UNSATURATED FATTY ACID VESICLES AS TOPICAL NANODELIVERY SYSTEMS TO IMPROVE THE EFFICACY OF NATURAL ANTIOXIDANTS

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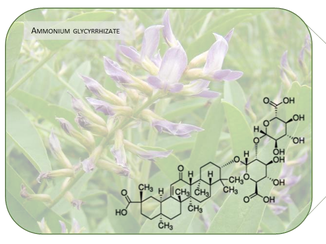
Oleic and linoleic acids are unsaturated fatty acids, which can be obtained from different natural sources. They are involved in several biological processes and show different beneficial properties for cutaneous diseases, due to their antioxidant effect. Considering the great properties of oleic acid for topical use and its efficacy as penetration enhancer, the aim of this study was to realize an all-natural delivery system based on oleic and linoleic acids for the cutaneous delivery of active compounds [1]. Ammonium glycyrrhizinate, well known as an anti-inflammatory and antioxidant compound, was delivered in the new realized natural system in the attempt to improve its cutaneous efficacy, severely limited by its physico-chemical properties. The natural system was deeply characterized, and efficacy studies were also performed. In detail, formulations made of only oleic and linoleic acid [1:1, molar ratio] or containing a small amount of Phospholipon 90 G [1:1:0.5, molar ratio] showed suitable values of mean size (lower than 300 nm), size distribution (polydispersity index value was around 0.2) and proper negative surface charge to be administered topically. These systems were able to encapsulate ammonium glycyrrhizinate for up to 80% of the dose loaded during the preparation phase (3 mg/mL). Further in vitro studies confirmed a great release of the active compound from the systems and the increase in the permeation of the active compound through the main skin barrier, the stratum corneum. The efficacy of ammonium glycyrrhizinate-loaded vesicles was tested on NCTC2544 keratinocyte cells, in terms of cell viability and LDH release before and after the oxidative stress induced using hydrogen peroxide. In vitro studies on cell lines highlighted the safe profile of empty vesicles and the ability of ammonium glycyrrhizinate-loaded vesicles to induce an improved antioxidant effect respect to free form of natural compound. In detail, their efficacy was shown by the reduction in LDH release (indicator of membrane damage) by 21% and 25% from formulation with and without Phospholipon 90G, respectively compared to 10% of reduction recorded from the free active. In vivo skin tolerability studies performed on human healthy volunteers also confirmed the good safety profile of the empty carriers for cutaneous administration up to 72 h. The obtained results [2] are encouraging and suggest that the new realized nanosystems represent promising tools for the treatment of oxidative stress as skin disease.



SAMPLE	Lipid composition (molar ratio)		
	OA	LA	PL®90G
Formulation A	1	1	0.5
Formulation B	1	1	-

OA:oleic acid; LA:linoleic acid;PL®90G:Phospholipon®90G

Sample	Size (nm)	PDI_1	Z Potential (mV)	DI_2	EE_3 (%)
Formulation A	189 ± 2	0.20 ± 0.02	-44 ± 1	9.71 ± 0.87	-
Formulation B	284 ± 2	0.22 ± 0.03	-42 ± 2	12.73 ± 1.01	-
Ag-Formulation A	146 ± 1	0.17 ± 0.01	-50 ± 1	9.55 ± 0.59	80.92 ± 1.03
Ag_4-Formulation B	153 ± 3	0.21 ± 0.01	-45 ± 1	10.02 ± 1.00	84.98 ± 1.20

1. Polydispersity index; 2. Deformability index; 3. Entrapment efficiency; 4. Ammonium glycyrrhizinate (3 mg/mL)

Table 1. Physico-chemical characterization of Ag-loaded unsaturated fatty acid vesicles. The addition of ammonium glycyrrhizinate leads to a reduction in vesicle size and increase of the negative surface charge. The low values of polydyspersity index confirmed that the addition of ammonium glycyrrhizinate didn't affect the stability and homogeneity of nanosystems. Studies concerning the deformability of the nanosystems highlighted good deformable properties, probably due to the presence of cis-double bonds in the structure of oleic and linoleic acid, which fluidify the bilayer of the system.

