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DSPC Liposomes Improve Transport of L-cysteine and Reduce Metabolic Activity

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RMP and NSC designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors NSC, MJP and SVA managed the analyses of the study. Author RMP also managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: In this work, we developed and characterized liposomal formulations that encapsulate L-cysteine to study their further application in drug delivery and amino acid supplementation. The lipids used were 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC).
Methodology: Encapsulation efficiency and amino acid release were determined. For biophysical characterization of the three formulations, the size, surface charge and surface packing were also studied. Cell viability was analyzed with MTT reagent after treatments with formulations ir order to study efficiency of these systems in induce changes in metabolism.
Results: Results showed that L-cysteine interacts at the polar head level and that this interaction stabilizes the surface charge and prevents aggregation. We also determined the influence on cell metabolism in all formulations. The presence of L-cysteine in the DSPC formulation induced deeper

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changes in metabolism, evidencing that this formulation provides better transport of this amino acid.

Conclusion: Liposomes developed herein are well suited for the application in the delivery of L-cysteine. Particularly, they can encapsulate nearly all the L-cysteine and can retain it for 6 hours. Also, L-cysteine stabilized liposomes, preventing their aggregation. L-cysteine encapsulated in the DSPC formulation induced deeper changes in cell metabolism, causing a decrease in metabolic activity; this was probably due to a higher entry, thus a better liposome-mediated transport. Considering that the smaller the particle, the better the circulation, we believe that the stabilization of the vesicle by L-cysteine may allow these transporters to have higher circulation times. Based on the above, we conclude that the DSPC formulation is the best suited for further application in L-cysteine delivery.

Keywords: Liposomes; L-cysteine; FTIR; cell metabolism; biophysics.

1. INTRODUCTION

L-cysteine is a semi-essential amino acid that plays a key role both directly in the oxidant/antioxidant balance as a glutathione precursor and indirectly in the regulation of metabolic processes. In occidental countries, the dietary consumption of L-cysteine is generally suboptimal and, sooner or later, everybody is likely to have cysteine deficiency. Evidence suggests that, in the human body, L-cysteine deficiency is related to the ageing process. This leads to a decrease in muscle and immune function, a decrease in plasma albumin concentration, and an increase in the concentration of the inflammatory cytokine tumor necrosis factor alpha (TNF-α) [1]. Several clinical trials have shown that L-cysteine supplementation improves skeletal muscle functions, decreases the body fat/lean body mass ratio, decreases the plasma levels of TNF-α, improves immune functions, and increases plasma albumin levels [1]. The best documented effects of L-cysteine supplementation on immunological functions in humans have been found in patients infected with viruses such as HIV and hepatitis B virus [1,2]. So an efficient delivery system that can increment the systemic concentration of L-cysteine and can enhance the aminoacid entrance to cells could serve as a backup therapy to treat a wide range of diseases. These delivery systems can be co-administrated orally with other specific treatments.

Unfortunately, L-cysteine is easily oxidized to cysteine and reaches low concentrations in plasma [1]. This low oxidative stability represents an issue for its supplementation and incorporation into cells.

Liposomes are widely used for drug delivery in the cosmetic and pharmaceutical industries. These vesicles are able to encapsulate unstable compounds and protect their function from oxidation and degradation [3]. They can also encapsulate hydrophobic and hydrophilic molecules [3], allowing a controlled release at the target site [4,5]. Liposomes are also able to increase cytosolic drug delivery [6] and decrease secondary effects [3]. Additionally, liposomes can increment drug absorption after incorporated by an oral route [7].

Due to their biocompatibility and biodegradability, liposomes were the first drug-delivery system approved for clinical purposes [8]. Since the first liposomal formulation Amphotericin B (Ambisome®), which is indicated for fungal infections [9], was approved by FDA in 1990, many other compounds such as nucleic acids, therapeutic molecules and proteins have been encapsulated in liposomes [8].

Amino acid derivates such as N-acetylcysteine, which is a mucolytic and has antioxidant properties, have also been encapsulated. It has been shown that liposomal N-acetylcysteine presents higher antioxidant activity than the non-encapsulated drug [10].

