

WATER STATE IN CONFINED REGIONS OF LIPID BILAYERS OBSERVED BY FTIR SPECTROSCOPY

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INTRODUCTION

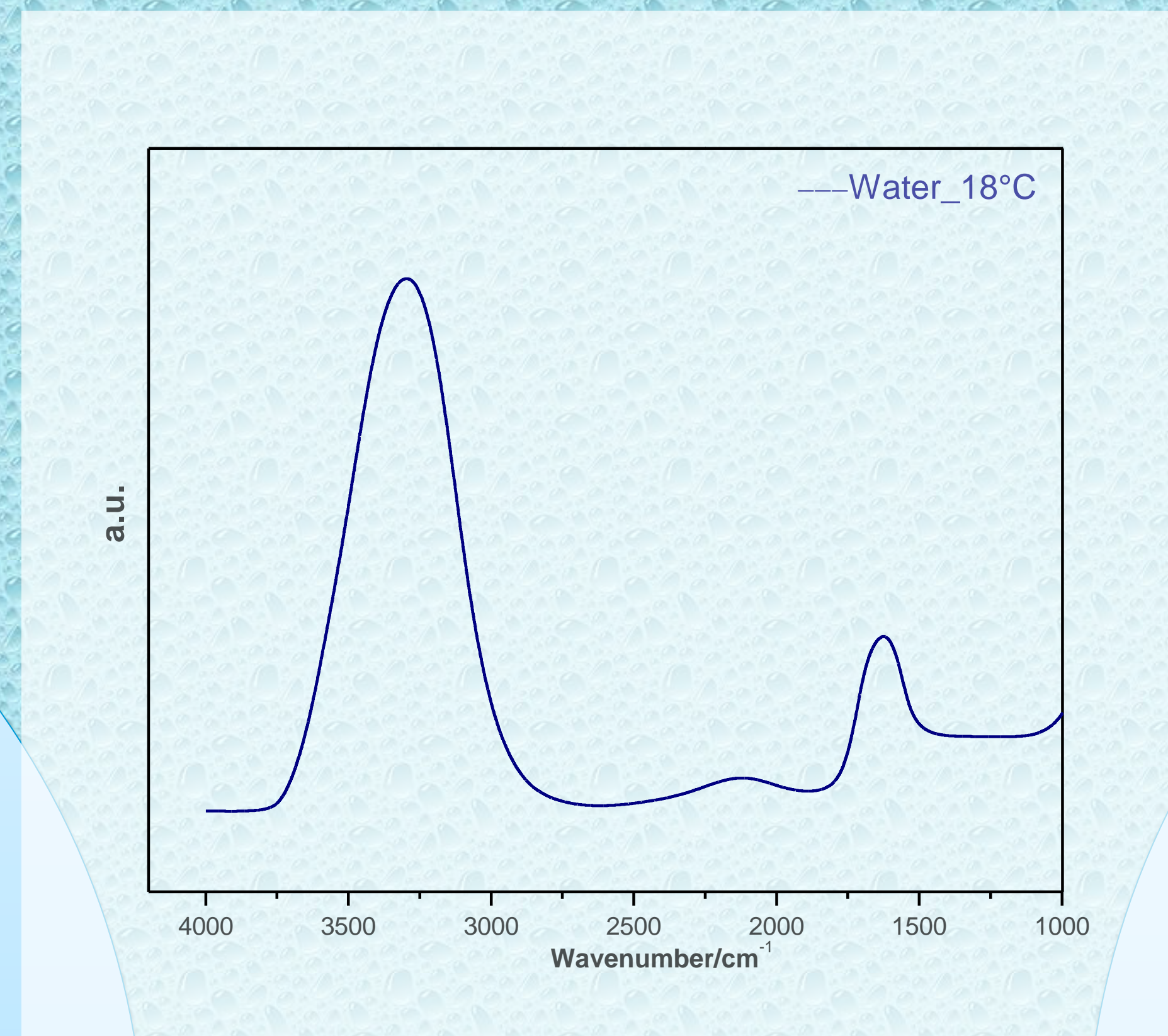
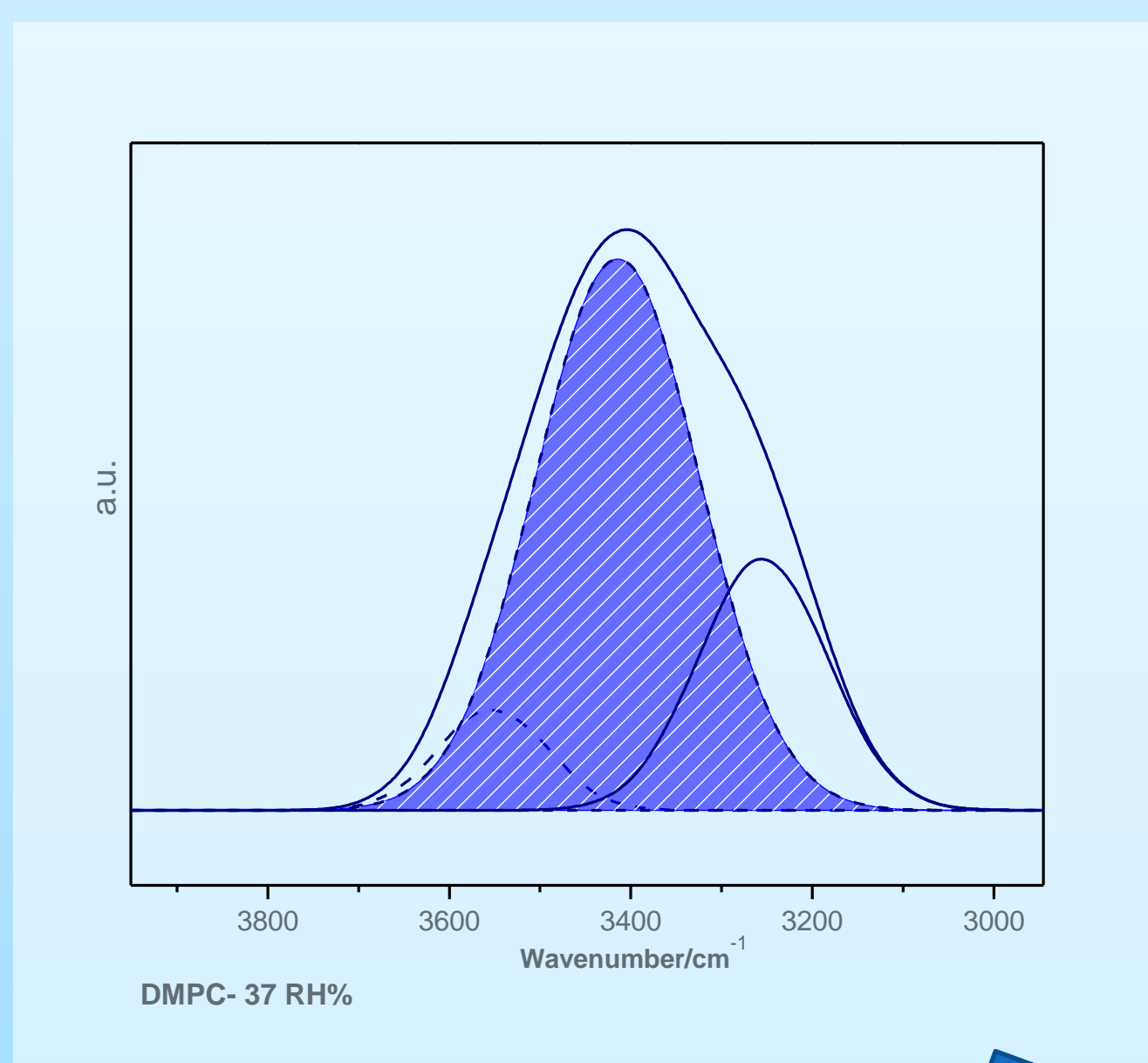
The presence of *water pockets* or *defects* in lipid bilayers has been proposed to explain the insertion of charged and polar peptides and aminoacids in membranes. The properties of these confined regions of water have not yet been analyzed. FTIR spectroscopy provides a direct visualization at molecular level of the order-disorder increase due to the trans-gauche isomers in the lipid acyl chains. It is assumed that the increase of conformational isomers enhances water penetration beyond the polar head group region. In this condition, water may be present in different states according to the hydrophobic-hydrophilic character of the wall it is facing. To gain insight into the interaction, the evolution of the water band (3600-3200 cm^{-1}) and the CH_2 vibration were monitored by FTIR as a function of temperature and saturation of lipids

