

**SPECTROPHOTOMETRIC DETERMINATION OF THE
PHOSPHATIDYLCHOLINE LIPOSOMES/WATER PARTITION
COEFFICIENTS AND THE PROTONATION EQUILIBRIUM
CONSTANTS OF PHENOTHIAZINE DERIVATIVES**

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Phenothiazines are known to be among the compounds that reverse or modulate the multidrug resistance (MDR) of cancer cells. There is much evidence that the interactions of these reversal agents with the lipid phase of the plasma membrane are involved in the mechanism of MDR reversal. In this study, two parameters of a set of commercial phenothiazines were estimated: the phosphatidylcholine vesicle (LUV-PC)/water partition coefficient (K_p), and the protonation equilibrium constant (pK_a). These two parameters were determined for five phenothiazine derivatives (PDs): trifluoperazine (TFP), thioridazine (TDZ), levomepromazine (LPZ), dextromepromazine (DPZ) and diethazine (DEZ), using UV-VIS absorption spectroscopy. The relatively high background signal appearing in PDs/LUV-PC absorption spectra caused by light scattering in the liposome suspension was entirely eliminated in the second derivative of the absorption spectra. The second derivative intensity change (ΔD) was measured at λ_{max} for each PD absorption spectrum, and the K_p values were calculated from the relationship between ΔD and the lipid concentration. The pK_a of PDs was determined by photometric titration and the second derivative of absorption spectra measurements at appropriate pH values. Phenothiazines, like aliphatic amines, visibly aggregated at basic pH. Since second derivative amplitudes (D) of absorption spectra linearly depend on pH, one can easily calculate the pK_a values.

The K_p values for all the studied phenothiazines (PD concentration - 15 μ M) were in the range 10^4 - 10^5 and they increased in the order DEZ<DPZ<LPZ<TDZ<TFP.

pK_a values for investigated phenothiazines (PD concentration - 35 μ M) obtained with photometric titration and the second derivative of absorption spectra method differ by less than 2% (only for TFP by 5%). The phenothiazines are weak acids; the mean pK_a values obtained in the experiments were: 7.51, 8.96, 9.67, 9.67, 9.73 for TFP, TDZ, LPZ, DPZ and DEZ, respectively.

With the exception of TFP (56%) all the investigated phenothiazines at pH = 7.4 are more than 97% in the protonated state (calculated from Henderson-Hasselbalch equation).