The delivery of the encapsulated drug depends on the nature of the lipid bilayer, the size of the drug molecules, their partition coefficient in oil/water, and their interactions with the lipid membrane [8]. The liposome delivery system is also strongly influenced by the nature and density of the charge (Z-potential) of the liposome surface. For this reason, it is essential to understand the biophysical properties of the formulation, including the surface charge and liposome size. Higher values of Z-potential (>±25 mV) may contribute to the stability and may decrease the aggregation and fusion rate of the formulation [11,12]. The liposome size is related to the stability and membrane fusion as well as to the localization of the liposome after administration [13].

Based on the above, encapsulation of L-cysteine in liposomes could represent a solution for the delivery of this amino acid, preventing its oxidation and allowing a higher amount of L-cysteine to reach target cells. In this study, we developed and biophysically and biologically characterized three liposomal formulations that encapsulate L-cysteine. The lipids used were 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC). We found that L-cysteine is encapsulated in liposomes through its interaction with the lipid polar head. We also observed that the amino acid stabilizes the liposomes by increasing the Z potential values. Finally, the decrease in metabolic activity induced by DSPC liposomes suggests that this formulation is the most appropriate for the delivery of L-cysteine and could be promoting higher incorporation of L-cysteine into cells.

2. MATERIALS AND METHODS

2.1 Materials

Lipids were purchased from Avanti Polar Lipids (Alabama, USA) and used without further purification. The reagents used in cell culture were purchased from Life Technologies (USA). All solvents were of analytical grade or higher.

2.2 Liposome Preparation

Lipids (2 mg) were dissolved in chloroform; the solvent was flash-evaporated with N_2 [14]. Large multilamellar vesicles were obtained by adding 1 mL of deionized water with or without L-cysteine. Sodium hydroxide was added to keep an alkaline pH to increase amino acid solubilization. Large unilamellar vesicles were obtained by passing the mixture 11 times through a Miniextruder (Avanti Polar Lipids). First, all formulations were passed through a 400-nm pore polycarbonate membrane, and then, only DMPC and DPPC were passed through a 100-nm pore membrane. This difference lies in the fact that the extrusion should be performed at a temperature above the transition temperature. Since the main transition temperature of DSPC was 60°C, the 100-nm pore membrane did not resist the pressure and collapsed.

2.3 Release Profile

The L-cysteine release profile of liposomes was studied in phosphate buffer (PBS). Non-encapsulated L-cysteine was separated by microdialysis [15]. Liposomes with 50 mol% of L-cysteine were prepared as described above and 0.5-mL aliquots were placed into a dialysis tube. The tube was placed in a 5 mL PBS buffer solution. At different times, an aliquot was withdrawn from the buffer. After withdrawing each sample, an aliquot of PBS was added to maintain the original concentration gradient. L-cysteine was quantified colorimetrically [16].

2.4 Surface Modifications

To determine the spectral characteristics of the membrane/solution interface, the visible spectra of the probe merocyanine 540 (MC540) were recorded between 400 and 600 nm at 10°C to maintain the gel phase and at 37°C. MC540 is sensitive to the polar environment. In water, the spectra show two maxima (a dimer at 500 nm and a monomer at 530 nm). In a hydrophobic environment, the maximum is shifted towards 530 nm (MC540 dimer) and 570 nm (MC540 monomer). The first two are the characteristic absorbance maxima when the membrane is in the gel phase, whereas the second two are the characteristic absorbance maxima when the membrane is in liquid crystalline phase [17]. The ratio of the absorbance at 570 nm with respect to that at 500 nm, called hydrophobicity factor (HF) [18], determines the degree of hydrophobic sites exposed to the interface.

2.5 FTIR Spectroscopy

Fourier-transform infrared (FTIR) spectroscopy was used to study the interaction between liposome formulations and L-cysteine, as previously described [19]. Briefly, 0.3 mL of the liposomal suspension with or without L-cysteine was pipetted onto ATR cells and dried with a hair drier. Infrared spectra were recorded from 3000 to 1000 cm^{-1} in an IRAffinity-1 FTIR spectrometer (Shimadzu). Data were analyzed, normalized and baseline corrected with the IRsolution software (Shimadzu). Frequencies were determined by the peak identification routine of the software.

2.6 Cell Culture Studies

To study the influence of the delivery systems proposed in a cell culture and analyze whether there was any cytotoxic effect, the HEK293T cell line, a human embryonic cell line [20], was chosen. This cell line was selected because it is

an established but not a tumor one. A human cell line derived from non-tumor tissue was necessary because these transporters are developed and studied to be applied in a wide range of illnesses.

