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Antioxidant Silver-based Biogenic Systems Generated from *Arctium lappa* Leaves

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Abstract*. This work aims at designing through a "green" bottom-up strategy, four types of bio-based systems containing artificial cell membranes (with or without vitamin C) and silver nanoparticles (AgNPs) phyto-generated from aqueous extract of Arctium lappa leaves. The "green" approaches for AgNP synthesis, by using the vast bioreducing potential of plants are low-cost and eco-friendly procedures.Exploiting vegetal wastes as precursors for phyto-metallic nanoparticles has received attention from scientific community in the last years due to its significant economic and environmental impacts. UV-Vis absorption spectra demonstrated the biosynthesis of metallic nanoparticles which exhibited a strong SPR peak located at 437 nm wavelengths. The formation of the biocomposites containing the "green" synthesized AgNPs has been demonstrated by UV-Vis absorption spectroscopy and Dynamic Light Scattering measurements. DLS results highlighted the nanoscale size of the prepared "green" AgNPs. The physical stability of the obtained silver-based systems was evaluated through zeta potential values, based on electrophoretic mobility. The systems loaded with vitamin C proved to be more stable and showed enhanced antioxidant activities (between 72.3% to 88% evaluated by chemiluminescence technique). Our results demonstrated that these developed silver-based biogenic systems generated from Arctium lappa leaves could be used as antioxidant agents in various biomedical applications.*

Keywords: *burdock-nanosilver, bio-inspired membranes, vitamin C, antioxidant activity.*

1.Introduction

This work develops a "green" bottom-up strategy to achieve antioxidant delivery systems based on vitamin C, biomimetic membranes (liposomes) and biogenic nano-silver synthesized from *Arctium lappa* leaves aqueous extract. Liposomes are self-assembling vesicles containing one or more lipid bilayers with a unique structure (similar to the structure of biological membranes) conferring them the ability to load both hydrophilic and lipophilic compounds, and also some other interesting properties such as: protection of active agents [1, 2], building block for optoelectronic devices [3], and also enhanced stability and improved biological properties (such as: antioxidant, antimicrobial and anti-proliferative activities) for silver-based composites [4-7]. The „green" synthesis of silver nanoparticles using aqueous vegetal extracts is a rapid eco-friendly technique which implies three stages: (i) reduction of silver ions; (ii) growth of AgNPs, and (iii) stabilization of developed metallic nanoparticles [8]. „Green" synthesized silver nanoparticles are recognized for their interesting biological activities. Thus, recent researches summarizes the antioxidant properties of AgNPs phytosynthesized from aqueous extract of *Glycyrrhiza glabra* (licorice, sweet wood) [9], cloves [4, 10] and *Citrus* peels [5, 11, 12]. The incorporation of AgNPs in polyurethane nanovesicles [13], liposomes [7, 14], or phytosomes [6] resulted in biocomposites with enhanced biological performances.

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In this study, the silver nanoparticles were phytosynthesized from *Arctium lappa* (known as *burdock*), a popular plant widely used in treatment of hepatitis, atherosclerosis, hypertension, geriatric diseases due to their polyphenolic constituents with antioxidant properties [15]. This wild plant was chosen due to its valuable bio-activities and therapeutic effectiveness, and also due to economic reasons, taking into account that burdock is one of the most widespread plant in our country, Romania. Valorization of our natural resources, in a "green" manner, is a good idea to keep clean the environment.

Barbinta-Patrascu and co-workers evaluated the antioxidant activity of AgNPs (obtained by "green synthesis" using edible plants) through chemiluminescence technique and $ABTS^{\cdot +}$ method, demonstrating that phytosynthesized silver nanoparticles exhibit superior activity of capturing short-lived free radicals compared to long-lived free radicals [16]. The antioxidant activity of the composites bio-developed in the present study was estimated by chemiluminescence assay - which is a rapid method of testing the ability to capture the short-lived free radicals [17-19]. The novelty of our study is the incorporation of AgNPs into liposomes with vitamin C that increases their bioactivity and stability; the developed biocomposites can serve as «building blocks» for the development of new bio-active materials that have a high potential for applicability in the biomedical field. Vitamin C or ascorbic acid is an antioxidant molecule which prevents and scavenges the formation of reactive oxygen species [20], while its incorporation in liposomes significantly improves their properties and applications [21]. The developed silver-based biocomposites were characterized by UV-Vis absorption spectroscopy and Dynamic Light Scattering measurements; their physical stability was estimated through zeta potential measurements, and the antioxidant properties by chemiluminescence technique. The presence of silver nanoparticles and of vitamin C in biocomposites was demonstrated by UV-Vis absorption spectra.

