

Proceeding

Nanosystems for Encapsulation and Release of Plant Extracts with Insecticidal Activity †

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† Presented at the 24rd International Electronic Conference on Synthetic Organic Chemistry, 15 November 2020–15 December 2020; Available online: <https://ecsoc-24.sciforum.net/>.

Received: date; Accepted: date; Published: date

Abstract: The encapsulation in suitable nanostructures is a relevant strategy for preservation and controlled release of bioactive compounds, allowing a safe application. In this work, dichloromethane extracts of *Mentha suaveolens* Ehrh. and *Phytolacca americana* L. were encapsulated in chitosan nanostructures and liposomes of soybean lecithin, considering their potential as biopesticides. Liposomes were prepared either by thin film hydration or ethanolic injection methods. For chitosan, the ionic gelation technique was used. The nanosystems were characterized regarding their size and polydispersity. Encapsulation efficiencies of the extracts were determined (being higher than 60%) and the release profiles were measured. Liposomes generally allowed a delayed release of the extracts, while chitosan nanosystems are suitable for a faster and complete release of the potential bioinsecticides.

Keywords: plant extracts; nanoencapsulation; liposomes; chitosan; green insecticides

1. Introduction

Besides the pharmaceutical potential of several plant extracts, their use as repellents and green insecticides has been described [1,2]. However, this application can be hampered by the high volatility and easy degradation of the active compounds. The encapsulation in suitable nanostructures is a relevant strategy for preservation and controlled release of bioactive compounds, allowing a safe application [3].

Extracts from *Mentha suaveolens* Ehrh. have been described as potent antimicrobials, together with analgesic, anti-inflammatory and antioxidant activities [4]. The insecticidal effect of extracts from *Mentha suaveolens* Ehrh. and *Mentha pulegium* L. towards *Toxoptera aurantii*, an insect pest of citrus, was also recently reported [5]. On the other hand, *Phytolacca americana* L. components have been described to exhibit anti-inflammatory, antioxidant, anticancer, anthelmintic, antimicrobial and insecticidal properties [6,7].

Following previous work [8], the extracts of *Mentha suaveolens* Ehrh. and *Phytolacca americana* L. in dichloromethane, after removal of chlorophylls, have demonstrated a high potential as biopesticides in assays using the *Sf9* (*Spodoptera frugiperda*) insect cell line. Encapsulation in chitosan nanostructures and lecithin liposomes were carried out as both nanosystems have been described as suitable carriers for plant extract components [9–11]. Preliminary release experiments were performed, keeping in mind future applications of these extracts as biopesticides.

2. Materials and Methods

2.1. Plant Material

The plant species were harvested in the North of Portugal. After drying, the vegetable matter was grounded with a shredder, and the resulting powder passed through a sieve until a particle size $\leq 900 \mu\text{m}$ was obtained. The material was stored under vacuum for further use. The dried materials (8 g) were subjected to Soxhlet extraction with dichloromethane (50 mL) for 4 h. The organic solvent was removed by evaporation under reduced pressure at $40 \text{ }^\circ\text{C}$. The evaporated extracts of dichloromethane were passed through a Chromabond C18 column, using methanol as eluent, for removal of chlorophylls. After the solvent evaporation under vacuum at $35 \text{ }^\circ\text{C}$, the resulting extracts were stored at $-20 \text{ }^\circ\text{C}$ for further use.

2.2. Biological Assays

The impact of the extracts in insect cell viability was evaluated using a resazurin-based assay, after 24 h of incubation. The cells used were *Sf9* (*Spodoptera frugiperda*) and they were plated at a density of 3×10^4 cells/well.

2.3. Nanoencapsulation and Release Studies

For nanoencapsulation studies, liposomes were prepared using soybean lecithin [12] (Sternchemie), by ethanolic injection and thin film hydration, as previously described [13,14]. Four cycles of extrusion (Lipex™ Extruder, Northern Lipids, Burnaby, BC, Canada) through polycarbonate membranes (200 nm pore size) were carried out. Chitosan nanocapsules were obtained by ionic gelation, as reported by Chauhan et al. [15]. Hydrodynamic diameters were obtained by Dynamic Light Scattering (DLS), with five independent measurements for each sample.

Encapsulation efficiencies were determined from the absorbance vs. concentration calibration curves. Absorption spectra were measured in a Shimadzu UV-3600 Plus UV-vis-NIR spectrophotometer (Shimadzu Corporation, Kyoto, Japan). Three independent measurements were performed for each system. The extracts release from nanoencapsulation systems to phosphate buffer (pH = 7.3) was followed for 24 h. at room temperature.

3. Results and Discussion

3.1. Insecticidal Activity

The activity of *Phytolacca americana* L. dichloromethane extracts against *Sf9* cells has previously shown promising results for application as a biopesticide [8], and we revisited here for comparison purposes (Sample A). These studies were expanded to include the dichloromethane extract of *Mentha suaveolens* Ehrh (B) (Figure 1).

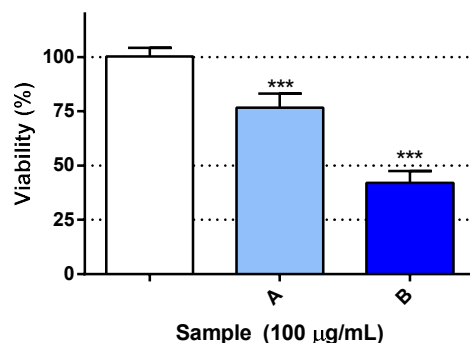


Figure 1. Viability of *Sf9* cells when exposed to *Phytolacca americana* (A) or *Mentha suaveolens* (B) dichloromethane extracts. *** $p < 0.001$.

