Capillary isoelectric focusing of simple liposomal models

Pavlína Dadajová
Institute of Analytical Chemistry of the CAS

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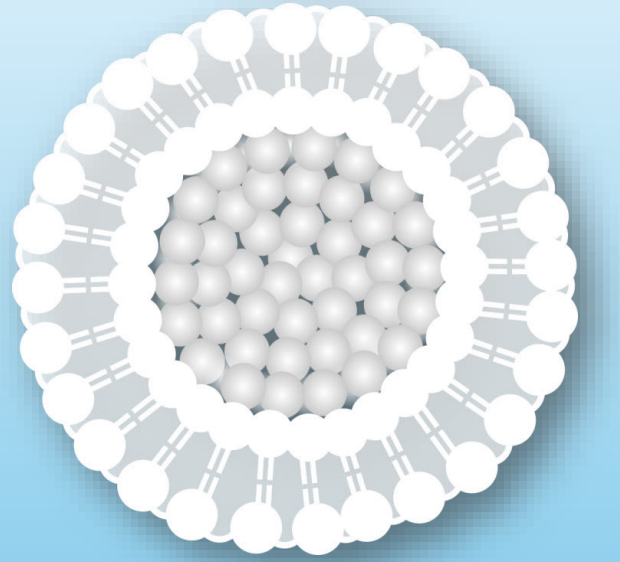
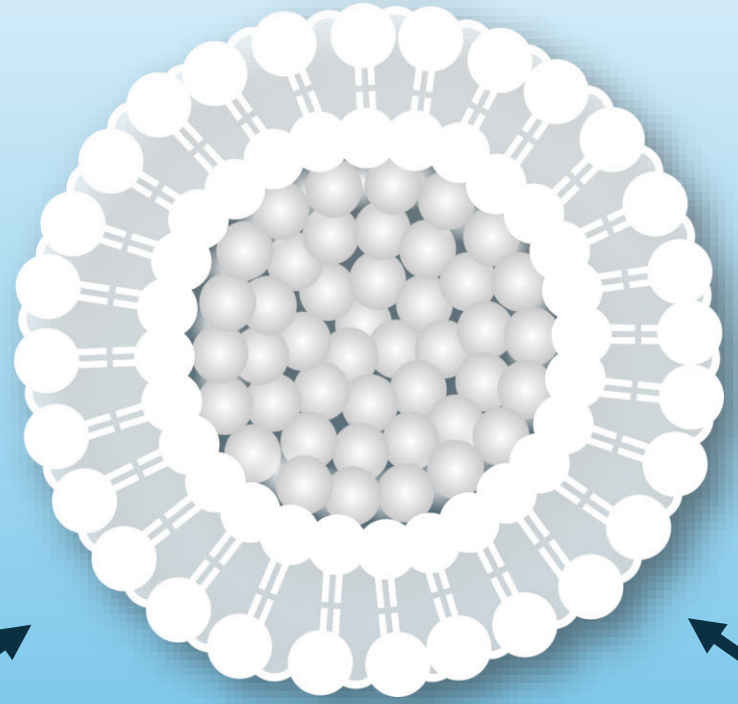
Introduction to the study



- Liposome is a self-assembling nano-sized biomimicking vesicle composed of an aqueous core enclosed by a lipid bilayer
- Versatile vesicles which can be modified, tweaked, or applied in food, cosmetic and pharmaceutical industry
- They serve protective, signaling, adsorbing, drug delivery, and other roles

Introduction to the study

Surface charge and stability (ELS, Zeta potential, IEF)



Size and morphology (DLS, TEM, Cryo-EM)

Encapsulation Efficiency (Fluorescence Spectroscopy, HPLC)

Composition and Interactions (FTIR, NMR, Nanoplasmonic Sensing)

