Nano Innovation 2021 Rome, 21-24 September Conference & Exhibition

Cationic ethosomes: new perspective for transdermal application

Maria Chiara Bruno (1), Antonia Mancuso (1), Martine Tarsitano (1), Donatella Paolino (2), Massimo Fresta (1), Maria Chiara Cristiano (2)
(1) Department of Health Sciences, School of Pharmacy and Nutraceuticals, University "Magna Græcia" of Catanzaro, Campus Universitario "S. Venuta"-Building of BioSciences, Viale S. Venuta, Germaneto-Catanzaro, I-88100, Italy. mariachiara.bruno@studenti.unicz.it; mchiara.cristiano@unicz.it
(2) Department of Experimental and Clinical Medicine, School of Pharmacy and Nutraceuticals, University "Magna Græcia" of Catanzaro, Campus Universitario "S. Venuta"-Building of BioSciences, Viale S. Venuta, Germaneto-Catanzaro, I-88100, Italy. mchiara.cristiano@unicz.it

Ethosomes® are nanocarriers composed by phospholipids, ethanol and water. They are one of the main nanocarriers studied for transdermal drug administration due to their ability to cross the *stratum corneum* thanks to the elasticity conferred by structural ethanol. The usual composition of ethosomes provides vesicles characterized by suitable size and polydispersity index and negative surface charge. The aim of this study was to add a phospholipid capable of conferring a positive surface charge, namely 1,2-Dioleoyl-3-trimethylammonium propane (DOTAP), thus maintaining the features of classical ethosomes. Three formulations, called A, B and C, with different concentrations of phospholipids were prepared (Table 1). The results of the physicochemical characterization was reported in table 2. The stability studies over time were carried out using the Turbiscan Lab® Expert and were reported in figure 2a and figure 2b. Cytotoxicity studies have been carried out using the NTCT 2455 cells, a human keratinocyte cell line. Various formulations resulted safe (Figure 3). Therefore, an amphipathic probe, i.e. bromophenol blue, a hydrophilic, i.e. rhodamine B, and a hydrophobic ones, i.e. oil red O were encapsulated to evaluate the potential drug encapsulation features. The results showed that the encapsulation efficiency was greater than 50 % in formulations A, B and C for rhodamine B and oil red O, while it was less than 50 % with bromophenol blue (Table 3). The satisfactory findings results suggest a possible future transdermal application of cationic ethosomes.

Table 1. Composition and physicochemical characterization of formulation A, B and C. The formulations had mean size of about 200 nm, *optimum* for transdermal application [1], a polidispersity index < 0.2 and the formulations B and C had a positive surface charge (+56 ± 1 mV and +52 ± 1 mV, respectevely) due to the presence of DOTAP.

Formulation	Lipid composition		Ethanol (% w/w)	Bidistilled water (% w/w)	Mean size (nm)	Polydispersity index	Zeta potential
	Lipid mixture	Molar ratio					
A	PL90G	-	40	59	224 ± 3	0.16 ± 0.01	−25 ± 0
B	PL90G:DOTAP	10:1	40	59	152 ± 2	0.16 ± 0.02	+56 ± 1
C	PL90G:DOTAP	10:0.5	40	59	189 ± 2	0.13 ± 0.01	+52 ± 1

Table 2. Physicochemical characterization of oil red O, rhodamine B and bromophenol blue loaded ethosomes A, B and C and their encapsulation efficiency. The mean size of different probes loaded formulations tended to reduce, instead the polidispersity index remained close to zero and the net surface charge in formulations B and C mantained positive values. The encapsulation efficiency is greater with rhodamine B (> 50 %). Probabily, there were greater interactions between the probe and the other ethosomes components.

Dye	Formulation	Mean size (nm)	Polydispersity index	Zeta potential (mV)	Encapsulation efficiency (%)
Oil Red O	**A**	100 ± 1	0.19 ± 0.02	−25 ± 1	43 ± 3
	B	112 ± 3	0.29 ± 0.01	+38 ± 2	55 ± 2
	C	105 ± 1	0.33 ± 0.01	+29 ± 3	50 ± 2
Rhodamine B	**A**	132 ± 2	0.17 ± 0.02	−14 ± 1	69 ± 4
	B	108 ± 2	0.13 ± 0.01	+33 ± 2	52 ± 2
	C	118 ± 1	0.08 ± 0.01	+31 ± 1	69 ± 6
Bromophenol blue	**A**	88 ± 3	0.18 ± 0.01	−16 ± 1	27 ± 2
	B	94 ± 2	0.16 ± 0.00	+28 ± 2	15 ± 6
	C	135 ± 2	0.08 ± 0.02	+13 ± 2	36 ± 3

