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Influence of the composition of liposomes on their stability and interactions with plasma proteins

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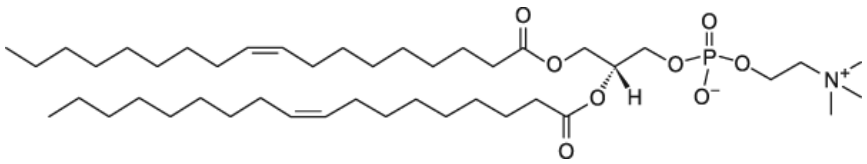
Aim of the study

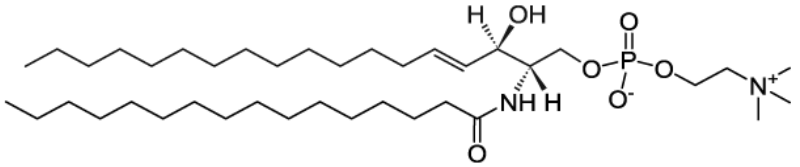
Evaluation of the influence of liposomes composition on their: stability, zeta potential and interaction with plasma proteins.

Tested lipids

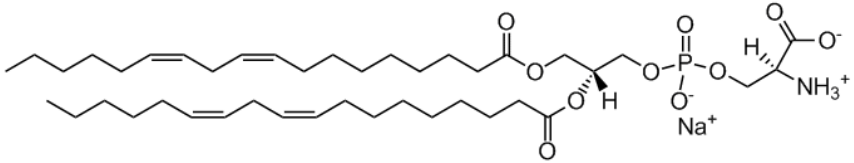
DOPC
1,2-dioleoyl-sn-glycero-3-phosphocholine



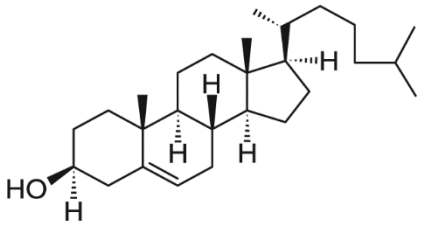
SM
sphingomyelin



PS
L-α-phosphatidylserine



CHOL
cholesterol



DOPC : CHOL	SM : CHOL	PS:CHOL
100 : 0	100 : 0	100 : 0
90 : 10	90 : 10	90 : 10
80 : 20	80 : 20	80 : 20
70 : 30	70 : 30	70 : 30
60 : 40	60 : 40	60 : 40
50 : 50	50 : 50	50 : 50

Tab.1. Presentation of mas ratio of tested liposomes compositions.
Figure source: https://avantilipids.com/

Methodology

- Synthesis method: thin film hydration fallowed by extrusion (pores diameter – 100 nm)
- Analysis right after synthesis: size, PDI, zeta potential, FTIR, cryo-TEM
- Incubation for 28 days in 4°C and 37°C
- Analysis in day 7, day 14, day 21 and day 28: size, PDI, FTIR
- Protein interaction analysis: evaluation of protein concentration and protein corona electrophoretic pattern

Cryo - TEM

- Vitrobot
- Grids:Quantifoil, holey carbon film, 400 mesh, Φ = 2 μm
- Humidity: 100%, blotting time: 3s
- Observation: JEOL JEM2100HT, Jeol Ltd, accelerating voltage 80 kV