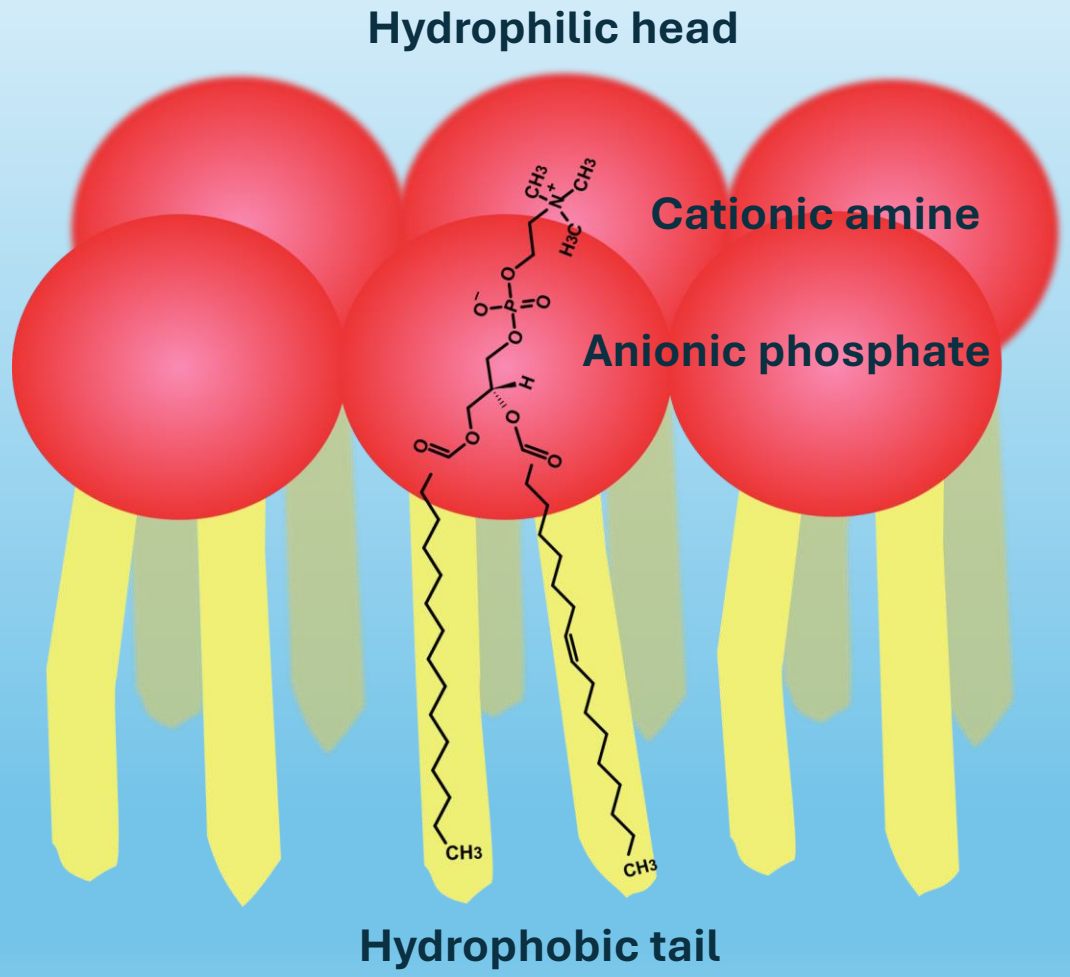
Motivation for an IEF-based analysis

Studying liposome behavior under isoelectric focusing (IEF) conditions as a function of their composition could provide valuable information on how the particular structural lipids and size influence the observed isoelectric point (p*I*) and contribute to the surface net charge of the liposome

Easier designing process of future liposome formulations

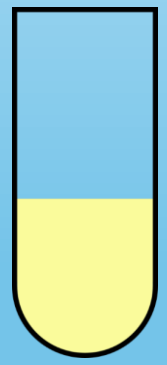
IEF analysis premise

- Depending on the lipid bilayer composition, liposomes manifest change in the net surface charge as the pH of the solution changes from acidic to basic
- Zwitterionic phospholipids (PC,PE)
- IEF can be used to study much larger structures including nanoparticles, viruses, and microorganisms



Liposome preparation

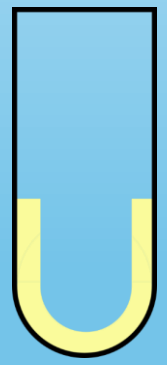
Phospholipid extract (Avanti) in chloroform 25 mg/ml



Evaporation

T = 25°C overnight

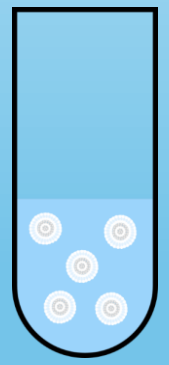
Dried phospholipid film



Hydratation

800 RPM T = 60 °C 1 h

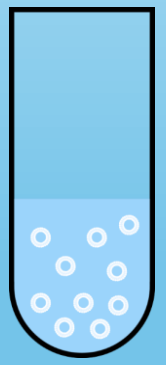
Dispersion of multilamellar vesicles in phosphate buffer 10 mM, pH=7.4