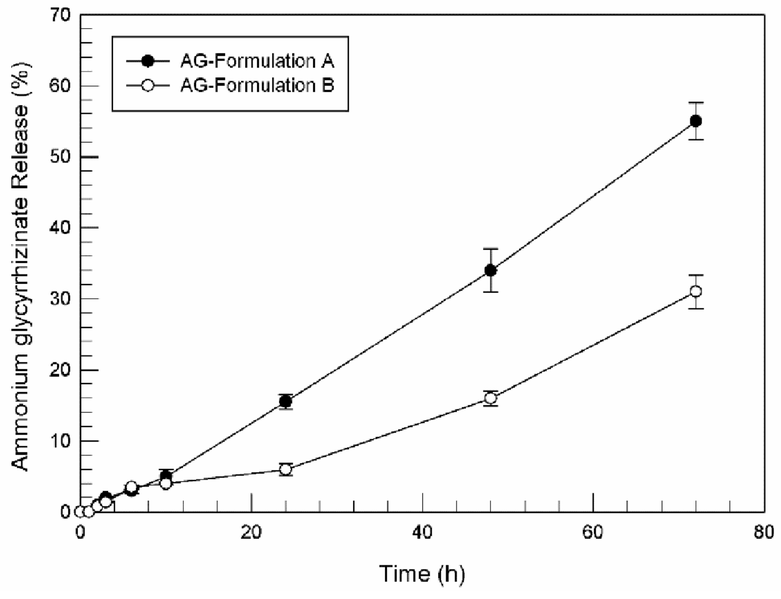


Figure 1. Release profile of Ag-loaded unsaturated fatty acid vesicles.
Franz diffusion vertical cells and synthetic membranes were used to perform experiments. Formulation B showed a biphasic release profile, whilst Formulation A presented the best releasing profile for ammonium glycyrrhizinate, which was recorded for up to 50 % of the loaded amount, within 72 h. However, both formulation showed a very low release profile (lower than 20%) and a great ability to retain the active compound within the vesicles, within 24 h.

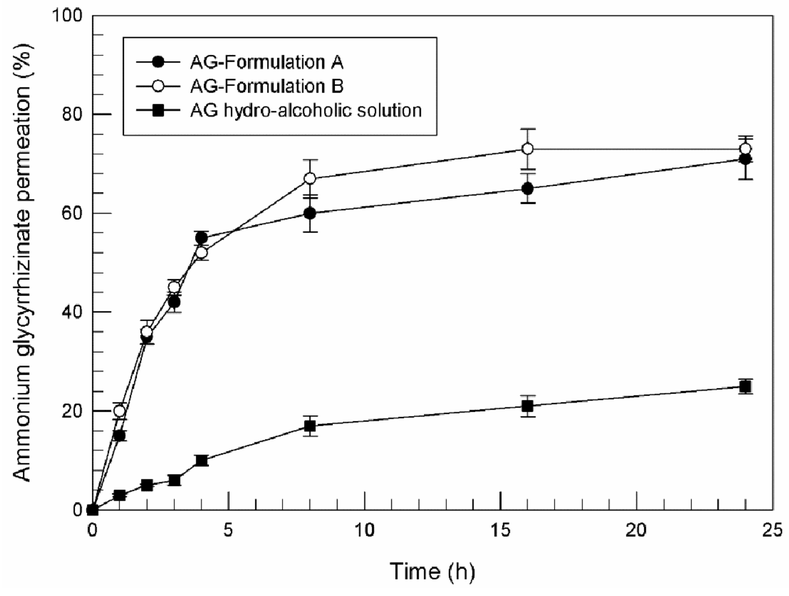


Figure 2. Percutaneous permeation profiles of Ag-loaded vesicles. Human SCE-membranes (stratum corneum and epidermis membranes) were isolated for this study and interposed between a donor and receptor compartment of Franz diffusion cells. Results showed that the ability of ammonium glycyrrhizinate to permeate membranes within 24 h was very low if compared to that recorded from Ag-loaded unsaturated fatty acid vesicles. Probably, a suitable interaction between vesicles and membrane occurs, thus increasing the release and permeation of the active compound.

