DOI: 10.7251/JEPM2202040j
UDC: 582.475:615.2.022

Journal of
Engineering & Processing Management

ORIGINAL PAPER

THE CHARACTERIZATION OF SILYMARIN AND SILIBININ LIPOSOMES

Aleksandra A. Jovanović[1] | Svetlana Dinić[2] | Aleksandra Uskoković[2] | Jelena Arambašić Jovanović[2] | Nevena Grdović[2] | Melita Vidaković[2] | Mirjana Mihailović[2]

[1]University of Belgrade, Innovation Centre of Faculty of Technology and Metallurgy, Belgrade, Serbia

[2]University of Belgrade, Institute for Biological Research "Siniša Stanković", Belgrade, Serbia

Correspondence
Aleksandra A. Jovanović, University of Belgrade, Innovation Centre of Faculty of Technology and Metallurgy, Belgrade, Serbia
Email: acancarevic@tmf.bg.ac.rs

Abstract

The aim of the present study was the characterization of silymarin and silibinin liposomes *via* determination of encapsulation efficiency, particle size, polydispersity index (PDI), zeta potential, mobility, and conductivity, as well as storage stability during 28 days at 4°C and stability after UV irradiation. Encapsulation efficiencies of silymarin and silibinin were 92.05±1.41% and 87.86±2.06%, respectively. Particle size and PDI of the liposomes with silymarin were changed from 3541.3±62.5 nm to 2677.0±44.2 nm and from 0.346±0.044 to 0.228±0.036, respectively, during the 28-day stability study; particle size and PDI of the liposomes with silibinin were changed from 2074.7±19.4 nm to 2704.0±35.0 nm and from 0.328±0.030 to 0.456±0.026, respectively. The Zeta potential of the silymarin-loaded liposomes and silibinin-loaded liposomes was changed from -27.0±0.7 mV to -26.4±0.4 mV and from -29.4±0.6 mV to -29.0±0.4 mV, respectively. Mobility and conductivity of the liposomes with silymarin were changed from -2.120±0.057 μmcm/Vs to -2.067±0.028 μmcm/Vs and from 0.017±0.005 mS/cm to 0.009±0.004 mS/cm, respectively. Mobility and conductivity of the liposomal particles with silibinin were changed from -2.307±0.053 μmcm/Vs to -2.110±0.033 μmcm/Vs and from 0.018±0.003 mS/cm to 0.060±0.001 mS/cm, respectively. UV irradiation did not affect particle size and PDI of all liposomes, but it caused a decrease in zeta potential: -23.9±0.8 mV for silymarin and -24.5±0.7 mV for silibinin, in mobility: -1.874±0.064 μmcm/Vs for silymarin and -1.920±0.057 μmcm/Vs for silibinin, and in conductivity: 0.014±0.001 mS/cm for silymarin and 0.007±0.003 mS/cm for silibinin. Overall, the obtained results qualify liposomes to be used as silymarin and silibinin carriers for application in functional foods and pharmaceutical products.

Keywords: silymarin, silibinin, liposomes, characterization.

1. INTRODUCTION

Silymarin represents the group of bioactive polyphenol compounds from milk thistle (*Silybum marianum*) and contains silibinin, isosilybin, silydianin, and silychristand. Silibinin is the most prevalent component (Zhang et al. 2022). According to previous studies, these polyphenolic compounds exhibit various biological activities that may promote human health and well-being, such as antioxidant, antimicrobial, anti-inflammatory(Zhang et al. 2022), antiviral (Liu, Jassey, Hsu, & Lin 2019), immunomodulatory (Zhao & Li 2015), and anticancer properties (Ahmad et al. 2017). Nevertheless, silymarin and silibinin are quite sensitive to temperature, light, and oxidation and have poor water solubility and low bioavailability; thus, their application in food, pharmaceutical, and cosmetic formulation is limited (Zhang et al. 2022). Silibinin, the flavonolignan, is the major active constituent of silymarin, a standardized extract of the milk thistle seeds mentioned above (Verdura et al. 2021; Zhang et al. 2022). Silibinin has been used traditionally as a chemopreventive and therapeutic agent in human lung cancer (Verdura et al. 2021). Song et al. (2022) sug-