MATERIALS

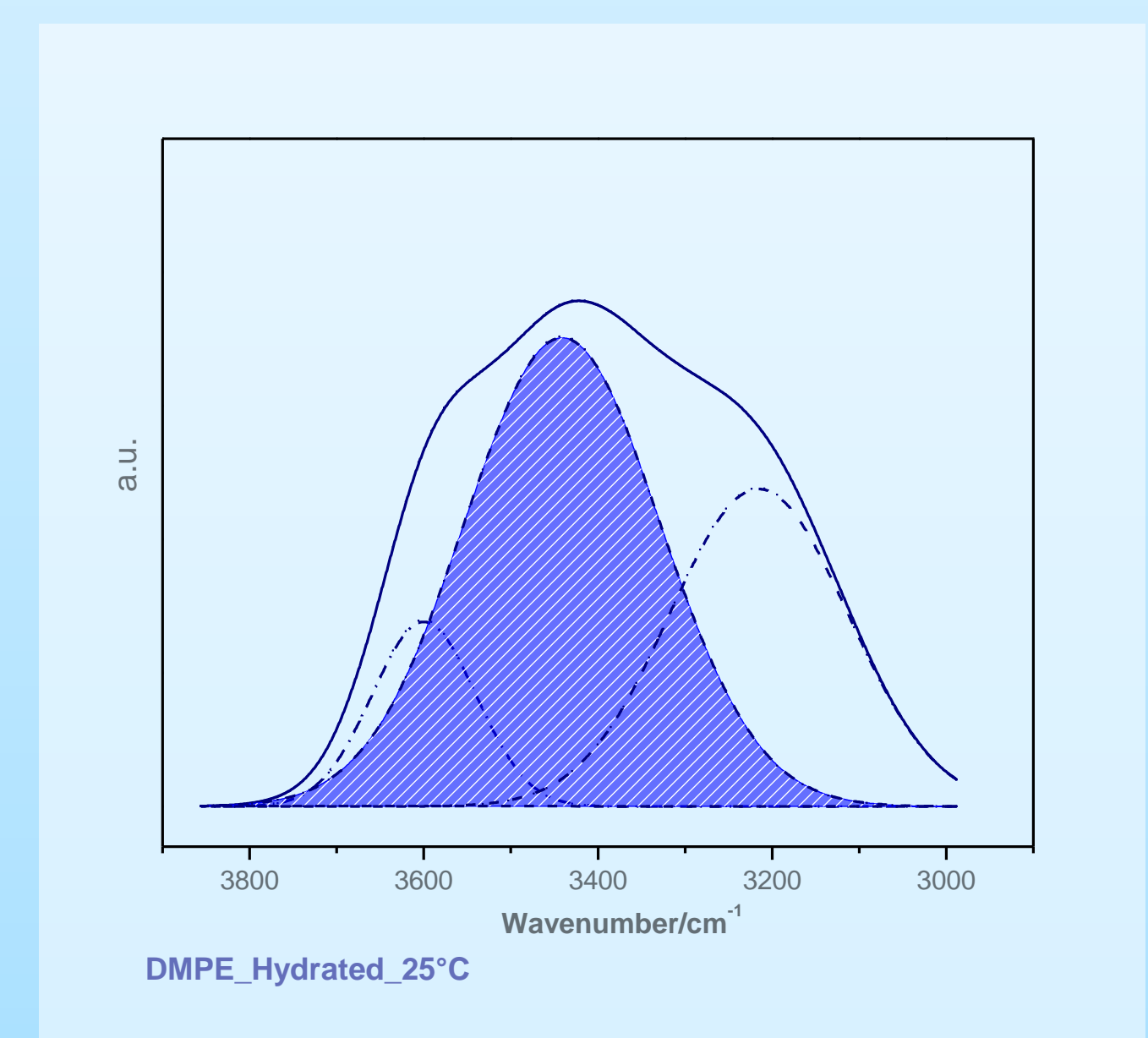
1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), 1,2-dimyristoylphosphatidylcholine (DMPC), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-dimyristoylphosphatidylethanolamine (DMPE) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) and purity (>99% pure) of the lipids was checked by thin layer chromatography and used without further purification.