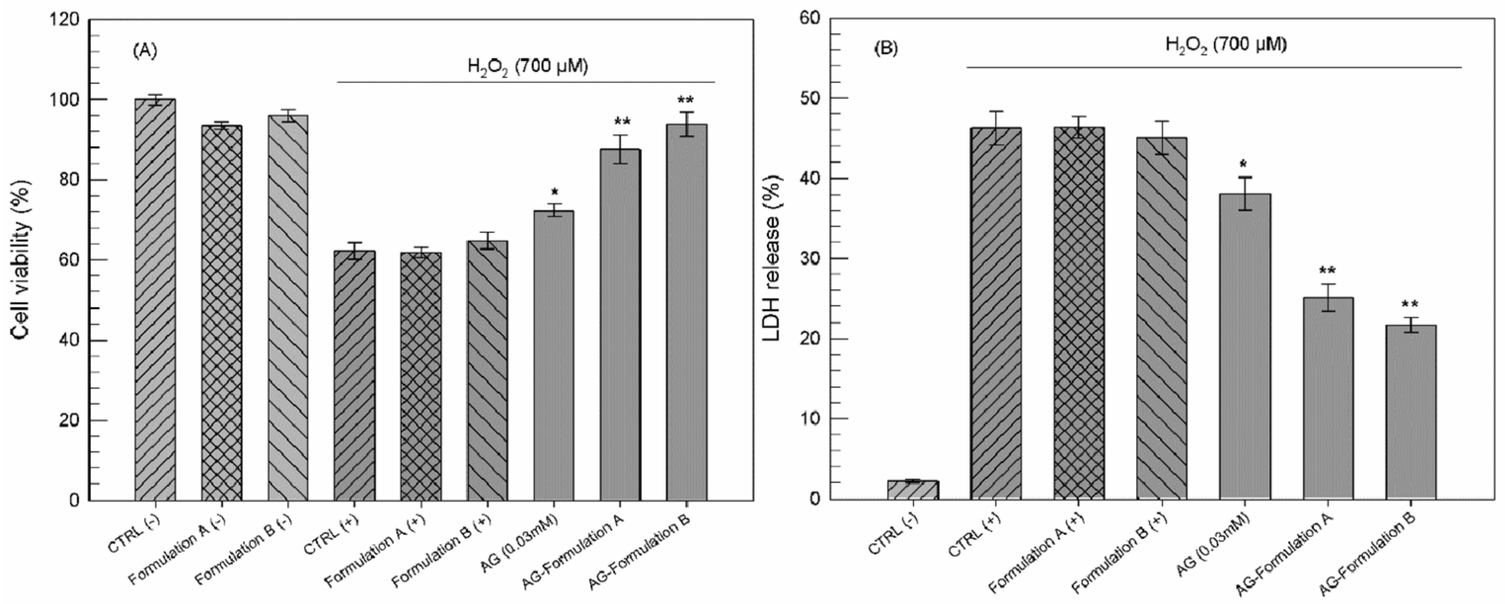


Figure 3. Antioxidant activity of Ag-loaded vesicles on human keratinocytes (NCTC-2544 cells). (A) The safety and efficacy of unsaturated fatty acid vesicles were tested in vitro on human keratinocytes, in terms of cell viability (MTT test) or LDH release, before (-) and after (+) the induction of the oxidative stress with H_2O_2 [700 µM]. Results showed that cell viability was retained up to 90% after the administration of empty vesicles, without oxidative stress. Empty vesicles seemed to be not able to induce an antioxidant effect by themselves but Ag-loaded unsaturated fatty acid vesicles protected cells against the oxidative stress, thus retaining a cell viability (%) significantly (*p< 0.05 and **p<0.001) higher if compared to that induced by the free active compound. **(B)** LDH-release assay confirmed that ammonium glycyrrhizinate was able to induce a reduction in LDH release (as indicator of membrane disruption) less than 10%, whilst Ag-loaded formulation A and formulation B reduced LDH release by about 21% and 25%, respectively.

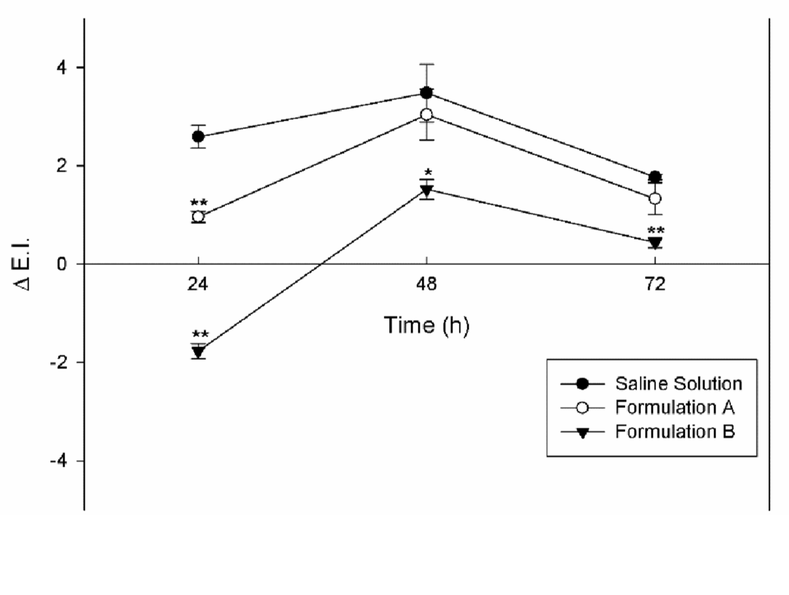


Figure 4. In vivo tolerability studies performed on healthy human volunteers. The cutaneous tolerability of empty vesicles was investigated using a reflectance spectrophotometer able to detect the variation of erythema index as a function of any skin color alteration. Erythema index (E.I.) values were compared with that recorded from the administration of saline solution, as control. Results showed the great safety of the nanovesicles for topical cutaneous administration.

[1] Cristiano, M.C.; Froiio, F.; Mancuso, A.; Cosco, D.; Dini, L.; Di Marzio, L.; Fresta, M.; Paolino, D. Oleuropein-Laded Ufasomes Improve the Nutraceutical Efficacy Nanomaterials 2021, *11*, 105.
[2] Cristiano, M.C.; Mancuso, A.; Fresta, M.; Torella, D.; De Gaetano, F.; Ventura, C.A.; Paolino, D. Topical Unsaturated Fatty Acid Vesicles Improve Antioxidant Activity of Ammonium Glycyrrhizinate Pharmaceutics 2021, *13*, 548.