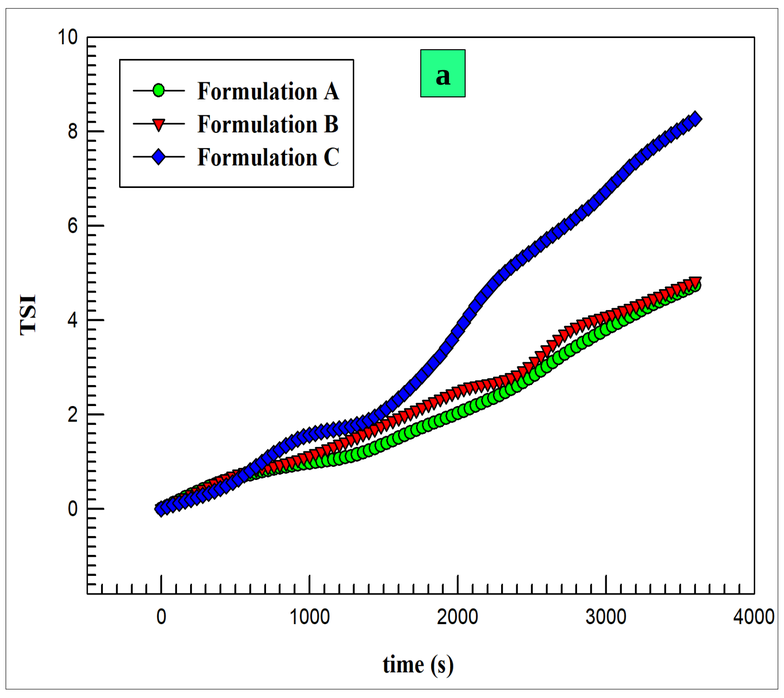

Figure 2a. Turbiscan stability index (TSI) of formulations A, B and C. The TSI of the three formulations had values below 8. In particular, the formulation B (with DOTAP) and the formulation A had almost overlapping TSI and the TSI had values below 4 .

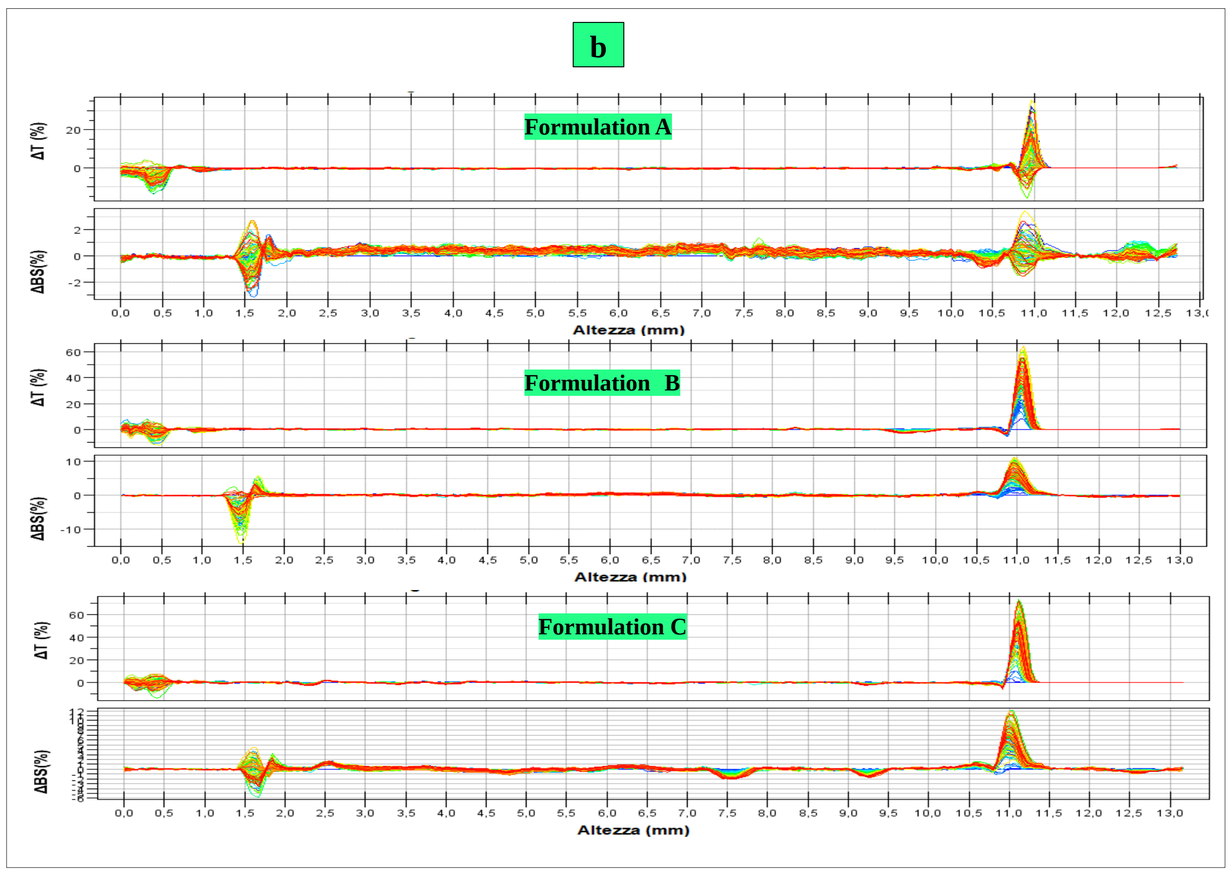

Figure 2b. Delta back scattering and delta trasmission profiles of formulations A, B and C. The delta back scattering and the delta transmission profiles remained constant throughout the analysis (1 h) confirming the stability of the formulations.

