Physicochemical characterization of the inclusion compounds of eugenol and β-caryophyllene in β-cyclodextrin**

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The present work was carried out to encapsulate the essential oil (EO) constituents (i.e., eugenol and β-caryophyllene) inside β-cyclodextrin. Encapsulates were subjected to physicochemical characterization. Absorption λ_{max} for eugenol and β-caryophyllene were observed at 280 nm and 230 nm, respectively. Scanning electron microscopy (SEM) analysis of encapsulates revealed rough surfaces, cracks and sharp edges; the particles were in the micrometre size range and are, therefore, referred to as microparticles. Characteristic peaks of eugenol were recorded at 3516 cm^{-1} (OH), 2842–3000 cm^{-1} (C-H stretching) and 1511 cm^{-1}, 1611 cm^{-1} and 1638 cm^{-1} (C=C aromatic ring) via Fourier transform infrared (FTIR) spectroscopy. The FTIR spectrum of β-caryophyllene showed peculiar bands at 3067–2856 cm^{-1} and 1671–885 cm^{-1}. Thermogravimetric analysis (TGA) data showed complete weight loss for eugenol and β-caryophyllene in the range of 30–215 °C. In contrast, encapsulated eugenol and β-caryophyllene showed weight loss in the range of 300–580 °C.

Keywords: encapsulation, eugenol, β-caryophyllene, FTIR, SEM, TGA

1. Introduction

β-cyclodextrin is a cyclic oligosaccharide containing seven glucopyranose units attached by α-1,4 glycosidic bonds. Glucopyranose units are spatially oriented forming a truncated cone and impart ring structure to cyclodextrins. This truncated cone has a hydrophilic exterior surface that helps cyclodextrins to dissolve in water. The hydrophobic interior space has a strong ability to form complexes with polar and nonpolar compounds. The guest molecules are held inside the cavity of host molecule by noncovalent interactions. The host–guest system is in

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** This paper was first presented at the International Conference on Advanced Materials, Energy & Environmental Sustainability (ICAMEES-2018), 14–15 December 2018 in Dehradun, India.

Nanotechnology Perceptions **15** (2019) 143–149
Received 24 January 2019; *accepted* 8 March 2019

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doi: 10.4024/N08SH19A.ntp.15.02

dynamic equilibrium and, therefore, offers slow release of guest molecules, thereby sustaining their action. Biocompatibility, effective degradability and low cost make β-cyclodextrin the most-used molecule as an encapsulant.

Eugenol (4-allyl-2-methoxyphenol), the principal component of clove oil, is a phenolic compound.[1] It is widely used in the pharmaceutical, food and active packaging industries because of its antioxidant and antimicrobial properties.[2,3] β-caryophyllene is a bicyclic sesquiterpene that is produced by plants in response to herbivore damage.

Although eugenol and β-caryophyllene are plant-derived compounds, they have certain limitations, such as a volatile nature, insolubility in water, rapid oxidation, degradation upon exposure to air, and change in stereochemistry in the presence of light.[4] These limitations restrict their use in various fields.

The objective of the present work was to improve the stability of plant-derived bioactive compounds (i.e., eugenol and β-caryophyllene) via encapsulation in β-cyclodextrin.

2. Material and methods

2.1 Chemicals

Eugenol (EU), β-cyclodextrin (β-CD), potassium bromide, dichloromethane (DCM), and β-caryophyllene (CAR) were from Loba Chemicals Pvt. Ltd.

2.2 Preparation of EU and CAR microparticles

Encapsulation of essential oils (EO) by β-CD was carried out using the inclusion complexation method.[4] 10% β-CD was dissolved in a 1:2 $^{v}/v$ mixture of ethanol and distilled water. 2% and 4% encapsulates of EU and CAR were prepared (Table 1). EO constituents were dissolved in ethanol followed by dropwise inclusion into the β-CD solution. The resulting mixture was further dispersed using an ultrasonicator at 90 W for 30 min. The solution was maintained for 12 h at 4 °C followed by recovery of encapsulated material. The encapsulated powder was stored in airtight glass vials at room temperature for physicochemical characterization.

