Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.


# Electronic Supplementary Information

## Acemetacin-phosphatidylcholine interactions are determined by the drug ionization state

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## 1. Partition coeffient of acemetacin by derivative spectrophotometry

As an example, the third-derivative spectra of increasing concentrations of DMPC LUVs in the absence and presence of acemetacin at pH 5.0 is displayed in Fig. S1A. The bathochromic shift observed with increasing concentrations of DMPC shows that the polarity of the acemetacin surrounding medium has changed, which indicates that the drug is translocating from the aqueous to the lipid phase. Moreover, the isosbestic points (for example, at 298 nm and 320 nm) also observed in the third-derivative spectra show that acemetacin is distributed between the aqueous and lipid media, and that the background signals that arise from liposome-induced light scattering were effectively eliminated. The same features were also observed in the third-derivative spectra obtained at pH 3.0 and 7.4.

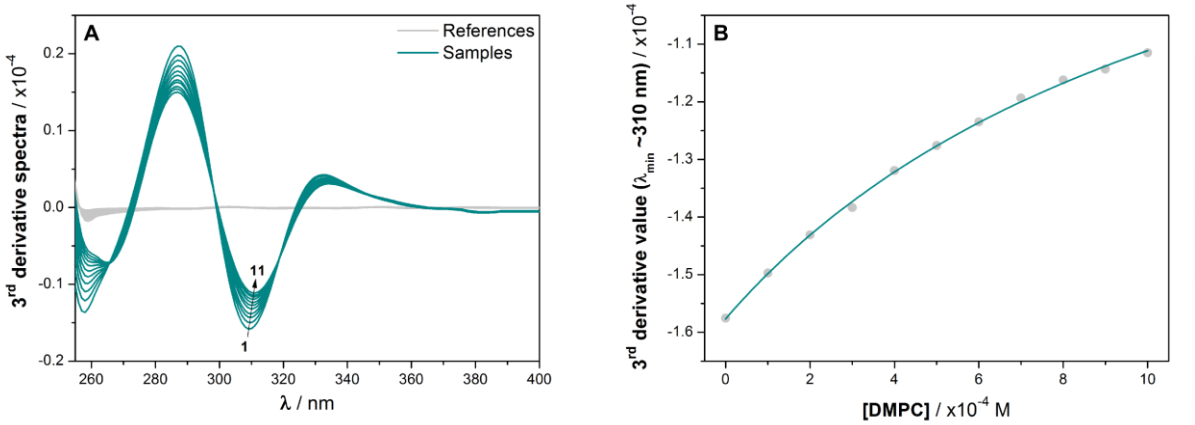


Fig. S1. (A) Third-derivative of the absorption spectra of DMPC LUVs (0 (1) to 1x10$^{-3}$ (11) M) in the absence or presence of acemetacin (references and samples, respectively) at 37 °C and pH 5.0. (B) Best fit of Eqn (1) (line) to experimental third-derivative data, at the minimum wavelength around 310 nm, as a function of DMPC concentration (dots).

## 2. Determination of the acidity dissociation constant of acemetacin in the DMPC bilayer

From the partition coefficient values obtained by derivative spectrophotometry, the molar percentage of the protonated (HA) and deprotonated ($A^-$) forms of acemetacin in the DMPC bilayer, in a range of pH values (2-8), were calculated. From the obtained distribution diagram (Fig. S2), the apparent acidity dissociation constant ($pK_m$) of acemetacin in the DMPC bilayer was determined considering the intersection of the two curves.

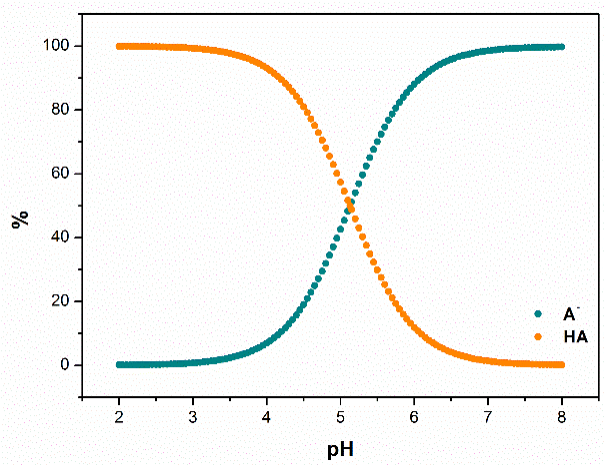


Fig. S2. Distribution diagram of the protonated (HA) and deprotonated ($A^-$) forms of acemetacin in the DMPC bilayer as a function of pH. The intersection of the curves yielded apparent $pK_m$.