Briefly, HEK293T cells were seeded in a 96-well plate. Cells were grown in Dulbecco's Modified Eagle Medium (DMEM) enriched with 10% v/v of bovine fetal serum (BFS), and a combination of antibiotics and antimycotics. All reagents were purchased from Life Technologies (USA). Cells were allowed to grow at 37℃ in a 5% CO_2 atmosphere. Upon cell confluence, liposomes were seeded in maintenance medium (without BFS) at two different lipid concentrations (1 and 2 mg/mL). After 4 or 24 hours, the medium was washed and the viability (or metabolic) assay was performed.

2.6.1 Viability assay

Viability was determined by staining cells that remained adhered to a 96-well plate with Crystal Violet dye [21]. The percentage of viability was calculated as:

> [(absorbance of treated cells/absorbance of untreated cells)]*100

3. RESULTS AND DISCUSSION

3.1 Entrapment Efficiency (EE)

The EE of L-cysteine was determined to study the amino acid retention capacity of the different liposomal formulations. We found no significant differences in the ability to retain the amino acid after extrusion and prior to any dilution of the three liposomal formulations. The assay showed an average EE of almost 100 for all formulations: 98.54±1.35% for DMPC, 98.27±0.68% for DPPC and 97.44±0.68% for DSPC. The three formulations showed similar EE at the studied lipid:amino acid molar ratio (1:0.5). The high EE could be due to the affinity of L-cysteine negatively charged at pH=11 by the lipid polar head [22].

3.1.1 Release Profile (RP)

After EE measurements, we studied the RP of L-cysteine by microdialysis in buffer pH=7.4 to emulate physiological conditions [23]. Fig. 1 shows the RP of the three liposomal formulations. After 24 hours, the RP was 71.9±2.7% for DMPC, 70.37±0.18% for DPPC, and 72.17±8.18% for DSPC. No significant differences were observed between formulations. Afterwards, liposomes were disaggregated with ETOH 30% to measure the unreleased amino acid after 24 hours [24]. We observed that 25.4±2.08% (DMPC), 21.3±3.9% (DPPC) and 24.7±0.8% (DSPC) of L-cysteine was retained in liposomes. The unreleased amino acid remained associated with the bilayer polar head or in the aqueous inner part of the liposome. These experiments provide evidence of an interaction between L-cysteine and the liposome surface.

It can be observed that at 6 hours, 50% of L-cysteine was linearly released every hour.

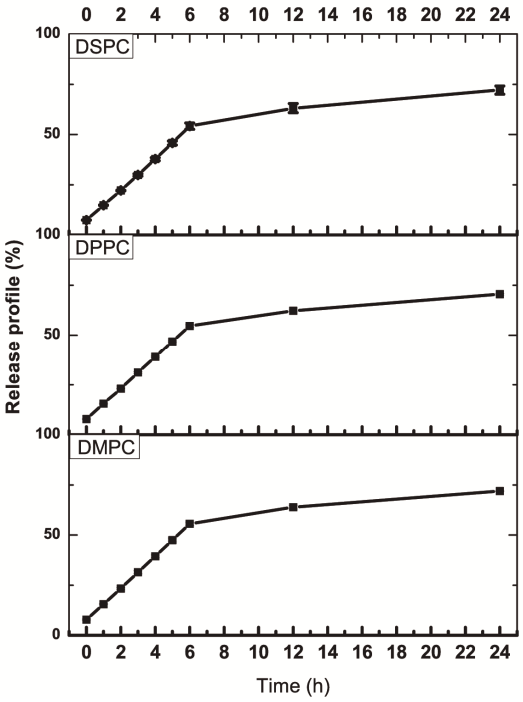

Fig. 1. Release profile of L-Cys entrapped in liposomes. Data is shown as mean ± SD of three independent measurements

3.2 Zeta Potential (ZP)

To determine the surface charge of the liposome formulations after the addition of L-cysteine, we measured the ZP. The results are shown in Table 1. All formulations had a negative ZP. A high ZP (±25 mV or higher) indicates more stability and lower rate of aggregation and fusion of the particles [11,12]. We observed a decrease in ZP after L-cysteine addition. This indicates that the amino acid has a stabilizing effect on the liposomal formulations, giving more evidence of an L-cysteine-surface interaction.

The ZP is a function of the surface charge that occurs when any material is placed in a liquid, and is useful to predict the stability of dispersion [23,25]. The control and prediction of the stability of liposomes are important when they are stored for a long period of time after preparation.