gest that silibinin has hepatoprotective activity through the protection of liver cells against toxins. According to the literature, silibinin can also inhibit amyloid beta aggregation by affecting the human islet amyloid polypeptide (García-Viñuales et al. 2022). However, its application is limited due to poor water solubility, limited intestinal resorption, and consequently low bioavailability (Mohammadi, Ariafar, Talebi-Ghane, & Afzali 2022). With the aim to overcome the disadvantages of bioactive components, numerous encapsulation techniques have been established (Jovanović et al. 2018; Kalušević et al. 2022; Zhang et al. 2022). The emphasis is on the protection of the target substances, the increase of oral or transdermal bioavailability, as well as the controlled release of the active molecules (Jovanović et al. 2018). Furthermore, the utilization of various carriers to improve the water dispersibility, chemical stability, and bioavailability of silymarin and silibinin, and consequently increase its implementation within functional foods, supplements, pharmaceuticals, and cosmetics is examined (Zhang et al. 2022). Liposomes are non-toxic, biodegradable, and biocompatible lipid micro- or nano-carriers with one or more phospholipid bilayers. Liposomes, as lipophilic and hydrophilic drug delivery systems, can provide controlled delivery of bioactive components, as well as their protection from degradation caused by light, oxygen, UV irradiation different pH values, and enzymes (Jovanović et al. 2019; Zhang et al. 2022). The main advantage of the mentioned encapsulation technology is the stability that liposomes provide in various food, pharmaceutical, and cosmetic products with a high amount of water (Isailović et al. 2013). In addition, lipids from the liposomes do not provoke a reaction with taste receptors, and, therefore, the liposomal bilayer is an appropriate carrier for covering the unpleasant taste of numerous polyphenols (Jovanović et al. 2019). Liposomes can be produced using the common thin film hydration method or proliposome method (Isailović et al. 2013; Jovanović et al. 2019). The common thin film hydration procedure is considered unsuitable for producing liposomes on an industrial scale, whereas the proliposome technique may be suitable for liposome production on a large scale (Isailović et al. 2013). Therefore, in the present research, silymarin- and silibinin-loaded liposomes were prepared using proliposome procedure and analyzed in terms of encapsulation efficiency, particle size, polydispersity index (PDI), zeta potential, mobility, and conductivity, as well as storage stability during 28 days at 4ºC and stability after UV irradiation.

2. MATERIALS AND METHODS

2.1. Materials

Phospholipon 90G (purified phosphatidylcholine from soybean, content ≥ 94.0%, granulated) was supplied by Natterman Phospholipids (Germany). The following reagent and standards were used: ethanol (Fisher Scientific, UK), silymarin, and silibinin (Sigma-Aldrich, Germany).

2.1.1. *Liposome preparation*

Liposomes with silymarin and silibinin were prepared using the proliposome method according to (Isailović et al. 2013). Specifically, a mixture of 1 g of phospholipids, 0.02 g of silymarin or silibinin, and 8 mL of ethanol was stirred and heated to 60°C for 10 min. After cooling to 25°C, 20 mL of distilled water was added in small portions. Subsequently, the mixture was stirred for 1 h at 800 rpm.

2.1.2. *Determination of extraction efficiency*

Free silymarin or silibinin were 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 silymarin or silibinin in the supernatant was determined spectrophotometrically at 280 nm (UV Spectrophotometer UV-1800, Shimadzu, Japan). Entrapment efficiency (EE%) was calculated as the content of silymarin or silibinin encapsulated in liposomal particles divided by the content of silymarin or silibinin used for the preparation of the liposome bilayer:

EE (%) = $(m_i\text{-}m_s)/m_i \cdot 100$, where m_i is the initial amount of silymarin or silibinin used for the liposomal preparation, and m_s is the amount of silymarin or silibinin determined in the supernatant.

2.1.3. *Analysis of particle size, polydispersity index, zeta potential, mobility, and conductivity*

The particle size, polydispersity index, zeta potential, mobility, and conductivity of the silymarin- or silibinin-loaded liposomes were determined by photon correlation spectroscopy in Zetasizer Nano Series, Nano ZS (Malvern Instruments Ltd., UK). Each sample was measured three times at room temperature.

2.1.4. *Storage and UV-irradiation stability of the liposomes*

The particle size, polydispersity index, zeta potential, mobility, and conductivity of the silymarin- or silibinin-

loaded liposomes were monitored for 28 days of storage at 4°C (on 1st, 7th, 14th, 21st, and 28th day) and immediately after UV irradiation. UV irradiation was performed in a laminar flow cabinet (AC2–4G8, ESCo, Singapore). Namely, the liposomal sample (2 mL) was exposed to UV-C irradiation (253.7 nm) for 20 min at 25°C in uncovered Petri dishes (Petrović et al. 2017; Yao et al. 2021). Subsequently, all measurements for physicochemical characterization were performed.