## 3. DMPC phase transition by differential scanning calorimetry

An example of the thermograms obtained with pure DMPC LUVs at pH 3.0, 5.0, and 7.4 by differential scanning calorimetry is presented in Fig. S3. The inset graphs present a closer look to the DMPC pretransition and the obtained values of temperature and enthalpy of the DMPC pretransition are also presented.

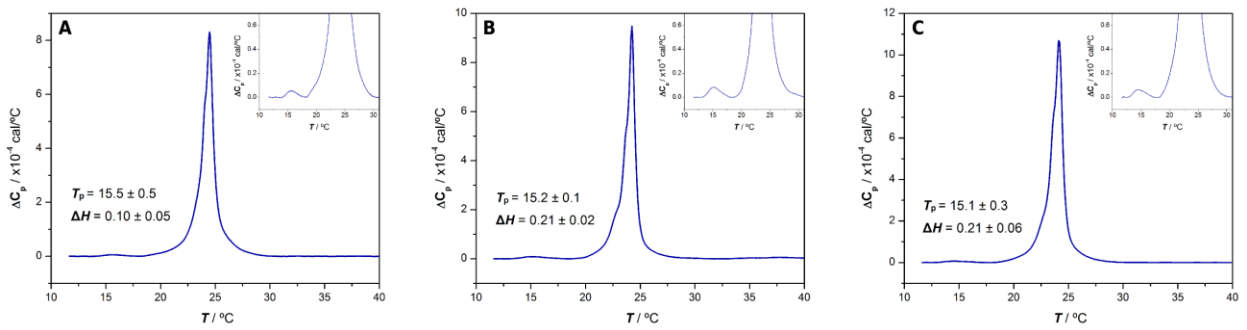


Fig. S3. Representative calorimetric curves of DMPC LUVs at pH 3.0 (A), 5.0 (B) and 7.4 (C). The inset graphs present a closer look to the DMPC pretransition. The mean values (± standard deviation) of the temperature ($T_p$) and enthalpy ($\Delta H$) of the DMPC pretransition are also shown.

## 4. Acemetacin-DMPC interactions evaluated by electron paramagnetic resonance

The EPR spectra of methyl 5-DOXYL-stearate (5-MeSL) incorporated in DMPC LUVs in the absence and presence of acemetacin at pH 5.0 and 7.4 and 10 and 37 °C are displayed in Fig. S4.

