UDK 66:54(05) CODEN HMIDA 8 ISSN 0367–598X

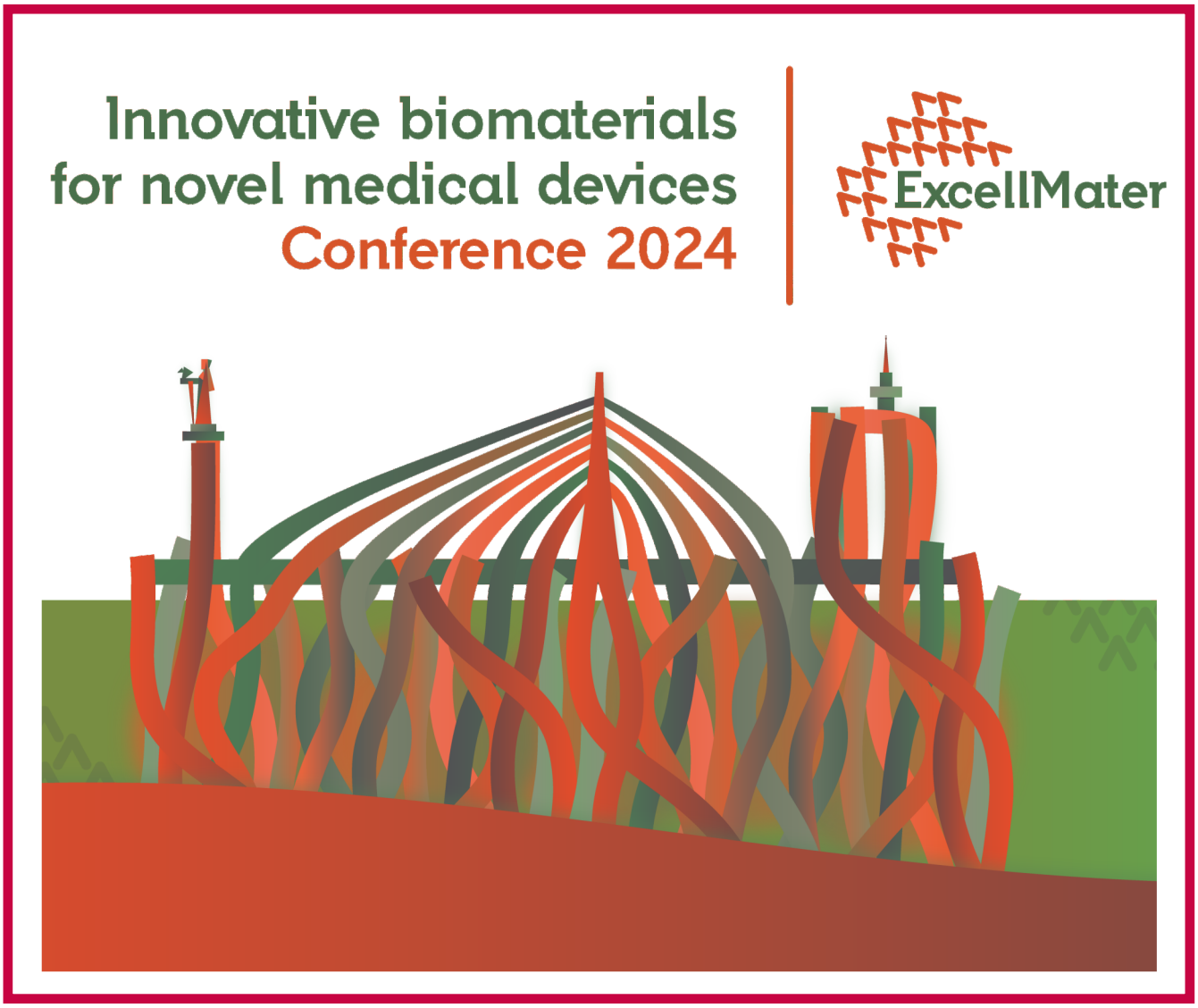
# 1s Hemijska industrija

Vol. 78

Časopis Saveza hemijskih inženjera Srbije

# Chemical Industry

**Supplementary Issue**
**ExcellMater Conference 2024 Abstracts**




BEOGRAD, MART 2024.

HMIDA 8, 78(1s) 1-79 (2024)

Chemical Industry
Химическая промышленность

# Hemijska industrija

Časopis Saveza hemijskih inženjera Srbije
Journal of the Association of Chemical Engineers of Serbia
Журнал Союза химических инженеров Сербии

**VOL. 78** **Beograd, mart 2024.** **Broj 1s**

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# Characterization of *Vaccinium myrtillus* leaf extract-loaded liposomes

**Muna Rajab Elferjane[1,2]*, Milena Milošević[3], Vojislav Ćirić[4,5], Petar Batinić[6], Branko Bugarski[2], Aleksandar Marinković[2] and Aleksandra A. Jovanović[7]**

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*Keywords:* Bilberry; encapsulation efficiency; liposomal particles; polydispersity index; size; stability

*Hem. Ind.* **78(1S)** 65 (2024)

*Available on-line at the Journal web address: http://www.ache.org.rs/HI/*

INTRODUCTION: *Vaccinium myrtillus* L. leaves contain bioactive components, such as polyphenols, stilbenes, iridoid glycosides, fatty acids, and fibers [1]. However, polyphenols possess low solubility, stability, and bioavailability, thus the encapsulation of the mentioned active principles in different carriers is necessary [2]. Liposomes are widely used as a carrier for the encapsulation, preservation, and controlled release of polyphenols in various products [3]. Therefore, the aims of the presented research are the development and characterization of *V. myrillus* leaf extract-loaded liposomes *via* the determination of encapsulation efficiency, particle size, polydispersity index (PDI), zeta potential, and mobility.