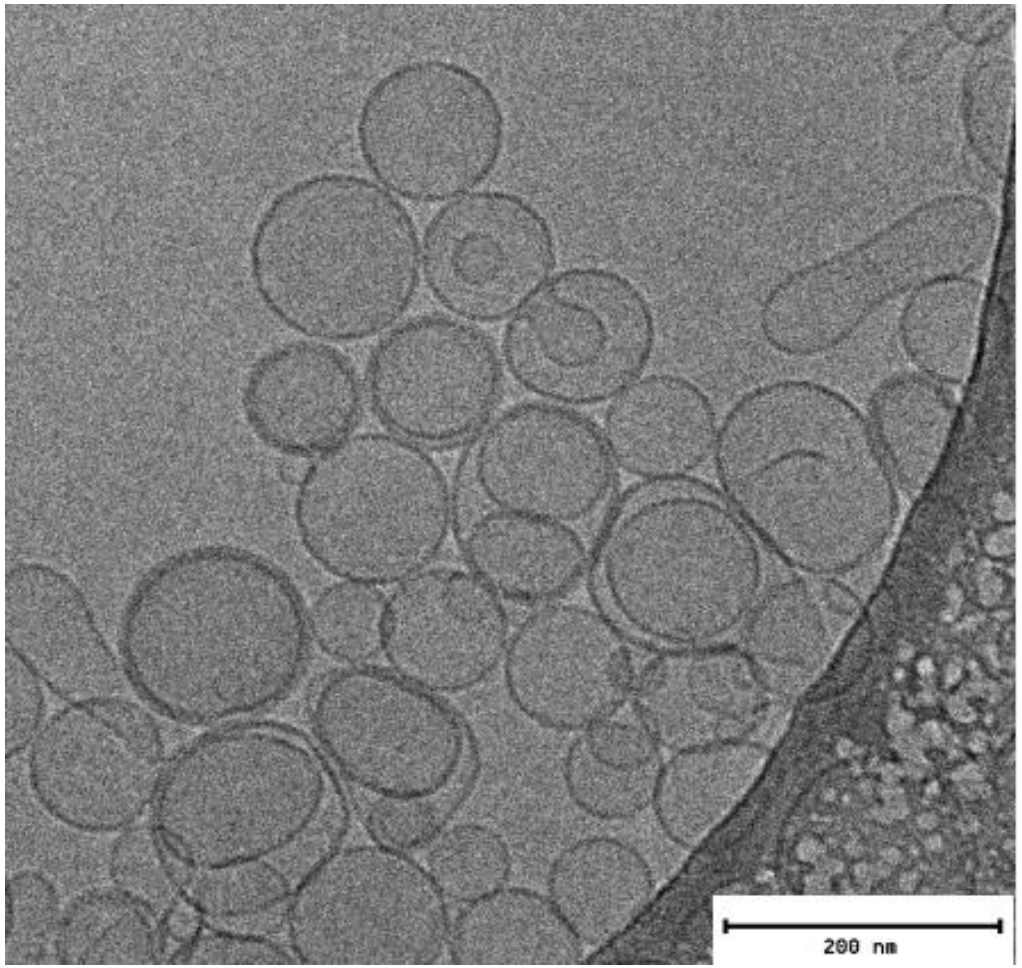


Fig.1. *DOPC:cholesterol liposomes images from cryo- TEM microscopy.*

DLS - DOPC

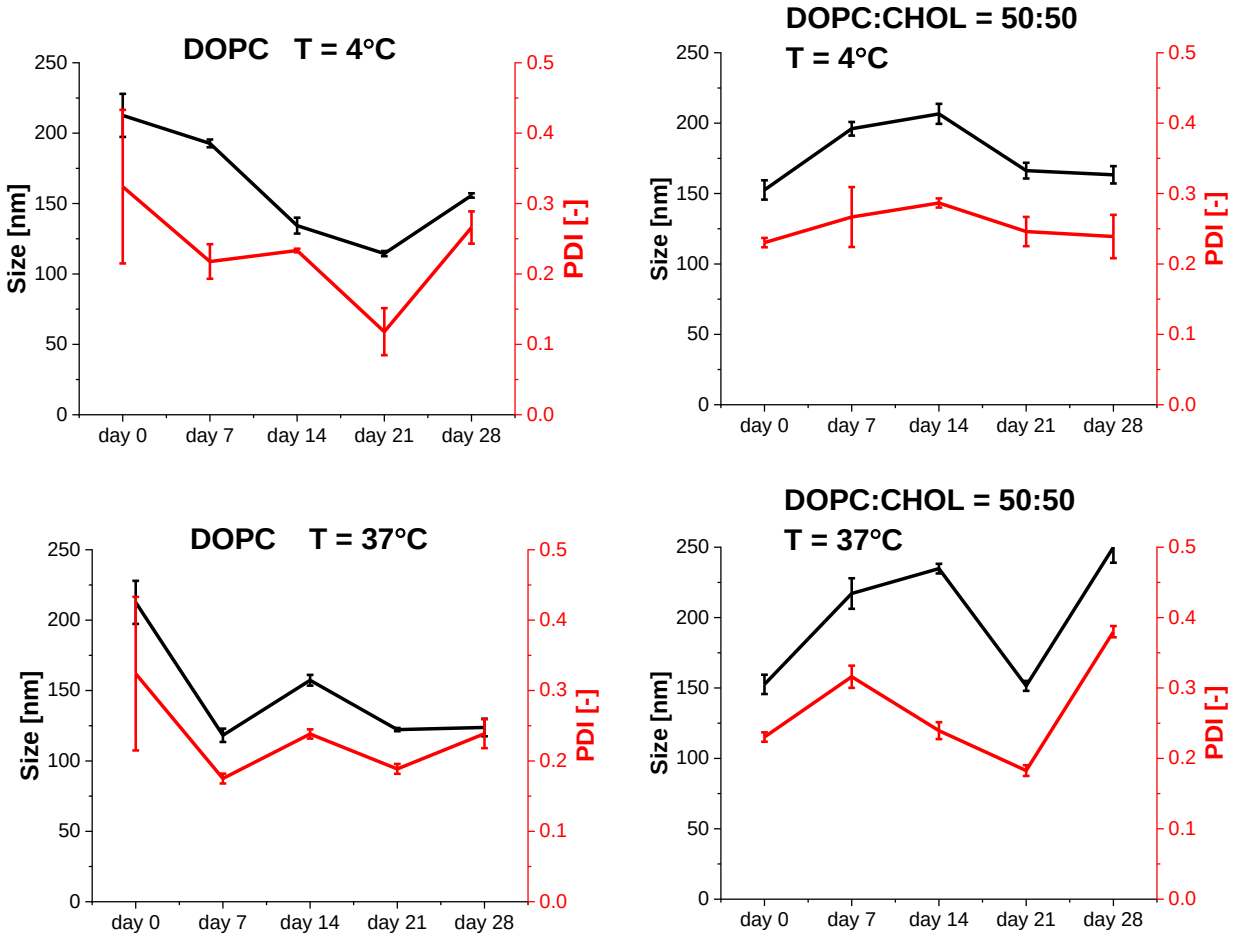


Fig. 2. Changes in liposomes size and PDI during incubation in different temperatures: a) DOPC, 37 °C, b) DOPC, 4 °C, c) DOPC: cholesterol = 50:50, 6 °C, d) DOPC: cholesterol = 50:50, 37 °C