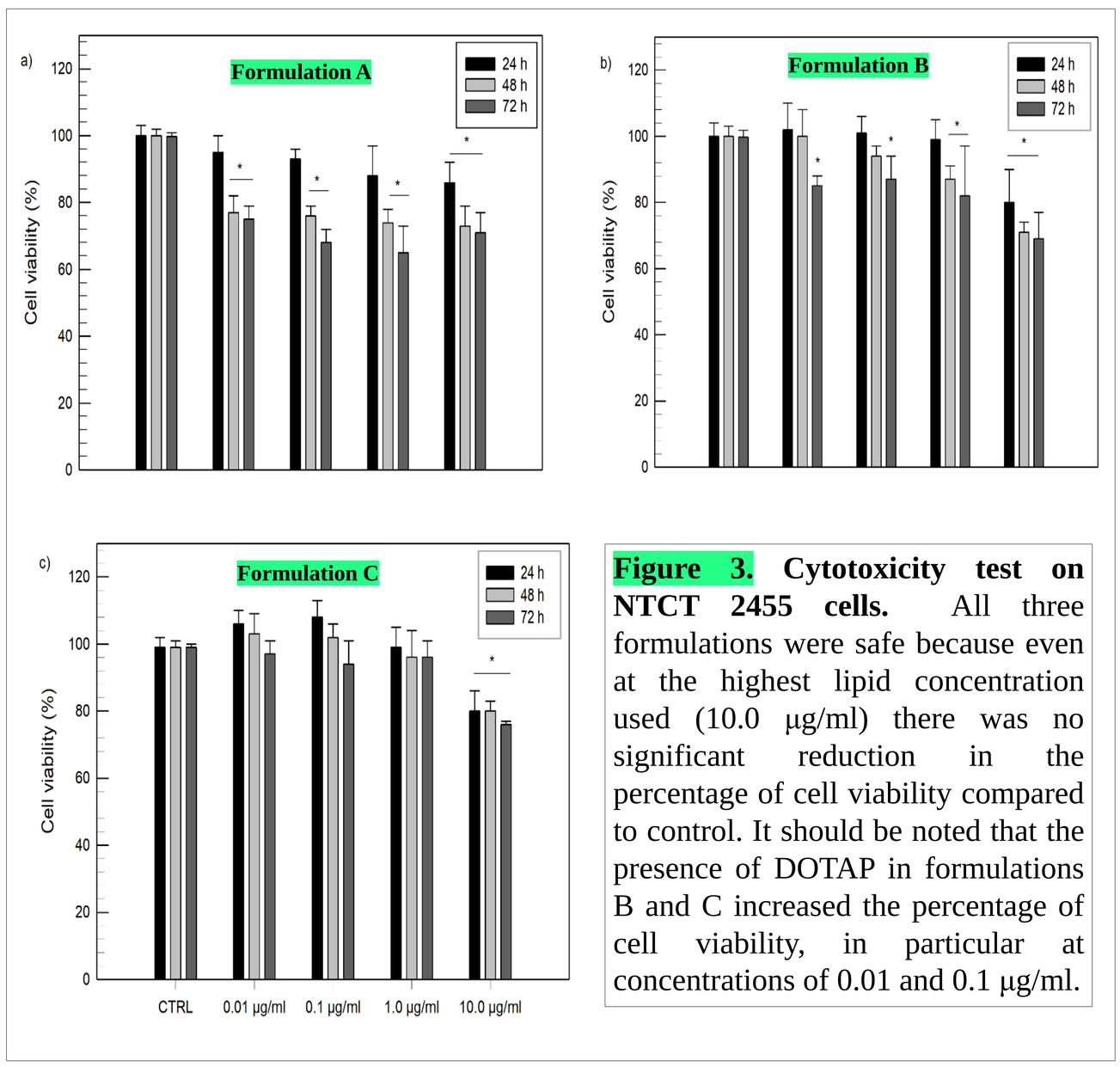

Figure 3. Cytotoxicity test on NTCT 2455 cells. All three formulations were safe because even at the highest lipid concentration used (10.0 µg/ml) there was no significant reduction in the percentage of cell viability compared to control. It should be noted that the presence of DOTAP in formulations B and C increased the percentage of cell viability, in particular at concentrations of 0.01 and 0.1 µg/ml.

[1] Fresta, M., Mancuso, A., Cristiano, M. C., Urbanek, K., Cilurzo, F., Cosco, D., Iannone, M., Paolino, D. (2020). Targeting of the pilosebaceous follicle by liquid crystal nanocarriers: In vitro and in vivo effects of the entrapped minoxidil. *Pharmaceutics*, *12*(11), 1127.