



## Regular Article

## Investigation of metal ion interaction with a lipid cubic phase using electrochemical impedance spectroscopy

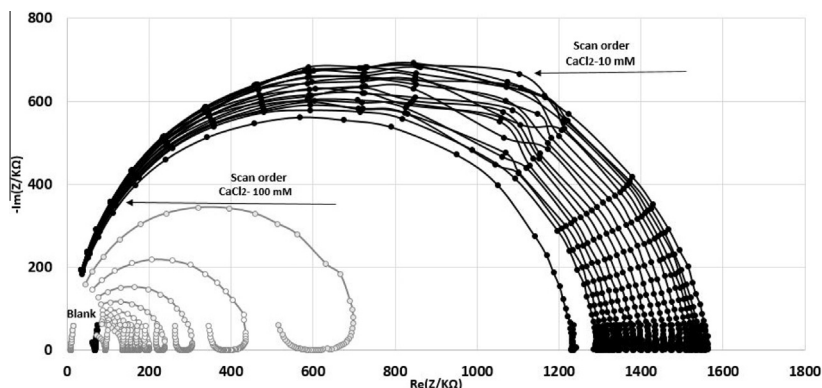


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## GRAPHICAL ABSTRACT



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## ABSTRACT

**Hypothesis:** Electrochemical impedance spectroscopy, EIS, can be used as a complementary technique to investigate ion interaction with the headgroup region in the aqueous channels of a lipid cubic phase, LCP. **Experiments:** A freestanding membrane made of monoolein LCP was formed by filling a small aperture that separates two cell compartments. The cell compartments were filled with electrolyte solutions at two different ionic strengths: i.e.: 10 and 100 mM, of KCl, CsBr and CaCl<sub>2</sub>. Electrochemical impedance spectroscopy was recorded between two platinum electrodes that were present at each side of the membrane.

**Findings:** The membrane resistance and capacitance were estimated from equivalent circuit fitting of the impedance data. It was confirmed that calcium ions interacts strongly with the headgroup region in the aqueous channels giving significantly higher membrane resistances compared to monovalent alkali metal ions. The membrane capacitance with Ca<sup>2+</sup>(aq) in solution was concentration dependent, which for the first time indicates formation of two different cubic phases at these conditions.

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**Abbreviations:** MO, monoolein; LCP, lipid cubic phase; NMR, nuclear magnetic resonance; SAX, small angle X-ray; DMPC, dimyristoylphosphatidylcholine; EIS, electrochemical impedance spectroscopy; CPE, constant-phase element; FT-IR, Fourier Transform Infrared Spectroscopy.

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## 1. Introduction

The interaction of biologically relevant cations and anions with lipid membrane surfaces has been of large research interest for a long period, as for example metal cations regulate membrane

related physiological process. Especially important is calcium ion interaction, that for instance affects membrane stability and structure [1] and play an important role in signal transduction in the interaction with neuron membranes [2].

The interaction of cations with lipid headgroup can be studied using monoolein (MO) lipid cubic phases (LCP) with or without addition of phospholipids. In pure MO the two hydroxyl groups in the glycerol give polar characteristics to the headgroup and form hydrogen bonds with the hydration shell of solvated cations. Ions with different sizes and charges have different corresponding hydration numbers in bulk, suggesting a competition between ion binding to water and lipids [3,4].

Ionic interactions have been investigated using molecular dynamic and metadynamic simulations [5], fluorescence measurements [6], nuclear magnetic resonance (NMR) [7], Fourier Transform Infrared Spectroscopic (FT-IR) [8], and small angle X-ray (SAX) [9]. Some of these findings are summarized below:

From molecular dynamic simulations and fluorescence measurements it is suggested that alkali metal ions predominantly interact with carbonyl groups and phosphates in the headgroup region of a phospholipid membrane [5,10]. Moreover potassium ion adsorption to the membrane was found to be weak especially when chloride is the counterion.

Metadynamic simulations were used to estimate the free energy landscape for some metal cations at dimyristoylphosphatidylcholine (DMPC) membrane surfaces [11]. It was suggested that the most favourable state for sodium and potassium ions is the fully hydrated state and therefore both these ions prefer to remain in the aqueous phase. In the case of calcium ions the most stable state is bound to the lipid headgroups coordinating four lipid oxygens and two water molecules.

It was early recognized that calcium ions are responsible for adhesion of cells to different substrates and to each other. Manery et al. reports on calcium bridging between carboxyl groups in the headgroup region and also that alkaline earth metals easily form stable chelates. The chelation induces a displacement of hydration water from the metal ion, and thereby causing dehydration of the membrane [12].