Table 1. Preparation of β-CD–CAR and β-CD–EU encapsulates.

Percentage	β-CD solution (mL)	EO constituents+ethanol mixture	Total volume
Control	27	3 mL ethanol	30 mL
2%	27	(0.6 mL EOs + 2.4 mL ethanol)	30 mL
4%	27	1.2 mL EOs + 1.8 mL ethanol)	30 mL

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2.3 Characterization of EU- and CAR-loaded microparticles

2.3.1 UV-vis absorbance

Inclusion complex formation was demonstrated by UV-vis spectrophotometry. Encapsulated powder (10 mg) of β-CD–CAR and β-CD–EU was dissolved in DCM (2 mL) and shaken for 15 min for releasing oil into the organic solvent. The supernatant was further subjected to serial dilution in DCM and scanned in the 200–800 nm range to obtain the absorption spectrum.

2.3.2 Scanning electron microscopy (SEM)

The morphology of β-CD–CAR and β-CD–EU inclusion complexes were analysed by SEM (ZEISS Merlin Compact) for resolving the surface structure. Samples were gold-coated and mounted on a stainless steel stub using double-sided sticky carbon tape.

2.3.3 Fourier transform infrared spectroscopy

Solid samples were ground and mixed thoroughly with KBr. Liquid samples were prepared using the KBr window technique. FTIR spectra were obtained on a Bruker (Tensor 27) instrument in the 4000–500 cm^{-1} range for the control and 3500–1000 cm^{-1} for the β-CD–CAR and β-CD–EU inclusion complexes and free oil.

2.3.4 Thermogravimetric analysis (TGA)

Differential thermal analysis was carried out with a Shimadzu DTG-60H instrument, using 10 mg of the sample in aluminum crucibles under nitrogen (slow flow rate of 40 mL/min) with a heating rate of 10 °C/min in the temperature range 30–580 °C.

3. Results and discussion

3.1 UV-vis absorbance

The content of eugenol loaded into the encapsulated particles was determined on the basis of EU's absorbance at 282 nm. Similarly, the absorbance of CAR had a λ_{max} of 230 nm. λ_{max} of eugenol was recorded at 283.5 nm in a comparable study.[5] A previously reported value of λ_{max} (205 nm) for the β-CD/β-caryophyllene complex was fairly near to the value (230 nm) observed in the present study.[6]

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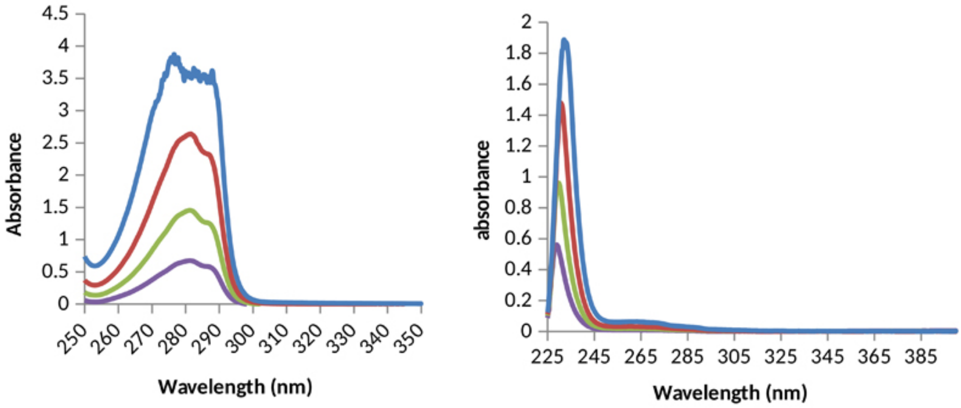

Figure 1. UV absorption spectra of eugenol (left) and β-caryophyllene, at a range of concentrations to bring the absorbance to unity for the maximum.