3. RESULTS AND DISCUSSION

3.1. Extraction efficiency in silymarin- and silibinin-loaded liposomes

In order to determine the silymarin and silibinin efficiency of encapsulation into liposomes, the concentration of silymarin or silibinin in the supernatant was quantified spectrophotometrically at 280 nm; the liposomes were separated from free silymarin and silibinin by centrifugation. The results are presented in Table 1. The encapsulation efficiencies of silymarin and silibinin were 92.05±1.41% and 87.86±2.06%, respectively. The obtained results are similar to the liposomes with resveratrol prepared using the proliposome method (97.36±2.00%), (Isailović et al. 2013), and, in addition, significantly higher in comparison to the liposomes with gentisic acid (∼ 54%), (Jovanović et al. 2019). In the present study, the liposomes contain only phospholipids (without the addition of sterols), which makes their bilayer more rigid (Jovanović et al. 2018), consequently preventing leakage of silymarin and silibinin, and providing higher encapsulation efficiency. The liposomal membrane containing sterols (cholesterol, ergosterol, lanosterol, ß-sitosterol, etc.) possesses higher permeability (Jovanović et al. 2018), and thus, encapsulation efficiency was lower, as in the case of the previously mentioned gentisic acid (Jovanović et al. 2019).

3.2. Particle size, polydispersity index, zeta potential, mobility, and conductivity

Since the average size of liposomal particles represents an essential and relevant parameter for liposome stability, biodistribution, as well as for the release of encapsulated compounds (Mozafari, Johnson, Hatziantoniou, & Demetzos 2008), the measurement of the mentioned variable was performed. The results are presented in Figure 1A. Additionally, PDI, as a measure of the particle size distribution, was determined as well, and the results are presented as the values above the bars in Figure 1A.

The average size of the liposomal particles with silymarin was 3541.3±62.5 nm, while the particle size of

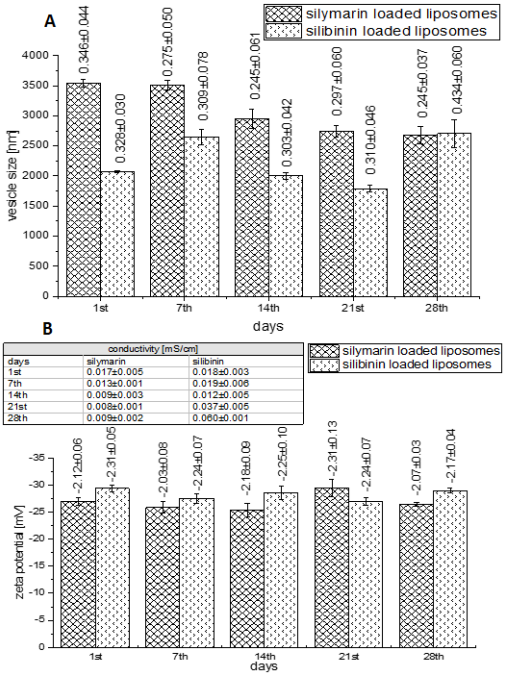

Figure 1. Particle size - bars and polydispersity index - numbers above bars (A) and zeta potential - bars, mobility - numbers above bars [µmcm/Vs], and conductivity - table (B) of silymarin- and silibinin-loaded liposomes, measured immediately after the liposomal preparation and for 28 days of storage at 4°C.

the liposomes with silibinin was 2074.7±19.4 nm (Figure 1A). The obtained values for liposome size are in agreement with the literature data, where pure phospholipid liposomes (without the sterols) had a diameter of 2974±140 nm (Jovanović et al. 2018). It can be noticed that silymarin liposomes had a larger diameter in comparison to silibinin sample. The explanation can be in the fact that some of the compounds from silymarin are probably incorporated within the liposomal bilayer, which causes the formation of inter-lipid space and membrane expansion, and consequently the increase of liposome size (Jovanović et al. 2018). Namely, the particle size of the liposomes is significantly affected by the lipid composition, liposomal preparation technique, and the nature of the encapsulated substances (Isailović et al. 2013; Jovanović et al. 2019; 2018).

