

ANALYSIS OF THE THERMOTROPIC PROPERTIES OF LIPOSOMES BY DIFFERENTIAL SCANNING CALORIMETRY (DSC) – COMPUTER SIMULATION

Theoretical introduction

Liposomes are spherical vesicles composed of one or more concentrically arranged phospholipid bilayers. The liposome membrane is structurally analogous to a biological membrane; therefore, liposomes constitute a good model of the cell membrane. Due to the **amphiphilic nature** of phospholipids, liposomes can simultaneously encapsulate (en-trap) hydrophilic substances within the aqueous core and hydrophobic compounds within the lipid bilayer. This property has made them one of the most important groups of nanocarriers used for the **controlled delivery of therapeutic substances** for many years. Modern medicine, particularly oncology, increasingly uses liposomes as systems for transporting drugs to their site of action. Encapsulation of an active substance in a liposome can reduce its systemic toxicity, prolong its circulation time in the bloodstream, and improve its bioavailability. Appropriately designed liposomes can also increase drug accumulation in tumor tissue due to the Enhanced Permeability and Retention (EPR) effect, which results from the characteristic structure of tumor blood vessels. Liposomes with controlled drug release are also becoming increasingly important. Their membranes can change their properties in response to specific factors such as temperature, pH, or the presence of selected enzymes.

The physicochemical properties of liposomes depend primarily on the lipid composition of their membrane. One of the most important methods used to study the thermotropic properties of lipid bilayers is **Differential Scanning Calorimetry (DSC)**. This method enables the measurement of the amount of heat absorbed or released by a sample during controlled heating or cooling. DSC can be used to analyze the transition of the lipid bilayer of liposomes from an ordered gel phase to a more fluid liquid-crystalline phase. This transition is observed on the thermogram as a characteristic endothermic peak.

Based on the thermogram, several parameters describing the properties of the lipid membrane can be determined: **the phase transition temperature (T_m)**, **the enthalpy change of the phase transition (ΔH)**, and **the full width at half maximum of the peak ($\Delta T_{1/2}$)**.

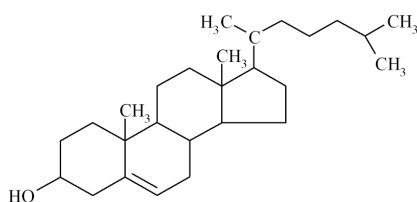
In this practical, a computer simulation of DSC measurements is used. Based on the generated thermograms, students determine the values of T_m , ΔH , and $\Delta T_{1/2}$ and then analyze the effect of cholesterol and DOPC content on the thermotropic properties and stability of the model lipid membrane. The final stage involves assessing which of the investigated liposome compositions may constitute the most beneficial carrier for drugs used in anticancer therapy.

Equipment

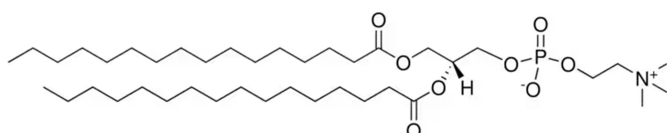
Computer software for the simulation of microcalorimetric measurements.

Aim of the Practical

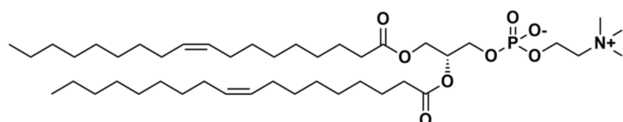
The aim of the exercise is to investigate the influence of the lipid composition of the liposome membrane on its thermotropic properties and to assess the suitability of different liposome formulations as carriers for anticancer drugs.



cholesterol



DPPC (1,2-dipalmitoyl-sn-glycero-3-phosphocholine)

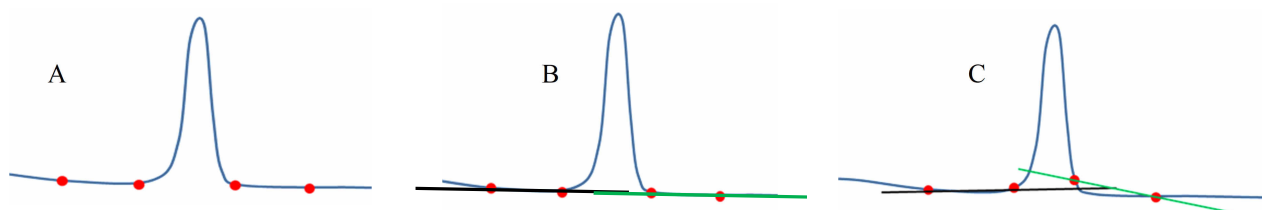


DOPC (1,3-bis(sn-3'-phosphatidyl)-sn-glycerol)