RESULTS

WATER BAND IN THE PRESENCE OF SOLID LIPIDS

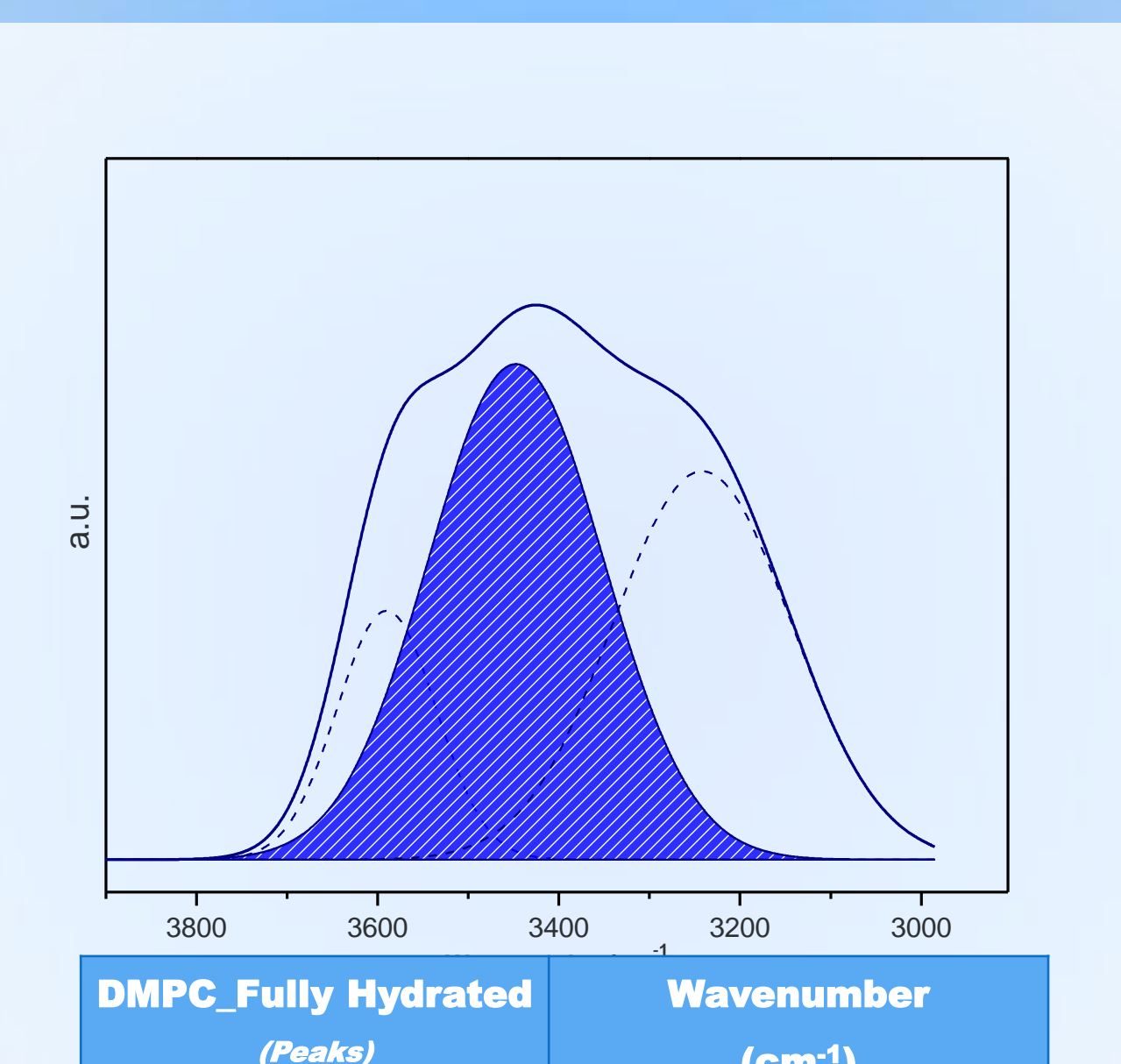


WATER BANDS ARE INDEPENDENT OF LIPID SPECIES IN THE GEL STATE



DMPE Fully Hydrated (Peaks) GEL PHASE	Wavenumber (cm^{-1})
1	3599,9
2	3441,1
3	3215,4
$\nu_{\text{asym}} \text{PO}_2^{-2}$	1222,7
$\nu_{\text{sym}} \text{PO}_2^{-2}$	1080,8