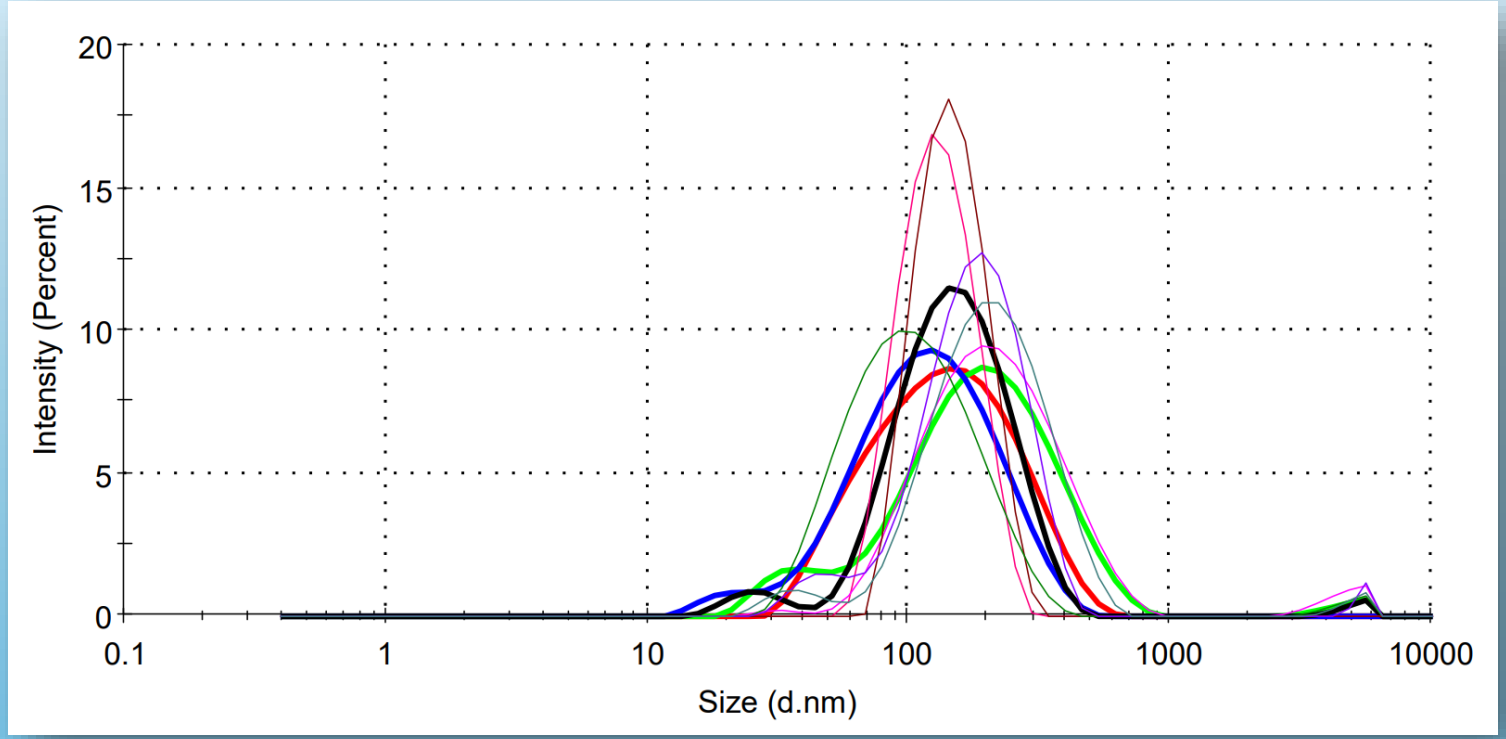
Sonication

T < 20°C 20 min

Small unilamellar vesicles, final concentration 5 mg/mL



Lipozome preparation



- The size distributions of the prepared vesicles were determined by a Zetasizer Nano ZS instrument
- The average vesicle size (Z-average) was 134 ± 31 nm

cIEF method

Conditions	
Instrument	Sciex P/ACE™ MDQ Plus
	Single vawelenght detector (280 nm)
Capillary	Neutral linear polyacryl amide (LPA) coating
	50 µm ID x 30 cm, 20°C
Focusing	Anolyte - 200 mM H_3PO_4 Catholyte - 300 mM NaOH
	10 min, 25 kV
Mobilization	350 mM CH_3COOH
	25 min, 30 kV

Sample	
Ampholytes	1.92% w/V SH AESlyte 3-10
Gel	80% V/V cIEF gel
Spacers	60 mM L-arginine and 1.6 mM iminodiacetic acid
Liposomes	0.39 mg/ml
Markers	Low-molecular-mass p/ markers

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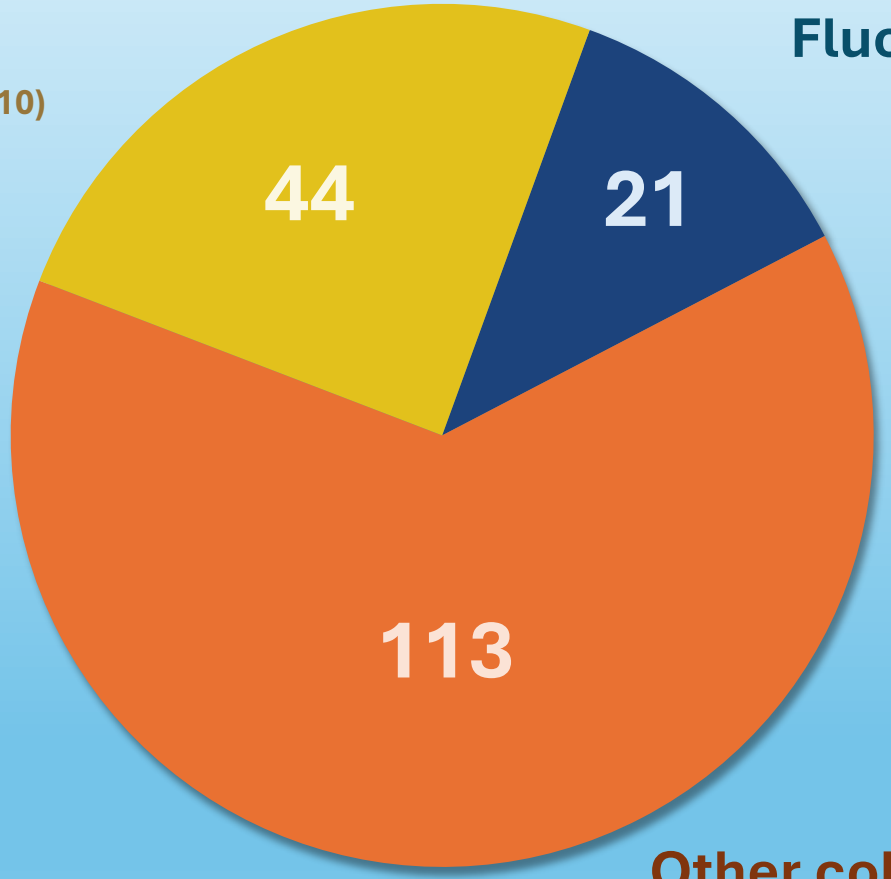
Low-molecular-mass isoelectric point markers

Yellow
Nitrophenol-based
(doi.org/10.1016/j.aca.2019.05.010)