DLS - sphingomyelin

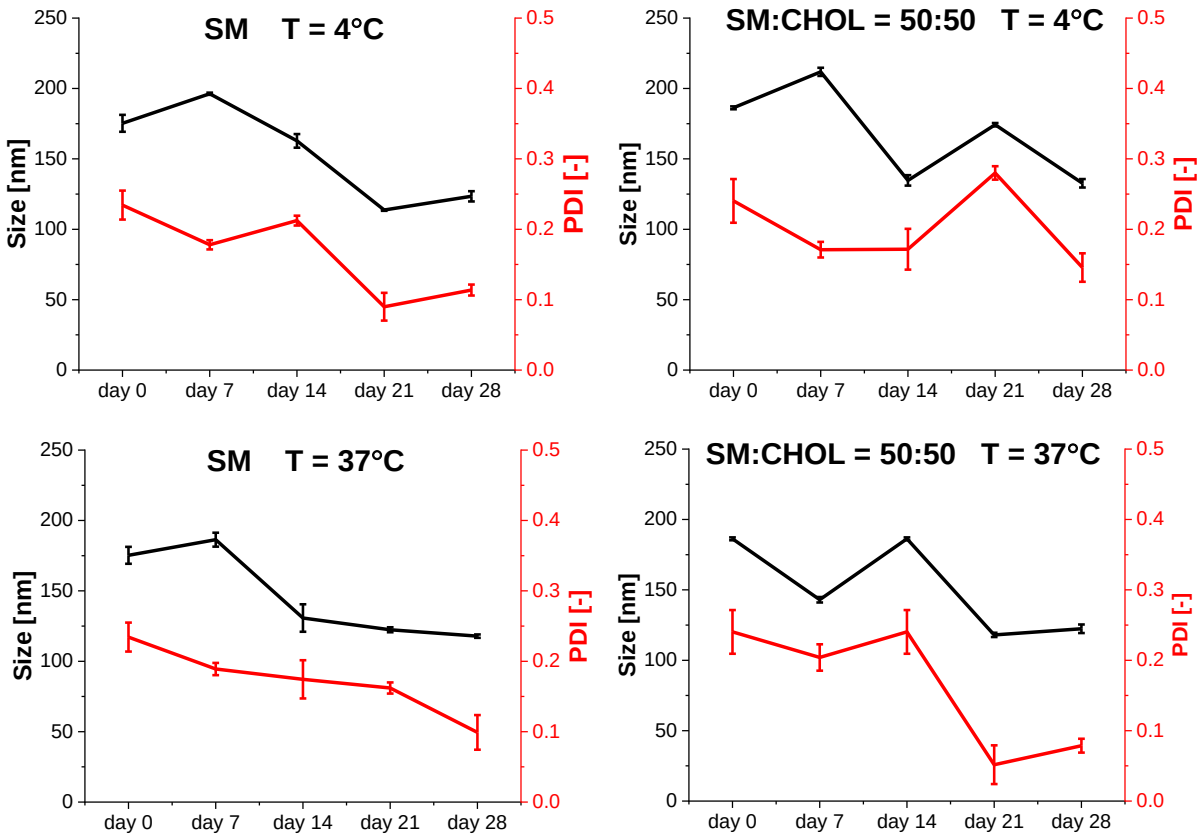


Fig. 3. Changes in liposomes size and PDI during incubation in different temperatures: a) sphingomyelin, 37 °C, b) sphingomyelin, 4 °C, c) sphingomyelin: cholesterol = 50:50, 6 °C, d) sphingomyelin: cholesterol = 50:50, 37 °C

