

Activities carried out in Stage 1 of the project
Understanding the Process and Formulation Variables in the Design of pH-Sensitive Liposomes for Anticancer Drug Delivery
(OncoSensLip)

Project code: PN-III-P3-3.6-H2020-2020-0105

Contract no.: 61PCBROMD/2025

Duration: 01.10.2025 – 30.09.2027

Budget: 750,000.00 RON

Stage no. 1/2025: WP1 – Formulation and optimization; WP4 – Management, dissemination, communication, promotion and exploitation (01.10.2025 – 31.12.2025)

Stage budget: 92,857.00 RON

Abstract

During this stage, which corresponds to the first three months of the project (October–December 2025), a bibliographic study was conducted on the development of pH-sensitive liposomes, and several pH-insensitive formulations were prepared using the microfluidic method, with the aim of observing the influence of critical formulation parameters, namely TFR (Total Flow Rate – the total flow rate, representing the sum of the flow rates of the organic and aqueous phases that are combined in the microchip) and FRR (Flow Rate Ratio – the ratio between the flow rates of the aqueous and organic phases), on liposome self-assembly and vesicle size. Nine liposomal formulations based on DOPC (1,2-dioleoyl-sn-glycero-3-phosphocholine) and cholesterol (CHOL) were prepared. The size of the obtained liposomes was characterized by DLS (Dynamic Light Scattering).

Theoretical aspects of liposomes

Liposomes were discovered by Bangham and his colleagues in the 1960s and are self-assembled nanometric structures ([Figure 1](#)), with a hydrophobic compartment (the lipid bilayer) and a hydrophilic one (the aqueous core), allowing the selective incorporation of hydrophilic, hydrophobic, or amphiphilic drugs.

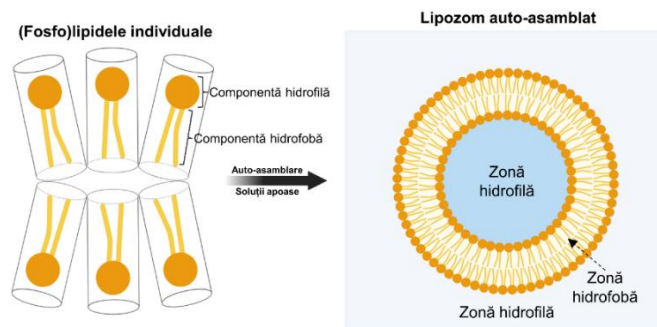


Figure 1. General scheme for obtaining a liposome through the self-assembly of (phospho)lipid molecules.

They protect drugs from the action of the biological environment and allow the administration of lower doses, increasing therapeutic efficiency and reducing systemic toxicity. Approximately 30 years after their discovery, liposomes were used in cancer treatment, the first formulation being *Doxil* (pegylated liposomal doxorubicin), approved by the Food and Drug Administration (FDA), USA, in 1995. Other formulations include *Myocet* (FDA 1996, metastatic breast cancer), *Lipusu* (China 2003, gastric and ovarian cancer), *Mepact* (European Medicines Agency – EMA 2009, osteosarcoma), *Onivyde* (FDA 2015, pancreatic cancer), and *Vyxeos* (FDA 2017, acute myeloid leukemia). However, the clinical use of liposomes still involves challenges such as immune system response, protein corona formation, lack of response to endogenous stimuli, inability to differentiate between normal and tumor cells, and difficulties in batch-to-batch reproducibility. Adjustment of physicochemical properties can reduce these limitations as follows: (i) PEGylation confers stealth properties to liposomes to avoid immune clearance, (ii) functionalization with antibodies or ligands directs the drug to target cells, (iii) standardization of protocols reduces inter-batch variations, and (iv) chemical modification of the lipid bilayer enables drug release in response to stimuli. For example, pH-sensitive liposomes remain stable at physiological pH but destabilize in acidic environments, promoting drug release. pH is the most studied of these stimuli, playing a crucial role in cancer treatment. Ionizable lipids, such as DOPE and CHEMS (Figure 2), are often used to form pH-sensitive formulations, with release at endosomal pH (~5.5).

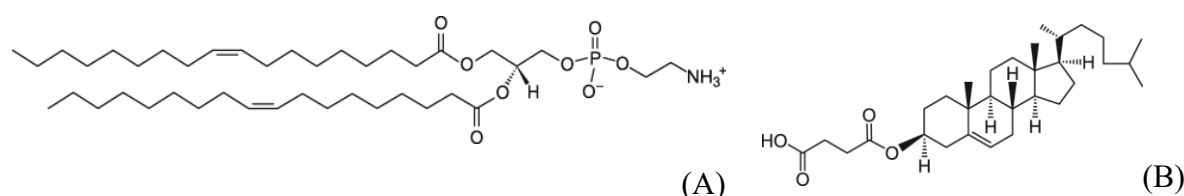


Figure 2. Chemical structure of the phospholipid DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine) (A) and of the lipid CHEMS (cholesteryl hemisuccinate) (B).

Liposomes can be obtained by various preparation methods, such as thin film hydration (TFH)

and the microfluidic method, as shown in Figure 3.

