FORMATION AND CHARACTERIZATION OF CHLOROPHYLL-A WITHIN TETHERED BILAYER LIPID MEMBRANE

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Introduction

In some recent biophysical and biochemical studies many different simplified biomimetic analogues of lipid membranes were widely used to evaluate/model structural and permeability/transport properties of more complex natural bio-membranes [1]. The tethered bilayer lipid membrane (tBLM) is a complex system that can be used as an experimental platform for fundamental studies of the structure and function of the biomembrane. Long-chain thiol compounds, such as 20-tetradecyloxy-3,6,9,12,15,18,22-heptaoxahexatricontane-1-thiol (WC14), can be utilized as "anchors" in self-assembled monolayer (SAM) synthesis, allowing the production of stable and repeatable phospholipid bilayers [2]. In this work, one of such models, tBLM on the gold surface is formed by using the fusion of vesicles [3]. This immobilized membrane alteration enables different biosensors to be produced by adding lipid components such as DOPC (1,2-dioleoyl-sn-glycero-3-phosphatidylcholine) and cholesterol [4], having stability that can be modified by adding molecules such as chlorophyll a (Chl-a). By including lipid components such as DOPC and Chol, which give extra stability, specific modifications of tBLMs are ideal for the construction of diverse biosensors.
The investigation is performed by applying electrochemical impedance spectroscopy (EIS) to measure dielectric capacity and conductivity changes. Fluorescence microscopy (FM) is used to estimate the morphology of the membranes.

Work objective

The goal of this research is to see if the photoactive compound Chl-a could be incorporated into a tethered bilayer lipid membrane to create a platform that could be used to develop tBLM photosensitive surface constructs in the future, potentially for the development of new biosensors and biomimetic artificial leaves.