NEW WATER BANDS APPEAR IN THE PRESENCE OF FULLY HYDRATED LIPIDS IN THE GEL STATE

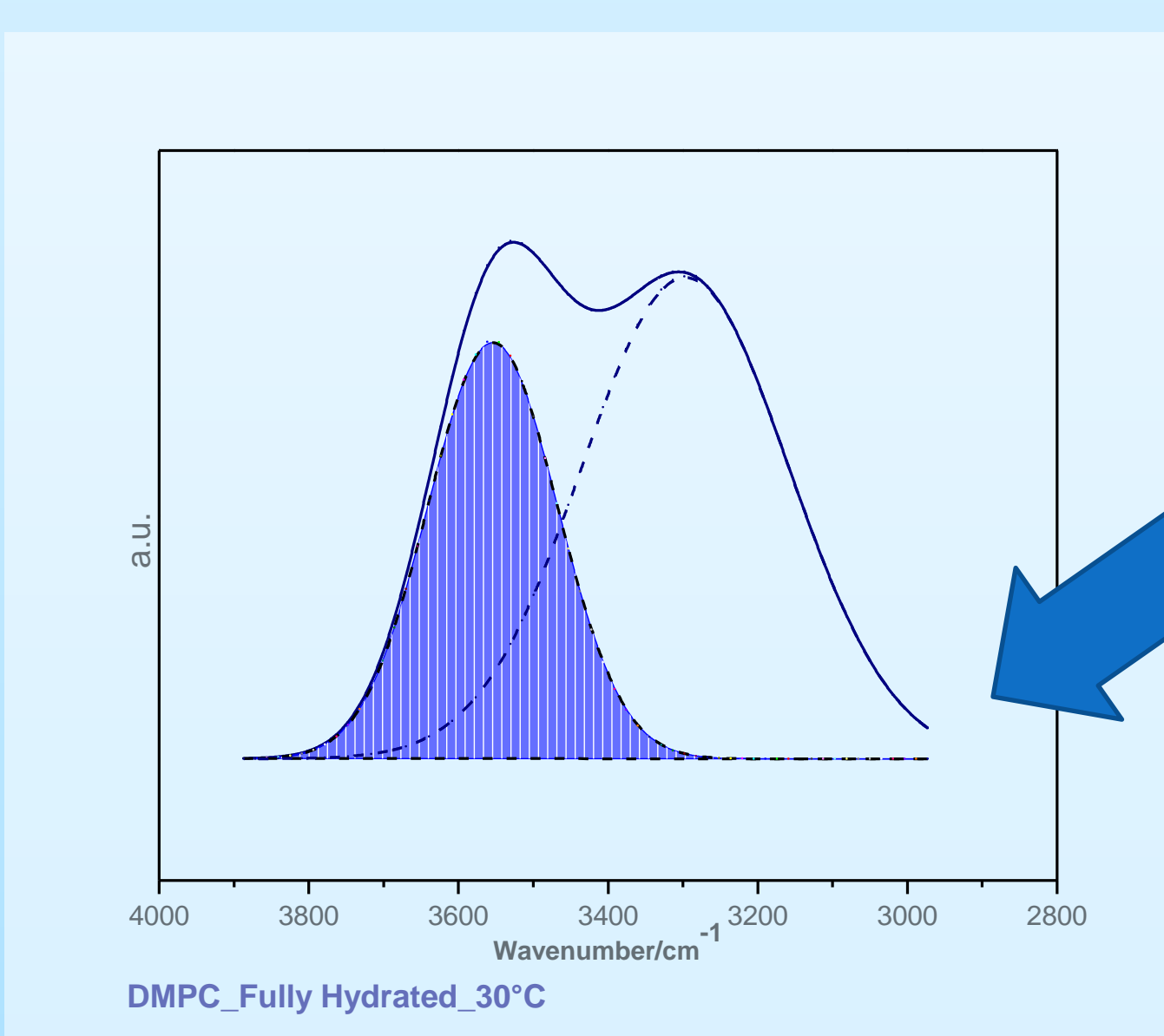


DMPC Fully Hydrated (Peaks) GEL PHASE	Wavenumber (cm^{-1})
1	3590,3
2	3447,4
3	3241,7
$\nu_{\text{asym}} \text{PO}_2^{-2}$	1231,8
$\nu_{\text{sym}} \text{PO}_2^{-2}$	1090,8