DLS - phosphatidylserine

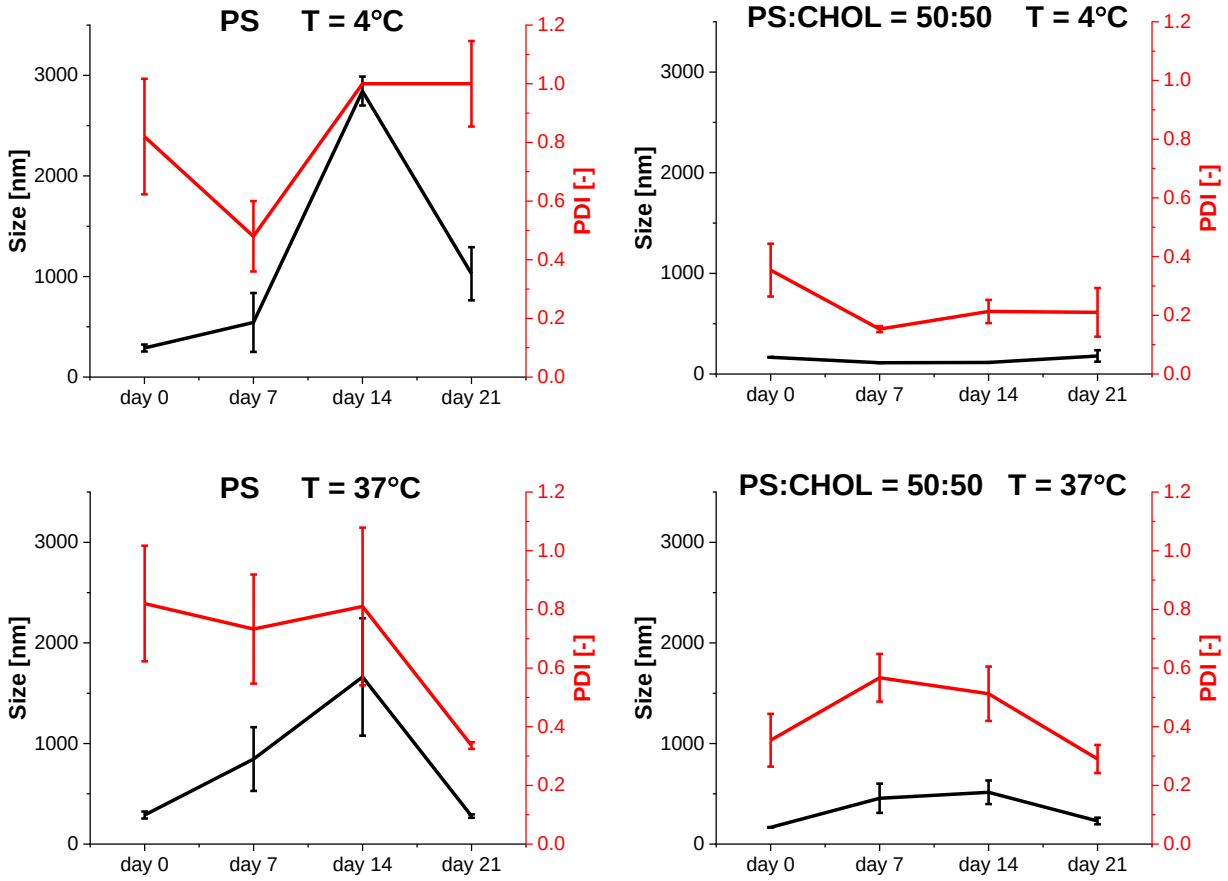


Fig. 4. Changes in liposomes size and PDI during incubation in different temperatures: a) phosphatidylserine, 37 °C, b) phosphatidylserine, 4 °C, c) phosphatidylserine: cholesterol = 50:50, 6 °C, d) phosphatidylserine: cholesterol = 50:50, 37 °C

