*chemistry proceedings*

MDPI

*Proceedings*

# Encapsulation in Lipid Nanosystems of Eugenol Oxirane Insecticide †

**Maria José G. Fernandes [1], David M. Pereira [2], A. Gil Fortes [1], M. Sameiro T. Gonçalves [1] and Elisabete M. S. Castanheira [3,*]**

[1] Centre of Chemistry (CQ/UM), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal; mjfernandes@quimica.uminho.pt (M.J.G.F.); gilf@quimica.uminho.pt (A.G.F.); msameiro@quimica.uminho.pt (M.S.T.G.)
[2] REQUIMTE/LAQV, Laboratory of Pharmacognosy, Department of Chemistry, Faculty of Pharmacy, University of Porto, R. Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal; dpereira@ff.up.pt
[3] Centre of Physics (CFUM), University of Minho, Campus of Gualtar, 4710-057 Braga, Portugal
* Correspondence: ecoutinho@fisica.uminho.pt
† Presented at the 24th International Electronic Conference on Synthetic Organic Chemistry, 15 November–15 December 2020; Available online: https://ecsoc-24.sciforum.net/.

Received: date; Accepted: date; Published: date

**Abstract:** Essential oils (EOs) play an important role in plant defense system and possess strong potential insecticides. However, they generally exhibit low water solubility, poor bioavailability, volatility, reduced stability, and low resistance to environmental stresses like light, oxygen, and temperature, which difficult their applications. To mitigate these limitations, EOs can be incorporated into nanoencapsulation systems. In the present work, an eugenol oxirane, namely ethyl 4-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)butanoate, with promising insecticidal activity, was submitted to encapsulation assays in lipid nanosystems, in order to boost its application as bioinsecticide.

**Keywords:** essential oils; eugenol oxirane; nanoencapsulation; bioinsecticides; natural products; bioinsecticides; insecticides

## 1. Introduction

The overuse of synthetic insecticides causes serious damage to the environment and agriculture. It becomes necessary to develop less aggressive and effective solutions. To replace conventional pesticides is urgent to consider the development of eco-friendly and alternative strategies [1].

Essential oils (EOs) have been studied owing to their properties, such as remarkable biological activities and health promoting benefits, including analgesic, anti-inflammatory, antibiotic, antioxidant and antimicrobial activities, as well as potential insecticides [2–4]. EOs have enhanced properties, namely high biodegradability, thus reducing environmental impact and making them species with large applications. Besides that, the structural modification of their chemical constituents has shown to enhance the biocidal effect of these substances by increasing their activity [5,6].

However, the low water solubility, high volatility and rapid oxidation of EOs constituents affect the possibility of biological application, decreasing their real potential [1,7]. To mitigate these problems, EOs can be incorporated into nanoencapsulation systems [3,7,8].

Eugenol is the major component of clove essential oil presenting relevant biological potential with well-known antimicrobial and antioxidant actions [9]. An eugenol derivative with promising insecticide activity was subject to a nanoencapsulation study, to boost its application as biopesticide.

## 2. Results and Discussion

Ethyl 4-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)butanoate was obtained by epoxidation of 3-(4-allyl-2-methoxyphenoxy)propan-1-ol with *m*-chloroperbenzoic acid in dichloromethane at room temperature, and its structure was confirmed by the usual analytical techniques.

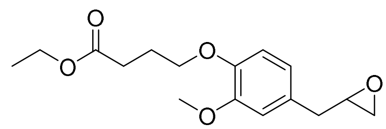

**Figure 1.** Structure of ethyl 4-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)butanoate.

Encapsulation assays in lipid nanosystems were carried out, using both the thin film hydration and ethanolic injection methods for the preparation of compound-loaded nanocarriers. Thin film hydration is one of the simplest ways to prepare liposomes, affording homogeneous small vesicles after extrusion, being especially suitable for hydrophilic compounds [10]. The ethanolic injection method has been shown to be adequate for enhanced encapsulation of poorly water-soluble compounds [11]. Table 1 shows the determined encapsulation efficiencies only for ethanolic injection preparation method.

