particle size, shape and moisture content

Particle size distribution data of the spray-dried product (EOSD) are presented in Table 1 as diameters D10, D50 and D90 corresponding to 10 %, 50 % and 90 % of the distribution. It can be seen that the particles formed a narrow and normal size distribution of low span [(D90-D10)/D50]=2.0, with mean diameter 11.0 µm. The narrow particle size distribution of EOSD as well as the 3.4 % moisture content (Table 1) is within the expected ranges for spray-dried products prepared using equipment of similar

capacity and operating conditions [11].

FT-IR and Raman spectroscopy

FT-IR spectra of carvacrol, oregano EO and of the spray-dried product without EO or with EO (EOSD) are presented in Figure 2. Carvacrol and oregano EO spectra looked the same even in the fingerprint region 1600-600 cm^{-1}, confirming that the consistency did not change after spray drying, and the carvacrol content remained very high. Several of the characteristic EO peaks present in the carvacrol and the oregano EO spectra (Figure 2a-b) also appeared in the EOSD spectrum (Figure 2c) at the same wavelengths: 2960 cm^{-1}, 1420 cm^{-1} and at 1249 cm^{-1} (indicated by vertical dotted lines), although at much lower intensities because of the relatively low EO content (10 %) in the product. These peaks, however, were absent from the spectrum of the spray-dried product without EO (Figure 2d), confirming that these were specific to oregano EO.

Similarly, from the Raman spectra shown in Figure 3 it can be seen that the peaks were almost identical for carvacrol and oregano EO reflecting the high carvacrol content in the EO. Several characteristic peaks present in the carvacrol and the oregano EO spectra (Figure 3a-b) also appeared distinctly in EOSD (Figure 3c) at the same wavelengths (marked by vertical dotted lines): 1621 cm^{-1}, 760 cm^{-1} and 570 cm^{-1} [22]. An additional peak at 1260 cm^{-1} in the carvacrol and EO spectra, appears to be shifted to the left at 1285 cm^{-1} (higher wavelength) in the spectrum of the EOSD, implying possible interaction of the EO with the wall materials. The above peaks were absent from the spectrum of the spray-dried product with wall materials alone (without EO).

In vitro release of oregano EO

The dialysis membrane method was used for testing the release of encapsulated EO from EOSD in order to avoid passage of colloidal particles of the high molecular weight (MW) polysaccharides into the dissolution medium and subsequent interference with UV absorption of EO. The release vs time profile of EO from the EO encapsulated spray-dried product (EOSD) is shown in Figure 4. As it can be seen, plateau is reached after about 60 min corresponding to release of about 7-8 mg, as expected due to loss during spray-drying [7]. The data were analyzed using different kinetic models. EO release should involve diffusion from the interior of the particles to the surface and additionally, relaxation and swelling of the hydrophilic wall materials due to hydration. Therefore, the simple power equation of Peppas and Ritger [23] for swelling-controlled release systems was initially tried but poor fitting of the data was found implying that a more complex release mechanism operates which, besides diffusion and swelling also involves progressive dissolution of the wall material.

For this reason the cube-root law [24-25], equation (1) was applied:

$$Mo^{1/3} - Mt^{1/3} = kt \quad (1)$$

Mt is the weight of unreleased EO at time *t* and *Mo* the initial weight of EO in the sample.

This is based on the assumptions that a) the dissolving particles are spherical and of narrow size distribution b) sink conditions prevail and c) there is no influence of stagnant layers forming in the neighborhood of the particles. These assumptions were obeyed in this study as demonstrated by the low content of EO in the dissolution fluid (about 7-8 mg in 200 mL) compared to the solubility of carvacrol (1.250 mg mL^{-1}) and, by providing sufficient agitation.