The ZP of the membrane is an important parameter that influences the membrane behavior. *In vivo* methods have shown that the surface charge density influences the biodistribution of the liposomes [26]. *In vitro* methods have also shown that a negative ZP may contribute to the physical stability of liposomes by reducing aggregation and fusion [11].

The results are shown in Table 1. All formulations had a negative ZP. A high ZP (±25 mV or higher) indicates more stability and lower rate of aggregation and fusion of the particles [11,12].

Table 1. Zeta potential of liposomal formulations with and without L-cysteine

Formulation	Z potential (mV)
DMPC	-29
DMPC CYS	-48
DPPC	-32
DPPC CYS	-44
DSPC	-34
DSPC CYS	-86

The addition of L-cysteine induced a considerable decrease in the ZP of the three formulations. This decrease was higher in the case of DSPC. This is because, at the working pH (pH = 11), L-cysteine has a net negative charge.

Change in ZP was particularly higher in DSPC formulation since this system has a larger size in comparison of the other two formulations. DSPC has a very high transition temperature and the obtaining of small vesicles is very difficult. So highly negatively charge L-cysteine could accommodate better in the surface of a higher liposome, stabilizing it by reducing its ZP and preventing fusion and leakage of the amino acid.

3.3 Particle Size

Liposome size was measured by dynamic light scattering. Having information about the particle sizes is of great interest due to the trend of membrane fusion and because the stability of the vesicles depends on this parameter [13]. Table 2 shows that the average diameter of the three liposomal formulations was below 200 nm. It is important to highlight the presence of a second population in DSPC liposomes (318.4±23.7*). This population disappeared when L-cysteine was added. This fact, coupled with the decrease in ZP after L-cysteine addition, strengthens the evidence that suggests that the amino acid exerts a stabilizing effect on the liposomal formulations.

Table 2. Average diameter of three liposomal formulations with and without L-Cys

Liposome formulation	Average diameter (nm)
DMPC	121,6±0,1
DMPC L-Cys	121,5±1,5
DPPC	108,3±1,3
DPPC L- Cys	140,3±4,0
DSPC	196,4±8,3 (318,4±23,7*)
DSPC L-Cys	145,9±2,0

**DSPC shows an extra population which disappear after L-Cys addition*

3.4 Hydrophobicity Factor (HF)

Hydrophobic defects on the surface of lipid bilayers may be related to their stability because the higher water ingress increases liposome destabilization [18]. Such defects can be studied using the probe Merocyanine 540 (MC540). This probe is sensitive to the polarity of the environment. When MC540 is in a hydrophobic environment, such as a liposome bilayer, the spectrum undergoes a shift to longer wavelengths. The ratio of absorbance at 570 nm and 500 nm is a parameter called Hydrophobicity Factor (HF) and gives an idea of the number of exposed hydrophobic sites at the water-liposome interface and the level of packing or rigidity on the liposome surface [17,18,21]. Samples were measured at 10℃ and 37℃ to evaluate changes in the membrane at the gel phase temperature (10℃) and physiological temperature (37℃).

To analyze the HF results, it is important to remember that in the bilayer there are different phases. When the temperature is below the pre-transition temperature, the phase is called rigid gel-crystalline and is very rigid. With increasing temperature, the rotational mobility of the lipid polar heads also increases and ripples appear on the surface of the bilayer. As a result, HF increases. Finally, the liquid crystalline phase is reached, where the polar heads and hydrophobic

chains have greater freedom and the bilayer becomes more fluid, further increasing HF [18]. This effect can be clearly seen in Fig. 2, where the increase in HF of lipid formulations without L-cysteine is observed after an increase in temperature. Table 3 shows the transition temperatures of the three lipids used [27].

