Colloids and Surfaces B: Biointerfaces 142 (2016) 199–206

Contents lists available at ScienceDirect

Colloids and Surfaces B: Biointerfaces

journal homepage: www.elsevier.com/locate/colsurfb

The use of zeta potential as a tool to study phase transitions in binary phosphatidylcholines mixtures

M.B. Sierra[a], V.I. Pedroni[a], F.E. Buffo[b], E.A. Disalvo[c], M.A. Morini[a,*]

[a] Laboratorio de Fisicoquímica, Dpto. de Química, INQUISUR, Universidad Nacional del Sur, Argentina
[b] Dpto. de Matemática, Universidad Nacional del Sur, Argentina
[c] Laboratorio de Biointerfases, CITSE, Universidad Nacional de Santiago del Estero, Argentina

ARTICLE INFO

Article history:
Received 27 November 2015
Received in revised form 10 February 2016
Accepted 26 February 2016
Available online 3 March 2016

Keywords:
Lipid mixtures
Unilamellar vesicles
Zeta potential
Transition temperatures
Ionic medium
Plot of phase boundaries

ABSTRACT

Temperature dependence of the zeta potential (ZP) is proposed as a tool to analyze the thermotropic behavior of unilamellar liposomes prepared from binary mixtures of phosphatidylcholines in the absence or presence of ions in aqueous suspensions. Since the lipid phase transition influences the surface potential of the liposome reflecting a sharp change in the ZP during the transition, it is proposed as a screening method for transition temperatures in complex systems, given its high sensitivity and small amount of sample required, that is, 70% less than that required in the use of conventional calorimeters. The sensitivity is also reflected in the pre-transition detection in the presence of ions. Plots of phase boundaries for these mixed-lipid vesicles were constructed by plotting the delimiting temperatures of both main phase transition and pre-transition vs. the lipid composition of the vesicle. Differential scanning calorimetry (DSC) studies, although subject to uncertainties in interpretation due to broad bands in lipid mixtures, allowed the validation of the temperature dependence of the ZP method for determining the phase transition and pre-transition temperatures. The system chosen was dipalmitoylphosphatidylcholine/dimyristoyl phosphatidylcholine (DMPC/DPPC), the most common combination in biological membranes. This work may be considered as a starting point for further research into more complex lipid mixtures with functional biological importance.

1. Introduction

Liposomes are bilayer microstructures composed of natural or synthetic lipids [1] whose polar head group and long hydrophobic tail form an amphipathic environment. Lipid membranes, which are the structural basis of biological membranes, can serve as an appropriate model for many biophysical studies [2]. During the last decades, the approach to overcome the complexity of biological membranes relies in the use simplified biomimetic models consisting primarily binary lipid mixtures or ternary [3,4].

It is still disputed that the partially exotic phases occurring in synthetic lipid mixtures, such as tilted gel phases have any relevance for native membranes consisting of tenth, hundreds components. About the interest in lipid phase studies, lateral phase separation in biological membranes could be functionally important [5]. Lipid rafts or ordered lipid domains may play a role in the localization, transport, and function of different proteins. In spite of the relevance of lipid-protein interactions, the phase behavior of the lipid components of the cell membrane is believed to be of great importance in the efforts to find out some the principles that underlie the membrane function [6]. The characterization of phase boundaries has been conducted for mixed lipid dispersions using various techniques such as differential scanning calorimetry (DSC) [7], nuclear magnetic resonance (NMR), [8] X-ray scattering [9] Fluorescence Microscopy [10] Laser Ultrasound [11] and ultrasonic velocimetry [12]. Pre-transition temperatures of lipid mixtures are usually determined with relatively large uncertainty especially due to the typical broad peaks, for instance, in DSC. Aqueous dispersions of polar lipids are known to form a large variety of phases depending on the chemical structure, temperature, and dispersing media. Their phase behavior is dominated by the main (order-disorder) phase transition associated with the melting of the lipid hydrocarbon chains. At temperatures above the main transition, lipids arrange in liquid crystalline structures. Below the main transition there is a multitude of different possible phases, a basic equilibrium structure is the subgel (crystalline) Lc phase. In addition, a large

* Corresponding author.
E-mail address: mamorini@criba.edu.ar (M.A. Morini).

http://dx.doi.org/10.1016/j.colsurfb.2016.02.061
0927-7765/

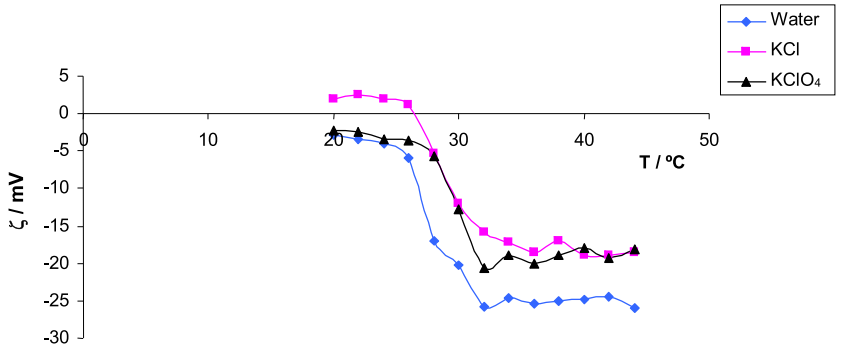

Fig. 1. Zeta potential as a function of T for $X_{DPPC} = 0.2$ mixture prepared and dispersed in water, KCl and $KClO_4$. In all cases, the standard deviation was lower than 10%, not shown for better viewing. Reported data were averaged over four different batches of liposomes.