SWELLING AND COOLING TO THE GEL STATE

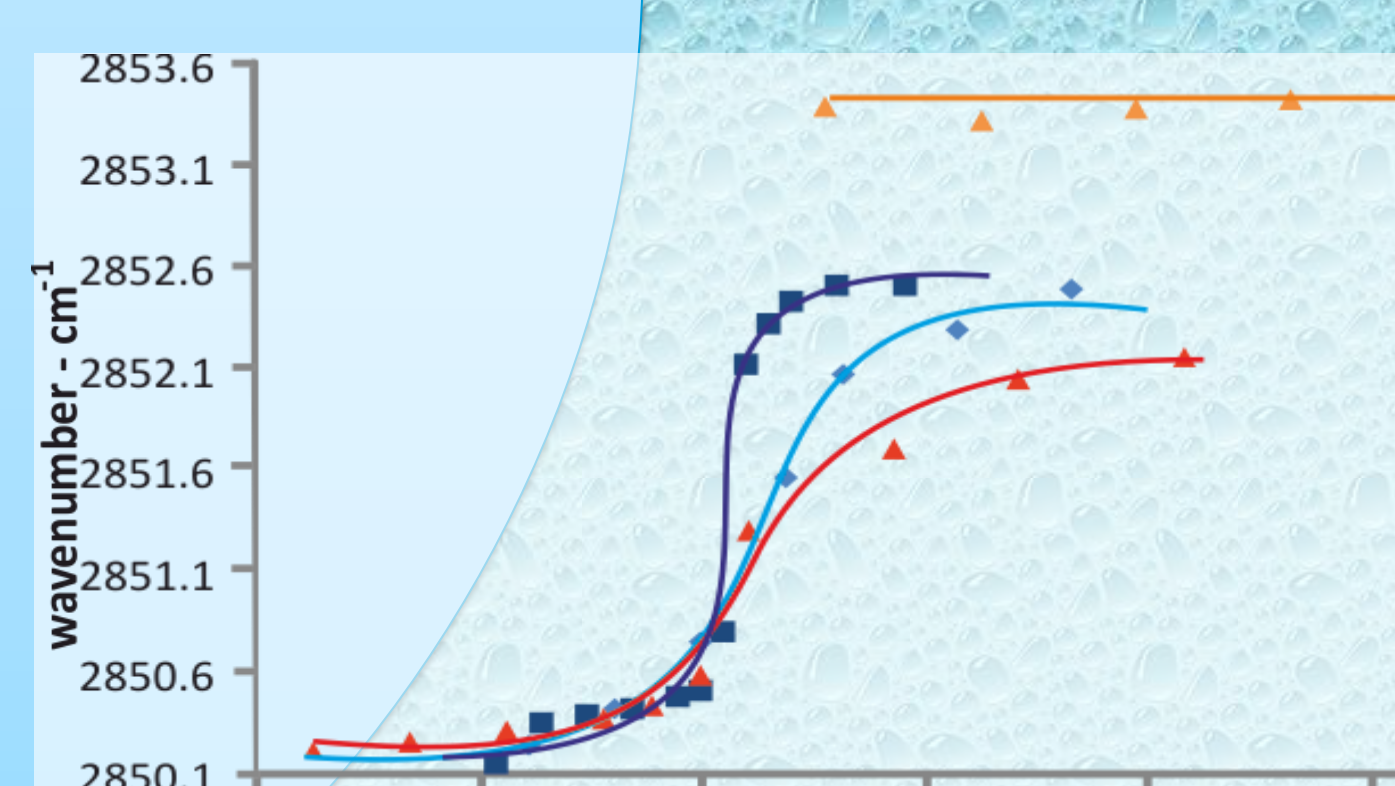
CHANGE IN POLAR HEAD GROUP

WATER BAND CHANGES DRASTICALLY WITH THE PHASE STATE OF THE LIPIDS