FTIR – DOPC

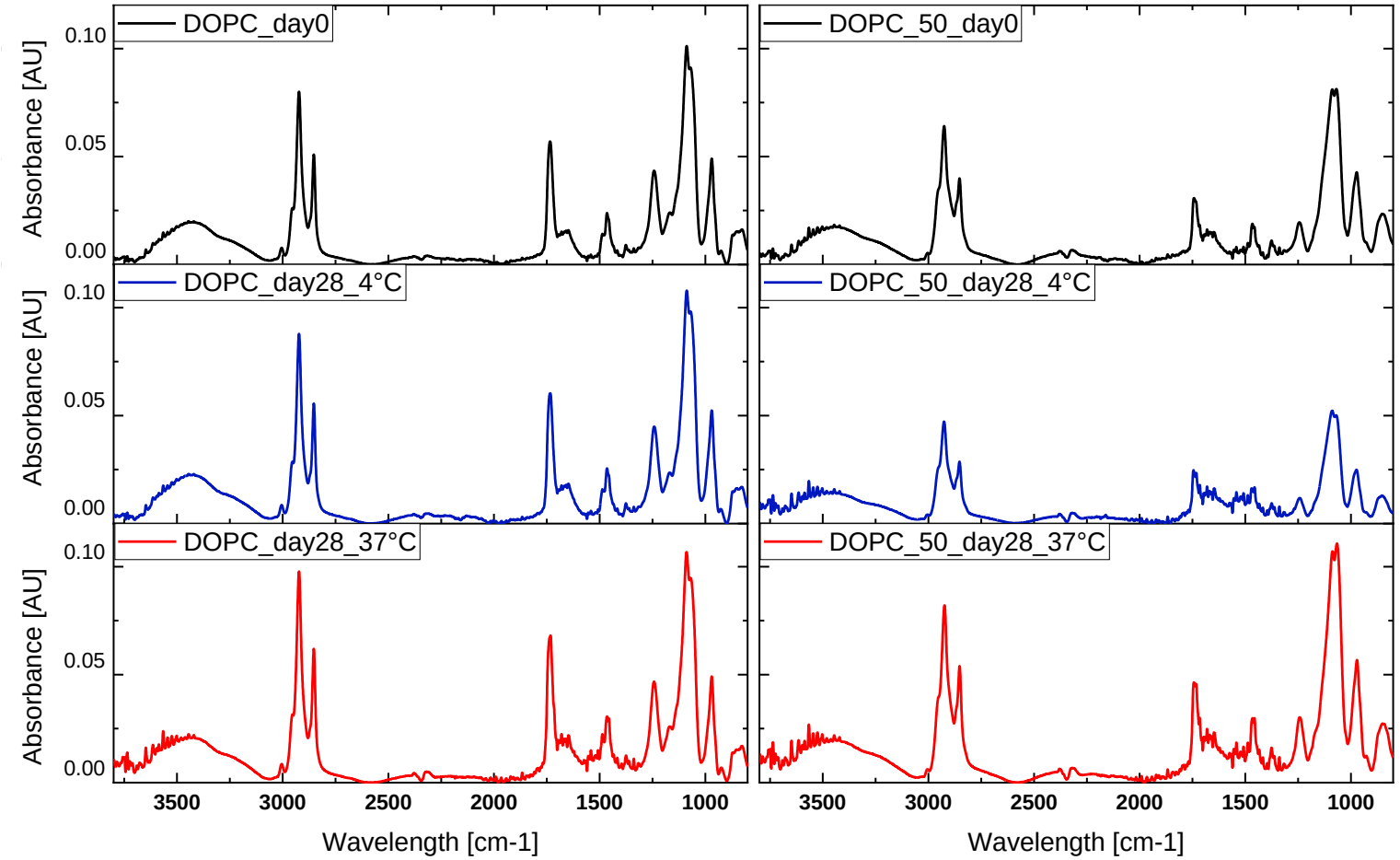


Fig.5. Changes in DOPC liposomes FTIR spectra after 28 days incubation in 4°C (blue yellow line) and 37°C (red line).

Fig.6. Changes in DOPC:CHOL = 50:50 liposomes FTIR spectra after 28 days incubation in 4°C (blue line) and 37°C (red line).

FTIR - sphingomyelin

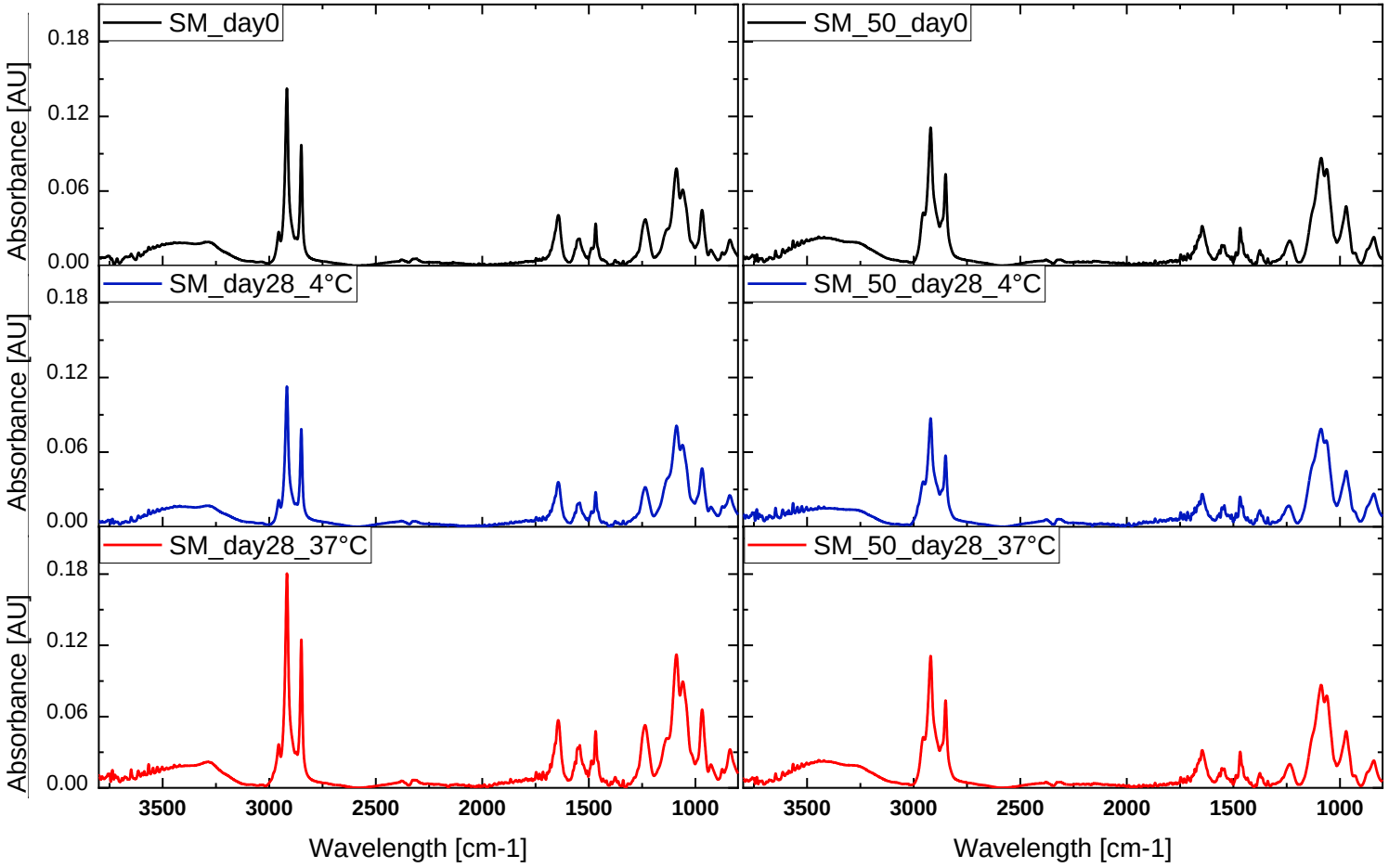


Fig.7. Changes in SM liposomes FTIR spectra after 28 days incubation in 4°C (blue line) and 37°C (red line).