3.2 Scanning electron microscopy

SEM analysis revealed that encapsulates possessed a rough surface and loss of spherical shape (Fig. 2). Encapsulates were observed to be rectangular-shaped and showed sharp edges and cracks. Furthermore, evidence of agglomeration (i.e., the attraction of smaller particles by large particles) is provided by these images. If a large number of pores and cracks are present on the external surface of the material, then loss and degradation of EOs is enhanced.[7,8] The present findings had some similarity to those of a previous study, in which a nonspherical morphology of microcapsules of β-CD–oregano EO was reported.[9]

In the present study, clusters were reported for encapsulates of the control, β-CD–EU and β-CD–CAR. The irregular shape of the encapsulated material as well as that of the control (β-CD only) has been reported previously.[10]

3.3 FTIR

The FTIR spectra of eugenol, β-CD and the inclusion complex are shown in Fig. 3.

The FTIR spectrum of eugenol showed characteristic peaks at 3516 cm^{-1} (OH), 2842–3000 cm^{-1} (C-H stretching), and 1511 cm^{-1}, 1611 cm^{-1} and 1638 cm^{-1} (C=C aromatic ring) (Fig 3a). Similar results were reported for the FTIR spectrum of eugenol.[5] FTIR spectrum of β-CD showed a band with an absorption maximum at about 3514 cm^{-1}. This band was formed due to stretching

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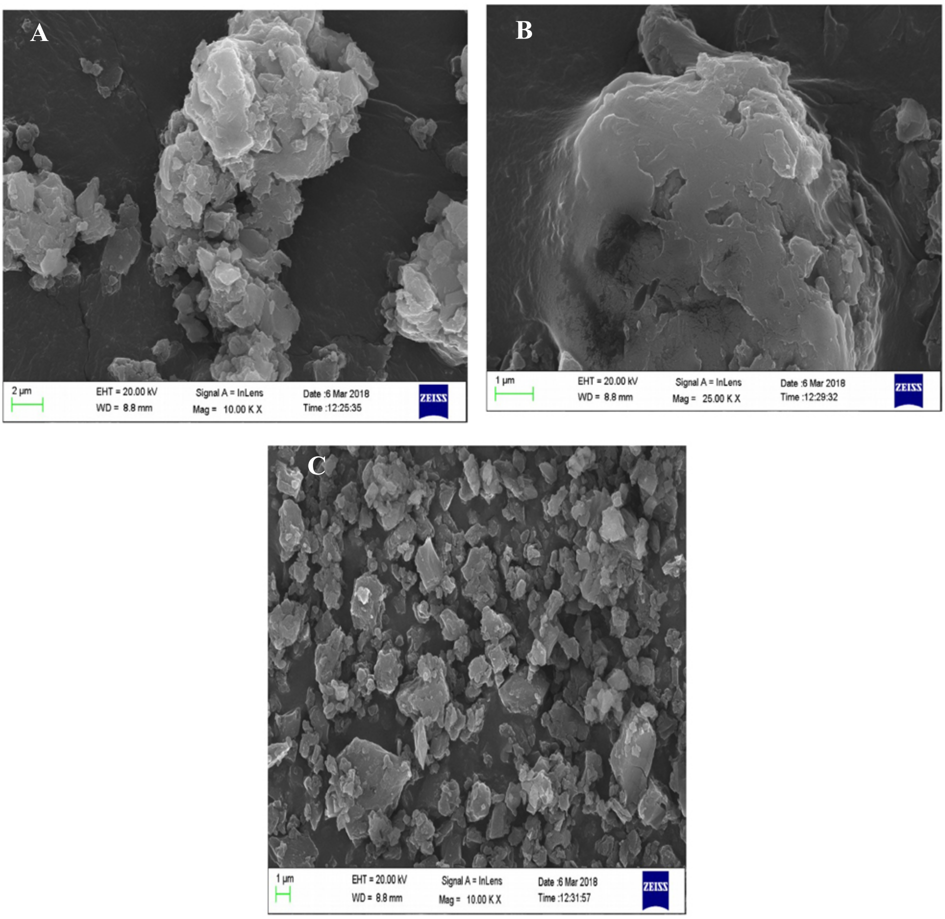