EXPERIMENTAL: The liposomes with encapsulated extract were prepared employing the proliposome procedure [3]. Encapsulation efficiency was indirectly calculated by the polyphenol concentration determined in the supernatant. Particle size, PDI, zeta potential, and mobility were measured by the photon correlation spectroscopy in Zetasizer. Every measurement was examined three times at 25°C.

RESULTS AND DISCUSSION: The encapsulation efficiency of polyphenols was >85 %. The liposomes contained only phospholipids resulting in a more rigid membrane [4] providing the prevention of the leakage of the encapsulated polyphenols, as well as a higher encapsulation efficiency. The diameter and PDI of the liposomes were 5408.7±56.4 and 0.249±0.049 nm, respectively confirming that higher liposomal vesicles possessed lower PDI values [4]. The zeta potential and mobility were -5.02±0.25 mV and -0.315±0.016 µmcm/Vs, respectively. Zeta potential possessed negative values that are related to the exposure of the phosphate group lying in an outer plane concerning the choline groups [4]. The mobility of liposomes represents a function of the size, zeta potential, and lipid composition. The liposomal vesicles with lower membrane fluidity also show low mobility. The changes in the mobility of the liposomes were attributed to the membrane fluidity and ability to deform. Additionally, when flavonoids (also presented in *V. myrtillus* extract) are adsorbed at the surface of the liposomes, it can decrease their mobility [5].

CONCLUSIONS: The beneficial effects of bioactive principles from *V. myrtillus* leaves on human health and their sensitivity highlight the application of liposomal particles as a carrier for *V. myrtillus* extract and their potential implementation in foods, functional foods, pharmaceutics, and cosmetics.

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# Stability of liposomal particles with encapsulated coumarin derivate

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*Keywords:* Liposomes; mobility; polydispersity index; vesicle size; zeta potential

*Hem. Ind.* **78(1S)** 66 (2024)

*Available on-line at the Journal web address: http://www.ache.org.rs/HI/*

INTRODUCTION: Coumarin derivates possess several biological effects, including anti-inflammatory, antioxidant, anticoagulant, antitumor, insecticidal, anthelminthic, hypnotic, antifungal, and HIV protease inhibition properties [1,2]. Due to their solubility in organic solvents and insolubility in water and body fluids, their bioavailability is significantly low. Thus, they can be encapsulated into liposomal particles with the aim of overcoming the mentioned disadvantage [3]. Hence, in the present study, coumarin derivate was encapsulated in phospholipid liposomes and their stability was monitored for 60 days in terms of vesicle size, polydispersity index (PDI), zeta potential, and mobility.

EXPERIMENTAL: Coumarin derivate-loaded liposomes were prepared by mixing 0.1 g of coumarin derivate, 1 mL of dimethyl sulfoxide, 2 mL ethanol, 1 g of phospholipids, and 7.5 mL of water in the proliposome technique [4]. Vesicle size, PDI, zeta potential, and mobility were determined during 60 days of storage at 4°C using the photon correlation spectroscopy and Zetasizer Nano Series, Nano ZS (Malvern Instruments, United Kingdom). Every measurement was performed in triplicates at room temperature. The statistical analysis was performed by using the analysis of variance and Duncan's *post hoc* test (STATISTICA 7.0). The differences were considered statistically significant at $p<0.05$, $n=3$.

RESULTS AND DISCUSSION: The vesicle size varied from 1669.0±55.1 (1$^{st}$ day) to 1583.5±78.8 nm (60$^{th}$ day). Due to a relatively high absolute value of zeta potential at the beginning, the absence of a significant change in the size was expected. PDI value, as a measure of the particle size distribution, significantly increased during the 60-day study, from 0.231±0.043 to 0.497±0.079 indicating the existence of a non-uniform system [5]. A single phospholipid provides the liposomal population with significantly lower PDI (better uniformity) in comparison to the commercial phospholipid mixture employed in the present study [3]. The zeta potential and mobility significantly decreased (absolute value), from -29.43±0.55 to -14.43±1.00 mV, and from -2.307±0.043 to -1.127±0.086 μmcm/Vs, respectively. After 60 days of storage, the liposomes had significantly higher PDI, but lower zeta potential and mobility, indicating their instability. However, even though the zeta potential value was low on the 60$^{th}$ day, there was no occurrence of fusion and fission confirmed by the absence of statistically significant changes in the diameter of liposomes.

CONCLUSIONS: Coumarin derivate-loaded liposomes were unstable during 60-day storage at 4°C, resulting in changes in PDI value, zeta potential, and mobility, therefore additional experiments for improving their stability should be performed.

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