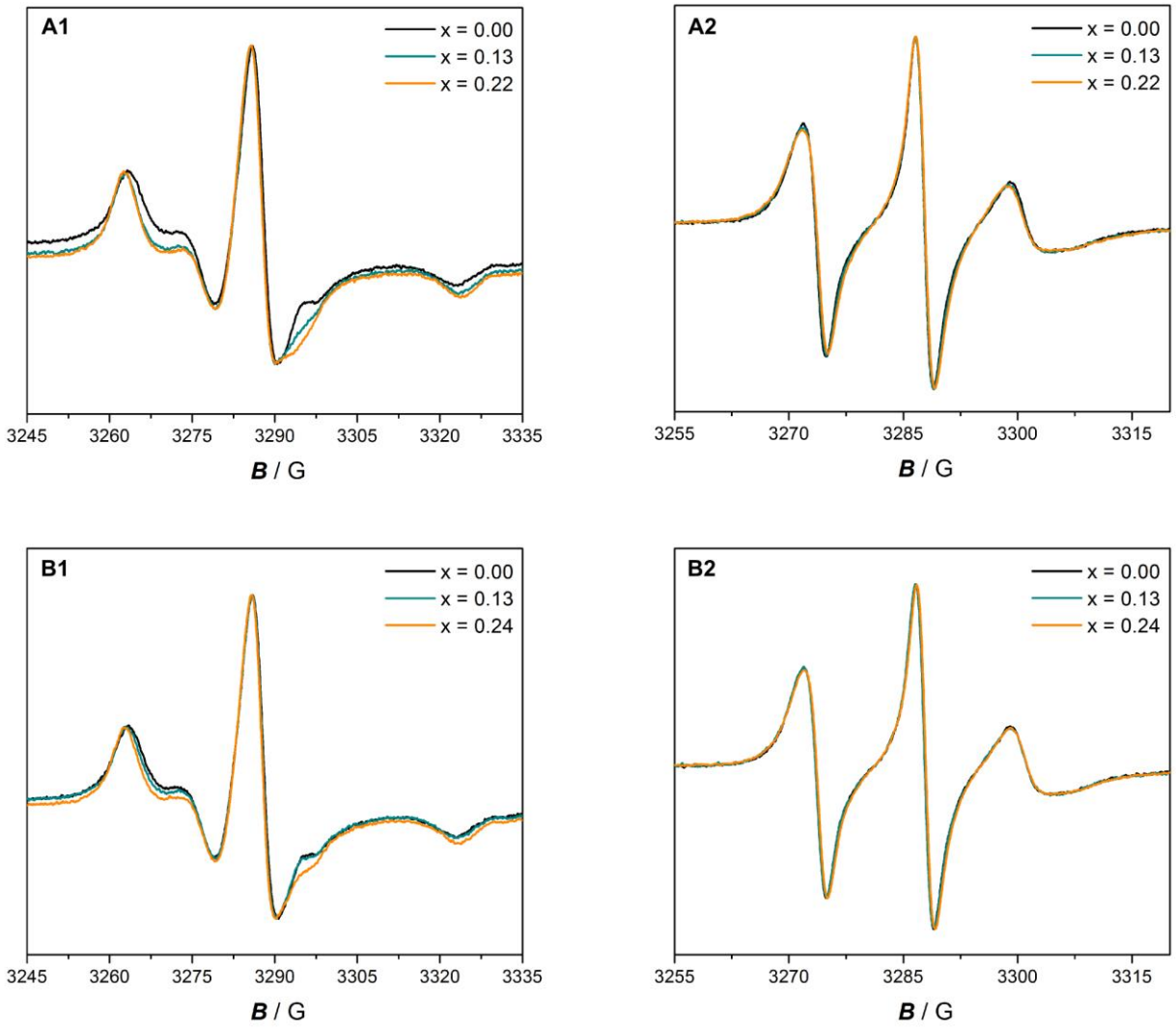


Fig. S4. Normalized EPR spectra of 5-MeSL labeled DMPC LUVs containing variable molar ratios (x) of acemetacin at pH 5.0 (A) or 7.4 (B) and at 10 °C (1) or 37 °C (2).

## 5. Acemetacin-DMPC interactions evaluated by small-angle X-ray scattering

The average SAXS patterns of DMPC bilayers in the absence and presence of acemetacin (molar ratio = 0.1) at pH 3.0, 5.0, and 7.4 and 37 °C are shown in Fig. S5.

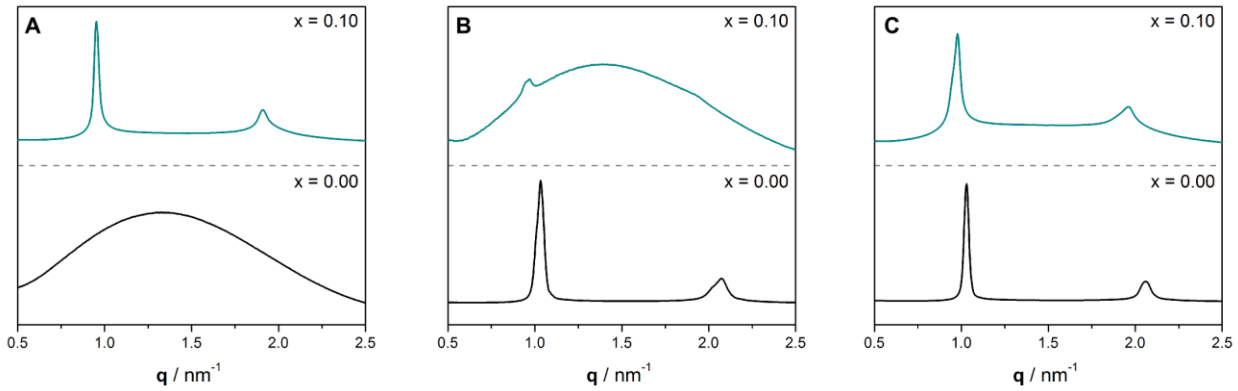


Fig. S5. Average SAXS patterns of DMPC bilayers in the absence and presence of acemetacin (molar ratio, x = 0.10) at 37 °C and at pH 3.0 (A), 5.0 (B) or 7.4 (C).