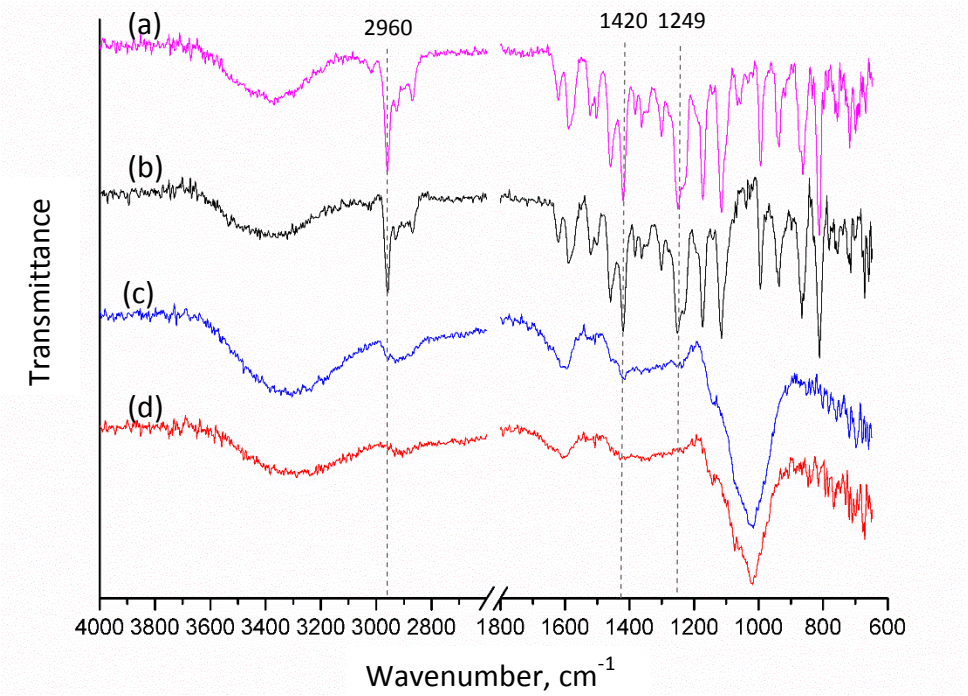

Figure 2. FT-IR spectra of (a) carvacrol, (b) oregano EO, (c) encapsulated EO spray-dried product and (d) spray-dried product without EO. Vertical dotted lines show presence of EO peaks in the EOSD product.

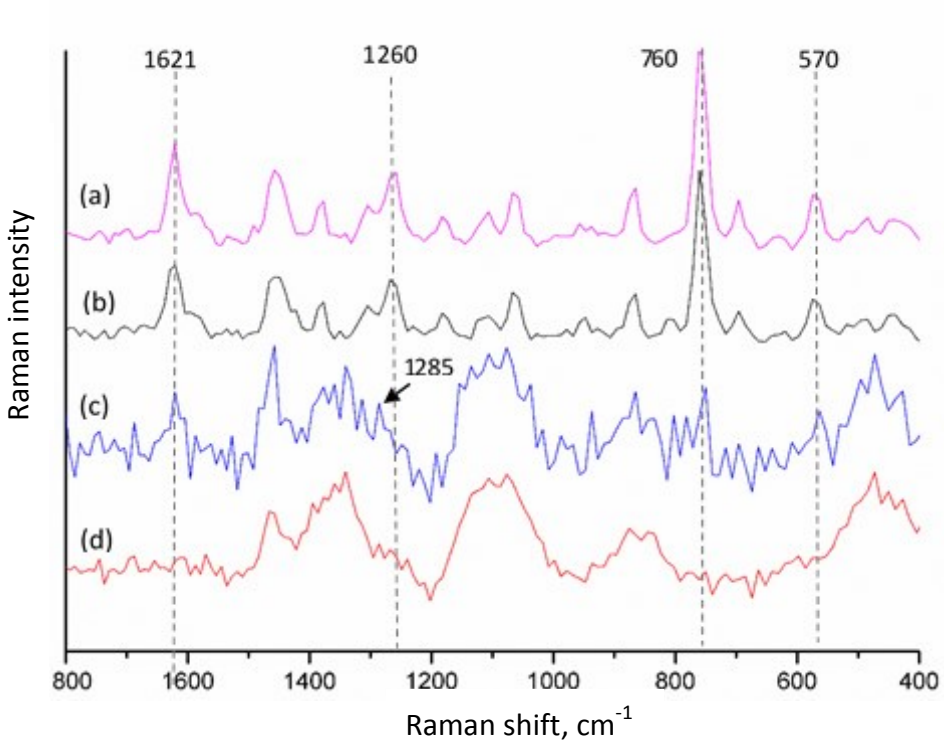

Figure 3. Raman spectra of (a) carvacrol, (b) oregano EO, (c) encapsulated EO spray-dried product and (d) spray-dried product without EO. Vertical dotted lines show presence of oregano EO peaks in the EOSD product.