Figure 2. SEM image of: (A) control (pure β-CD); (B) eugenol encapsulated inside β-cyclodextrin; (C) β- caryophyllene encapsulated inside β-cyclodextrin.

vibrations of the several hydroxyl groups (OH) of the β-cyclodextrin. Another band present at 164 cm^{-1} was correlated to the vibrations of these -OH groups. The spectrum also showed the presence of a prominent band mainly at 2925 cm^{-1} due to C-H vibrations and stretching of the C-H and C-H_2 bonds. Various numbers of bands due to CH stretching vibrations were also present at 1414, 1368, 1335, 1301 and 1248 cm^{-1}. The presence of bands at 1156–1000 cm^{-1} was attributed to the C-O stretching vibrations due to the linkage between ether and hydroxyl groups. The band found in the region 1000–700 cm^{-1} corresponds to the vibrations of the C-H bonds and C-C skeletal vibrations in the glucopyranose ring. The results showed similarity to earlier studies.[11] However, the spectrum of the control (β-CD) microparticles was similar to that of the β-CD–EU microparticles. All bands of the eugenol spectrum were totally obscured by the β-CD bands. It is

[11] De Sousa Oliveira F, De Freitas TS, Da Cruz RP, Do Socorro Costa M, Pereira RL, Quintans-Junior LJ, De Araujo Andrade T, Dos Passos Menezes P, De Sousa BM, Nunes PS, Serafini MR. Evaluation of the antibacterial and modulatory potential of α-bisabolol, β-cyclodextrin and α-bisabolol/ β-cyclodextrin complex. *Biomed. Pharmacother.* **92** (2017) 1111–1118.

possible that eugenol entered the cavity of β-CD and an inclusion complex was formed, but infrared spectroscopy cannot provide evidence of that.

The FTIR spectrum of β-caryophyllene showed bands at 3067–2856 cm^{-1} and 1671–885 cm^{-1}. Similar findings with respect to the spectrum of β-caryophyllene were reported previously.[6] No remarkable difference was seen between the spectra of β-cyclodextrin and the β-caryophyllene–β-cyclodextrin inclusion complex. This was probably due to the complete obscuring of the β-cyclodextrin bands by the β-caryophyllene bands, consistent with the inference that β-caryophyllene had formed a complex with β-cyclodextrin.