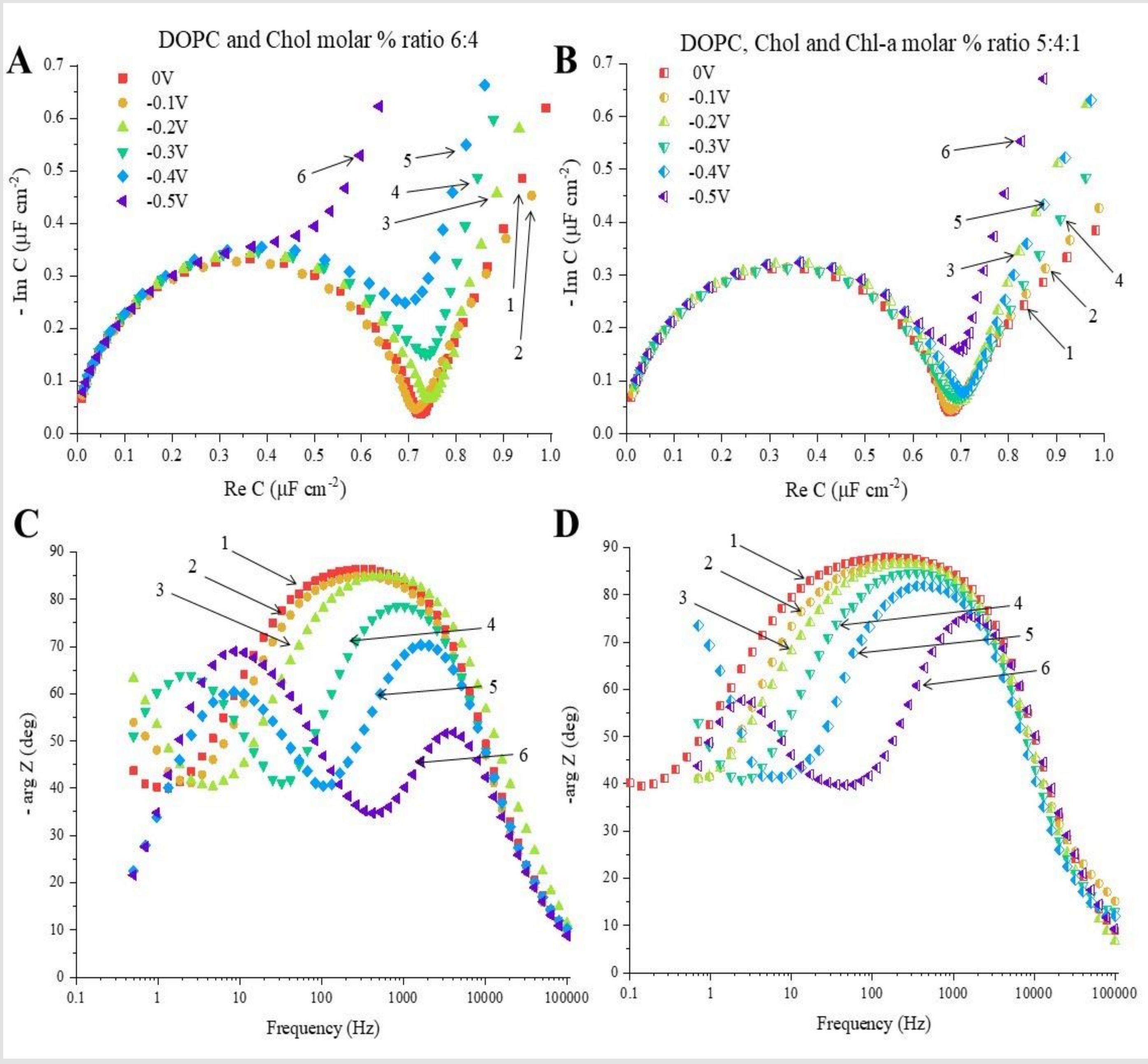

Figure 3. A and C – EIS spectra of the tBLM containing DOPC and Chol molar % ratio of 6:4, respectively in Cole-Cole complex capacitance and Bode coordinates at different potentials in the range from -0.1 to -0.5 V vs Ag/AgCl/NaClsat. Curve 1 (squares) – at 0 V vs Ag/AgCl/NaClsat, the curves 2-6 are marked by changing the potential from -0.1 to -0.5 V vs Ag/AgCl//NaClsat, respectively. **B and D** – tBLM containing DOPC, Chol and Chl-a molar % ratio 5:4:1, respectively, in Cole-Cole complex and Bode coordinates at different potentials in the range from -0.1 to -0.5 V vs Ag/AgCl/NaClsat. Curve 1 (squares) – at 0 V vs Ag/AgCl/NaClsat, the curves 2-6 registered by changing the potential from -0.1 to -0.5 V vs Ag/AgCl/NaClsat, respectively.

Results

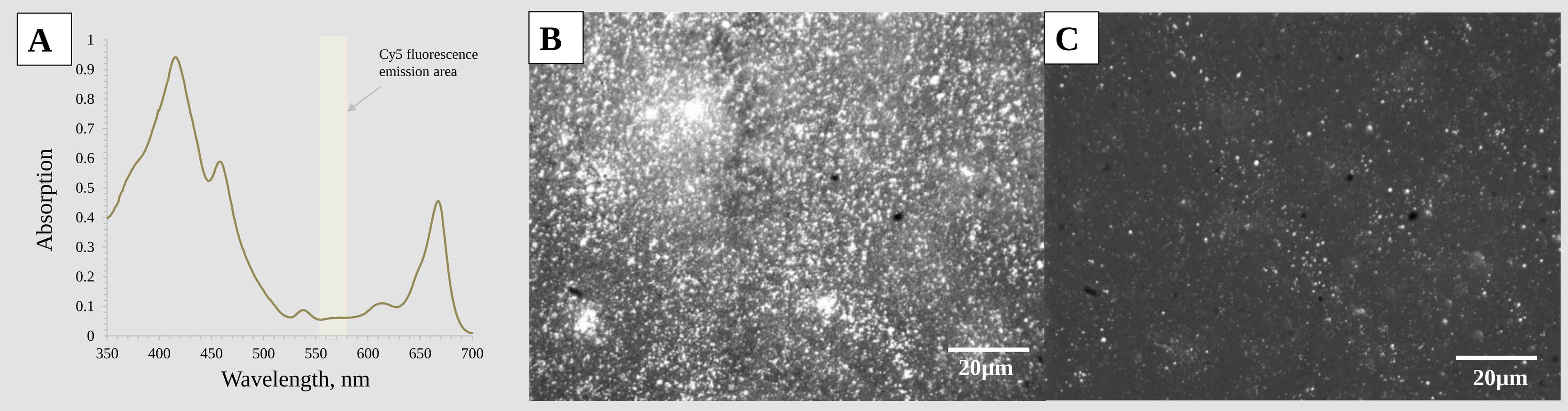

Figure 1. A – Optical absorption spectra of Chl-a; Fluorescence microscopy images obtained by observing tBLM-based structure of different composition with cholesterol marked by fluorophore Cy5. **B**, – tBLM-based structure containing DOPC and Chol at molar % ratio of 6:4, respectively; **C** – tBLM-based structure containing DOPC, Chol and Chl-a at molar % ratio of 5:4:1, respectively.