2. Materials and methods

2.1. Materials

L(+)Ascorbic acid (vitamin C), KH_2PO_4, Na_2HPO_4, tris (hydroxymethylaminomethane base), HCl, H_2O_2, luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), were procured from Merck (Germany). Soybean lecithin and NaCl were supplied by Sigma Aldrich (Germany), and silver nitrate from Gatt Koller - GmbH Austria. The burdock leaves were purchased from a local Romanian garden in Ilfov county.

2.2 Methods

2.2.1 Bio-Preparation of silver nanoparticles from burdock extract

An amount of 40 g fresh leaves of *Arctium lappa* harvested in June month, in Ilfov county, were inserted into 210 mL boiling water and kept in there for 15 minutes. After cooling, this mixture was filtered through Whatman paper no. 1, resulting in a clear burdock extract (sample BE). A volume of 300 μL of this extract was added into 30 mL of 1 mM $AgNO_3$ solution, and the resulted solution was continuously stirred for 24 h; the colour turned from pale yellow to reddish brown after 40 min., highlighting the formation of silver nanoparticles (sample nS).

2.2.2 Preparation of bio-inspired membranes

The biomimetic membranes were obtained by hydration of a thin film of soybean lecithin, with a phosphate buffer saline Na_2HPO_4-KH_2PO_4-NaCl (PBS) solution, at physiological *p*H (7.4), according to the method of Barbinta-Patrascu *et al*. [22]. The resulted multilamellar lipid vesicles (0.5 mM) were denoted "L".

An appropriate volume of a 200 fold diluted stock solution of vitamin C 0.05 g/mL, was inserted into 15 mL suspension of the as-prepared soybean lecithin liposomes, by ultrasound assisted treatment (5 min. three times, on ultrasound bath Elmasonic S60H) resulting in lipid vesicles loaded with vitamin C (sample LC). During sonication steps, the liposomes undergo a structural reorganization, because the lipid membranes are broken and then re-assembled, resulting smaller vesicles, thus incorporating the dispersing medium containing vitamin C. The final concentration of vitamin C in liposomal suspension was 11.9 µg/mL. It should be mentioned that vitamin C is water-soluble drug, so it is found both in the aqueous interior of liposomes and in the dispersed aqueous environment of these lipid vesicles.

2.2.3 Preparation of silver-based biocomposites

In this paper we designed for the first time silver-based composites based on liposomes, vitamin C, and AgNPs synthesized from burdock, with two silver:liposomes ratios. The biocomposites were prepared by ultrasonic irradiation (15 minutes with breaks, on ultrasound bath Elmasonic S60H) of a mixture containing burdock-derived AgNPs (nS) and liposomes (L or LC) in a volumetric ratio of 1:1 and 2:1, resulting in 4 types of composites: nSL1, nSL2, nSLC1 and nSLC2.

In addition, liposomes confer stability and protection to the components of biocomposites: AgNPs and vitamin C. Marsanasco et al. highlighted the protective role of liposomes as vehicles for vitamins E and C [23].

In previous studies, TEM images showed that the silver nanoparticles are coated with liposomes [4], and could be located both in the intervesicular medium and in the vesicular multilamellar structures [24].

A summary presentation of all the samples obtained together with their abbreviations is shown in Table 1.

Table 1. The abbreviations of the obtained samples

Sample	**Code**
Burdock extract	BE
Nano-silver (AgNPs) phytogenerated from burdock extract	nS
Soybean lecithin MLVs	L
Soybean lecithin MLVs – vitamin C	LC
Liposomes/AgNPs biohybrids (1:1, v/v)	nSL1
Liposomes/AgNPs biohybrids (1:2, v/v)	nSL2
Liposomes–vitamin C /AgNPs biohybrids (1:1, v/v)	nSLC1
Liposomes–vitamin C /AgNPs biohybrids (1:2, v/v)	nSLC2

2.3 Characterization methods

The absorption spectra of the samples were recorded on a Jasco V-570 (LSE-331) double beam UV-Vis spectrophotometer, operating at 1 nm resolution, in the 200-600 nm wavelength range.