Table 3. Transition temperatures of lipids used [28]

Lipid	Temperature (ºC)	
	Pre-transition	Main transition
DMPC	15.3	24.0
DPPC	35.5	41.5
DSPC	51.0	54.3

The results of HF are shown in Fig. 2. Lower HF values mean higher membrane rigidity. When the bilayers were in liquid-crystalline phase and pre-transition temperature, the addition of L-cysteine induced a significant decrease in HF (DMPC and DPPC at 37ºC respectively). When the bilayers were in gel phase, L-cysteine addition tended to increase HF values (DMPC and DPPC at 10ºC respectively). We also observed a significant decrease in the HF value of DSPC in the gel phase (10ºC), which indicates lower membrane rigidity and greater fluidity. To summarize, L-cysteine addition increased the membrane rigidity when the bilayer was in the liquid-crystalline phase and pre-transition temperature. This was the case of DSPC and DPPC. Also, amino acid decreased membrane rigidity and increased fluidity at the gel phase (DMPC). This effect is due to the interaction between the negatively charged amino acid and the bilayer polar head. Effect was similar to the one reported in the case of cholesterol: amino acid is able to increase order in the disordered phase (liquid-crystalline) and decrease order in the ordered phase (gel) [29].

3.5 Infrared Spectroscopy

To study changes which can be induced by the addition of L-cysteine in the lipid bilayer and identify possible lipid-amino acid interactions, we next measured the FTIR of the three liposomal formulations (DMPC, DPPC and DSPC). In all cases, the control was the liposomal formulation without the amino acid. Bands corresponding to the functional groups present in the lipid polar head and in the aliphatic chains were analyzed [30-33]. Results are shown in Table 4.

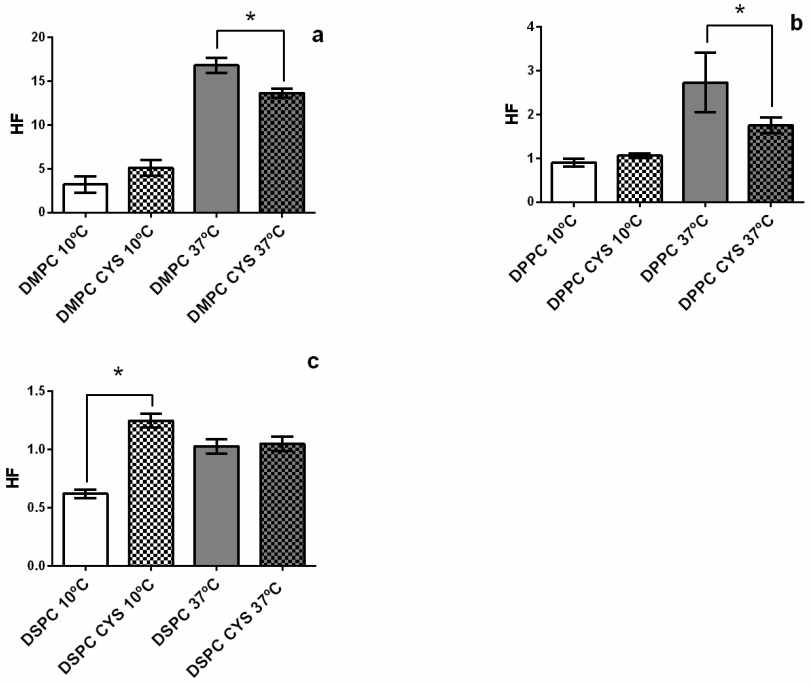

Fig. 2. HF of liposome formulation with and without L-cysteine. Assays were performed at two different temperatures: 10 and 37ºC. Data are shown as mean ±SD of four independent measurements

**Significant differences after performed ANOVA and Tukey´s post test (P<0.01)*

Table 4. FTIR Vibration of the different functional groups

Group (Vibrational type)	DMPC	DMPC CYS	DPPC	DPPC CYS	DSPC	DSPC CYS
υ_a(+)N-(CH_3)$_3$ asymmetric stretching	968	968	968	968	968	968
υ_s(+)N-(CH_3)$_3$ symmetric stretching	925	923	925	921	923	921
R-O-P-O-R	1062	1068	1060	1064	1060	1064
υ_sP=O symmetric stretching	1087	1089	1089	1091	1089	1091
υ_aP=O asymmetric stretching	1232	1236	1240	1246	1238	1249
CH_2 scissoring	1467	1467	1467	1467	1467	1467
C=O stretching	1735	1735	1735	1734	1735	1734
$\upsilon_s CH_2$ symmetric stretching	2850	2850	2848	2848	2848	2850
$\upsilon_a CH_2$ asymmetric stretching	2920	2920	2916	2916	2916	2918
$\upsilon_a CH_3$ symmetric stretching	2956	2956	2954	2956	2956	2956

The bands corresponding to the symmetric (2850 cm^{-1}) and asymmetric (2920 cm^{-1}) stretching of the methylene groups (-CH_2) of the aliphatic chains of phosphatidylcholine are associated with conformational changes in the lipid order [30]. The shift to higher frequencies of these bands indicates a decrease in the lipid order. In the present study, we observed that after the addition of L-cysteine, the three formulations remained unchanged at the level of the aliphatic chains.

