



University of Belgrade, Technical Faculty in Bor



ECOENTER

**30th International Conference Ecological Truth
& Environmental Research
2023**

Proceedings

**Editor
Prof. Dr Snežana Šerbula**





University of Belgrade, Technical Faculty in Bor



ECOTER

30th International Conference Ecological Truth
& Environmental Research
2023

Proceedings

Editor
Prof. Dr Snežana Šerbula



PROCEEDINGS

30th INTERNATIONAL CONFERENCE

ECOLOGICAL TRUTH AND ENVIRONMENTAL RESEARCH – EcoTER'23

Editor:

Prof. Dr Snežana Šerbula

University of Belgrade, Technical Faculty in Bor

Editor of Student section:

Prof. Dr Maja Nujkić

University of Belgrade, Technical Faculty in Bor

Technical editors:

Jelena Milosavljević, PhD, University of Belgrade, Technical Faculty in Bor

Asst. prof. Dr Ana Radojević, University of Belgrade, Technical Faculty in Bor

Sonja Stanković, MSc, University of Belgrade, Technical Faculty in Bor

Cover design:

Aleksandar Cvetković, BSc, University of Belgrade, Technical Faculty in Bor

Publisher: University of Belgrade, Technical Faculty in Bor

For the publisher: Prof. Dr Dejan Tanikić, Dean

Printed: University of Belgrade, Technical Faculty in Bor, 100 copies, electronic edition

Year of publication: 2023

This work is available under the Creative Commons Attribution-NonCommercial-NoDerivs licence (**CC BY-NC-ND**)

ISBN 978-86-6305-137-9

CIP - Каталогizacija u publikaciji
Narodna biblioteka Srbije, Beograd

502/504(082)(0.034.2)

574(082)(0.034.2)

INTERNATIONAL Conference Ecological Truth & Environmental Research (30 ; 2023)

Proceedings [Elektronski izvor] / 30th International Conference Ecological Truth & Environmental Research - EcoTER'23, 20-23 June 2023, Serbia ; organized by University of Belgrade, Technical faculty in Bor (Serbia) ; co-organizers University of Banja Luka, Faculty of Technology – Banja Luka (B&H) ... [et al.] ; [editor Snežana Šerbula]. - Bor : University of Belgrade, Technical faculty, 2023 (Bor : University of Belgrade, Technical faculty). - 1 elektronski optički disk (CD-ROM) ; 12 cm

Sistemski zahtevi: Nisu navedeni. - Nasl. sa naslovne strane dokumenta. - Preface / Snežana Šerbula. - Tiraž 100. - Bibliografija uz svaki rad.

ISBN 978-86-6305-137-9

а) Животна средина -- Зборници б) Екологија – Зборници

COBISS.SR-ID 118723849



**30th International Conference
Ecological Truth and Environmental Research – EcoTER'23**

is organized by:

**UNIVERSITY OF BELGRADE
TECHNICAL FACULTY IN BOR (SERBIA)**

Co-organizers of the Conference:

**University of Banja Luka, Faculty of Technology,
Banja Luka (B&H)**

**University of Montenegro, Faculty of Metallurgy and Technology,
Podgorica (Montenegro)**

University of Zagreb, Faculty of Metallurgy, Sisak (Croatia)

**University of Pristina, Faculty of Technical Sciences,
Kosovska Mitrovica**

Association of Young Researchers Bor (Serbia)

Prof. Dr Fernando Carrillo-Navarrete
(Spain)

Prof. Dr Pablo L. Higuera
(Spain)

Prof. Dr Mustafa Cetin
(Turkey)

Prof. Dr Mauro Masiol
(Italy)

Prof. Dr George Z. Kyzas
(Greece)

Prof. Dr Mustafa Imamoğlu
(Turkey)

Prof. Dr Petr Solzhenkin
(Russia)

Prof. Dr Dejan Tanikić
(Serbia)

Prof. Dr Milan Trumić
(Serbia)

Dr Jasmina Stevanović
(Serbia)

Dr Dragana Randelović
(Serbia)

Dr Viša Tasić
(Serbia)

Dr Ljiljana Avramović
(Serbia)

Dr Stefan Đorđievski
(Serbia)