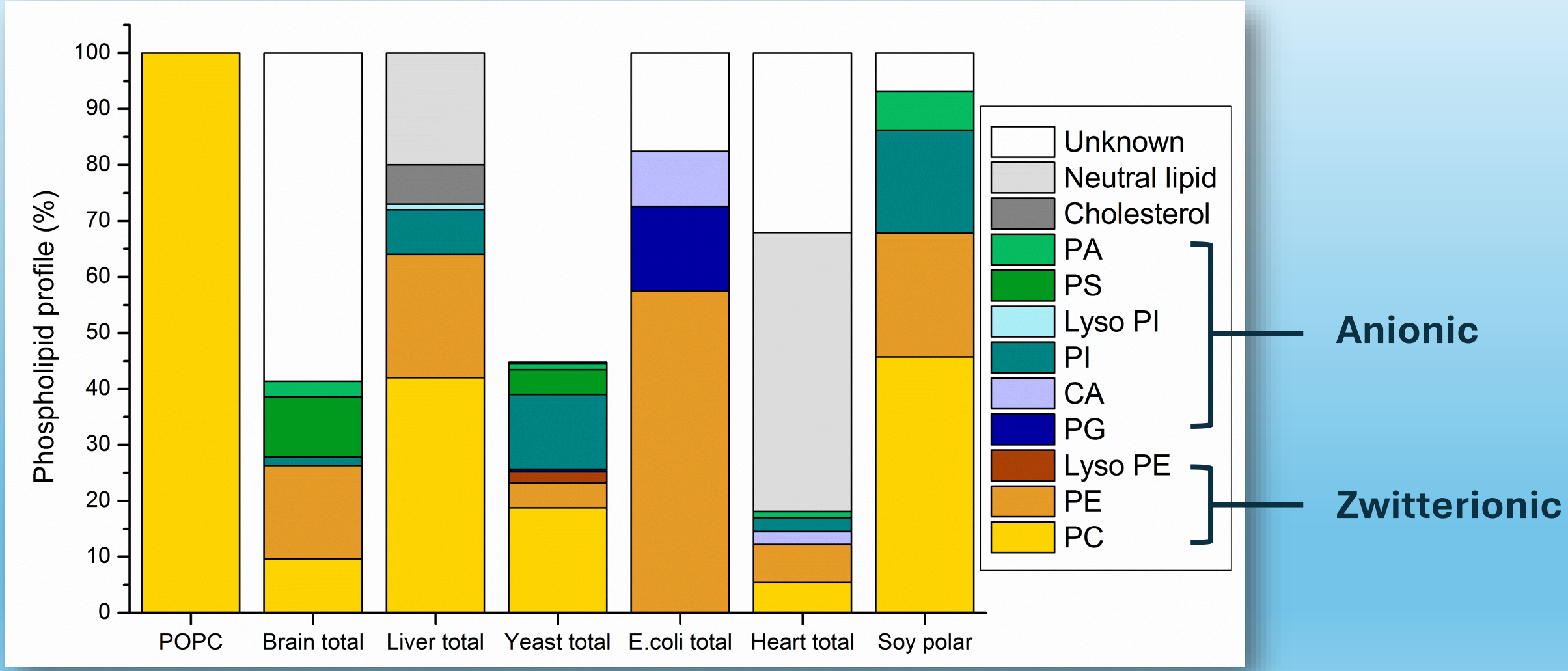
Fluorescent
Fluorescein-based
(TBA)



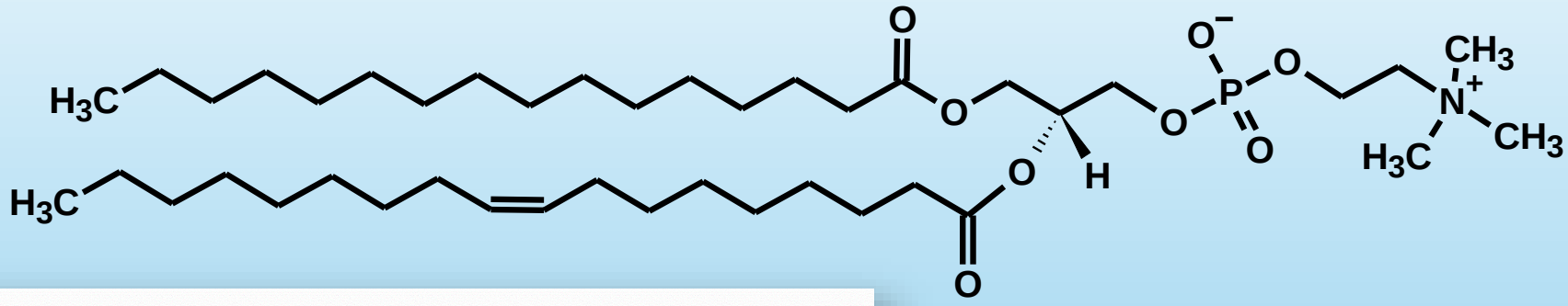
Other colorful:
orange, red, violet
(doi.org/10.1016/j.aca.2022.340035)

