Near infrared spectroscopy and multivariate analysis for the study of water in lipidic membranes

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**Keywords:** Membranes; Water; Near Infrared Spectroscopy; Principal Component Analysis.

Introduction

The ability of near infrared spectroscopy to inform about the vibrations of the covalent O-H, by means of the first overtone of water around 1450/55 nm, was exploited to examine the water in membranes and the influence of the constitutive phospholipids on hydrogen bonding. The less the number and/or the strength of H-bonds, the more the strength of the O-H bonds and so they vibrate and absorbs radiation at higher frequencies. This shift is employed to evaluate the water status in membranes composed of two phospholipids having the same acyl chain but different polar head, the part of the molecule typically associated in the interaction with water.

Materials and Methods

Membrane production: solvent from phospholipids solution was removed with nitrogen flux while rotating the container, in order to obtain a thin film. MiliQ water was next added, vortexing and sonicating to form the vesicles, working at temperatures higher than the highest transition temperature of the lipids. NIR spectra and analysis: absorbance of the suspensions was registered in the 1100-2300 nm interval, between 13-58 °C every 5°C. Principal Components Analysis was employed to disclose the spectral changes [1].

Results

Figure 1 shows the sixty spectra, quite overlapped and unfeasible to discriminate according to phospholipid (DMPA or DMPC) or concentration (250 or 500 µM). Only the effect of temperature can be barely advised in some regions (shown by arrows). The localization of the first overtone of water (1450/55 nm) matches with previous reports [2-4]. The isosbestic region at 1425-1430 nm, is not far from the 1440-1442 nm reported by [5] and 1446 nm reported by [4] for pure water.
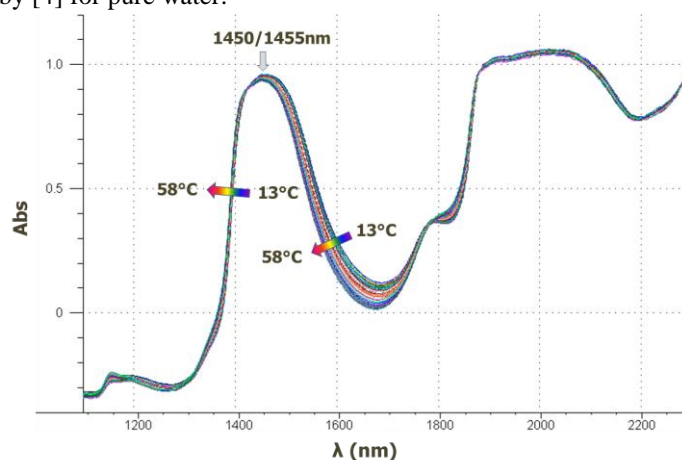

Figure 1. The sixty NIR spectra includes: two water control samples (Milli-Q), a sample of DMPA (1,2-dimyristoyl-sn-glycero-3-phosphate) and a sample of DMPC

31   (1,2-dimyristoyl-sn-glycero-3-phosphocholine) at two concentrations (250 and 500 μM). All samples measured
32   at ten temperatures (every 5°C in the 13-58 °C interval).

33 The simultaneous analysis of scores (Figure 2, left) and loadings (Figure 2, right) discloses useful information.
34 Scores along PC1 capture the effect of temperature on the hydrogen bonding, whereas scores on PC2 capture
35 the effect of phospholipids. By far, temperature in the assayed range is the dominating affecting factor (PC1 ≈
36 97 %). As temperature increases, the differences in the effect of phospholipids become smaller, as series
37 become closer at the highest temperatures.

38 Loadings reveal that increasing temperatures and the presence of phospholipids (particularly DMPC) weaken
39 water H-bonds, as the O-H vibration band (first overtone) shift to higher frequencies, i.e. higher energy. As
40 temperature increase, the absorbance increases at shorter (1391 nm) and it decreases at longer (1546 nm)
41 wavelengths of the water band. To a lesser extent, phospholipids cause similar changes around 1385 and 1494
42 nm, respectively.
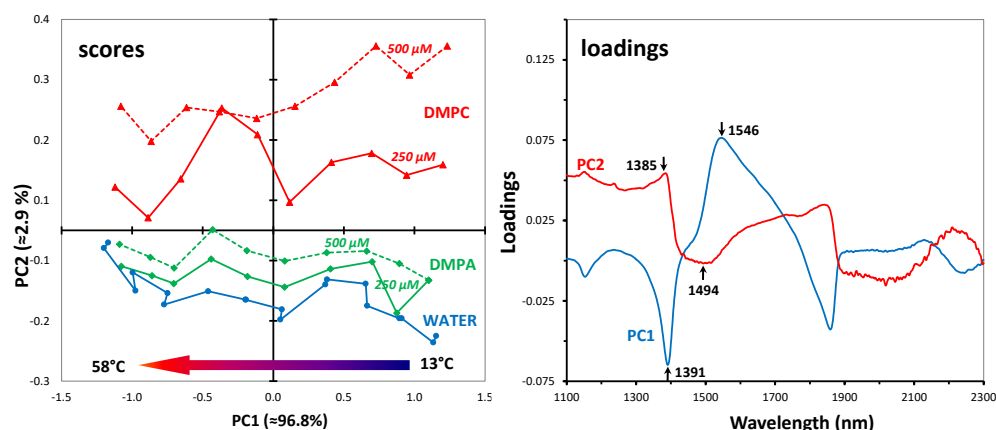
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44 **Figure 2.** Output of the PCA, considering the first two PCs. Left panel: scores, where the connecting lines
45   denote the evolution of samples with temperature, increasing from right to left every 5°C, as indicated by the
46   arrow. Right panel: loadings plotted against the original variables.

Conclusions

48 As occurs with increasing temperature, DMPA and DMPC disrupt H-bonding in membranes, and the effect
49 increases with the concentration.
50 Having identical acyl chain (myristoyl), the absence of OH in the polar head of DMPC may explain why it has
51 a stronger disrupting effect on H-bonds than DMPA (OH acts as a H-bond donor).

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