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UV Irradiation's Influence on Fumitory Extract-Loaded Liposomes †

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Abstract: The aim of the present study was the characterization of fumitory extract-loaded liposomal vesicles after UV irradiation *via* the determination of the encapsulation efficiency, size, polydispersity index (PDI), zeta potential, mobility, and conductivity. The encapsulation efficiency was the same before and after UV irradiation (>69%). The particle size and PDI of the UV-irradiated liposomes with the fumitory extract were 294.2 ± 4.1 nm and 0.387 ± 0.011, respectively. The zeta potential after UV irradiation was −5.51 ± 0.4 mV. The mobility and conductivity of the obtained liposomal particles were −0.429 ± 0.012 μmcm/Vs and 0.468 ± 0.005 mS/cm, respectively. The results indicate the existence of nanoparticles and a non-uniform system, while a negative zeta potential value is related to the organization of phospholipids. Since UV irradiation did not cause significant changes in all of the mentioned parameters of the fumitory extract-loaded liposomes, it can be employed as a sterilization step in the preparation of liposomes.

Keywords: *Fumaria officinalis*; liposomes; particle size; UV irradiation; zeta potential

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1. Introduction

Fumaria officinalis L. (fumitory, Fumariaceae family) is a scrambling annual plant, distributed and cultivated throughout Europe, and represents smooth, slender, and branched stems of variable height (10–100 cm), with grey-green, feathery, and alternate leaves, lateral and terminal racemes of purplish pink, and a smoky appearance. The plant is a component of various phytotherapeutic formulations in European ethnobotany, used for hepatobiliary dysfunction, illnesses of the gastrointestinal and urogenital tracts, cancer, rheumatism, high blood pressure, and skin disorders [1,2]. The biological activities of the plant can be attributed to its high content of polyphenols and alkaloids. However, the application of the mentioned compounds is limited due to their poor water solubility, intestinal resorption, and low bioavailability. Liposomal vesicles, as non-toxic, biodegradable, and biocompatible carriers, can provide the controlled delivery of bioactive components, protection from degradation, and improved bioavailability. In addition, phospholipids from the liposomal membrane do not provoke a reaction with taste receptors, and, therefore, the liposomal bilayer is an appropriate carrier for obscuring the unpleasant taste of numerous polyphenols and alkaloids [3–5]. Moreover, liposomes can encapsulate lipophilic, hydrophilic, and amphiphilic compounds [6]. UV irradiation, as a sterilization method, is highly effective

against a wide range of bacterial strains. Therefore, the aim of the present study was the characterization of fumitory extract-loaded liposomal vesicles after UV irradiation via the determination of the encapsulation efficiency (EE), size, polydispersity index (PDI), zeta potential, mobility, and conductivity.

2. Materials and Methods

F. officinalis was from the Institute for Medicinal Plants Research "Dr Josif Pančić" (Serbia). The Simplicity UV® water purification system from Merck Millipore (Merck KGaA, Darmstadt, Germany) was used to prepare ultrapure water. Ethanol (used for extract preparation) was from Fisher Science (Leicestershire, UK), while soy phospholipids (used for liposome preparation) were from Lipoid (Ludwigshafen, Germany).

The extraction from the fumitory plant (grinded aerial part) was performed at room temperature in the incubator shaker KS 4000i control (IKA, Staufen, Germany) using 50% ethanol and a solvent-to-solid ratio of 30:1 mL/g for 60 min. The samples were filtered through filter paper.

Liposomes with fumitory extract were prepared using the proliposome method [7]. Ethanol fumitory extract (10 mL) and phospholipids (1 g) were stirred and heated to 60 °C for 15 min. After cooling, distilled water (20 mL) was added, and the mixture was stirred for 2 h at 800 rpm.

UV irradiation was performed in a laminar flow cabinet (AC2-4G8, ESCo, Singapore) for 20 min. Subsequently, measurements of EE, size, PDI, zeta potential, mobility, and conductivity were performed.

The free extract was removed from liposome dispersions by centrifugation at 17,500 rpm for 45 min at 4 °C in a Thermo Scientific Sorval WX Ultra series ultracentrifuge (Thermo Scientific, Waltham, MA, USA). The amount of extract in the supernatant was determined spectrophotometrically (UV Spectrophotometer UV-1800, Shimadzu, Japan). EE was calculated as the content of fumitory extract polyphenols encapsulated in liposomal particles divided by the content of extract polyphenols used for the preparation of the liposome bilayer: EE (%) = $(m_i - m_s)/m_i \times 100$. Here, m_i is the initial amount of polyphenols used for liposomal preparation, and m_s is the amount of polyphenols determined in the supernatant.