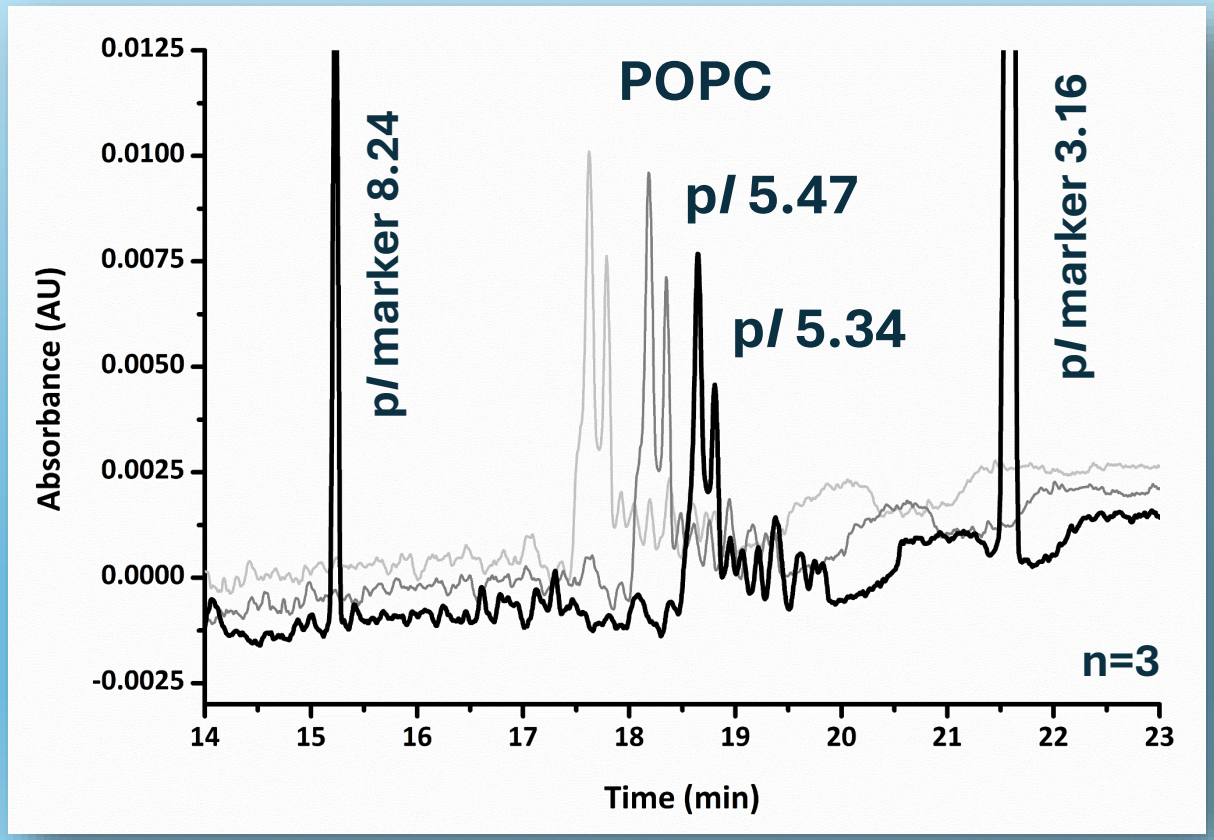
Dr. Filip Duša
Czech Academy of Sciences
Institute of Analytical Chemistry
Veveří 97, 60200 Brno
Czech Republic
Phone: +420 532 290 211
E-mail: dusa@iach.cz
http://www.iach.cz/en/
Commercially available on request

