Design and Evaluation of a Delivery System Based on Liposomes for *Armoracia rusticana* Extract

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The aim of this paper was the design and evaluation of delivery system for Armoracia rusticana leaves extract with the purpose to use such systems in food or cosmetic field. Liposomes loaded with Armoracia rusticana were prepared by film hydration method and presented good entrapment efficiency, nano-sizes (<150 nm), low polydispersity index and good stability over 90 days at 4°C. In vitro drug release study showed the ability of liposomes to provide slow release of extract with reduced burst effect compared to free extract. These promising results suggest that liposomes could be exploited as carriers for herbal ingredients.

Keywords: Armoracia rusticana, liposomes, drug delivery systems, encapsulation

Liposomes are self-organizing colloidal nanoparticles which contain an aqueous inner compartment with 25 nm -2.5 µm molecules, separated by one or more lipid bilayers, composed of amphiphilic phospholipids. Liposomes can encapsulate both hydrophobic and hydrophilic compounds due to the amphiphilic nature of phospholipids, unlike other carriers, which can load compounds having only specific characteristics. Apart from this feature they possess biodegradability, biocompatibility, non-toxicity, non-immunogenicity and have been extensively used in pharmaceutical, food and cosmetic industries [1-4].

Liposomes have been employed as an effective method to encapsulate natural compounds and whole plant extracts to improve stability, solubility, and therefore bioavailability [5]. Other carriers like: micro-/nano-emulsions, microspheres, micro-/nanoparticles, have been also successfully applied as carries [6,7]. The advantages of encapsulation in liposomes are: better absorption, decrease of toxicity and side effects, decrease of administration frequency, decrease of administration dose and increase of therapeutic effectiveness [8-10]. Several preparation methods of liposomes loaded with plant extracts or natural compounds were described in literature, such as: thin-film hydration technique [11], micro-emulsification [12], sonication [13], reverse-phase evaporation [14] and ethanol injection [15].

The aim of the study was the design and evaluation of liposome-based system for *Armoracia rusticana* leaves extract with the purpose to use such systems in cosmetic field. *A. rusticana*, known as horseradish, is a perennial plant from the Brassicaceae family with antioxidant, antimicrobial, chemopreventive, anti-inflammatory, gastroprotective and hypocholesterolaemic effects, used in food industry and cosmetic products [16]. To fulfill the aim of this paper, liposomes loaded with *A. rusticana* were prepared by film hydration method followed by sonication and extrusion. Entrapment efficiency, particle size, polydispersity index, stability were assessed for the characterization of liposomes. The evaluation of liposomes loaded with *A. rusticana* as delivery systems was performed.

Experimental part

Materials

Phosphatidylcholine (PC), sodium cholate, sodium carbonate, Triton X-100, gallic acid (95%), Folin-Ciocalteu reagent, Phosphate-Buffered Saline (PBS), were purchased from Sigma-Aldrich Co (Germany). *A. rusticana* (leaves) were harvested from Dambovita County, Romania, and identified by the botanical team of INCDCF-ICCF Bucharest. A voucher specimen, coded as AR_leaves_AA2016 was stored at INCDCF-ICCF Plant Material Storing Room. An *A. Rusticana* leaves extract in 50% (v/v) ethanol was used in this study; the extraction procedure was: forty grams (40 g) of raw material, shade dried at room temperature and ground to a powder, was extracted with 500 mL ethanol (50% v/v), at reflux temperature for one hour under continuous stirring. The resulted solution was paper filtered and concentrated at 72-75 mm Hg, 60°C and further solved in 200 mL ethanol (extraction yield ~ 23.43%.) The extract had a significant value for phenol content of 21.16 ± 0.010 mg GAE/g dry material and its main component was rutin (55%). The extract was stored at 4°C until analysis.

Preparation of liposomes loaded with A. rusticana extract

Liposomes loaded with *A. rusticana* were prepared using thin-film hydration method followed by sonication and extrusion. Briefly, *A. Rusticana* extract, phosphatidylcholine and sodium cholate (table 1) were dissolved in 10 mL methanol and the lipid solution was evaporated using a

Sample code	PC (mg)	Sodium cholate (mg)	Extract (mg)	EE (%)	Size (nm)	Polydispersity index
L1	80	-	-	-	85.40 ± 0.34	0.400 ± 0.01
L2	80	20	-	-	105.20 ± 2.04	0.379 ± 0.02
AR_L1	80	-	25	79.01 ± 0.23	140.30 ± 0.31	0.343 ± 0.03
AR_L2	80	20	25	72.01 ± 0.57	138.30 ± 0.11	0.319 ± 0.02