The size, PDI, zeta potential, mobility, and conductivity of the UV-irradiated liposomes were determined by photon correlation spectroscopy in a Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., Malvern, UK). Each sample was measured three times at room temperature.

3. Results and Discussion

In the present research, the influence of UV irradiation on the EE, size, PDI, zeta potential, mobility, and conductivity of the fumitory extract-loaded liposomes was investigated. The results are presented in Table 1. The EE did not change after UV irradiation and amounted to >69% (>73% for the non-treated parallel samples). The factors affecting the encapsulation efficiency in the liposomes are various, including the liposome composition, procedure, drug characteristics, solvent properties, pH, ionic strength, processing conditions, etc. [8,9]. The vesicle size and PDI of the UV-irradiated liposomes with the fumitory extract were 294.2 ± 4.1 nm and 0.387 ± 0.011, respectively. The size and PDI of the non-treated samples were 274.0 nm and 0.307 [8], respectively. The zeta potential after UV irradiation was low and amounted to −5.51 ± 0.40 mV. The mobility and conductivity of the UV-irradiated fumitory extract-loaded liposomes were −0.429 ± 0.012 μmcm/Vs and 0.468 ± 0.005 mS/cm, respectively. The zeta potential, mobility, and conductivity of the non-treated parallels were −6.34 ± 0.71 mV, −0.491 ± 0.011 μmcm/Vs, and 0.465 ± 0.009 mS/cm, respectively.

Table 1. Encapsulation efficiency, size, polydispersity index, zeta potential, mobility, and conductivity of the fumitory extract-loaded liposomes.

Sample	EE (%)	Size (nm)	PDI	Zeta Potential (mV)	Mobility (μmcm/Vs)	Conductivity (mS/cm)
Non-treated liposomes	73.1 ± 2.3 [a] *	274.0 ± 10.5 [a]	0.387 ± 0.041 [a]	−6.34 ± 0.71 [a]	−0.491 ± 0.011 [a]	0.465 ± 0.009 [a]
UV-irradiated liposomes	69.0 ± 1.8 [a]	294.2 ± 9.1 [a]	0.317 ± 0.036 [a]	−5.51 ± 0.40 [a]	−0.429 ± 0.012 [a]	0.468 ± 0.005 [a]

* The same letter indicates the absence of differences that are considered statistically significant (analysis of variance, i.e., one-way ANOVA and Duncan's post hoc test; $p < 0.05$, n = 3).

Since the liposomes contain only phospholipids (without sterols), the liposomal bilayer was more rigid [9], preventing the leakage of the encapsulated compounds, and thus improving the EE. The measured particle size value agrees with those reported in the literature, where phospholipid liposomes containing plant extracts can have diameters over 200 nm [10–12]. The obtained PDI value indicates the existence of a non-uniform system. A negative zeta potential value is related to the organization of phospholipids, whereas a low value suggests that the liposomal suspension is not electrostatically stabilized. The mobility of the liposomal particles represents a function of the size, total charge, and composition of the liposomal membrane. At the same time, the fluidity/rigidity and deformable properties of the liposomes also significantly impact their mobility [13]. In the case of the fumitory extract-loaded liposomes, a higher amount of the added extract resulted in a higher conductivity value. Namely, the measurement of conductivity values can be used for further simple assays of the concentrations of bioactives in release experiments [14]. Additionally, the enhancement in the conductivity of liposomal suspension can be associated with the leakage of the entrapped bioactives, and since the conductivity correlates with the concentration of the plant extract, the encapsulation efficiency can be indirectly examined without time-consuming and expensive analytical procedures [15].

4. Conclusions

In the present study, fumitory extract-loaded liposomes were developed, UV-irradiated, and characterized in terms of their encapsulation efficiency, size, PDI, zeta potential, mobility, and conductivity. The results indicate the existence of nanoparticles and a non-uniform system with very low values of zeta potential and mobility. The beneficial effects of bioactive principles from *F. officinalis* on human health highlight the application of liposomes as carriers for its extracts and their potential implementation in food, functional food, pharmaceuticals, and cosmetic formulations. Since UV irradiation did not cause significant changes in the measured parameters of fumitory-loaded liposomes, it can be employed as a sterilization step in the preparation of liposomes. However, future studies should be directed toward the improvement of liposomal stability.

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