Phospholipid profile of each prepared extract



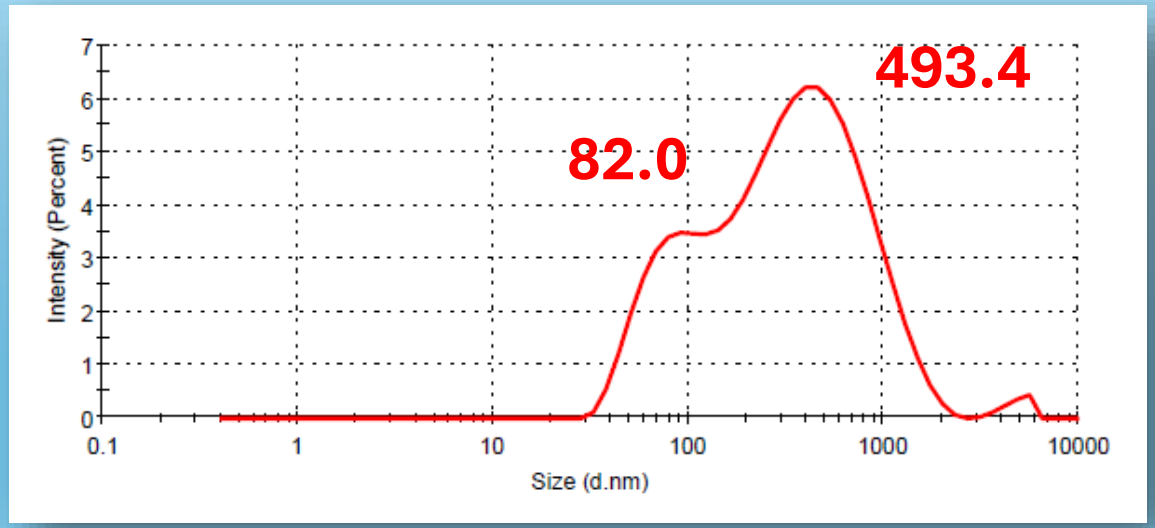
cIEF of prepared phospolipid extracts



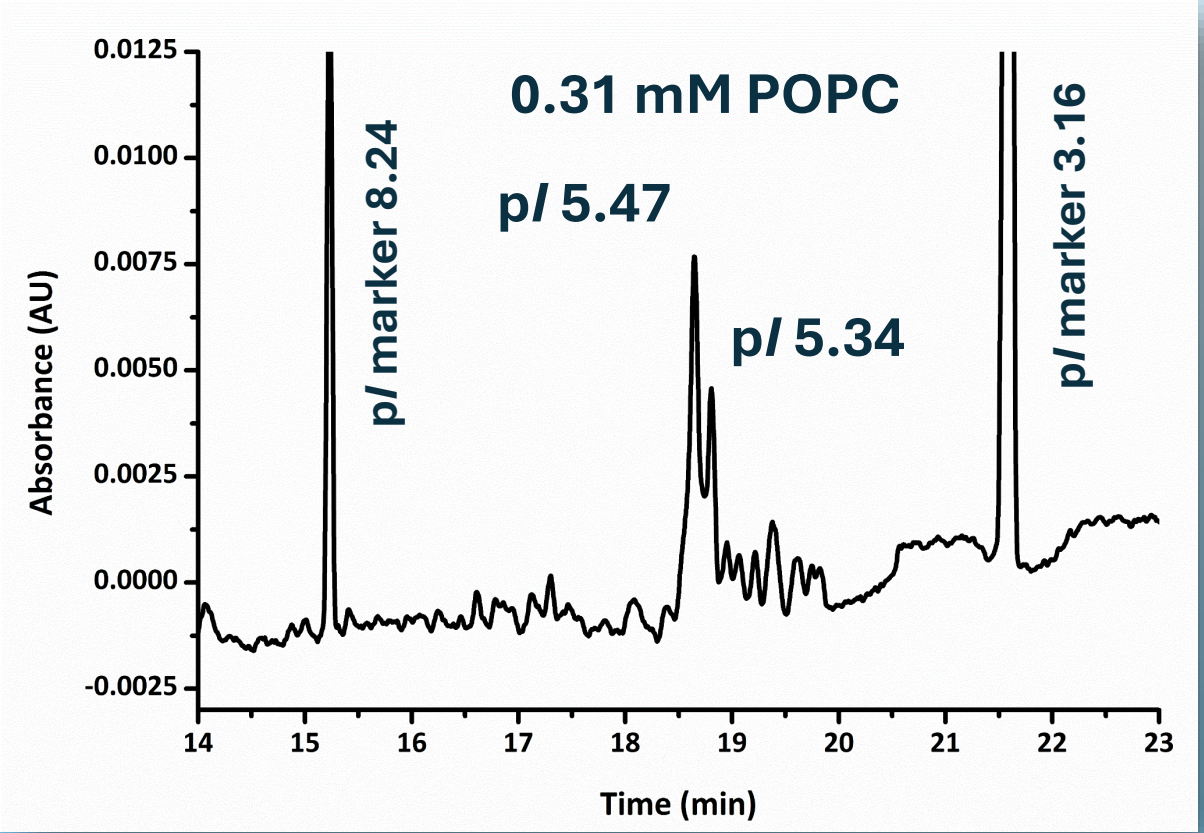


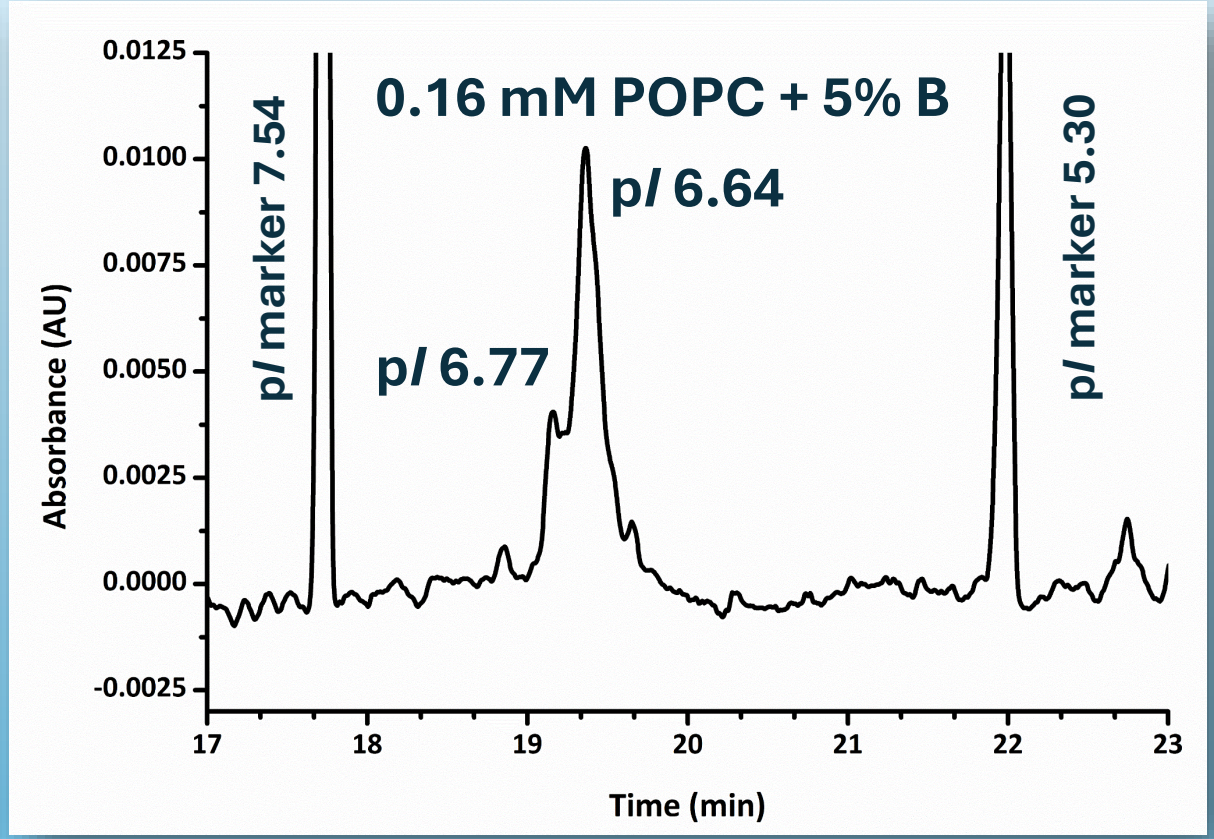
POPC (16:0-18:1 PC)