Dynamic Light Scattering (DLS) method (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.) was used to evaluate the average size, Z_{av}, of the particles (the particle diameter plus the double-layer thickness), and also the polydispersity index, PdI. The samples were analysed as previously described [25]. The mean values ± standard deviations of Z_{av} and PdI were reported for each sample, from three different measurements.

Zeta potential (ZP, mV) measurements were performed in triplicate, with a special dispositive of Zetasizer Nano ZS (Malvern Instruments Ltd., UK) by applying an electric field across the tested aqueous suspensions.

Estimation of antioxidant activity by chemiluminescence method

The antioxidant properties of the samples were checked by chemiluminescence technique, by using luminol-based free radical generator system (containing 10^{-3} M luminol, 10^{-5} M H_2O_2, in TRIS-HCl buffer solution pH 8.6), according to [26]. The *in vitro* antioxidant activity (AA%) values were calculated from three different determinations, by using the following equation:

$$AA\% = [(I_0\text{-}I)/ I_0] \cdot 100\%\% \qquad (1)$$

where I_0 and I are the maximum CL intensities at t=5 s, for the standard and for each sample, respectively.

3. Results and discussions

3.1 Spectral characterization of silver-based composites

UV-Vis absorption spectroscopy and Dynamic Light Scattering measurements were used to demonstrate the formation of silver nanoparticles and silver–based composite systems.

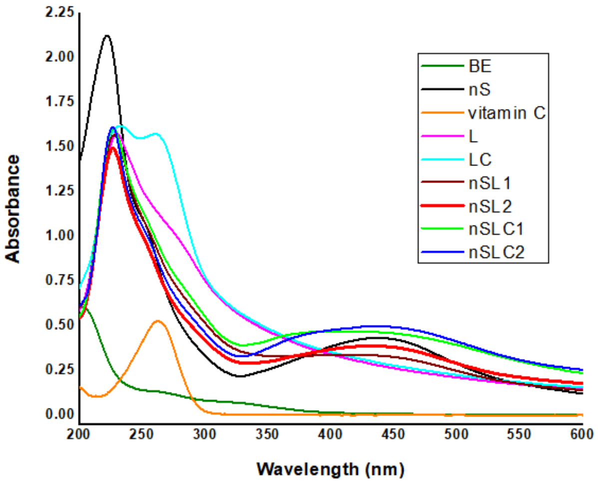

Figure. 1. Comparative UV-Vis absorption spectra of the obtained samples

The UV-Vis absorption spectrum of AgNPs "green" synthesized from *Arctium lappa* leaves (sample nS in Figure 1) showed a strong SPR peak located at 437 nm wavelength (peak not present in the BE spectrum), occurring after addition of burdock extract (BE) in 1 mM $AgNO_3$ solution, thus demonstrating the biosynthesis of silver nanoparticles.

The characteristic peak of vitamin C located at 263 nm was shifted to 259 nm in absorption spectrum of liposomes LC.

The spectral fingerprint of AgNPs in silver-based biocomposites was blue-shifted from 437 nm (for nS) to 409 nm for nSL1, 431 nm for nSL2, 417.5 nm for nSLC1, and 435 nm for nSLC2, confirming the synthesis of biocomposites. These shifts are more pronounced for samples nSL1 and nSLC1, with liposomes:AgNPs ratio of 1:1 (v/v) as compared to the samples with liposomes:AgNPs ratio of 1:2 (v/v). On the other hand, it could be observed that absorption spectra of composites containing ascorbic acid display a slight blue shift. This different behaviour could be explained by a different structural organization of biocomposites loaded with vitamin C.

The presence of vitamin C and AgNPs in liposome-based biocomposites was demonstrated by the presence of spectral signatures of AgNPs and vitamin C in the UV-Vis absorption spectra of liposome-based biocomposites, as well as through the "shifts" of these "spectral fingerprints".