Fig.8. Changes in SM:CHOL = 50:50 liposomes FTIR spectra after 28 days incubation in 4°C (blue line) and 37°C (red line).

FTIR - phosphatidylserine

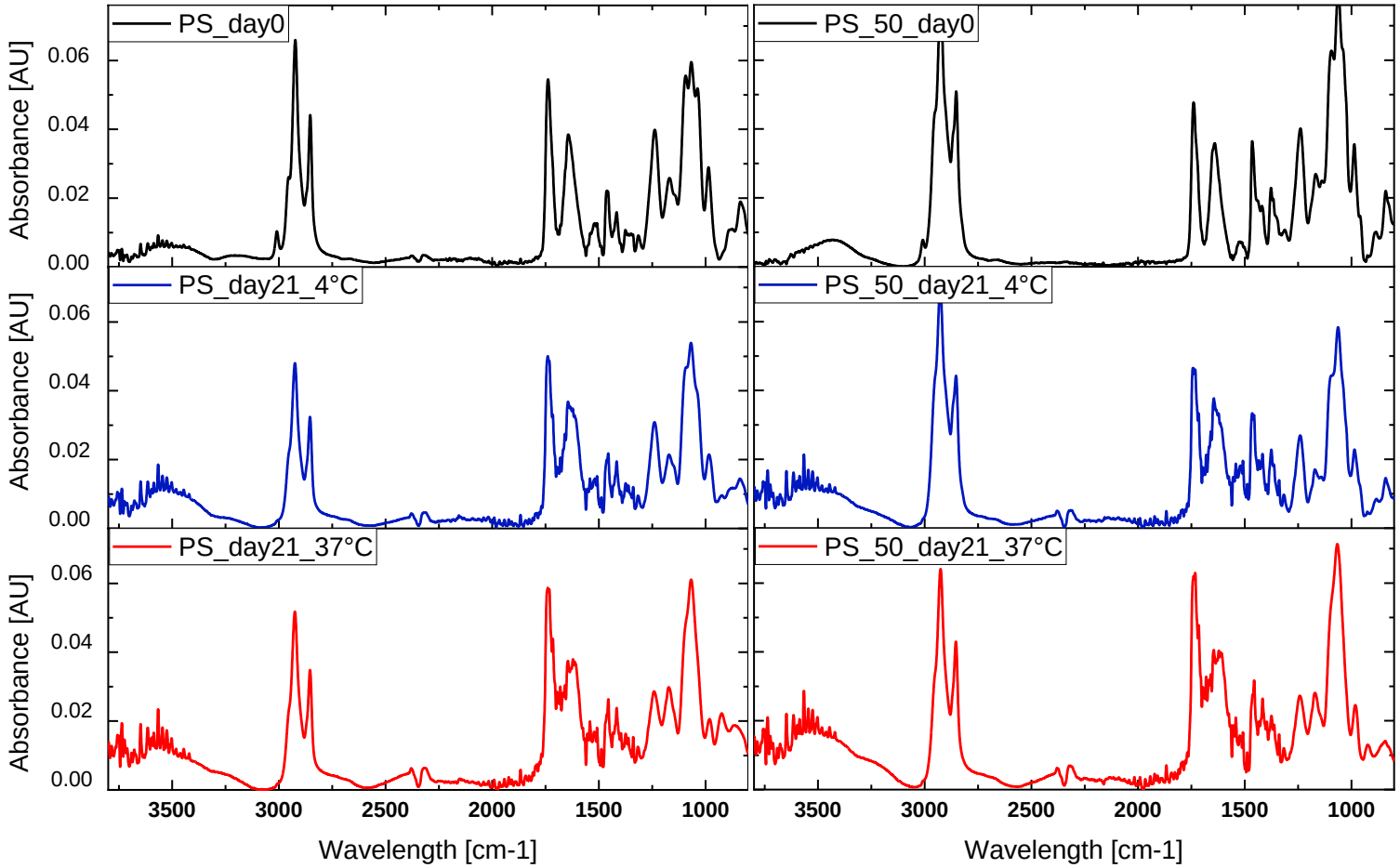


Fig.9. Changes in PS liposomes FTIR spectra after 28 days incubation in 4°C (blue line) and 37°C (red line).

Fig.10. Changes in PS:CHOL = 50:50 liposomes FTIR spectra after 28 days incubation in 4°C (blue line) and 37°C (red line).

Electrophoresis

- Incubation with plasma : 60 min, 37 ° C
- Ultracentrifugation – removal of unbounded proteins
- DLS – zeta potential and FTIR

	ζ-potential before incubation [mV]	ζ-potential after incubation [mV]
DOPC	-17.6±2.3	-10.1±0.8
DOPC 50	-21,0±0.7	-8.3±0.4
SM	-20.3±5.4	-10.7±0.9
SM 50	-50.0±14.3	-14.3±1.1
PS	-10.6±0.68	-5.8±0.2
PS 50	-29.0±2.3	-14.9±0.9

Tab.2. Changes in liposomes ζ-potential after incubation with plasma proteins.