PHOSPHOLIPID LIPOSOMES AS A CARRIER FOR *Aloe vera* WASTE EXTRACT

Aleksandra A. Jovanović^{1*}, Muna Rajab Elferjane^{2,3}, Marija Gnjatović¹,
Branko Bugarski², Aleksandar Marinković²

¹University of Belgrade, Institute for the Application of Nuclear Energy INEP, Banatska 31b,
11080 Zemun Belgrade, SERBIA

²University of Belgrade, Faculty of Technology and Metallurgy, Karnegijeva 4,
11000 Belgrade, SERBIA

³University of Misurata, Faculty of Nursing and Health Sciences, Misurata, LIBYA

*ajovanovic@inep.co.rs

Abstract

Aloe vera L. leaf waste contains biologically active compounds, including polyphenols, anthraglycosides, free anthraquinones, resins, mono- and polysaccharides, polypeptides, terpenoids, sterols, chromones, lectins, fatty, amino and organic acids, enzymes, saponins, vitamins, and minerals. However, the application of the mentioned bioactive components is rather limited because of their low solubility, stability, integrity, permeability, and consequently bioavailability. Thus, the encapsulation of the active principles of *A. vera* into liposomes can be advantageous. In the present study, *A. vera* waste extract-loaded liposomes were developed and characterized via the determination of particle size, polydispersity index (PDI), zeta potential, conductivity, mobility, storage stability, density, surface tension, and viscosity. The particle size of liposomes with *A. vera* extract amounted to 335.0 ± 20.5 nm, while the PDI was 0.505 ± 0.056 . The zeta potential, conductivity, and mobility were 1.81 ± 0.32 mV, 0.485 ± 0.013 mS/cm, and 0.142 ± 0.026 $\mu\text{mcm/Vs}$, respectively. The vesicle size and PDI changed during 21 days of storage. The zeta potential, conductivity, and mobility did not vary in the liposomal suspension during the stability study. The density, surface tension, and viscosity were 0.991 ± 0.003 g/mL, 22.8 ± 0.2 mN/m, and 22.9 ± 0.1 mPa·s, respectively. The beneficial effects of bioactive principles from *A. vera* on human health highlight the application of liposomes as a carrier for *A. vera* leaf waste extract and their potential implementation in food, functional food, pharmaceutical, and cosmetic formulations.

Keywords: *Aloe vera*, encapsulation, extract, liposomes, waste.

INTRODUCTION

Aloe vera L. (Liliaceae) and its leaf waste (plant material without aloe gel) contains biologically active compounds, including polyphenols (flavonoids, tannins, and phenolic acids), anthraglycosides, free anthraquinones, resins, mono- and polysaccharides, polypeptides, terpenoids, sterols, chromones, lectins, fatty, amino and organic acids, enzymes, saponins, vitamins, and minerals [1,2]. Nevertheless, the application of the mentioned bioactive compounds is rather limited because of their low solubility, stability, integrity, permeability, and consequently bioavailability [3]. Therefore, the encapsulation of the active principles of *A. vera* leaf waste can be advantageous. Liposomes, micro- or nanovesicles usually constituted by phospholipids (but different kinds of other lipids can be used), can be

used for encapsulation of various hydrophilic, lipophilic, or amphiphilic compounds [4,5]. Liposomes have been widely used due to their high structural integrity, stability during storage, and controlled release capability. Additionally, they are easy to prepare and readily functionalized for active targeted delivery. Also, liposomal particles have long been perceived as the ideal drug delivery vehicles because of their superior biocompatibility. Namely, a liposomal bilayer is an analog of a biological membrane and can be prepared from both natural and synthetic phospholipids [6].

In the present study, phospholipid liposomes with *A. vera* leaf waste extract were developed and characterized via particle size, polydispersity index (PDI), zeta potential, conductivity, mobility, storage stability, density, surface tension, and viscosity.

MATERIALS AND METHODS

Plant material and reagents

A. vera leaf waste was purchased in ASC Garden d.o.o., Belgrade, Serbia. The aloe gel was removed from the leaves. Subsequently, clean and empty leaves that represent the waste were cut and freeze-dried in Beta 2-8 LD plus (Christ, Germany). Ethanol (Merck, Germany) and Phospholipon 90 G (unsaturated diacyl-phosphatidylcholine) (Lipoid GmbH, Germany) were also used. Distilled water was purified through a Simplicity UV[®] water purification system (Merck Millipore, Merck KGaA, Germany).