As shown in Figure 1, both extracts were capable of eliciting loss of cell viability in the cells under study, when compared to control conditions. Among the two samples, B is more active, resulting in cell viability around 40%, while A resulted in ca. 75%.

3.2. Encapsulation Systems

Encapsulation assays in liposomes and chitosan nanocapsules were carried out. Chitosan has been reported for encapsulation of natural compounds and essential oils [9,15]. Regarding the lipid-based systems, ethanolic injection method has been described to be suitable for compounds with a very low solubility in water [13]. On the other hand, thin film hydration technique allows obtaining homogeneous liposomes after extrusion and is adequate for hydrophilic substances [14]. Table 1 shows the hydrodynamic diameters of the several nanosystems and the encapsulation efficiencies for both plant extracts. The nanosystems present sizes around or below 200 nm, with low standard deviation values.

Table 1. Hydrodynamic sizes of nanosystems and encapsulation efficiencies, EE(%) \pm SD (%), of plant extracts (SD: standard deviation).

Nanosystem	Hydrodynamic Diameter \pm SD (nm)	EE(%) \pm SD (%)	
		<i>Phytolacca americana</i> L.	<i>Mentha suaveolens</i> Ehrh.
Liposomes (ethanolic injection)	121 \pm 28	73.3 \pm 5 [8]	84.0 \pm 6
Liposomes (thin film hydration)	203 \pm 17	77.5 \pm 6 [8]	81.4 \pm 13
Chitosan nanocapsules	118 \pm 23	63 \pm 19	60.7 \pm 4

The encapsulation efficiencies are higher for liposomes than for chitosan nanocapsules. The lipid nanocarriers prepared by ethanolic injection present the highest encapsulation efficiency for *Mentha suaveolens* extract (EE(%) = 84%), while thin film hydration is better for encapsulation of *Phytolacca americana* L. extract. Nevertheless, the encapsulation efficiency in chitosan nanostructures is higher than 60% for both extracts.

Preliminary assays of extract release from the nanoencapsulation systems towards phosphate buffer (pH near 7) were performed and the release profiles are displayed in Figures 2 and 3. It can be observed that the release of encapsulated extracts from chitosan nanocapsules is faster and complete, while liposomes allow a more retarded release. Extension of the release assays to 40 h. in case of *Mentha suaveolens* encapsulated in liposomes prepared by ethanolic injection evidence that the release is continuous, attaining almost 70% at 40 h.

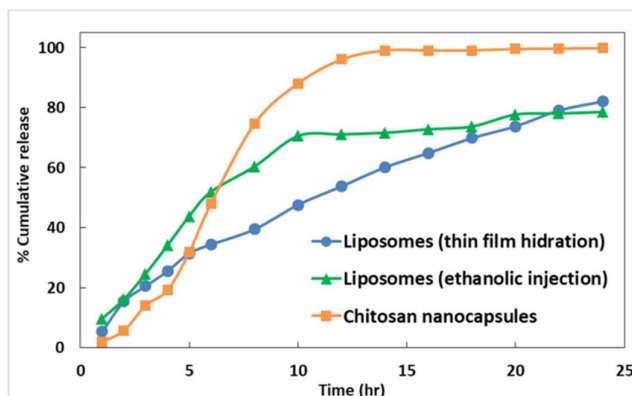


Figure 2. Release profiles of *Phytolacca americana* L. extract from liposomes and chitosan nanostructures, for 24 h.

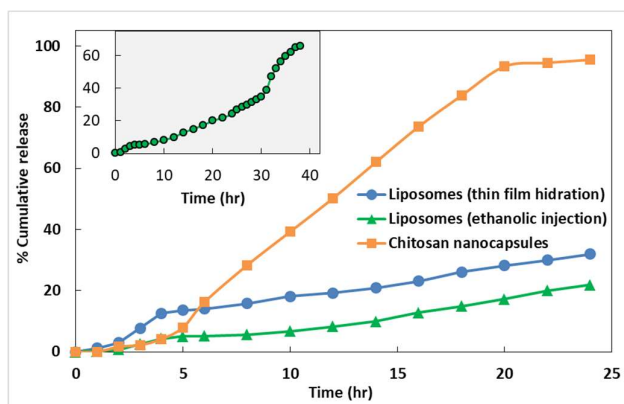


Figure 3. Release profiles of *Mentha suaveolens* Ehrh. extract from liposomes and chitosan nanostructures, for 24 h. Inset: Release profile from liposomes prepared by ethanolic injection up to 40 h.

4. Conclusions

Extracts of *Mentha suaveolens* Ehrh. and *Phytolacca americana* L. exhibit promising insecticidal activity against *Spodoptera frugiperda* cells. The extracts were successfully encapsulated in liposomes and chitosan nanocapsules. Liposomes prepared by ethanolic injection present the highest encapsulation efficiency for *Mentha suaveolens* extract (EE(%) = 84%), while for *Phytolacca americana* the thin film hydration method is advantageous. Chitosan nanocapsules display lower efficiencies of encapsulation and are suitable for a faster release of the extracts. These results anticipate a promising use of these plant extracts in biopesticide nanoformulations.

Funding: This research was funded by Foundation for Science and Technology (FCT) for financial support to the research centres CQ/UM (UIDB/00686/2020), CF-UM-UP (UIDB/04650/2020) and REQUIMTE (UIDB/50006/2020). Funding by FCT, COMPETE2020 and FEDER through project PTDC/ASP-AGR/30154/2017 (POCI-01-0145-FEDER-030154) is also acknowledged. 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.

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