The PDI for silymarin- and silibinin–loaded liposomes was similar, 0.346±0.044 and 0.328±0.030, respectively (the values above the bars in Figure 1A). However, a single phospholipid, such as 1,2-dipalmitoyl-sn-glycero-3-phosphocholine, provides better uniformity (PDI of ∼0.1) compared to the mixture of phospholipids (such as Phospholipon 90G, commercial phospholipid mixture used in the present research). Namely, a single phospholipid eliminates the imperfect packing that can occur in the case of various hydrophobic fatty acyl chain

lengths, head groups, and degrees of saturation present in the mixture (Jovanović et al., 2018). Nevertheless, the obtained values for PDI are acceptable from the point of view of further application of the liposomes. Additionally, according to Jovanović et al. (2018), larger liposomes (multilamellar vesicles, such are silymarin–and silibinin-loaded liposomes) possessed lower PDIs in comparison to smaller liposomes (small unilamellar vesicles). The applied technique for liposomal preparation also influenced uniformity of the system (Isailović et al. 2013; Jovanović et al. 2019). The PDI values for liposomal samples loaded with gentisic acid prepared using the thin film method were higher than in the case of silymarin and silibinin liposomes where proliposome methods were used (0.4 and 0.5), (Jovanović et al. 2019). Isailović et al. (2013) reported that resveratrol-loaded liposomes produced by the proliposome method had the PDI of ~0.2, while the PDI of the same sample prepared by the thin film method was ~0.4.

According to the literature data, in an aquatic environment, phosphatidylcholines are neutral lipids. However, the reorientation groups belonging to the lipid heads cause the presence of a surface charge, which depends on the phase state and types of the lipids (Jovanović et al. 2018). Thus, the zeta potential (as a measure of system stability) of the obtained silymarin and silibinin loaded liposomes was examined and the results are presented in Figure 1B. The results of the liposome mobility are presented as the values above the bars, while conductivity values are presented in the tables, within Figure 1B.

The zeta potential of the silymarin liposomes was -27.0±0.7 mV, whereas the zeta potential was -29.4±0.6 mV for silibinin liposomes (Figure 1B). The negative values of zeta potential are related to the exposure of the phosphate group lying in an outer plane concerning the choline groups (Jovanović et al. 2019). The obtained results of zeta potential are in agreement with the literature data, where the liposomes with resveratrol prepared using the proliposome technique had the zeta potential of ~-25 mV (Isailović et al. 2013). The zeta potential of silibinin liposomes was slightly higher in comparison to silymarin liposomes, due to the changes in the space between the head groups of phospholipids within the bilayer membrane (Jovanović et al. 2019).

According to the literature, conductivity represents an indicator of total dissolved compounds and a predictor of the antioxidant capacity of a sample as well (Suliman et al. 2015). Further, the number of ions per unit volume and their drift velocity affect the electrical conductivity of a liquid. The drift velocity of an ion changes depending on the strength of the electric field, the ion mass, the temperature of the solution, as well as on other variables. The electrical conductivity of various liquids may thus be anticipated to have a wide range of values (Rhoades, Raats, & Prather 1976). The conductivity of the liposomes with silymarin and silibinin immediately after the preparation was 0.017±0.005 and 0.018±0.003 mS/cm, respectively, while mobility was -2.12±0.06 and -2.31±0.05 (Figure 1B). According to Azarbayjani, Jouyban, and Chan (2009), higher capture volume corresponds to a decrease in conductivity. Lidgate, Hegde, and Maskiewicz (1993) also reported that greater lipid concentrations = higher capture volume = the effective removal of ions from the liposome dispersions=the reduction in conductivity. Indeed, the liposomes containing silymarin and silibinin possessed a high concentration of lipids (50 mg/mL), showed higher encapsulation efficiency, and had lower conductivity (Figure 1B). The mobility of liposomes is a function of vesicle size, zeta potential, and bilayer membrane composition (Duffy et al. 2001). Therefore, the obtained differences among various liposomal populations were expected. Namely, liposomes with lower zeta potential correspondingly possess lower mobility, which was the case with silymarin- and silibinin-loaded liposomes. Furthermore, some bilayer membranes are rigid, whereas others are highly permeable, flexible, and deformable, which depends on the composition of the bilayer, as well as the encapsulated compounds. The liposomes that have higher membrane fluidity also show better mobility. Since any changes in liposome mobility were attributed to the mechanical rigidity, or the ability of the liposomes to deform (Pysher & Hayes 2004), it can be concluded that liposomes with silibinin (slightly higher mobility) were softer and more fluid (consequently lower extraction efficiency, Table 1) than liposomes with silymarin, which exhibited lower mobility and probably higher rigidity (consequently higher extraction efficiency, Table 1). In addition, when flavonoids (among which are silymarin and silibinin) are adsorbed at the surface of the liposomes, this can reduce liposome mobility (Yang et al. 2015).