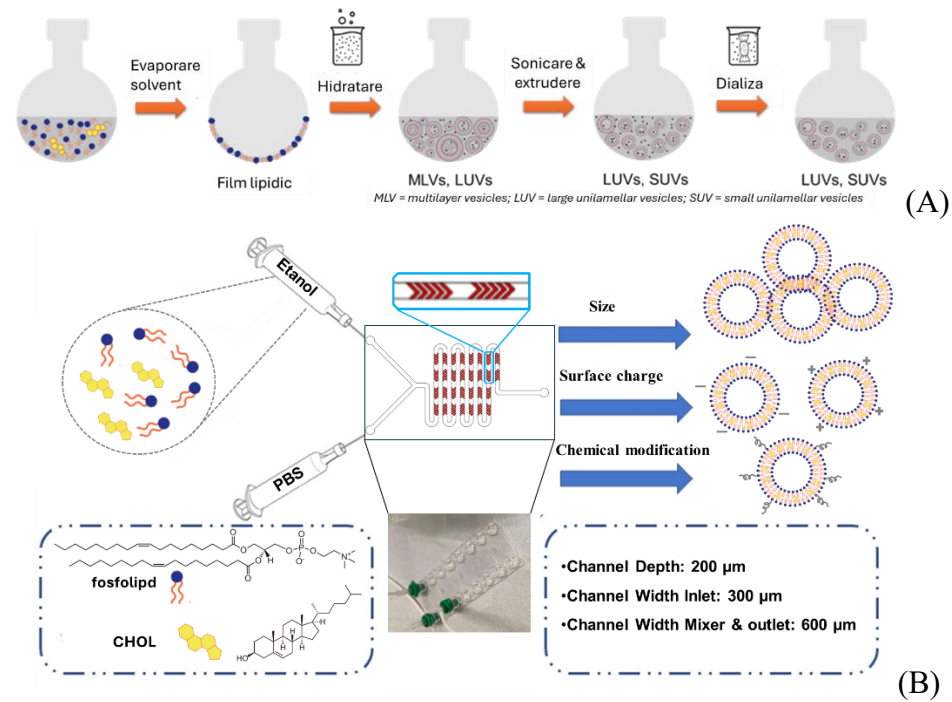


Figure 3. Method for obtaining liposomes by thin film hydration (A) and by the microfluidic method (B).

Thin film hydration (TFH) is a simple method, but it generates a broad size distribution of liposomes and requires additional steps, such as extrusion. Microfluidics allows very good control over liposome size and provides batch-to-batch reproducibility, but solvent removal is necessary for pharmaceutical or biological applications.

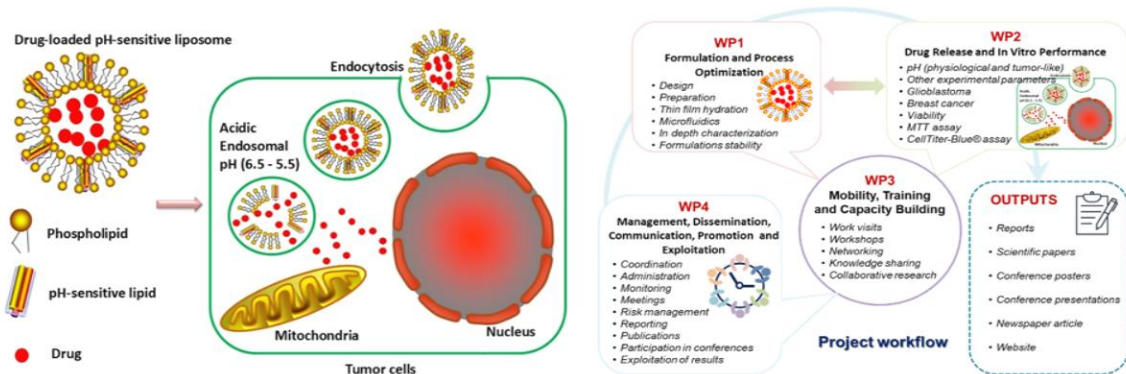
Management, communication, and promotion activities



On September 30, 2025, the official project launch (kick-off meeting) took place in Iași. The event was held in the Nanotechnology Laboratory within the TRASCEND Research Center, Regional Institute of Oncology. Representatives of both project partners attended the meeting. Key aspects for the project implementation were discussed, and the Risk Management Plan and the Communication, Dissemination, Promotion, and Exploitation Plan were developed. Additionally, the schedules of the team from the Republic of Moldova in the Nanotechnology Laboratory during stages 2 and 3 of the project, as well as internal procedures for progress monitoring, were established. The governance plan was also defined, including management structures, communication channels, and decision-making processes. To promote the project, a **flyer** was created for both the **scientific community and the general public** (*on the next page*).

OncoSensLip

Understanding the Process and Formulation Variables in the Design of pH-Sensitive Liposomes for Anticancer Drug Delivery



The overall aim of the OncoSensLip project is to develop a pH-sensitive liposomal formulations for anticancer drug delivery by adjusting formulation and process variables, while building technical capacity among project partners.

Specific objectives (SO):

SO1. Formulation and Process Optimization

SO2. Drug Release and In Vitro Performance

SO3. Training and Capacity Building



This project has received funding from the UEFISCDI under grant agreement PN IV – PCB-RO-MD-2024, 61PCBROMD/01.10.2025



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