The scissoring vibration mode of –CH_2 is much more sensitive to changes in the packing of the aliphatic chain [34]. None of the three formulations presented changes in this band after L-cysteine addition (1467 cm^{-1}), which, added to the small changes in the symmetric and asymmetric stretching of methylene, provides strong evidence to suggest that L-cysteine prefers to interact with the liposome surface rather than with the hydrophobic core.

The position of the peaks belonging to the phosphate group (P = O) is sensitive to the formation of hydrogen bonds and coulombic interactions [32]. If the band shifts towards lower frequencies, it indicates increased formation of such interactions, and consequently greater hydration. The asymmetric phosphate has a band at 1240 cm^{-1} when dehydrated and this band shifts to 1220 cm^{-1} when fully hydrated. The addition of L-cysteine generated dehydration in the phosphate group in all three formulations; this was evidenced by the shifting of the bands corresponding to the symmetric (1089 cm^{-1}) and asymmetric (1236 cm^{-1}) stretching to higher frequencies.

The shift of the bands to a lower frequency for the carbonyl group (1732 cm^{-1}) indicates greater hydration of the polar head in the region near the beginning of the aliphatic chain [31]. The DMPC formulation showed no changes at the C=O level, while DPPC and DSPC showed slight hydration in this region after the addition of L-cysteine.

The band corresponding to the stretching of the amino group gives information concerning the level of rigidity on the liposome surface. A lower frequency indicates an increase in the mobility [35]. The three liposomal formulations showed a shift towards lower frequencies in the band corresponding to the symmetric stretching of the amino group. This suggests higher mobility and therefore less rigidity on the liposome surface.

L-cysteine induces an increase in order of the liposome surface. The phosphate and amino groups forming part of the dipole of the lipid polar head are sensitive to electrostatic interactions. When the interaction is with a negatively charged molecule, such as L-cysteine at pH = 11, it induces the exposure of the amino group, reducing the angle of the head relative to the polar aliphatic chains bilayer order [22].

Finally, a strong band at 1590 cm^{-1} has been reported by other authors [36]; this band corresponds to the asymmetric vibration of the carbonyl group of L-cysteine. The FTIR spectra recorded for formulations with L-cysteine had a very strong band at 1585 cm^{1} (data not shown). These peaks are of great importance to confirm the presence of the amino acid in the liposomal formulation. In addition, since a shift was also observed, this strongly suggests that the part of the amino acid that interacts with the liposome surface is the carbonyl group.

3.5.1 Raman spectroscopy

Raman and FTIR spectroscopy are complementary techniques to study vibrational frequencies of a molecule. Results are shown in

Table 5. The vibrational frequencies of the amine group (715 cm^{-1}) decreased as the bilayer rigidity increased**.** We also observed a peak at 662 cm^{-1} belonging to the C-N stretching of L-cysteine, absent in control formulations.

The results of Raman and FTIR spectroscopy support the evidence observed in the previous experiments and allow us to conclude that negatively charged L-cysteine interacts with the liposome surface, especially with the polar head. This interaction induces major changes in vibrational frequencies when the bilayer shows less rigidity, as we can be observed in DMPC formulations (C-N stretching). When the bilayer was in a less rigid conformation, as in the case of DMPC, deeper changes in C-N stretching were observed. On the other hand, when the bilayer was in a more rigid conformation, as in the case of DSPC formulations, C-N stretching showed no changes.

3.6 Biological Characterization

3.6.1 Metabolic activity assay

Metabolic processes, especially the mitochondrial respiratory chain, can be evaluated by studying mitochondrial dehydrogenases. MTT is a colorimetric reagent, which turns yellow when oxidized and purple after reduction by mitochondrial enzymes. Absorbance at 570 nm is a quantitative analysis used to detect reduced

Table 5. Raman vibration of different functional groups

Group (vibration type)	DMPC	DMPC CYS	DPPC	DPPC CYS	DSPC	DSPC CYS
C-S stretching	-	662	-	664	-	663
S-S	-	501	-	501	-	493
C-N stretching	716	711	718	715	713	713
$_{Us}CH_2$ symmetric stretching	2850	2850	2848	2848	2848	2850
$_{Ua}CH_2$ asymmetric stretching	2920	2920	2916	2916	2916	2918