Extraction procedure

Heat-assisted extraction of bioactive principles from *A. vera* leaf waste was performed at 60°C using the incubator shaker KS 4000i control (IKA, Germany) at a solid-to-solvent ratio of 1:25 g/mL, 50% ethanol, as an extraction medium for 30 min. The extract was prepared in the Erlenmeyer flasks covered by aluminum foil to avoid light exposure and evaporation of solvent. After the extraction, the sample was filtered using filter paper and stored at 4°C until further experiments.

Preparation of extract-loaded liposomes

Phospholipid liposomes containing *A. vera* leaf waste extract were obtained using the proliposome method and a mixture of phospholipids (Phospholipon) [7]. Phospholipids (4 g) and ethanol *A. vera* extract (50 mL) were stirred at 50°C with the aim to homogenize a mixture and evaporate ethanol. After cooling to 25°C, ultrapure water (20 mL) was added and the formulation was stirred at 800 rpm for 1 h.

Determination of particle size, PDI, zeta potential, conductivity, and mobility

The measurements of particle size, PDI, zeta potential, conductivity, and mobility were performed using photon correlation spectroscopy in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). The measurement was repeated on the 1st, 3rd, 5th, 7th, 14th, and 21st days after the liposomal preparation with the aim to monitor their stability at 4°C. Each sample was diluted 500 times and measured in triplicate at room temperature.

Measurement of density, surface tension, and viscosity

The density and surface tension of the extract-loaded liposomes were determined using Force Tensiometer K20 (Kruss, Germany). The sample (20 mL) was examined three times at room temperature.

The viscosity of the sample was examined using Rotavisc lo-vi device (IKA, Germany). The sample (6.7 mL) was examined three times at room temperature.

Statistical analysis

The statistical analysis of the results obtained in storage stability study was done by using analysis of variance (one-way ANOVA) and Duncan's *post hoc* test in STATISTICA 7.0. The differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

A. vera extract-loaded liposomes was developed and particle size, PDI, zeta potential, conductivity, mobility, density, surface tension, and viscosity of the sample were determined. The results are presented in Table 1 (the values measured after the liposomal preparation). In addition, storage stability study was performed and the results are shown in the graphs of Figure 1.

Table 1 Particle size, polydispersity index (PDI), zeta potential, conductivity, and mobility of phospholipid liposomes with *Aloe vera* leaf waste extract measured immediately after the liposomal preparation

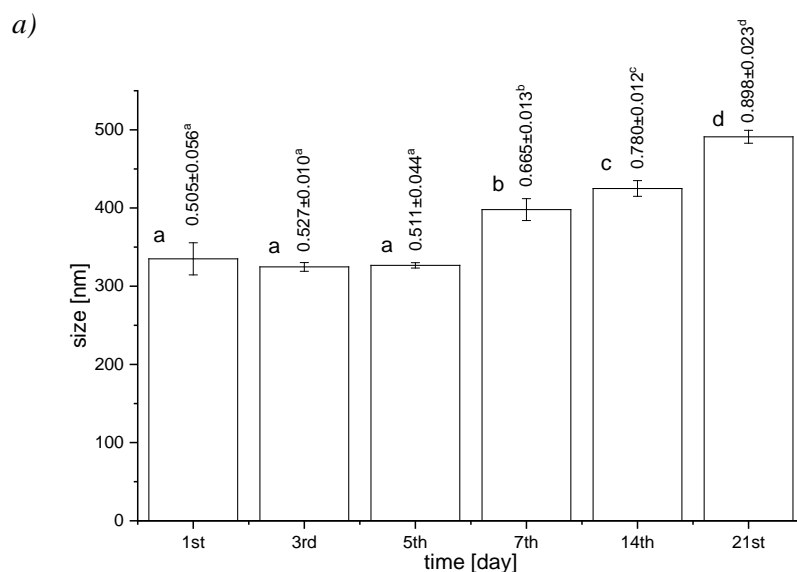
Variables	Liposomes with extract
Particle size [nm]	335.0±20.5
PDI	0.505±0.056
Zeta potential [mV]	1.81±0.32
Conductivity [mS/cm]	0.485±0.013
Mobility [$\mu\text{mcm/Vs}$]	0.142±0.026
Density [g/mL]	0.991±0.003
Surface tension [mN/m]	22.8±0.2
Viscosity [mPa·s]	22.9±0.1