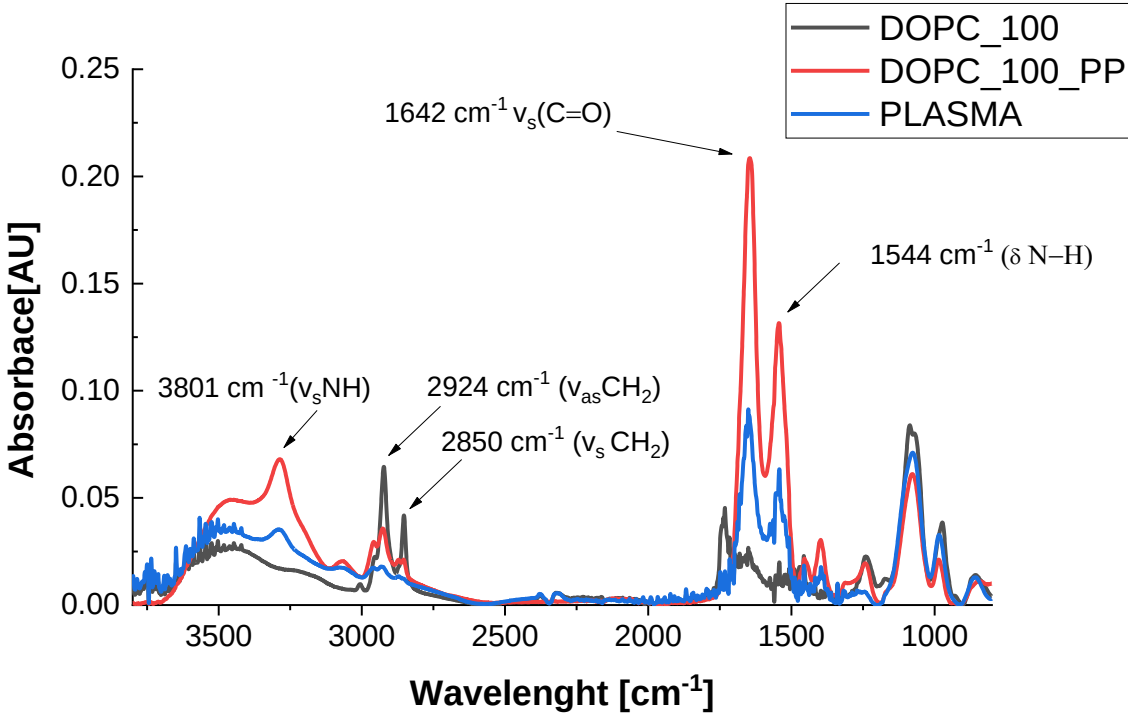


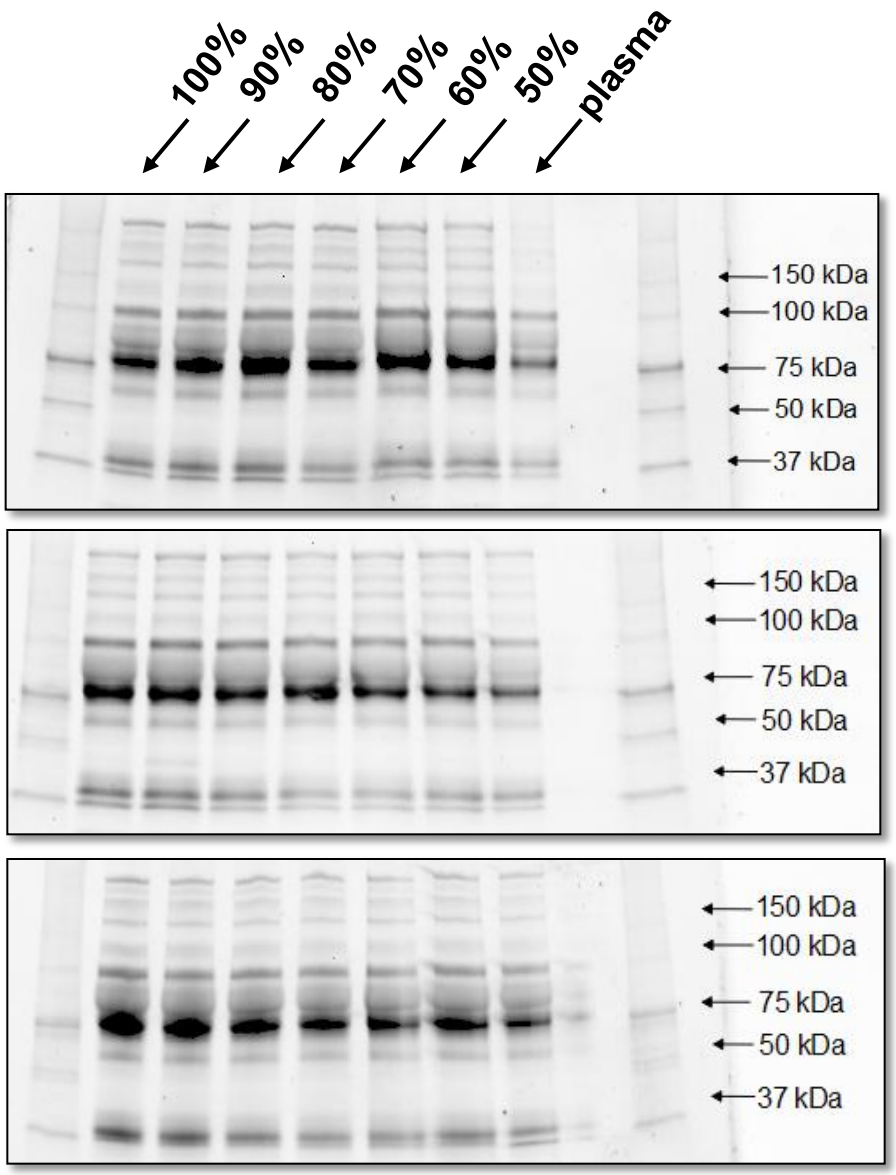
Fig.11. FTIR spectra of DOPC liposomes (black line), DOPC liposomes incubated with plasma proteins (red line) and plasma (blue line).