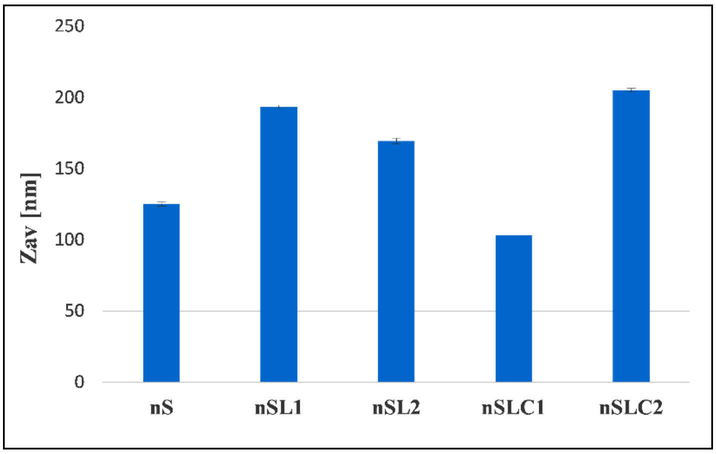

Figure 2. The mean size values of silver-based composites

Mean diameter of nanoparticles estimated by DLS measurements (Fig. 2) ranged from 103 to 194.5 nm. Among all developed biocomposites, those containing burdock-nanosilver and bio-inspired membranes loaded with vitamin C (1:1, v/v) presented the smallest size, with a mean diameter of 103 nm ± 4.25 (Figure 2).

3.2 Estimation of physical stability of bio-based composites

The physical stability of the bio-based composites was estimated in terms of zeta potential, ZP (Fig. 3), based on electrophoretic mobility; ZP value is related to the surface particle charge [27].

All the samples presented a negative surface charge that induces repulsive forces among particles (Fig. 3), assuring short-term stability for nanosilver nS (ZP = -17 mV) and liposomes L (ZP = -20.45 mV), and good stability for the other samples as well. Samples loaded with ascorbic acid have more negative ZP values, therefore they are more stable.

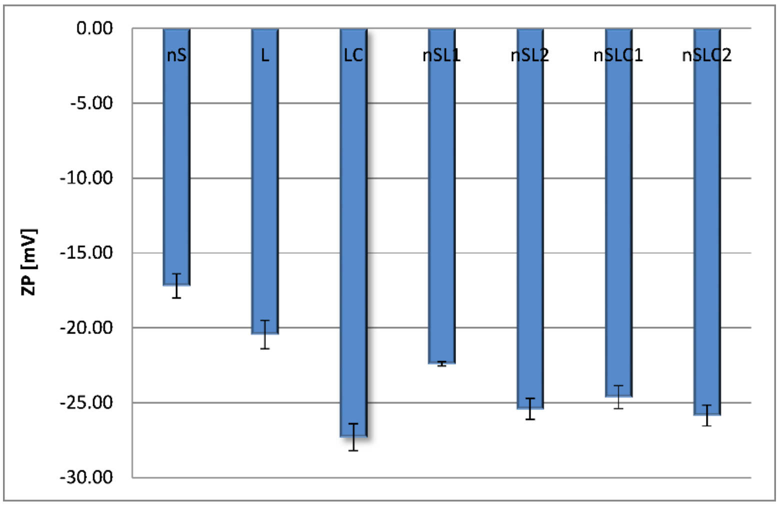

Figure. 3. Zeta potential values of the burdock-nanosilver, liposomes, and their biocomposites

Liposomal formulation of vitamin C showed good stability as compared to liposomes alone (ZP_{LC}=-27.3 mV; ZP_L = -20.45 mV). Thus, addition of vitamin C resulted in enhancing the ZP magnitude, from |-22.4| mV to |-24.63| mV for systems containing AgNPs and liposomes in a ratio of 1:1, v/v, and from |-25.43| mV to |-25.85| mV for systems containing AgNPs and liposomes in a ratio of 2:1, v/v. Combination of biomimetic membranes with burdock-derived silver nanoparticles (nS) assured an enhancement of physical stability of nS. These results are in agreement with our previous works [4-7].

3.3 Evaluation of antioxidant activity of silver-based bionanosystems generated from Arctium lappa leaves

The antioxidant properties of the samples were tested through chemiluminescence technique, and the values of AA% (calculated using equation 1) are comparatively displayed in Fig.4.