3.3. Storage stability of silymarin and silibinin loaded liposomes

The size of the liposomes, PDI, zeta potential, mobility, and conductivity of silymarin- and silibinin-loaded liposomes were measured on the 1st, 7th, 14th, 21st, and 28th day after preparation. As can be seen from Figure 1A, the particle size of the liposomes with silymarin was changed from 3541.3±62.5 nm (1st day) to 2677.0±44.2 nm (28th day) during the 28-day stability study, while PDI varied from 0.346±0.044 to 0.228±0.036. The decrease in the diameter of silymarin-loaded liposomes was detected on the 14th day of storage in refrigeration, to 2954.0±55.9 nm, and it continued to decrease contin-

uously up to the 28th day. On the other hand, particle size and PDI of the liposomes with silibinin were changed from 2074.7±19.4 nm (1st day) to 2704.0±35.0 nm (28th day) and from 0.328±0.030 to 0.456±0.026, respectively. The results presented in Figure 1A show that liposomes were physically stable during 28 days of storage, i.e., there was no occurrence of agglomeration or significant changes in uniformity of the liposomal system.

The zeta potential of the silymarin-liposomes did not change; it amounted to -27.0±0.7 mV (1st day) and -26.4±0.4 mV (28th day). The zeta potential of silibinin-loaded liposomes did not vary either and was -29.4±0.6 mV (1st day) and -29.0±0.4 mV (28th day) (Figure 1B). The results of zeta potential presented in Figure 1B prove that liposomes were stable during 28 days of storage at 40°C, i.e., there were no changes in the values of zeta potential. Mobility and conductivity of the liposomes with silymarin were changed from -2.120±0.057 μmcm/Vs (1st day) to -2.067±0.028 μmcm/Vs (28th day) and from 0.017±0.005 mS/cm (1st day) to 0.009±0.004 mS/cm (28th day), respectively (Figure 1B). Mobility and conductivity of the liposomal particles with silibinin were changed from -2.307±0.053 μmcm/Vs (1st day) to -2.110±0.033 μmcm/Vs (28th day) and from 0.018±0.003 mS/cm (1st day) to 0.060±0.001 mS/cm (28th day), respectively (Figure 1B). The conductivity of silymarin-loaded liposomes and silibinin-loaded liposomes differs only between some measurements during the 28-day stability study, but the values of conductivity are low, and thus some differences are minor (Figures 1B). Additionally, conductivity was continuously decreased in silymarin-loaded liposomes during the 28-day stability study, whereas in silibinin-loaded liposomes, conductivity increased from the 1st to the 28th day. This can be explained by the fact that silibinin-loaded liposomes are more fluid in comparison to silymarin liposomes, and, therefore, the leakage of encapsulated compounds (i.e. silibinin) into the surrounding water medium can occur, increasing conductivity (Lidgate et al. 1993). The interactions between liposomes, which depend among other things on the concentration of liposomes, cause modifications in liposomal particles and changes in mobility. Thus, liposome fusion or fission can cause a redistribution of phospholipids between liposomes and variations in mobility (Duffy et al. 2001).

3.4. UV irradiation stability of silymarin- and silibinin-loaded liposomes

UV irradiation stability of silymarin- and silibinin-loaded liposomes was examined by measuring vesicle size, PDI, zeta potential, mobility, and conductivity of the liposomes after 20 min of UV irradiation. The results are presented in Table 1.

Table 1. Particle size, polydispersity index (PDI), zeta potential, mobility, and conductivity of UV-irradiated silymarin- and silibinin-loaded liposomes, measured immediately after the 20-min UV irradiation (253.7 nm).