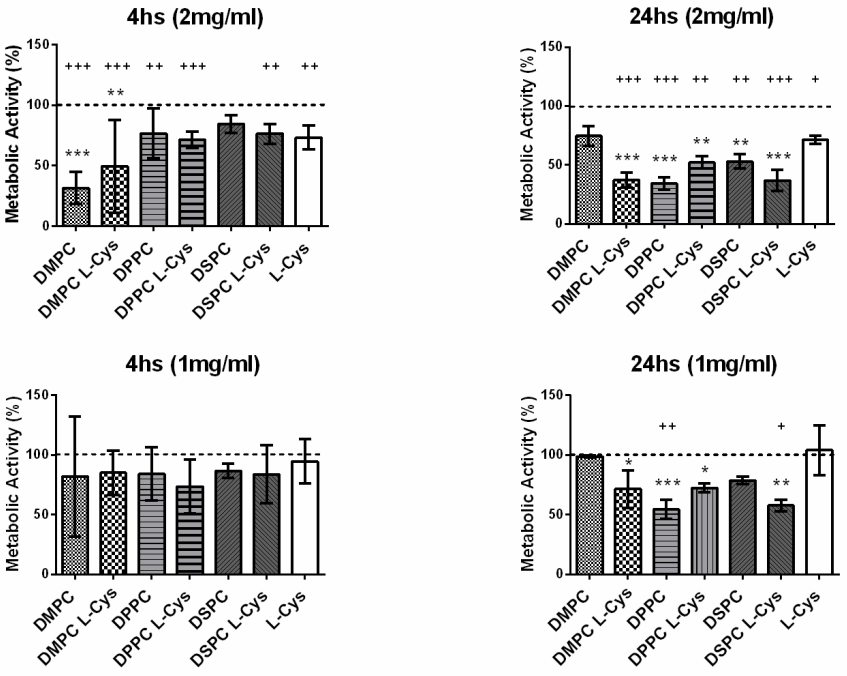

Fig. 3. MTT determination in HEK293T cell line. Data is shown as mean ± SD of eight determinations. Cells with no treatment were taken as 100% of metabolic activity

**Significant differences after perform ANOVA and Dunnet post test taking cells with no treatment as control*
+Significant differences after perform ANOVA and Dunnet post test taking cells treated with free L-cysteine as control

MTT [37]. A higher amount of reduced MTT (high absorbance at 570 nm) reveals high reductase activity, thus high metabolic activity. The MTT assay was performed in HEK293T cells treated with liposome formulations. Reduced MTT by cells treated with L-cysteine was also measured. Cells without liposomes or amino acid were used as controls [19].

The liposome concentration in all assays was 1 and 2 mg/mL. Untreated cells were used as controls. Liposomes and liposomes without L-cysteine were also tested. Results are shown in Fig. 3.

At high lipid concentrations (2 mg/mL), the formulations decreased metabolism both after 4 and 24 hours.

When the incubation time was 4 hours, we found no changes in metabolism respect to untreated cells. In contrast, after 24 hours, the formulations decreased metabolism except DMPC and DSPC without L-cysteine. When cells treated with L-cysteine were taken as control (Dunnet test was performed, statistical differences marked with +), DSPC was the only formulation that decreased metabolism. As explained before, DSPC without L-cysteine had no effect on cell metabolism; but in the presence of the aminoacid, metabolism was modified. These data suggest that L-cysteine can enter the cell more efficiently when it is transported by DSPC than when it is transported by DMPC or DPPC, since L-cysteine transported by DMPC or DPPC had no influence on cell metabolism.

4. CONCLUSION

Liposomes developed herein are well suited for the application in the delivery of L-cysteine. Particularly, they can encapsulate nearly all the L-cysteine and can retain it for 6 hours. Also, L-cysteine stabilized liposomes, preventing their aggregation. L-cysteine encapsulated in the DSPC formulation induced deeper changes in cell metabolism, this was probably due to a higher entry, thus a better liposome-mediated transport.

Considering that the smaller the particle, the better the circulation, we believe that the stabilization of the vesicle by L-cysteine may allow these transporters to have higher circulation times.

Based on the above, we conclude that the DSPC formulation is the best suited for further application in L-cysteine delivery.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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