Procedure

I. Investigation of the Effect of Cholesterol on the Thermotropic Properties of the Lipid Membrane

1. Launch the program by double-clicking the KALORYMETR folder.
2. Click the “start” button and then select the L1 file. It corresponds to a lipid bilayer composed exclusively of DPPC.
3. Set the initial temperature to 285, the final temperature to 350, and the scanning rate to 99999. Then select “confirm.”
4. By single-clicking in the appropriate locations, mark the starting and ending points of the baseline (straight line) before and after the main phase transition peak (Figure A).
5. After clicking “analysis,” straight lines will be drawn through the selected points. Their purpose is to indicate the appropriate microcalorimetric peak to the program and isolate it from the rest of the thermogram (Figure B). If the points are selected incorrectly (Figure C), the lines will intersect without isolating the appropriate part of the graph. In this case, correct the position of the points by clicking again in the appropriate locations on the thermogram and selecting “analysis.”



6. Read the values of the phase transition temperature (T_m), phase transition enthalpy (ΔH), and full width at half maximum ($\Delta T_{1/2}$) and enter them in Table 1.
7. Click the “BACK” button to return to the initial settings.
8. Read the values of ΔH , T_m , and $\Delta T_{1/2}$ for liposomes labeled L2 to L6, following the procedure described in steps 2–7. The composition of the liposomes is presented in Table 1.
9. Based on the results obtained, prepare graphs showing the dependence of T_m , ΔH , and $\Delta T_{1/2}$ on the cholesterol content for the following liposomes: DPPC, DPPC:cholesterol (80:20), and DPPC:cholesterol (60:40).
10. Answer the questions provided.

II. Liposome Stability

1. Generate thermograms and corresponding data for liposomes with the same composition as those used in Part I, but after 2 weeks of use (files labeled L7–L12), following the instructions given in steps 2–7 of Part I of the experiment.
2. Enter the results in Table 2 and compare them with the data from Table 1.
3. Answer the questions provided.

Required Theoretical Knowledge

1. Structure and properties of biological membranes – organization of the lipid bilayer, the role of phospholipids and cholesterol.
2. Liposomes as lipid-based drug carriers.
3. Lipid phase transitions and their biological significance – factors affecting the thermotropic properties of lipid membranes.
4. Differential Scanning Calorimetry (DSC) – the operating principle of a differential scanning microcalorimeter, applications of DSC in lipid and liposome studies, and interpretation of thermograms.
5. Parameters characterizing a DSC thermogram – phase transition temperature (T_m), enthalpy change of the phase transition (ΔH), full width at half maximum ($\Delta T_{1/2}$), and their significance in assessing the properties and stability of liposomes.

Recommended Literature

Wroclaw Medical University Department of Biophysics and Neuroscience	Practical No 9 Analysis of the thermotropic properties of liposomes by differential scanning calorimetry (DSC) – computer simulation	
..... Student names		Faculty: Group number: Date:
Grade:	Tutor's signature:	

Table 1

Liposome	Composition	T _m (K)	ΔT _{1/2} (K)	ΔH (kJ/mol)
L1	DPPC			
L2	DPPC: cholesterol(80:20)			
L3	DPPC: cholesterol (60:40)			
L4	DPPC: DOPC (70:30)			
L5	DPPC: DOPC: cholesterol (50:30:20)			
L6	DPPC: DOPC: cholesterol (60:20:20)			

How does cholesterol affect the phase transition temperature, transition enthalpy, and peak full width at half maximum? Explain the observed changes based on the interactions between cholesterol and membrane lipids.

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What effect does the presence of DOPC have on the properties of the lipid membrane? Explain why the addition of DOPC causes changes in the thermogram parameters.

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Which of the analyzed liposomes would be the most suitable as a carrier for an anticancer drug, and why?

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Table 2

Liposome	Composition	T _m (K)	ΔT _{1/2} [K]	ΔH (kJ/mol)
L7	DPPC			
L8	DPPC: cholesterol (80:20)			
L9	DPPC: cholesterol (60:40)			
L10	DPPC: DOPC (70:30)			
L11	DPPC: DOPC: cholesterol (50:30:20)			
L12	DPPC: DOPC: cholesterol (60:20:20)			

Which formulations demonstrated the greatest stability during storage?

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Which changes in DSC parameters indicate a decrease in liposome stability?

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Is the most stable liposome also the best carrier for an anticancer drug? Justify your answer.

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