**Table 1.** Encapsulation efficiency, *EE(%) ±SD(%)*, of the eugenol derivative in liposomes prepared by the ethanolic injection method (SD: standard deviation).

| Assay | EE(%) | EE (%) ± SD (%) |
|---|---|---|
| 1 | 89.7 | |
| 2 | 91.6 | 88.8 ± 2.7 |
| 3 | 85.1 | |

The encapsulation efficiencies are very good for the ethanolic injection method. The results of encapsulation efficiencies for thin film hydration method were not possible to be determined because the percentage of encapsulation was very low.

Nevertheless, the high *EE%* values obtained (Table 1) point to promising future applications of the extract-loaded soybean liposomes as green insecticides, with the possibility of controlled release of the encapsulated compounds.

## 3. Materials and Methods

### *3.1. Nanoencapsulation Studies*

For nanoencapsulation studies, the compound ethyl 4-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)butanoate was used. Liposomes were prepared using a commercial lipid mixture used in food industry, soybean lecithin [12] (Sternchemie), containing 22% phosphatidylcholine, 20% phosphatidylethanolamine, 20% phosphatidylinositol and 10% phosphatidic acid as major components at 1 mM concentration. In thin film hydration method [10], a lipidic film was obtained from evaporation of ethanolic lipid solution and addition of the extract, followed by hydration, bath sonication and extrusion (Lipex™ Extruder, Northern Lipids, Canada) through polycarbonate membranes (200 nm pore size). In ethanolic injection method [11], simultaneous injection of the compound and lipid were carried out, under vigorous vortexing, in an aqueous buffer solution.

### *3.2. Encapsulation Efficiency*

To determine a calibration curve (absorbance vs. concentration), concentration dilutions of $1 \times 10^{-6}$–$1 \times 10^{-5}$ mg/mL were performed. Loaded liposomes were subjected to centrifugation at 3000 rpm for 10 min using Amicon® Ultra centrifugal filter units 100 kDa. Then, the filtrate (containing the nonencapsulated compound) was pipetted out, the water was evaporated and the same amount of ethanol was added. After vigorous agitation, its absorbance was measured, allowing to determine compound concentration using a calibration curve previously obtained in the same solvent. Absorption spectra were performed in a Shimadzu UV-3600 Plus UV-vis-NIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Three independent measurements were performed for each system. The encapsulation efficiency, $EE(\%)$, was obtained through Equation (1),

$$EE(\%) = \frac{Total\ amount\ -\ Amount\ of\ nonencapsulated\ extract}{Total\ amount} \times 100 \quad (1)$$

## 4. Conclusions

A eugenol derivative, ethyl 4-(2-methoxy-4-(oxiran-2-ylmethyl)phenoxy)butanoate, with promising insecticidal activity, was submitted to encapsulation assays in lipid nanosystems, in order to boost its application as bioinsecticide. The encapsulation efficiency was very good for the ethanolic injection method, pointing to possible future applications of the encapsulated compound as green insecticide with the possibility of controlled release.

**Funding:** FCT under project PTDC/ASP-AGR/30154/2017 (POCI-01-0145-FEDER-030154) of COMPETE 2020, co-financed by FEDER and EU. FCT- Portugal and FEDER-COMPETE/QREN-EU for financial support to the research centres CQ/UM (UIDB/00686/2020), CF-UM-UP (UIDB/04650/2020) and REQUIMTE (UIDB/50006/2020). The NMR spectrometer Bruker Avance III 400 (part of the National NMR Network) was financed by FCT and FEDER.