number of intermediate stable, metastable, and transient lamellar gel structures are adopted by different lipids—with perpendicular or tilted chains with respect to the bilayer plane, with interdigitated, partially interdigitated, or non-interdigitated chains, rippled bilayers with various ripple periods, etc. Even so, the number of reported phases continues to grow. The formation of a subgel phase usually requires a prolonged low-temperature equilibration. The lipid polymorphism at low temperatures still appears to be far from clear [13].

Furthermore, liposomes pack concentrically hydrophilic and lipophilic portions thus rendering an internal vacuole which can serve as a storage compartment for an active agent. The rate limiting step in the use of this versatile system, for example for drug delivery, is the physical and chemical stability of liposomes [14]. Regarding stability, the surface charge of the aggregate is very important. This is also a factor to consider that influences the interaction of the liposome with a substrate.

In a previous work from our research group [15] it was shown that the surface charge of the liposome mainly depends on the kind of lipid and conditions such as temperature, phase state of the liposome and the presence or absence of ions in the medium.

Membrane properties such as surface potential, the dipole potential [16], structure and mechanical strength [17–19] are closely associated with ions present within the cell and its environment. Therefore, the study of the interactions of ions with the lipid bilayer is of considerable interest [20].

Temperature dependent Zeta Potential Studies are proposed in this work to analyze the thermotropic behavior of mixtures of synthetic phospholipids in unilamellar aqueous suspensions in the presence or absence of ions. Since the lipid phase transition influences the surface potential of the liposome reflecting a sharp ZP change during the phase transition [21], we propose this technique as a screening method for transition temperatures in complex systems, given its high sensitivity and small amount of sample required, that is, 70% less than that required in the use of conventional calorimeters. With data intervals transition temperatures obtained from zeta potential it was possible to construct plots of phase boundaries of the DPPC-DMPC mixture, similar to that obtained by calorimetric studies in literature [22–24]. The proposed use of ZP studies relies on the importance of continuous and gradual stabilization of the sample temperature, which ensures thermal equilibrium and thermodynamic study lipid mixture. The immediate consequence is to allow the system its conformational organization at a given temperature. This is particularly relevant in the transition zone where the system experiences continuous and large organizational changes in small temperature ranges. Based on the above mentioned, it is affirmed that the proposed technique allows to obtain accurate information about the phase transition of the system.

Despite the vast variety of lipid mixtures [25], this paper focuses on phospholipids, the most abundant structural elements present within cell membranes. In order to validate this approach, a kind of mixture of phospholipids was chosen, for which the phase behavior has already been studied experimentally [22,23,26–29] and computer simulations have been performed [23,27–29]. The chosen system is a binary mixture of saturated phospholipids with the same polar head and different length of the alkyl chain, such as 1,2-dimyristoyl-sn-glycero- 3-phosphocholine (DMPC, chain length n = 14, temperature Tm melting: 24° C) mixed with 1,2-dipalmitoyl-snglycero-3-phosphocholine (DPPC, n = 16, Tm: 41 °C). The mixture is an isomorphic system, both of its lipids components are miscible in both crystalline and liquid crystalline solid [23,30].

This work maybe considered as a starting point for further research into more complex lipid mixtures with functional biological importance.

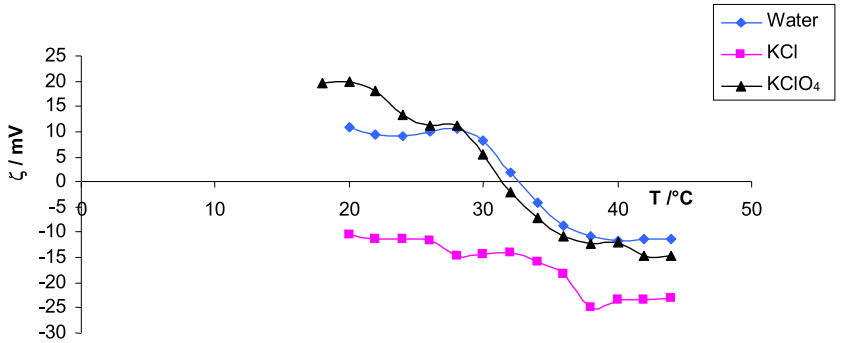

Fig. 2. Zeta potential as a function of T for $X_{DPPC} = 0.5$ mixture prepared and dispersed in water, KCl and $KClO_4$. In all cases, the standard deviation was lower than 10%, not shown for better viewing. Reported data were averaged over four different batches of liposomes.