Table 1
COMPOSITION AND CHARACTERISTICS OF LIPID VESICLES

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rotary evaporator (Laboranta 4000 Rotary evaporator, Heidolph Instruments GmbH & Co. KG) for 2 h, under vacuum, at 35°C. After the complete solvent removal, the lipid film was hydrated for 1h with distilled water at 35°C. Obtained liposomes were left 2 h at room temperature for the stabilization of lipid membranes. After stabilization, the liposomes were sonicated in a sonication bath (Sonorex Digital 10P, Bandelin Electronic GmbH & Co) filled with ice for 20 min and then extrudated using 0.4 μm and 0.2 μm pore size filters (five extrusions for each pore size) to reduce pore size. Loaded liposomes were separated from free extract by centrifugation at 10 000 rpm, 5°C for 30 min. The clear supernatant was siphoned off carefully and the sediment was re-suspended in distilled water. This procedure of centrifugation was repeated twice. All samples were stored at 4°C until analysis.

Characterization of liposomes loaded with A. rusticana extract

Size and polydispersity index were measured by Dynamic Light Scattering technique (DLS) using a particle size analyzer (Beckman Coulter N4 PCS Submicron, Coulter Company). Measurements were made at 25°C (detector position: angle 90°, solvent refractive index: 1.332; solvent viscosity: 0.871 cP) using 10 runs for each measurement on diluted samples (1:10). The entrapment efficiency (EE, %) was calculated using equation 1, where M was the amount of extract loaded in liposomes and M_t was the amount of extract initially added in formulation. The amount of extract loaded in liposomes was determinated using equation 2, as the difference of the amount of extract initially added in formulation (M_t) and the amount of free extract (M_{fe}). The amount of free extract was assessed by spectrophotometry at maximum absorbance of extract using rutin calibration curve (264 nm wavelength; concentration range 5×10^{-6}-50×10^{-6} g/mL; $y=6830.5x+0.0245$, $R^2=0.9992$).

The formulations were subjected to a stability study for a period of 90 days at a temperature of 4°C. Samples were analyzed at 30, 60 and 90 days by evaluating extract content.

$$EE(\%) = \frac{M}{M_t}\times 100 \quad (1)$$

$$M = M_t - M_{fe} \quad (2)$$

In vitro release of A. rusticana extract from loaded liposomes

In vitro release of *A. rusticana* extract from loaded liposomes was assessed using dialysis membrane method under sink conditions [17-19]. A sample of 1.0 mL loaded liposomes or free extract, was placed in a dialysis bag with 14 000 molecular weight cut-off (Sigma Aldrich, USA) and immersed into 200 mL PBS 0.1 M *p*H 7.4, with a stirring speed of 100 rpm/min and the temperature was maintained at 37°C. At predetermined times (15, 30, 45, 60, 120, 180, 240, 300, 360, 600 min and 24 h) samples of 1.0 mL were taken. After a sample collection the release medium taken was replenished with the same sample volume of fresh medium.The cumulative release rate (CDR) was calculated using equation 3, where Q_n was the amount of extract released at time n and Q_t was the amount of extract initially entrapped in liposomes. The amount of extract released was assessed by spectrophotometry at maximum absorbance of extract using rutin calibration curve. The mechanism of extract release was investigated by applying several kinetics models (Zero-order, First order, Higuchi and Hixson-Crowell). The models were compared using the correlation coefficient (R^2).

$$CDR(\%) = \frac{Q_n}{Q_t}\times 100 \quad (3)$$

Statistical analysis

Statistical analysis of the data was performed with SPSS 18.0 (SPSS Inc., Chicago, IL, USA). Values are represented as mean ± standard deviation (SD) of thee replicates. Differences were considered significant at $p < 0.05$.

Results and discussions

The main aim of our work was to develop a delivery system for an antioxidant plant extract based on liposomes with potential application in cosmetic field. *A. rusticana* (leaves) was selected as plant material due to its significant antioxidant activity. Also, from our best knowledge no previous studies regarding the incorporation of *A. rusticana* leaves extract in liposomes were described in literature.

The liposomes loaded with *A. rusticana* extract were prepared using thin-film hydration method and characterizated in terms of size, polydispersity index, entrapment efficiency and stability over 90 days. The liposomes loaded with *A. rusticana* extract had good entrapment efficiency values, 79.01 ± 0.23% for AR_L1 and 72.01 ± 0.57% for AR_L2, confirming that thin-film hydration method is a suitable method for plant extract encapsulation in liposomes. Similar results were presented in other papers, eg. an entrapment efficiency of ~ 84% for *Glycyrrhiza glabra* L. [20] or ~ 88% for grape-seed [21, 22]. Also, the liposomes loaded with *A. rusticana* were stable at 4°C for at least 90 days with minimal plant material loss (~ 0.36 plant material loss after 30 days; ~ 0.7 plant material loss after 90 days). All formulations had nanometer size range, with values below 150 nm. Also, it can be observed an increase in size at the incorporation of *A. rusticana* extract in liposomes with ~ 33.1 for AR_L1, and respectively ~ 54.9 for AR_L2. The small difference between liposomal formulations is due to the addition of sodium cholate, an edge activator that entered in competition with the extract and therefore only a smaller quantity of extract was accommodated in the liposomes containing sodium cholate, resulting in a decrease of size and entrapment efficiency. Also, all samples had polydispersity index values lower than 0.4 indicating a less pronounced tendency to aggregation.