Electrophoresis

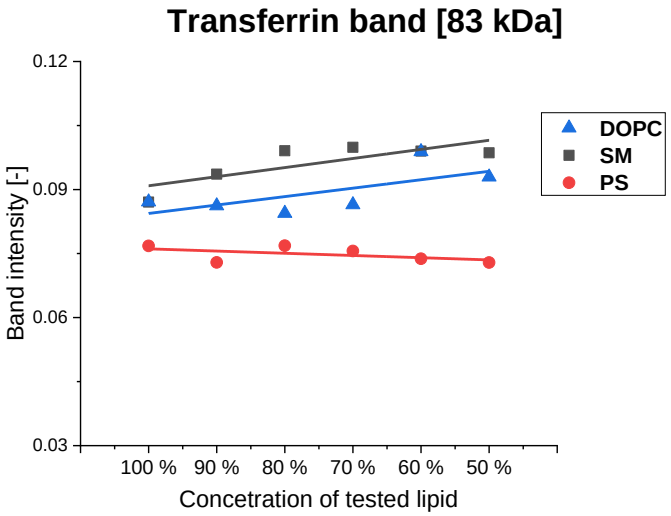
DOPC – **nicely visible bands for proteins with molecular weight greater than 150 kDa**

SM – **linear changes in interaction with proteins in albumin band**

PS – **visible strong interaction with proteins with low molecular mass**



Electrophoresis



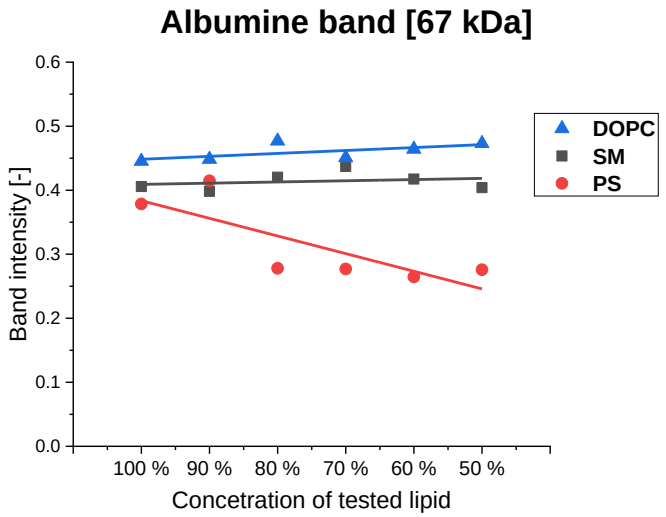
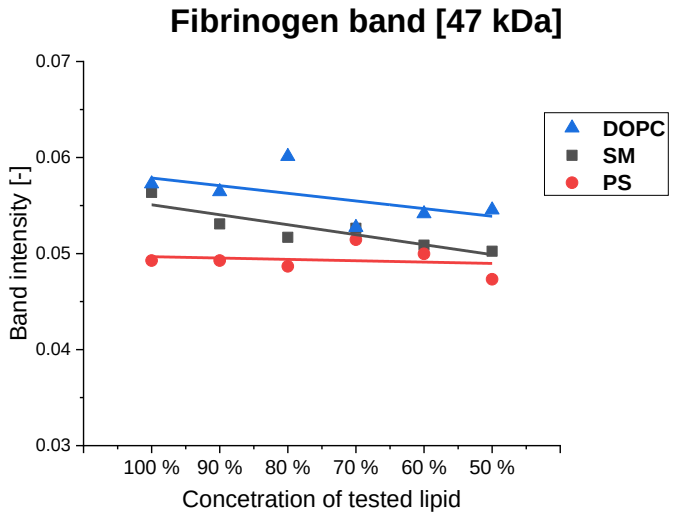


Fig.12. Comparison of concentration of three the most abundant proteins in human plasma in liposomes proteins corona.



Conclusions

- Lipid:cholesterol ratio in liposomes influence their stability and plasma protein interactions pattern
- The most stable are liposomes composed of DOPC, the less stable liposomes with phosphatidylserine
- Protein corona electrophoretic pattern changes with liposomes composition

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