As can be seen in Table 1, the particle size of extract-loaded liposomes was 335.0 ± 20.5 nm, whereas the PDI, as a measure of particle size distribution in the liposomal formulation, was 0.505 ± 0.056 . The measured values of vesicle size are in agreement with the literature, where phospholipid liposomes had a diameter of ~ 400 nm [8]. The particle size of liposomes is significantly affected by the type of lipids, the method used for the liposomal formulation, as well as the physicochemical properties of the encapsulated molecules [7,9]. The obtained PDI values indicate the existence of a non-uniform system (0.505 ± 0.056). The zeta potential, conductivity, and mobility were 1.81 ± 0.32 mV, 0.485 ± 0.013 mS/cm, and 0.142 ± 0.026 $\mu\text{mcm/Vs}$, respectively. The zeta potential was very low indicating the presence

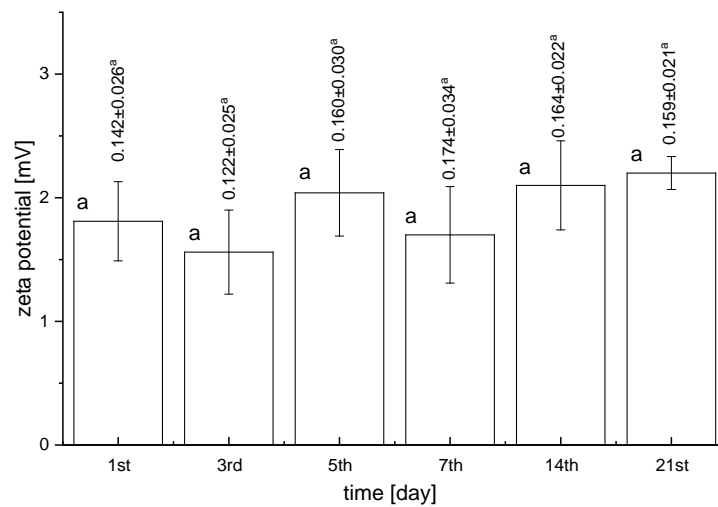
of a very unstable system. According to Lidgate *et al.* [10], a lower capture volume corresponds to an increase in conductivity. Namely, a lower amount of ions are removed as liposome capture volume decreased with decreasing lipid concentration, thus conductivity of the liposome dispersions increased as the lipid concentration decreased. Also, conductivity values increase in the case of leakage of encapsulated compounds [10]. In the case of *A. vera* extract-loaded liposomes, a higher amount of the added extract resulted in a higher value of conductivity. According to the literature, the mobility of liposomal particles represents a function of particle size, total charge, and composition of the liposomal membrane [11]. Additionally, some liposomes are fluid, flexible, and deformable, whereas others are rigid that depend on the bilayer composition, as well as entrapped components. Their fluidity/rigidity and deformable properties also significantly impact mobility [12,13]. The density, surface tension, and viscosity of the liposomes were 0.991 ± 0.003 g/mL, 22.8 ± 0.2 mN/m, and 22.9 ± 0.1 mPa·s, respectively (Table 1). A higher viscosity of the liposomes is an indication of smaller vesicle size [14] which was the case with the obtained *A. vera* extract-loaded liposomes.

In order to examine the stability of phospholipid liposomes with *A. vera* leaf waste extract, particle size, PDI, zeta potential, conductivity, and mobility were measured during 21 days of storage at 4°C (Figure 1).

As can be seen from Figure 1a, the particle size and PDI did not change in the first 5 days of storage, whereas a slight increase appeared after the 7th day and continued to increase up to the 21st day. The obtained results were expected due to very low values of zeta potential and a high probability of particle fusion. The zeta potential and mobility did not vary and kept low values in the liposomal suspension during the stability study (Figure 1b). Considering that there was no increase in the conductivity of the liposomes (Figure 1c), it can be concluded that there was no leakage of encapsulated compounds from the liposomal bilayer.



b)



c)