Variable	Sample	
	Silymarin liposomes UV	Silibinin liposomes UV
Particle size [nm]	3552±45	2234±50
PDI	0.36±0.03	0.32±0.02
Zeta potential [mV]	-23.9±0.8	-24.5±0.7
Mobility [μmcm/Vs]	-1.87±0.06	-1.92±0.06
Conductivity [mS/cm]	0.014±0.001	0.007±0.003

As can be seen from Table 1, UV irradiation did not have an influence on vesicle size and PDI of all liposomes, but it caused a decrease in zeta potential: -23.9±0.8 mV for silymarin liposomes and -24.5±0.7 mV for silibinin liposomes, in mobility: -1.874±0.064 μmcm/Vs for silymarin- and -1.920±0.057 μmcm/Vs for silibinin liposomes, and in conductivity: 0.014±0.001 mS/cm for silymarin and 0.007±0.003 mS/cm for silibinin.

4. CONCLUSION

In the present research study, silymarin- and silibinin-loaded liposomes were developed using proliposome procedures and characterized via encapsulation efficiency, vesicle size, PDI, zeta potential, mobility, and conductivity, as well as storage stability and stability after UV irradiation. The encapsulation efficiencies of silymarin- and silibinin-loaded liposomes were 92.05±1.41% and 87.86±2.06%, respectively. Silymarin-loaded liposomes had a higher diameter in comparison to silibinin-liposomes, while PDIs and conductivity were similar. The zeta potential and mobility (absolute value) of silibinin-loaded liposomes were slightly higher in comparison to silymarin-loaded liposomes. The obtained liposomes were physically stable during 28 days of storage, i.e., there was no occurrence of agglomeration and no significant changes in uniformity and zeta potential of the liposomal system. UV irradiation did not cause changes in vesicle size and PDI of liposomes, but it caused a decrease in zeta potential, mobility, and conductivity. The obtained results qualify silymarin- and silibinin–loaded liposomes for application in functional foods and pharmaceutical products; however, future experiments should deal with the biological activities of the developed liposomes.

REFERENCES

Ahmad, U., Akhtar, J., Singh, S. P., Badruddeen, Ahmad, F. J., Siddiqui, S., & Wahajuddin. (2017). Silymarin nanoemulsion against human hepatocellular carcinoma: development and optimization. *Artificial Cells, Nanomedicine,*

and Biotechnology, *46*(2), 231–241. https://doi.org/10.1080/21691401.2017.1324465

Azarbayjani, A. F., Jouyban, A., & Chan, S. Y. (2009). Impact of surface tension in pharmaceutical sciences. *Journal of Pharmacy &amp Pharmaceutical Sciences*, *12*(2), 218. https://doi.org/10.18433/j32p40

Duffy, C. F., Gafoor, S., Richards, D. P., Admadzadeh, H., O'Kennedy, R., & Arriaga, E. A. (2001). Determination of properties of individual liposomes by capillary electrophoresis with postcolumn laser-induced fluorescence detection. *Analytical Chemistry*, *73*(8), 1855–1861. https://doi.org/10.1021/ac0010330

García-Viñuales, S., Ilie, I. M., Santoro, A. M., Romanucci, V., Zarrelli, A., Fabio, G. D., … Milardi, D. (2022). Silybins inhibit human IAPP amyloid growth and toxicity through stereospecific interactions. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, *1870*(5), 140772. https://doi.org/10.1016/j.bbapap.2022.140772

Isailović, B. D., Kostić, I. T., Zvonar, A., Đorđević, V. B., Gašperlin, M., Nedović, V. A., & Bugarski, B. M. (2013). Resveratrol loaded liposomes produced by different techniques. *Innovative Food Science &amp Emerging Technologies*, *19*, 181–189. https://doi.org/10.1016/j.ifset.2013.03.006

Jovanović, A. A., Balanč, B. D., Djordjević, V. B., Ota, A., Skrt, M., Šavikin, K. P., … Ulrih, N. P. (2019). Effect of gentisic acid on the structural-functional properties of liposomes incorporating -sitosterol. *Colloids and Surfaces B: Biointerfaces*, *183*, 110422. https://doi.org/10.1016/j.colsurfb.2019.110422

Jovanović, A. A., Balanč, B. D., Ota, A., Grabnar, P. A., Djordjević, V. B., Šavikin, K. P., … Ulrih, N. P. (2018). Comparative effects of cholesterol and -sitosterol on the liposome membrane characteristics. *European Journal of Lipid Science and Technology*, *120*(9), 1800039. https://doi.org/10.1002/ejlt.201800039

Kalušević, A., Salević, A., Jovanović, A., Trifković, K., Veljović, M., Pravilović, R., & Nedović, V. (2022). Encapsulation of plant extracts. , 172–203. https://doi.org/10.1201/9780429324918-7