The *in vitro* release of *A. rusticana* extract from liposomes was presented as cumulative percent release over an 24 h study period in PBS at *p*H 7.4, 37°C, 100 rpm; the results of this study were presented in Fig. 1. The release profile from the free extract presented a *burst release* phenomenon with 50.65 ± 0.13% released in the initial 30 minutes; the burst effect was restricted drastically by loading the *A. Rusticana* extract in liposomes (19.66 ± 0.13% was released in the initial 30 minutes from AR_L2 and respectively 34.67 ± 0.33 % from AR_L1). After 10 hour almost all amount of *A. Rusticana* from free extract was released (98.61 ± 1.09%), while liposomal formulations provided a much slower release, for example after 10 hours, the percentage release of extract was found out to be 67.39 ± 3.65% and 30.51 ± 0.32% in case of AR_L1 and AR_L2 formulation, respectively; ultimately after 24 hours reaching 72.98 ± 3.90% and 48.25 ± 3.90% for AR_L1 and AR_L2 liposomes, respectively. In order to evaluate the mechanism of *in vitro* extract release, several

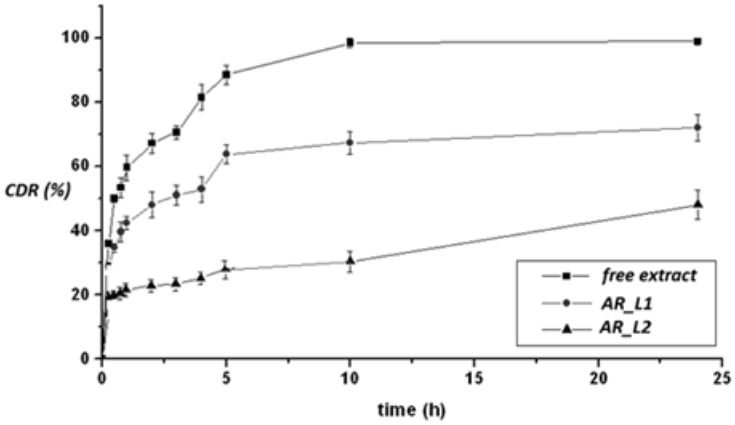

Fig. 1. Release of polyphenols from liposomes loaded with *A. rusticana* vs. free extract.

Model	Model equation	Sample code	R^2
Zero order	$Q_t = Q_0 + k_0 \cdot t$	*A. rusticana*	0.7122
		AR_L1	0.8437
		AR_L2	0.9202
Hixon	$Q_0^{1/3} - Q_t^{1/3} = k_{HC} \cdot t$	*A. rusticana*	0.7199
		AR_L1	0.9209
		AR_L2	0.9562
Higuchi	$Q = k_H \cdot t^{1/2}$	*A. rusticana*	0.8696
		AR_L1	0.9555
		AR_L2	0.9747
First order	$\ln(Q_t / Q_0) = -k_1 \cdot t$	*A. rusticana*	0.9901
		AR_L1	0.9261
		AR_L2	0.9337

Table 2
RELEASE KINETIC MODELING OF LIPOSOMES LOADED WITH *A. RUSTICANA* AND FREE EXTRACT.

kinetics models were applied (Zero-order, First order, Higuchi and Hixson-Crowell) and the model that best fitted our data was determined by comparison of the correlation coefficient (R^2). It was observed that release from free extract followed an exponential equation (First order), with $R^2 > 0.99$, while release from liposomal formulations followed a Higuchi model driven mainly by a diffusion-controlled mechanism ($R^2 > 0.95$), results confirmed by other studies [23].

Conclusions

Liposomes loaded with *A. rusticana* were prepared using thin-film hydration method and characterizated for entrapment efficiency, size, polydispersity index and stability. Liposomes loaded with *A. rusticana* presented good entrapment efficiency, nano-sizes (lower than 150 nm), narrow polydispersity index and good stability over 90 days at 4°C. The liposomal formulations reduced burst release of extract and provided a much slower release compared with free extract. These promising results suggest that liposomes could be exploited as carriers for herbal ingredients.

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