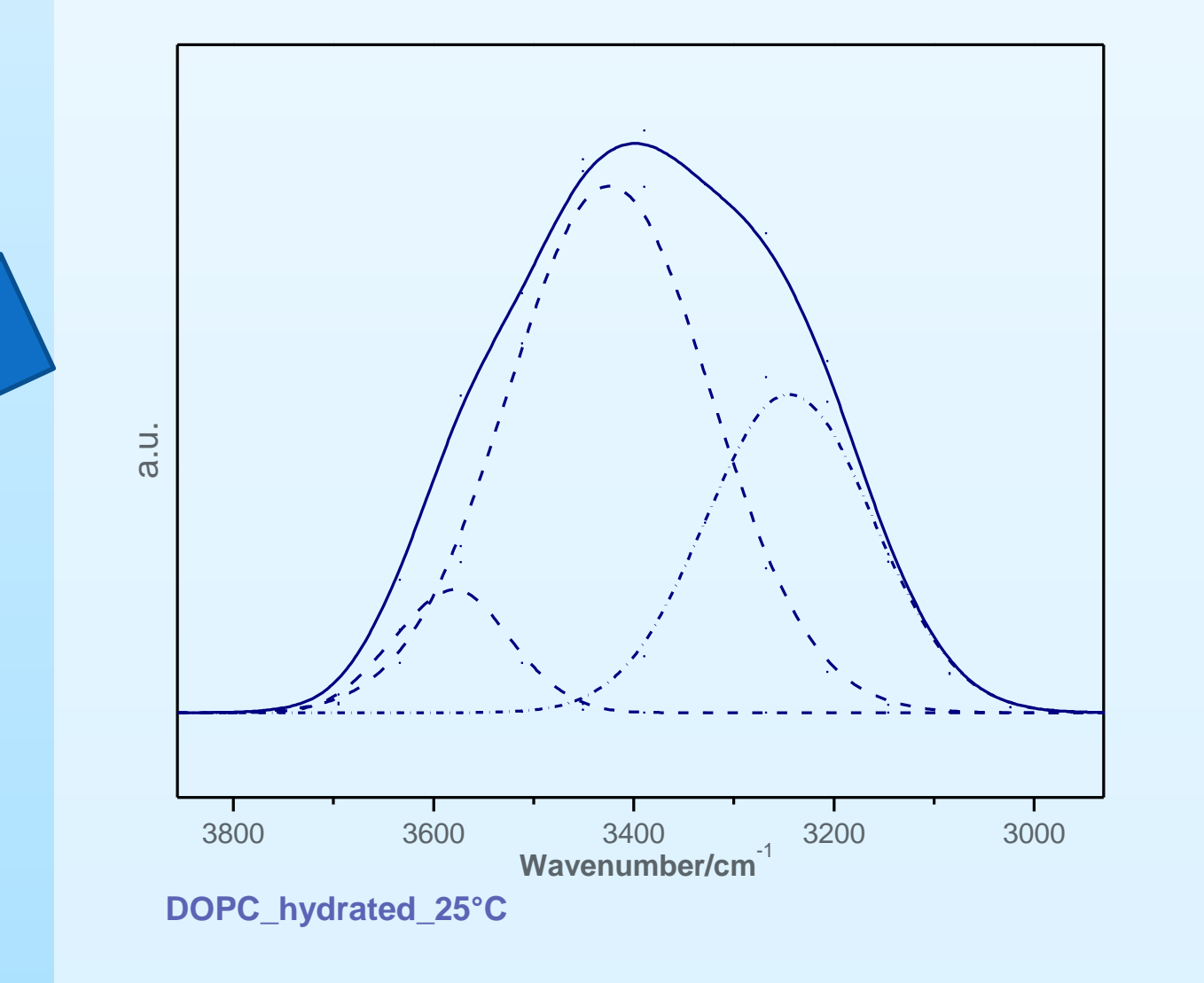


DMPC Fully Hydrated (Peaks) FLUID PHASE	Wavenumber (cm^{-1})
1	3554,2
2	3296,8
$\nu_{\text{asym}} \text{PO}_2^{-2}$	1231,8
$\nu_{\text{sym}} \text{PO}_2^{-2}$	1090,8

