For the 5-DSA marker, the order parameter S was determined according to the formula:

$$S = \frac{(T_{II} - T_{\perp})a}{(T_{ZZ} - T_{XX})a'}$$

where:

a – the relationship between the isotropic splitting constants for nitroxide molecules in the crystal:

$$a = \frac{1}{3}(T_{XX} + T_{YY} + T_{ZZ})$$

a' – the relationship between the isotropic splitting constants for nitroxide molecules in the membrane:

$$a' = \frac{1}{3}(T_{II} + 2T_{\perp})$$

The quantities T_{XX}, T_{YY}, T_{ZZ} are components of the tensor T measured for the crystal; they are constants with values T_{XX} = 6,16; T_{YY} = 6,1; T_{ZZ} = 32,46

$2T_{II}$ – is the distance between the outer maxima of the hyperfine splitting,

$2T_{\perp}$ – is the distance between the inner maxima of the hyperfine splitting.

For the 16-DSA marker, the mobility of the marker was determined by calculating the rotational correlation times. The rotational correlation time is the average time required for a marker molecule to rotate through a specific angle in Brownian motion, usually 2π radians. The rotational correlation times were determined according to the formula:

$$\tau_B = K^* \Delta W_0 \left[\left(\frac{h_0}{h_{+1}} \right)^{\frac{1}{2}} - \left(\frac{h_0}{h_{-1}} \right)^{\frac{1}{2}} \right]$$

$$\tau_C = K^* \Delta W_0 \left[\left(\frac{h_0}{h_{+1}} \right)^{\frac{1}{2}} - \left(\frac{h_0}{h_{-1}} \right)^{\frac{1}{2}} - 2 \right]$$

gdzie: $K = 6{,}5 \times 10^{-10}$ s/mT, a coefficient dependent on the type of marker,

h_0 – height of the central line,

h_{-1} – height of the high-field line,

h_{+1} – height of the low-field line,

ΔW_0 – width of the central line,

τ_B – characterizes the speed of the molecule's movement in a direction perpendicular to the long axis of the lipid molecule,

τ_C – characterizes the speed of the molecule's movement along the axis parallel to the long axis of the lipid molecule.

The rotational correlation times provide information about the microviscosity of membranes in hydrophobic regions.