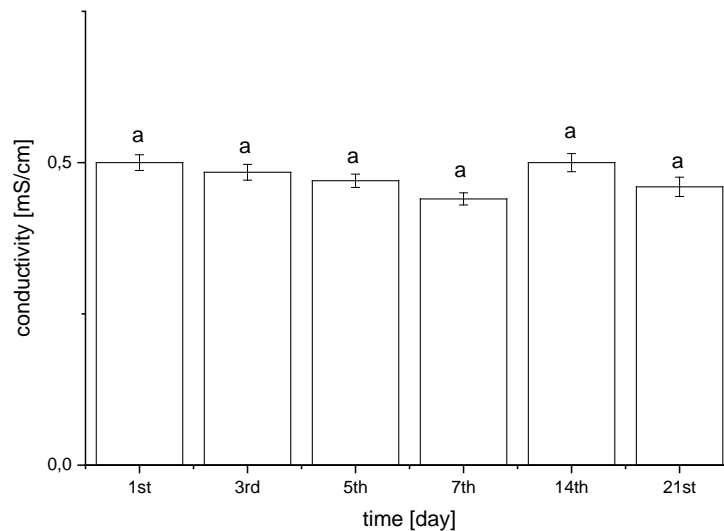


Figure 1 a) Particle size – bars and polydispersity index – numbers above bars, b) zeta potential – bars and mobility – numbers above bars ($\mu\text{mcm/Vs}$), and c) conductivity of phospholipid liposomes with Aloe vera leaf waste extract for 21 days; values with different letters (a–c) showed statistically significant differences ($p < 0.05$; $n = 3$; analysis of variance, Duncan's post-hoc test)

CONCLUSION

In the present study, *A. vera* waste extract-loaded liposomes were developed and characterized in terms of physical properties. The results indicate the existence of nanoparticles and a non-uniform system with very low values of zeta potential and mobility. The results of the stability study showed that particle size and PDI values increased during storage, while zeta potential, conductivity, and mobility did not change. The beneficial effects of bioactive principles from *A. vera* on human health highlight the application of liposomes as a carrier for *A. vera* leaf waste extract and their potential implementation in food, functional

food, pharmaceutical, and cosmetic formulations. However, future experiments and perspectives should be directed toward the improvement of liposomal stability.

ACKNOWLEDGEMENT

The authors acknowledge their gratitude to the Ministry of Education, Technological Development and Innovation of Serbia, contract numbers 451-03-47/2023-01/200019 and 451-03-47/2023-01/200135.

REFERENCES

- [1] Khan I. A., Abourashed E. A. Leung's encyclopedia of common natural ingredients: used in food, drugs and cosmetics, John Wiley & Sons, New York (2011), p.25, ISBN: 978-0-471-46743-4.
- [2] Heś M., Dziedzic K., Górecka D., *et al.*, Plant Foods Hum. Nutr. 74 (2019) 255–265.
- [3] Fang Z., Bhandari B. Trends Food Sci. Technol. 21 (2010) 510–523.
- [4] Ribeiro H., Schuchmann H., Engel R., *et al.*, Encapsulation of carotenoids in Encapsulation technologies for active food ingredients and food processing, Editors: Zuidam N. J. and Nedović V., Springer, New York (2010), 211–252, ISBN: 978-1-4419-1007-3.
- [5] Ricci, M., R. Oliva, Del Vecchio P., *et al.*, Biochim. Biophys. Acta. 1858 (2016) 3024–3031.
- [6] Hadinoto K., Sundaresan A., Cheow W. S. Eur. J. Pharm. Biopharm. 85 (2013) 427–443.
- [7] Isailović B., Kostić I., Zvonar A., *et al.*, Innov. Food Sci. Emerg. Technol. 19 (2013) 181–189.
- [8] Jovanović A., Petrović P., Čujić D., *et al.*, Proceedings of the VIII International Congress “Engineering, Environment and Materials in Process Industry“, 20–23 March, Jahorina, Bosnia and Herzegovina (2023).
- [9] Jovanović A., Balanč B., Djordjević V., *et al.*, Colloids Surf. B. 183 (2019) 110422.
- [10] Lidgate D., Hegde S., Maskiewicz R. Int. J. Pharm. 96 (1993) 51–58.
- [11] Duffy C., Gafoor S., Richards D., *et al.*, Anal. Chem. 73 (2001) 1855–1861.
- [12] Pysher M., Hayes M. Langmuir 20 (2004) 4369–4375.
- [13] Jovanović A., Balanč B., Ota A., *et al.*, Eur. J. Lipid Sci. Technol. 120 (2018) 1–41.
- [14] Shashidhar G. M., Manohar B. RSC Adv. 8 (2018) 34634–34649.