The Miscibility Gap in the Fluid Phase of DMPC:Cholesterol

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The understanding of the structural properties of lipid/lipid mixtures is a first step to understanding biological membranes. In view of applications we mention vesicles as drug delivery systems and lipid mixtures as biosensors. Even a two-lipid system shows properties none of the two components alone possesses, owing to the incomplete compatibility of the two lipid species.

Of particular interest are binary mixtures with only moderately different main transition temperatures because they are expected to mix quite well. Here, we study the binary mixture of dimyristoylphosphatidylcholine (DMPC) and cholesterol, both lipids are very common in biological membranes. A phase diagram based on SANS, DSC, ESR and theoretical modeling has been put forward [1] (Fig.1). We here pay special attention to the miscibility gap in the fluid-fluid binary mixture,

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{Figure1.png}
\caption{The phase diagram of the binary mixture DMPC:cholesterol; adapted from [1].}
\end{figure}

which is the loop displayed in the center of the phase diagram. This miscibility gap is located in a temperature and cholesterol range typical for cell membranes. This gap is supposed to be a universal feature of all cholines and many other lipid mixtures. A hidden critical point should be expected to terminate the gap.

We have tested this phase diagram by time-resolved small- and wide angle x-ray diffraction close to and far away from equilibrium. Referring to the phase diagram (Fig.1) a sample containing 15\% cholesterol should cross the miscibility gap upon heating - entering at about 19\(^\circ\)C and leaving at

\begin{figure}[h]
\centering
\includegraphics[width=0.4\textwidth]{Figure2.png}
\caption{The x-ray diffraction pattern recorded for a binary mixture DMPC:15\% cholesterol on heating and cooling through the miscibility gap.}
\end{figure}
about 48°C. A temperature cycle between 10°C and 80°C has been measured. Fig. 2 shows the data recorded.

With increasing temperature the intensity of the two reflections decreases drastically. Upon cooling, the two reflections regain their initial intensity. The sequence of diffraction pattern reveals a splitting of the reflections, nicely seen on the second order, at higher temperatures. This splitting appears to be fully reversible, too. The structural parameters, integral intensity, maximum intensity, the lattice d-spacing and the integral width, of the first and second lamellar order have been determined using the in-house software MIX13. The ratios of the respective parameters are seen in Fig. 3: The ratios show some peculiarities: The ratio of the integral intensities does not have the same

![DMPC:15% Cholesterol - Ratio 1st/2nd order reflection]

Figure 3: Temperature dependence of the structural parameters of the binary mixture DMPC:15% cholesterol.

The one-sided cusp-like functional form of the lattice parameter ratio is caused by the larger splitting effect on the second order. Therefore, the splitting begins obviously in the miscibility gap region. In the close vicinity of the predicted hidden critical point at 48°C, we do not observe any remarkable changes in any of the parameters analyzed. The parameters, however, show extrema at temperatures above the temperature predicted for the critical point.

The analysis in progress of the data collected under non-equilibrium conditions may shed some more light on the driving interactions of the demixing.

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**References**