1-palmitoyl-2-oleoyl-glycero-3-phosphocholine



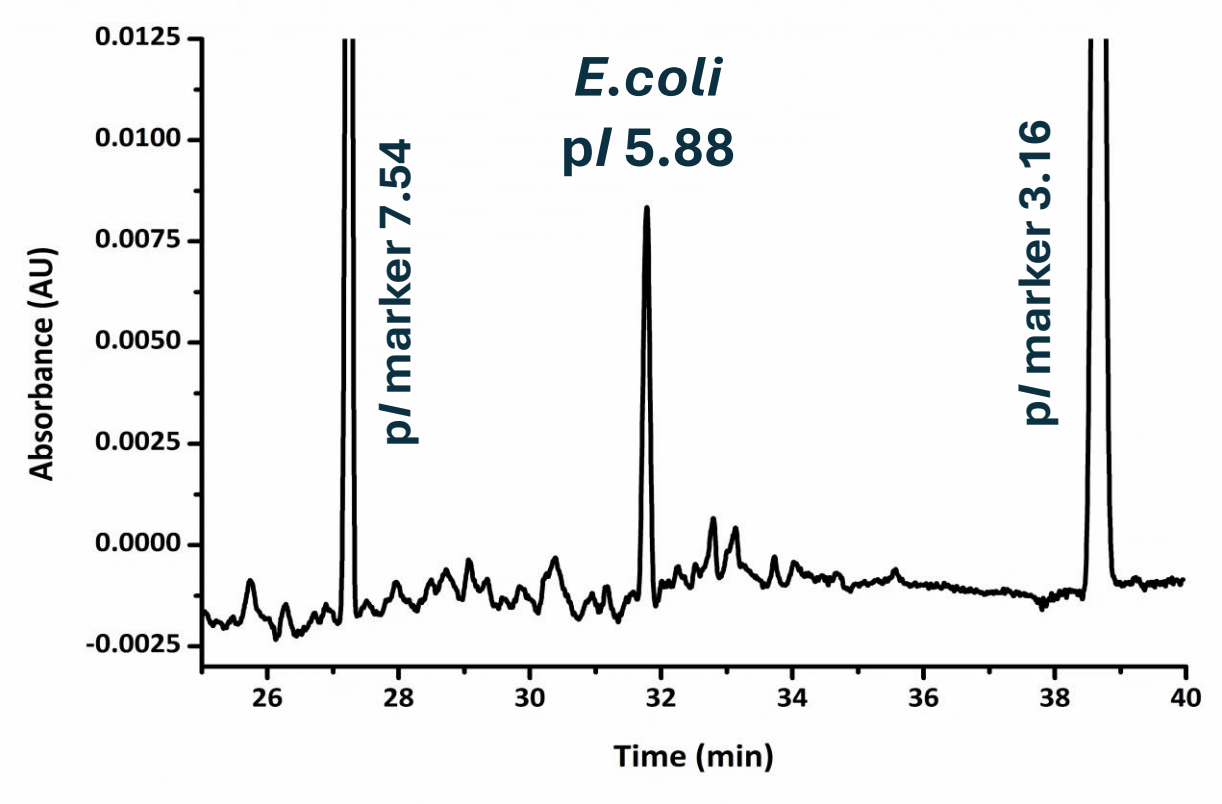
cIEF of prepared phospolipid extracts

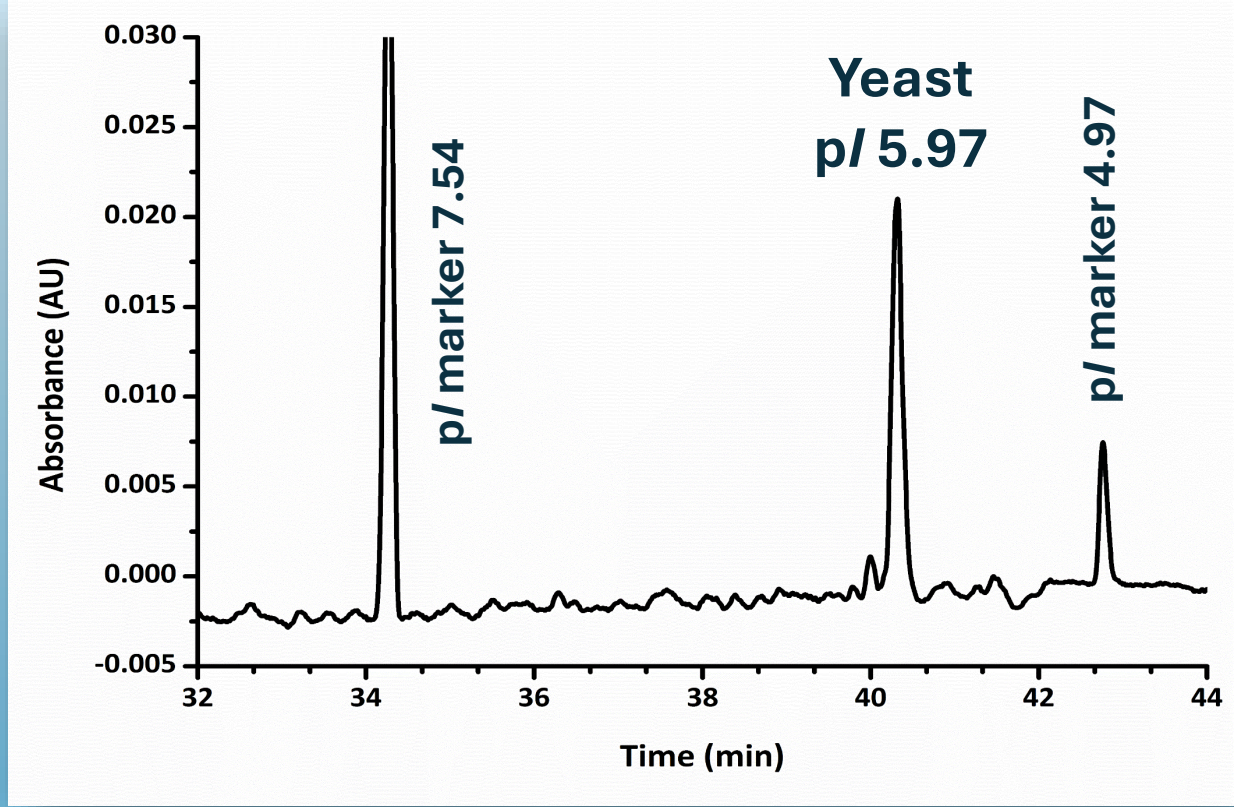




- p*/* shift and 2.5x stronger signal with POPC + 5% Butenafine compared to bare POPC

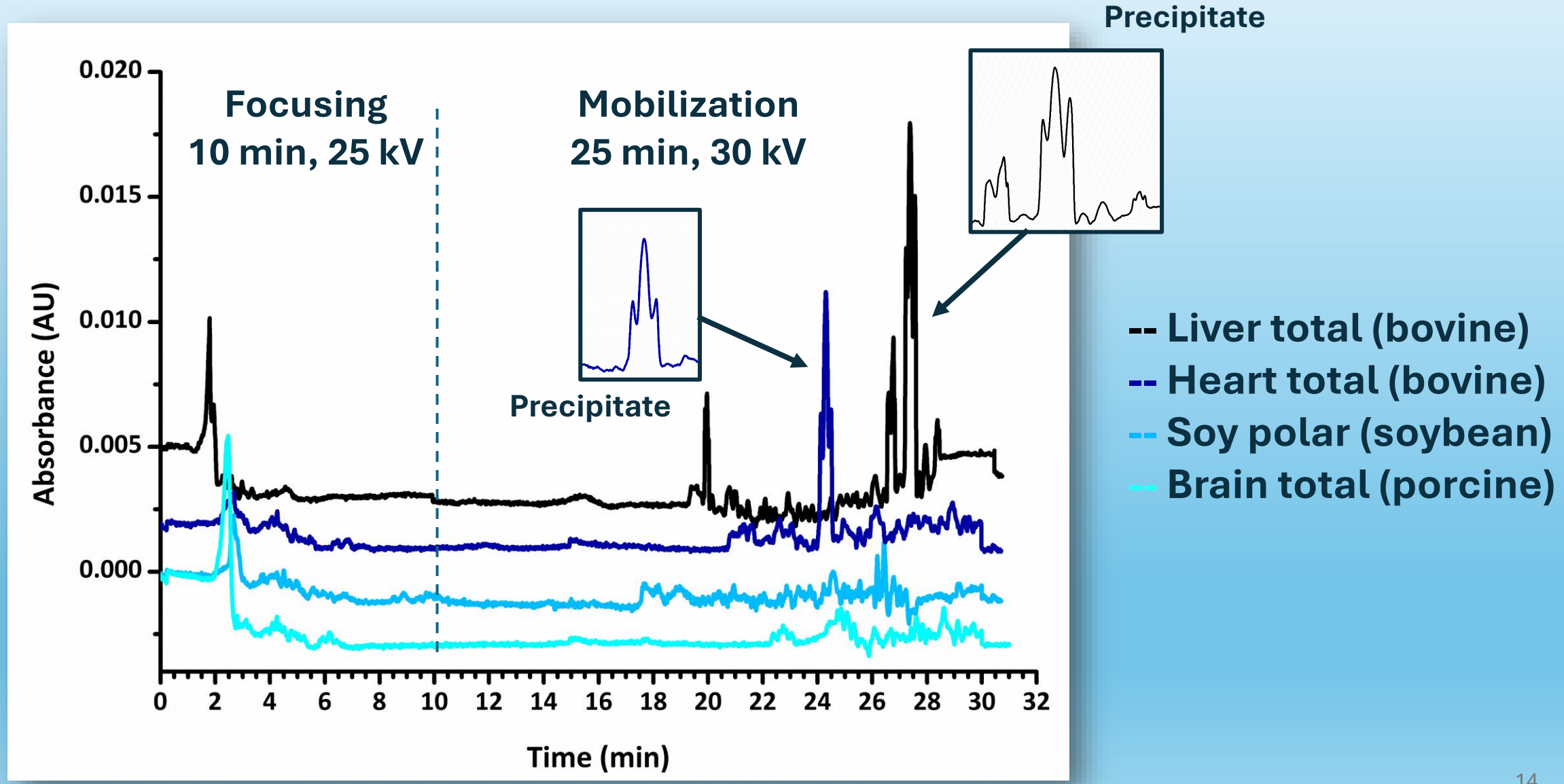
cIEF of prepared phospolipid extracts





- Single peak observed both with *E.coli* and Yeast (*S. cerevisiae*) extract
- CA, PG, PE prevalent in *E.coli* vs PC, PI prevalent in Yeast extract

cIEF of prepared phospolipid extracts



Conclusion

Findings ☑

POPC, Yeast and *E.coli* lipozomes are a good working model for cIEF

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Indications that populations with different sizes could be differentiated

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Improved signal and p*I* change in bare vs vesicles with additive

Obstacles ☒

Mobilization not succesfull with all samples (p*I* too acidic, charged phospholipids)

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Observed precipitation

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Low absorbance at 280 nm (background ampholytes, light scattering on spherical particles)

Thank you for your attention !

Acknowledgements

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