The results were plotted according to equation (1) in Figure 5 where it can be seen that the points fall on a straight (R^2=0.986) with slope line k=0.0213 ($mg^{1/3}$ / min) representing the rate constant of the Hixson-Crowell equation known as 'cube-root law' [24], expressed by equation:

$$k = (4/3 \times \pi\rho N)^{1/3} \times (D_{app} Cs / \rho h) \quad (2)$$

ρ=0.95 g/cm^3 is the density of oregano EO, Cs its solubility in water (taken as that of carvacrol 1.250

mg/ml), *h* the thickness of diffusion layer (taken as the mean particle radius 5.5 μm, Table 1, assuming uniform EO distribution in the particles) and N=1.387x10^4 the number of EO releasing particles in the 100 mg sample, calculated from the mean particle diameter and its density (1.34 g/cm^3, equal to the weighted average of the components). D_{app} is an 'apparent' diffusion coefficient representing the composite release mechanism involving diffusion, swelling and particle dissolution. By substituting the values of the above parameters into equation (2), D_{app}=8.3x10^{-10} m^2/s. Although the estimation of diffusion may be affected due to the passage of EO through the pores of the dialysis membrane, this effect is not expected to be significant due to the low M_W of the ingredients of EO (i.e. Carvacrol 155.22, p-Cymene 139.21, γ-Terpinene 136.23 and Thymol 150.22). Besides, previous estimations of the diffusion coefficients [20, 21] were also obtained using the dialysis method and therefore comparisons of the present with the previous data can be made.

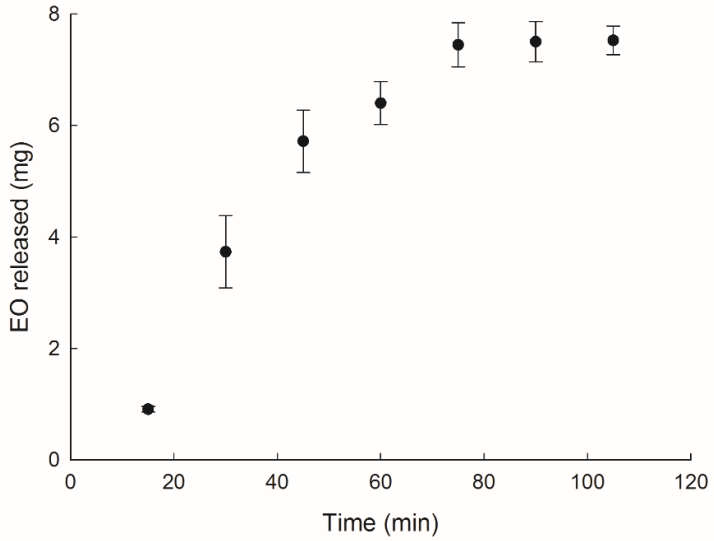

Figure 4. Release-time profile of oregano EO from the spray–dried powder product (error bars show standard deviation, n=3)

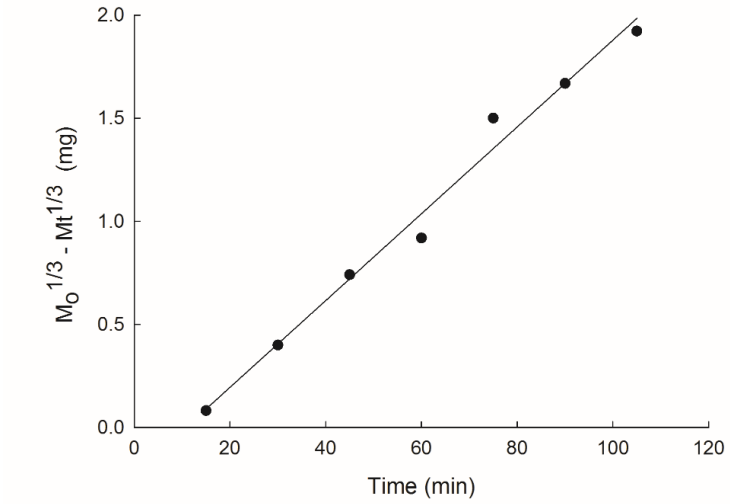

Figure 5. Release data of oregano EO from the spray-dried powder product plotted according to the Hixson-Crowell 'cube-root law' equation.