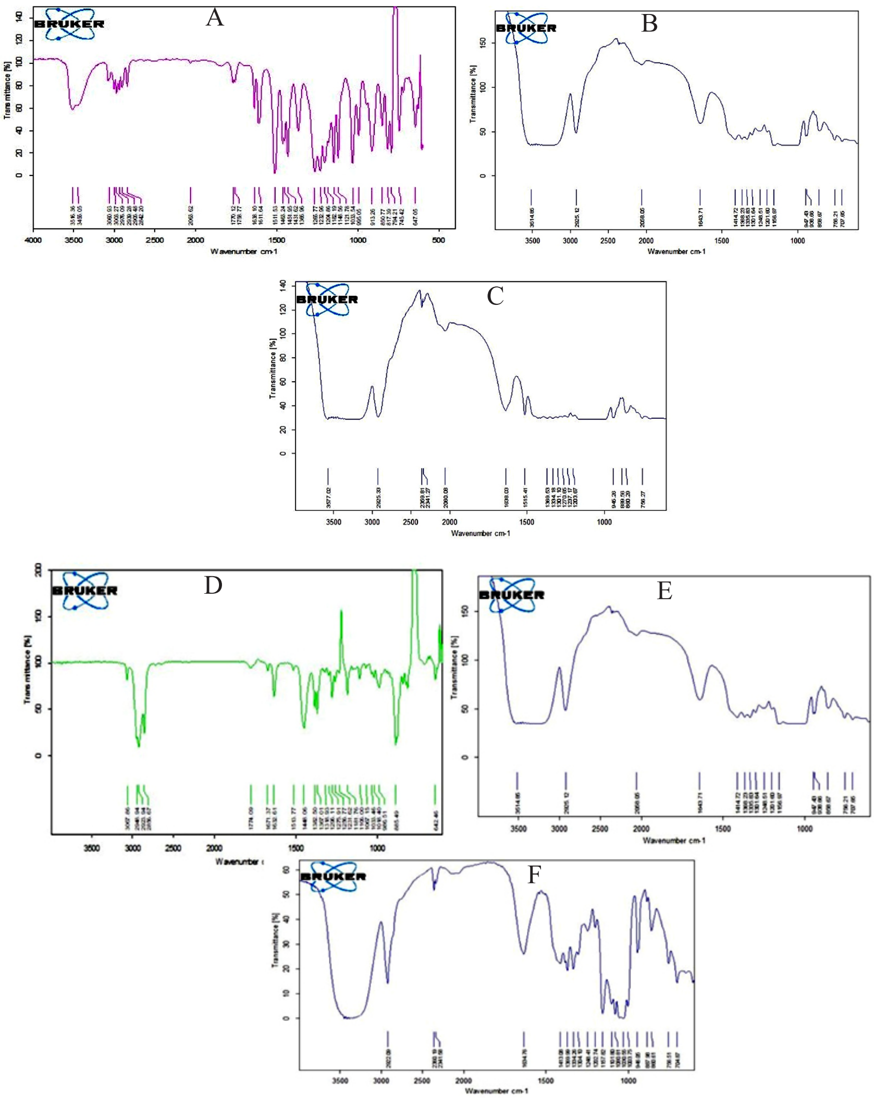

Figure 3. FTIR Spectra of (A) eugenol; (B) β-cyclodextrin; (C) eugenol–β-cyclodextrin inclusion complex; (D) β-caryophyllene; (E) β-cyclodextrin; and (F) β-caryophyllene–β-cyclodextrin inclusion complex.

3.4 Thermogravimetric analysis (TGA)

Fig. 4 shows TGA of β-CD, β-CD–EU, β-CD–CAR, free eugenol and β-caryophyllene. The TGA curve for eugenol and β-caryophyllene showed 100% weight loss in the range of 30–215 °C. However, the initial weight loss of β-CD took place at 100 °C and the final weight loss occurred around 300–580 °C. Similarly, the weight loss of β-CD–EU and β-CD–CAR occurred in the range of 300–580 °C. The experimental results led to the conclusion that cyclodextrin requires a high temperature for degradation and acts as a protective covering for the encapsulated material, thereby preventing its early degradation at lower temperatures. The results obtained were homologous to the previous findings.[12–14]

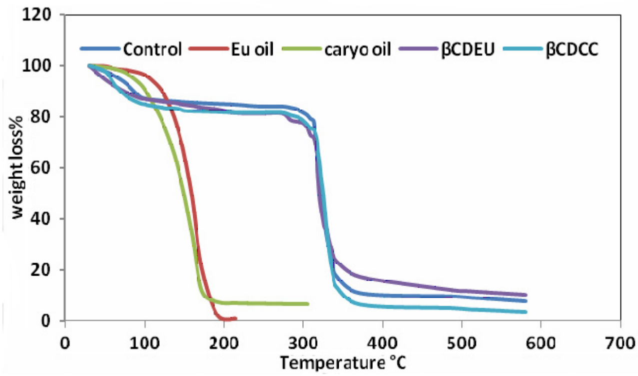

Figure 4. TGA curves of β-CD, β-CD–EU, β-CD–CAR, free eugenol and β-caryophyllene (colour online).

4. Conclusion

The aim of present work was to improve the physical stability of EU and CAR by encapsulation. SEM, FTIR and TGA showed that β-CD–EU and β-CD–CAR had physicochemical characteristics different from those of unencapsulated EU and CAR. The results led to the conclusion that EU- and CAR-loaded microparticles could possibly be used for various applications.

Acknowledgment

The authors are thankful to the Indian Council of Medical Research, New Delhi for providing financial assistance.

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