The “green” synthesized silver nanoparticles, nS, presented high value of antioxidant activity: 89.3%, and the liposomal formulations of vitamin C showed an AA% value of 72.3%, greater than liposomes alone (AA% = 47.8%). The biogenic silver nanoparticles, nS, exhibited an amplified value of antioxidant activity (89.3%) as compared to vegetal extract alone (83%), this amplification being assessed to the nano-scaled size of metallic nanoparticles which offers an increase in total surface area providing many reaction centers for free radical scavenging [7]. This enhancement of AA% value was highlighted also in our previous studies [4, 7].

Moreover, the presence of ascorbic acid gave rise to biosystems with enhanced antioxidant activities (83% and 88% for nSLC1 and nSLC2, respectively) as compared to liposomes free of vitamin C (75% and 85% for nSL1 and nSL2, respectively).

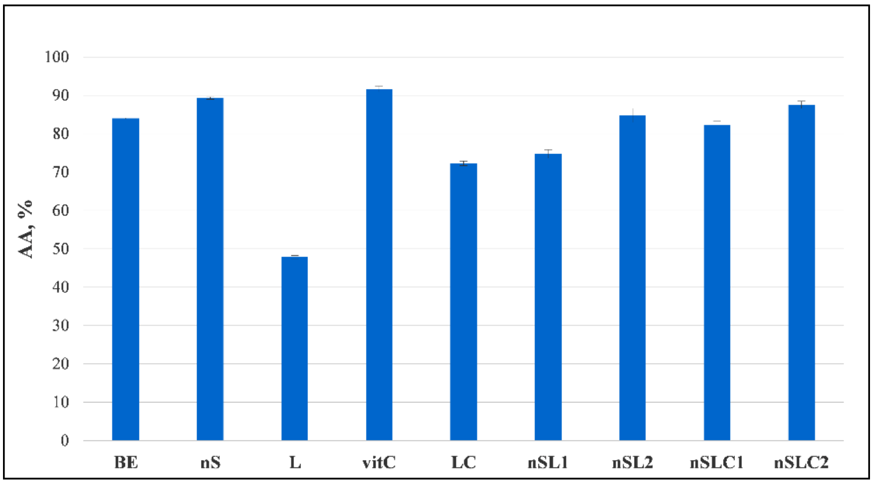

Figure 4. The antioxidant activity of the obtained silver-based bionanosystems generated from *Arctium lappa* leaves

Even in dilution of 1.5 or 2 fold, in lipid vesicles, the silver nanoparticles bio-generated from burdock exhibited high antioxidant activity. The AA% values of silver – based composites containing vitamin C, developed in this study, are higher than for nanosilver/lecithin – biocomposites derived from *Geranium* (72% [14]) and sage (86.5% [28]).

The antioxidative potential of burdock-derived nanoparticles is conferred by the presence of antioxidant biocomponents in the burdock leaf composition: flavone derivatives (such as rutin, quercetin, luteolin, crocin, and cyanine) and polyphenol carboxylic acids (such as chlorogenic and caffeic acids) [15, 29].

4. Conclusions

Artificial cell membranes and phytogenic silver nanoparticles “green” generated from *Arctium lappa* extract were used to bottom-up design of antioxidant biogenic systems with and without vitamin C. UV-Vis absorption spectra demonstrated the formation of burdock-generated silver nanoparticles and their composites with bio-inspired lipid membranes loaded or not with ascorbic acid. DLS measurements displayed the nano-scaled size of the developed silver – based composite systems.

The shifts of spectral fingerprints of vitamin C and “green” silver nanoparticles in the UV-Vis absorption spectra of liposome-based biocomposites proved the presence of AgNPs and vitamin C in the obtained biocomposites.

The addition of vitamin C in biocomposites improved the properties of the obtained materials. Thus, silver-based systems containing vitamin C are more stable than silver-loaded systems without vitamin C, so bio-based systems containing vitamin C showed enhanced physical stability as compared to systems without vitamin C (the increase was 33.5% for liposomes alone, 10% for biocomposites with liposomes:AgNPs ratio of 1:1, v/v, and 1.7% for biocomposites with liposomes:AgNPs ratio of 1:2, v/v). In addition, the insertion of vitamin C in the burdock-derived silver-based composites significantly improved the antioxidant efficiency of such composites.

The liposomal components of biocomposites developed in our study, assured an enhancement of “green” nanosilver stability.

These developed materials could be applied in biomedical field as free radical scavenger, and as adjuvants in treatment of oxidative stress – related diseases.

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