Discussion

Antimicrobial resistance is an emerging health problem which is enlarged by the shortage of new antibiotic agents. At the same time, interest in antimicrobials obtained from natural sources has increased

due to their accepted safe status [1-2, 3-4]. Essential oils derived from aromatic plants and, oregano EO in particular fall into a group of antimicrobials that has attracted major attention as shown by the numerous reports in the literature [26-27]. However, since pure oregano EO is highly irritating to taste and cannot be easily consumed as such, its microencapsulation into a spray-dried product offers a convenient way for oral administration besides providing protection from environmental factors as well.

The peaks that are common in the FT-IR spectra of carvacrol, oregano EO and EOSD (Figure 2) are ascribed at: 2960 cm^{-1} to antisymmetrical -CH_3 stretching vibration, 1420 cm^{-1} to antisymmetric -CH_3 bending and 1249 cm^{-1} to C-O-C stretching. Since these peaks are absent from the spectrum of the spray-dried product without EO they could be potentially used (especially the more distinct at 1420 cm^{-1} and 1249 cm^{-1}), for identification purposes. Raman spectroscopy is complementary to FT-IR and was used to add more information about the state of the EO in the product. The peak at 760 cm^{-1} in the EOSD corresponds exactly to the position in the carvacrol spectrum, with no shifting towards lower wavenumbers that might be caused by the presence of thymol confirming carvacrol as the main constituent and that substances present at low percentages do not influence the EO spectrum [25].

The distinct peaks that are common in the Raman spectra of carvacrol, unprocessed oregano EO and EO in the EOSD (Figure 3) are ascribed at: 1621 cm^{-1} to conjugated C=C stretching vibration, 760 cm^{-1} to breathing vibration mode and 570 cm^{-1} to aromatic ring vibration. Since they are absent from the spectrum of the spray-dried product without EO, they can be used for identification of EO [22]. The shifting of the peak at 1260 cm^{-1}, which is assigned to the stretching vibration of the C-O bond of the phenol, to the left at 1285 cm^{-1} implies interaction of the EO and the wall material, probably between the aromatic –OH of carvacrol and the hydroxyl groups of maltodextrin, resulting in lower vibration frequency and shifting of the peak to higher wavelength. From the above discussion it appears that Raman spectroscopy provides more information on the state of EO in the product by revealing more distinct peaks of EO in the product and possible interactions, thus offering a better alternative for identification and the state of EO in the EOSD than FTIR.

Contrary to previous works [20, 21] the results in the present study could not be described by Fick's diffusion models due to progressive dissolution of the wall material resulting in change of available particle surface. For this reason the release model cube-root law of Hixson-Crowell [24] was applied which is derived on the basis of surface change, expressed in equation (1) as mass change with time. The good fitting of the data to this model (R^2=0.986) indicates distribution of EO in the wet jelly matrix of spray-dried particles during release, so that mass change at different times corresponds to release of proportional amounts of EO. The rather high value of the apparent diffusion coefficient D_{app} = 8.3x10^{-10} m^2/s in equation (2) compared to the values of about 10^{-13} to 10^{-16} in previous works [20-21], is attributed to the different wall materials used and different types of diffusion mechanisms. Also, in their studies [20], interactions between EO and the wall material were reported whereas only minor interactions were seen in the present work.

Conclusions

Microencapsulated oregano EO by spray drying using Arabic gum, starch and maltodextrin as wall materials has been previously reported by da Costa et. al [7] to offer good retention and encapsulation efficiency. It was therefore considered worthwhile investigating the state of microencapsulated EO and its release profile from the spray-dried product (EOSD). FT-IR and Raman spectroscopy clearly identified the presence of EO in the EOSD, with a small shift of the peak at 1260 cm^{-1} in the Raman spectrum to 1285 cm^{-1},

implying only minor chemical interaction with the wall materials. Release of the EO from EOSD was completed within 1 – 1.5 h and was well described by the Hixson-Crowell equation (R^2=0.986) with apparent diffusion coefficient $8.3x10^{-10}$ m^2/sec. The above findings encourage formulation of oregano EO into spray-dried product for oral administration for therapeutic purposes.

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