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EPR-Ghost Characterization of Spin Labeled Alkylphospholipid Liposomes with Different Concentration of Cholesterol.

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Characterization of biological membranes is important to understand physiological aspects of cellular functions. Liposomal bilayers can be used as model system, in which the composition can be varied easily in order to follow the effect of a particular lipid component on the mem-brane domain structure and modes of molecular motions within these domains. Electron paramagnetic resonance (EPR) together with spectral simulation is one of the methods by which it can be obtained. The number of spectral parameters needed for the characterization of EPR spectra increases substantially due to the membrane heterogeneity. The problem of accurate determination of EPR spectral parameters, together with personal influence of the spectroscopist, therefore implies the usage of an automatic optimization procedure. In this work a newly developed characterization procedure (hybrid evolutionary optimization HEO and GHOST condensation method) was applied to the EPR spectra of alkylphospholipid liposomes, with different concentration of cholesterol (CH). The results were compared to those obtained previously for the same system, as well as to the theoretical predictions of possible membrane structure in presence of cholesterol and the measurements obtained by other authors. This is the first time that the HEO-GHOST method was applied to characterize the membrane properties of liposomes in more detail. For this purpose a four-spectral-compo-nent model was used to simulate the EPR spectra and HEO procedure was applied 200 times. The solutions are presented on GHOST diagrams as the basis for determination of spectral parameters and relative proportion of spin probe motional and polarity modes within the domains. It was found that OPP liposomes with less than 45% of CH can be described with four modes of spin probe motions. An increase in CH resulted in a shift of solutions to higher ordering of the membrane domains and increased proportion of the most ordered domain with complementary disappearance of one of the disordered domains. Based on these results we assume that domains with random lipid distribution coexist with domains with regular lipid distribution in OPP liposomes with CH concentration below 0.45 mol%.