Lidgate, D. M., Hegde, S. G., & Maskiewicz, R. (1993). Conductivity measurement as a convenient technique for determination of liposome capture volume. *International Journal of Pharmaceutics*, *96*(1-3), 51–58. https://doi.org/10.1016/0378-5173(93)90211-w

Liu, C.-H., Jassey, A., Hsu, H.-Y., & Lin, L.-T. (2019). Antiviral activities of silymarin and derivatives. *Molecules*, *24*(8), 1552. https://doi.org/10.3390/molecules24081552

Mohammadi, M., Ariafar, S., Talebi-Ghane, E., & Afzali, S. (2022). Comparative efficacy of silibinin and nanosilibinin on lead poisoning in male wistar rats. *Toxicology*, *475*, 153242. https://doi.org/10.1016/j.tox.2022.153242

Mozafari, M. R., Johnson, C., Hatziantoniou, S., & Demetzos, C. (2008). Nanoliposomes and their applications in food nanotechnology. *Journal of Liposome Research*, *18*(4), 309–327. https://doi.org/10.1080/08982100802465941

Petrović, S., Tačić, A., Savić, S., Nikolić, V., Nikolić, L., & Savić, S. (2017). Sulfanilamide in solution and liposome vesicles in vitro release and UV-stability studies. *Saudi Pharmaceutical Journal*, *25*(8), 1194–1200. https://doi.org/10.1016/j.jsps.2017.09.003

Pysher, M. D., & Hayes, M. A. (2004). Examination of the electrophoretic behavior of liposomes. *Langmuir*, *20*(11), 4369–4375. https://doi.org/10.1021/la0362730

Rhoades, J. D., Raats, P. A. C., & Prather, R. J. (1976). Effects of liquid-phase electrical conductivity, water content, and surface conductivity on bulk soil electrical conductivity. *Soil Science Society of America Journal*, *40*(5), 651–655. https://doi.org/10.2136/sssaj1976.03615995004000050017x

Song, X.-Y., Liu, P.-C., Liu, W.-W., Zhou, J., Hayashi, T., Mizuno, K., … Ikejima, T. (2022). Silibinin inhibits ethanol- or acetaldehyde-induced ferroptosis in liver cell lines. *Toxicology in Vitro*, *82*, 105388. https://doi.org/10.1016/j.tiv.2022.105388

Suliman, R., Asmani, F., Suliman, M., Hussain, M., Khan, J., Kaleemullah, M., … Yusuf, E. (2015). A new approach for predicting antioxidant property of herbal extracts. *International Journal of Pharmacognosy and Phytochemical Research*, *7*, 166–174.

Verdura, S., Cuyàs, E., Ruiz-Torres, V., Micol, V., Joven, J., Bosch-Barrera, J., & Menendez, J. A. (2021). Lung cancer management with silibinin: A historical and translational perspective. *Pharmaceuticals*, *14*(6), 559. https://doi.org/10.3390/ph14060559

Yang, D., Wang, X.-Y., Gan, L.-J., Zhang, H., Shin, J.-A., Lee, K.-T., & Hong, S.-T. (2015). Effects of flavonoid glycosides obtained from a ginkgo biloba extract fraction on the physical and oxidative stabilities of oil-in-water emulsions prepared from a stripped structured lipid with a low omega-6 to omega-3 ratio. *Food Chemistry*, *174*, 124–131. https://doi.org/10.1016/j.foodchem.2014.11.036

Yao, W., Liu, C., Wang, N., Zhou, H., Shafiq, F., Yu, S., & Qiao, W. (2021). O-nitrobenzyl liposomes with dual-responsive release capabilities for drug delivery. *Journal of Molecular Liquids*, *334*, 116016. https://doi.org/10.1016/j.molliq.2021.116016

Zhang, Z., Li, X., Sang, S., McClements, D. J., Chen, L., Long, J., … Qiu, C. (2022). A review of nanostructured delivery systems for the encapsulation, protection, and delivery of silymarin: An emerging nutraceutical. *Food Research International*, *156*, 111314. https://doi.org/10.1016/j.foodres.2022.111314

Zhao, F., & Li, X. (2015). Evaluation of immunomodulatory activity of silymarin extract from silybum marianum in mice of health food. *Advance Journal of Food Science and Technology*, *8*(4), 278–282. https://doi.org/10.19026/ajfst.8.1508