THERMO TROPIC TRANSITION OF DMPC. CHANGES IN THE CONFORMATIONAL STATE



WATER BANDS FOR DOPC ARE SUPERPOSABLE WITH WATER BANDS FOR DMPC IN THE GEL STATE

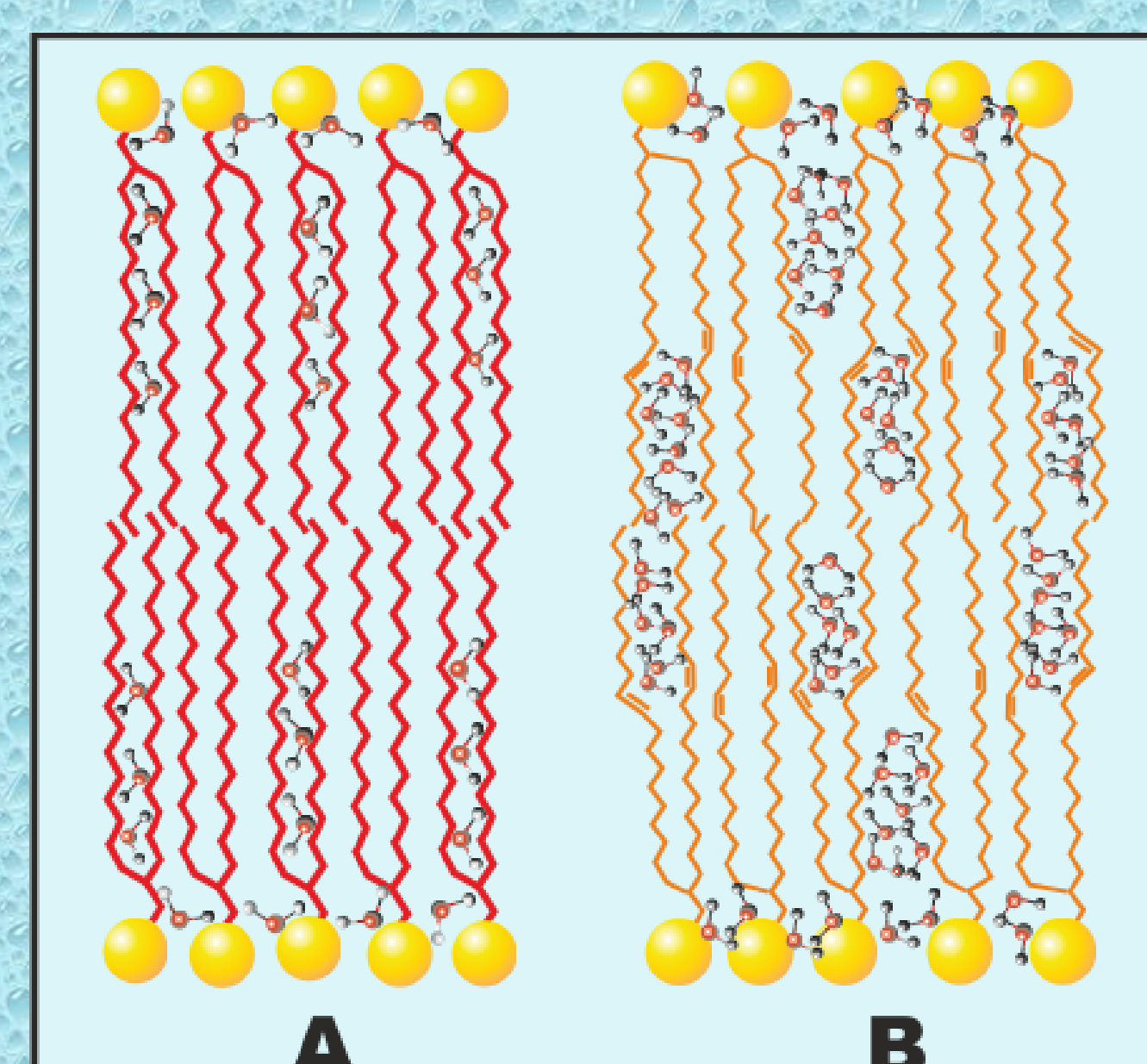


DOPC Fully Hydrated (Peaks) FLUID PHASE	Wavenumber (cm^{-1})
1	3580,4
2	3425,3
3	3238,7
$\nu_{\text{asym}} \text{PO}_2^{-2}$	1231,8
$\nu_{\text{sym}} \text{PO}_2^{-2}$	1090,8

CHANGE IN THE SATURATION OF THE ACYL CHAINS

CONCLUSION

THE BAND OBSERVED AT 3400 CM^{-1} IN THE GEL STATE DISAPPEARS ABOVE THE PHASE TRANSITION TEMPERATURE AND NEW ONES CENTERED AT 3200 CM^{-1} AND 3600 CM^{-1} ARE VISUALIZED. THE PEAK OBSERVED AT LOWER FREQUENCIES IN THE FLUID STATE, IS CONGRUENT WITH THE STRENGTHENING OF H-BONDS BETWEEN WATER MOLECULES.



REINFORCEMENT OF H BONDS IN WATER STRUCTURE IN THE PRESENCE OF NON POLAR RESIDUES SUCH AS THE CH_2 RESIDUES (A).

WATER CLUSTERING IN BETWEEN UNSATURATED LIPID ACYL CHAINS IS REVEALED BY THE SIMILARITY OF THE BAND OBTAINED FOR DMPC IN THE GEL STATE (B).