Infrared spectroscopy showed that divalent ions such as magnesium and calcium penetrate deeply into the polar headgroup region and cause partial dehydration. A complex rearrangement of the carbonyl region is following calcium ion binding involving both hydration and conformational changes. The hydration shell becomes less structured than the pure hydrated lipid [13]. In accordance to this Sinn et al. found that calcium binding to the lipid membrane headgroup is entropy driven because of the dehydration. Moreover, the dehydration following calcium ion binding leads to a tighter packing of the headgroups and the hydrocarbon chains [14].

Cook et al. observed that each calcium ion is coordinated to the hydroxyl groups of lipid bilayer which can lead to an arrangement of hydroxyl groups suitable for binding calcium ions. Calcium ions are most stable when bound to the lipid oxygens in the membrane, and the corresponding bond thereby a condensing effect on the membrane [3]. The condensing effect of calcium might induce pore formation in a planar lipid membrane of fixed area. This is probably not the case in the LCP.

The radii of the hydrated potassium were reported by different authors giving values that vary between 0.201 and 0.331 nm [15–18]. The corresponding values for cesium ions were 0.295 and 0.329 [19,20]. Thus, the hydrated potassium ion is somewhat smaller than the hydrated cesium ion at infinite dilution. The aqueous ionic radii of the  $\text{Br}^-$ (aq) and  $\text{Cl}^-$ (aq) anions are both 0.30 nm [21]. The inner diameter of the water channels within the monoolein LCP saturated with pure water is estimated to

5–10 nm [4,22,23]. Therefore, no physical hindrance is expected for hydrated anions and cations to move in the aqueous channels in the MO-LCP. The differences in ionic mobility can therefore be explained by interactions with the membrane headgroup region in the LCP channels [24].

Electrochemical impedance spectroscopy (EIS) was first introduced to investigate dielectric properties of tissues and other biological samples for more than a century ago [25,26] and references cited therein. Thus one of the first estimations of the molecular thickness of cell membranes, was performed by Fricke [27] with dielectric relaxation studies in cell suspensions. Later, EIS was used at black lipid membranes for investigation of antigen-antibody and enzyme-substrate reactions by del Castillo et al. [28] and some similar early work were presented by de Levie et al. and Coster et al. [29–31]. Since black lipid membranes are very fragile, stabilisation of artificial lipid membranes on solid support was suggested. Hence, polymer cushioned electrodes were used by for instance [32,33] or lipid bilayers on thiolipid support by Vogel et al. [34]. Supported monolayers on mercury provide a nice and reproducible model that was employed for metal ion interaction investigations with the lipid headgroup region [35]. In the present work we extend the use of EIS to investigation of metal ion interaction within the ion channels of a free-standing membrane of a LCP.

A monoolein/water LCP was covering a small circular aperture that separates two cell compartments containing one platinum electrode each. The compartments were filled with different electrolyte solutions at two ionic strengths, 10 mM and 100 mM. Electrochemical impedance spectra were recorded continuously with this two-electrode setup to monitor changes in membrane properties as the electrolyte ions diffuse into the aqueous channels. The dielectric properties of the LCP membrane were estimated from circuit fitting, i.e.: membrane resistance and membrane capacitance.

EIS is a new method for detection and tracking ionic interactions with the membrane surfaces in the aqueous channels inside LCP. EIS is inexpensive and simple to use as compared to existing techniques. The hypothesis is that EIS can enable disclosure of novel findings on lipid head group interactions with ions.

The condensing effect of calcium ions was observed from an increased membrane resistance, which confirms earlier findings with other techniques. The two concentrations of  $\text{CaCl}_2$  gave significantly different membrane capacitances, which might suggest the prevalence of two different cubic phases.

## 2. Experimental

### 2.1. Materials

Monoolein (1-oleoly-*rac*-glycerol,  $\geq 99\%$ , Sigma), calcium chloride, potassium chloride, and Cesium bromide (99%, Sigma-Aldrich) were used as received. Water was obtained from Milli-Q water purification system; Millipore Corp. 18 M $\Omega$  cm.

### 2.2. Instrumentation

Modulab (Solartron Analytical) was used for Electrochemical Impedance Spectroscopy measurements (EIS); amplitude 10 mV, frequency range 0.1–100,000 Hz. A two-electrode arrangement was used and the platinum electrodes were at open circuit potential with a bias of Zero Volt between them. All electrochemical measurements were carried out at room temperature. Zview version 3.4 was used for quantitative evaluation of the data.

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