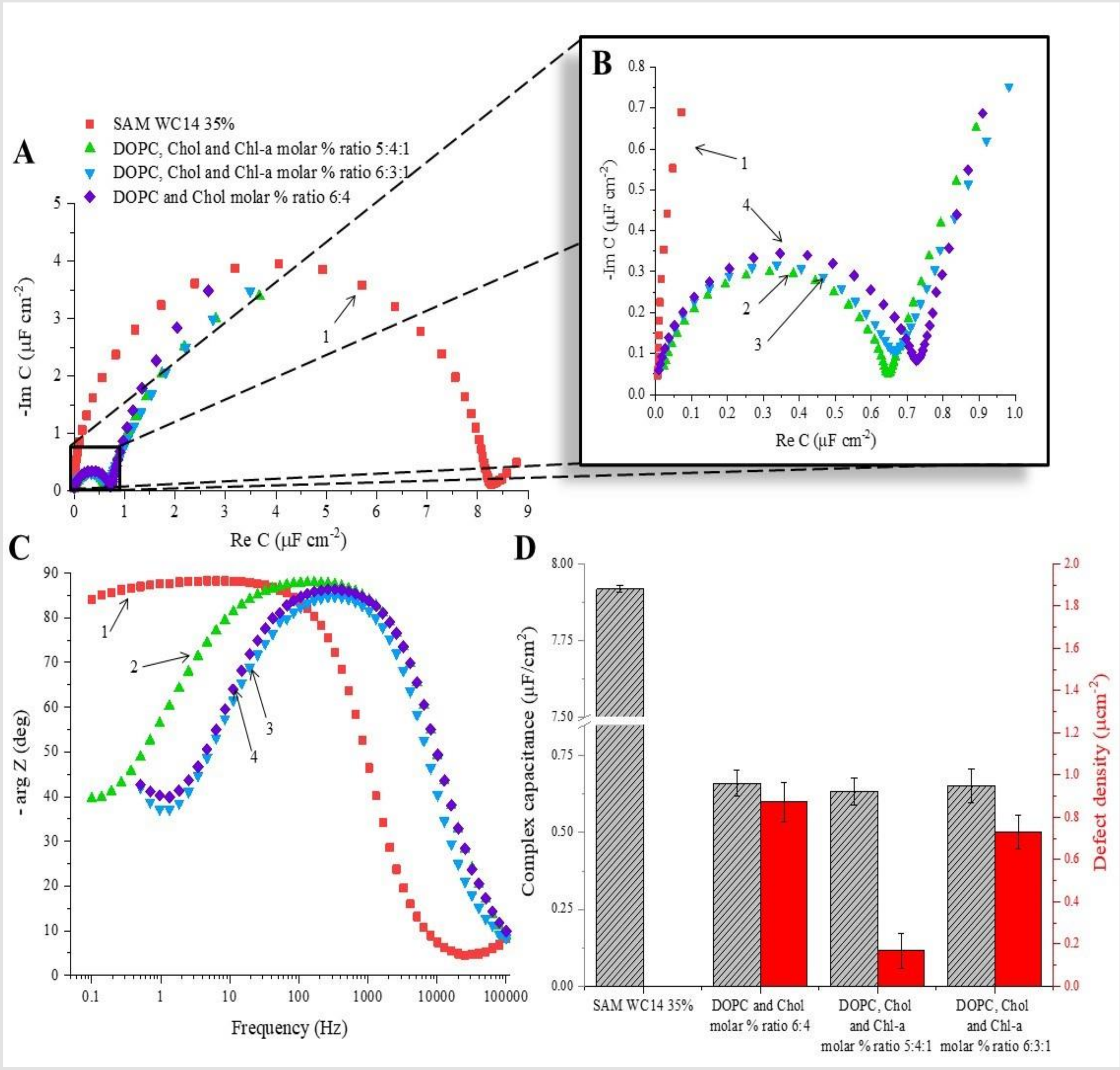

Figure 2. A, B – Cole-Cole plot of EIS spectra of (i) self-assembled monolayer curve 1 and (ii) tBLM structure curve 2-4 formed at different molar % ratios of various components: curve 2 – DOPC, Chol and Chl-a at molar % ratio of 5:4:1, respectively, curve 3 – DOPC, Chol and Chl-a at molar % ratio of 6:3:1, respectively, curve 4 – DOPC and Chol at molar % ratio of 6:4, respectively. Bode coordinates: spectrum **C** – frequency dependence on complex phase shift (frequency was applied until phase minimum was reached), **D** – complex capacitances and defect density of various components tBLM-based structures.

Conclusion

- The images registered by fluorescence microscopy are well revealing the results obtained by the EIS method and are proving that the incorporation of Chl-a into tBLM-based structure is rather efficient.
- Due to presence of Chl-a the capacitance of the electrical layer decreased. The tBLM-based structure DOPC, Chol and Chl-a had the least number of defects, which were determined in the range of 0.1-0.5 Hz, with defect density lower than 1 µm-2.
- Therefore, immobilized Chl-a suggests changes in post membrane reservoir which can result in Chl-a tetrapyrrole ring situated in post membrane reservoir.

References

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Acknowledgements

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