**Conflicts of Interest:** The authors declare no conflict of interest.

## References

1. Yeguermana, C.; Jessera, E.; Massirisc, M.; Delrieuxc, C.; Murrayd, A.P.; Werdin González, W.J.O. Insecticidal application of essential oils loaded polymeric nanoparticles to control German cockroach: Design, characterization and lethal/sublethal effects. *Ecotoxicol. Environ. Saf.* 2020, *189*, 110047, doi:10.1016/j.ecoenv.2019.110047.
2. Salman, M.; Abbas, R.Z.; Israr, M.; Abbas, A.; Mehmood, K.; Khan, M.K.; Sindhu, Z.D.; Hussaind, R.; Saleemie, M.K.; Shaha, S. Repellent and acaricidal activity of essential oils and their components against *Rhipicephalus* ticks in cattle. *Vet. Parasitol.* **2020**, *283*, 109178, doi:10.1016/j.vetpar.2020.109178.
3. Mishra, P.A.; Devkota, H.P.; Nigam, M.; Adetunji, C.O.; Srivastava, N.; Saklani, S.; Shukla, I.; Azmi, L.; Shariati, M.A.; Coutinho, H.D.M.; Khaneghah, A.M. Combination of essential oils in dairy products: A review of their functions and potential benefits. *LWT* **2020**, *133*, 110116, doi:10.1016/j.lwt.2020.110116.
4. Patial, P.P.; Cannoo, D.S. Evaluation of volatile compounds, phenolic acids, antioxidant potential and DFT study of essential oils from different parts of Araucaria columnaris (G. Forst.) Hook. from India. *Food Chem. Toxicol.* 2020, *141*, 111376, doi:10.1016/j.fct.2020.111376.
5. Novato, T.; Gomes, G.A.; Zeringóta, V.; Franco, C.T.; Oliveira, D.R.; Melo, D.; Carvalho, M.G.; Daemon, E.; Monteiro, C.M.O. In vitro assessment of the acaricidal activity of carvacrol, thymol, eugenol and their acetylated derivatives on *Rhipicephalus microplus* (Acari: Ixodidae). *Vet. Parasitol.* **2018**, 260, 1–4, doi:10.1016/j.vetpar.2018.07.009.
6. Chen, C.-H.; Tung, S.-H.; Jeng, R.-J.; Abu-Omar, M.M.; Lin, C.-H. A facile strategy to achieve fully bio-based epoxy thermosets from eugenol. *Green Chem.* **2019**, *21*, 4475–4488, doi:10.1039/C9GC01184F.
7. Lammari, N.; Ouahida Louaer, Q.; Meniai, A.H.; Elaissari, A. Encapsulation of essential oils via nanoprecipitation process: Overview, progress, challenges and prospects. *Pharmaceutics* **2020**, *12*, 431, doi:10.3390/pharmaceutics12050431.
8. Kujur, A.; Kumar, A.;Yadav, A.; Prakash, B. Antifungal and aflatoxin $B_1$ inhibitory efficacy of nanoencapsulated *Pelargonium graveolens* L. essential oil and its mode of action. *LWT* **2020**, *130*, 109619, doi:10.1016/j.lwt.2020.109619.
9. Silva, F.F.M.; Monte, F.J.Q.; Lemos, T.L.G.; Nascimento, P.G.G.; Costa, A.K.M.; Paiva, L.M.M. Eugenol derivatives: Synthesis, characterization, and evaluation of antibacterial and antioxidant activities. *Chem. Cent. J.* **2018**, *12*, 34, doi:10.1186/s13065-018-0407-4.

10. Zhang, H. Thin-film hydration followed by extrusion method for liposome preparation. *Methods Mol. Biol.* **2017**, *1522*, 17–22, doi:10.1007/978-1-4939-6591-5_2.
11. Jaafar-Maalej, C.; Diab, R.; Andrieu, V.; Elaissari, A.; Fessi, H. Ethanol injection method for hydrophilic and lipophilic drug-loaded liposome preparation. *J. Liposome Res.* **2010**, *20*, 228–243, doi:10.3109/08982100903347923.
12. Peters, S.E.; Brain, C.H. Benefits of a soy lecithin based nanotechnology for the animal and human food industry. *ACS Symp. Ser*. **2009**, *1007*, 183–197, doi:10.1021/bk-2009-1007.ch012.