Nano Innovation 2024 Rome, 9-13 September
Conference & Exhibition

Sapienza Università di Roma

Enhancing Liposome Formation at Low Lipid Concentrations: Advancing Potential with Microdroplet Deposition over Thin Layer Evaporation

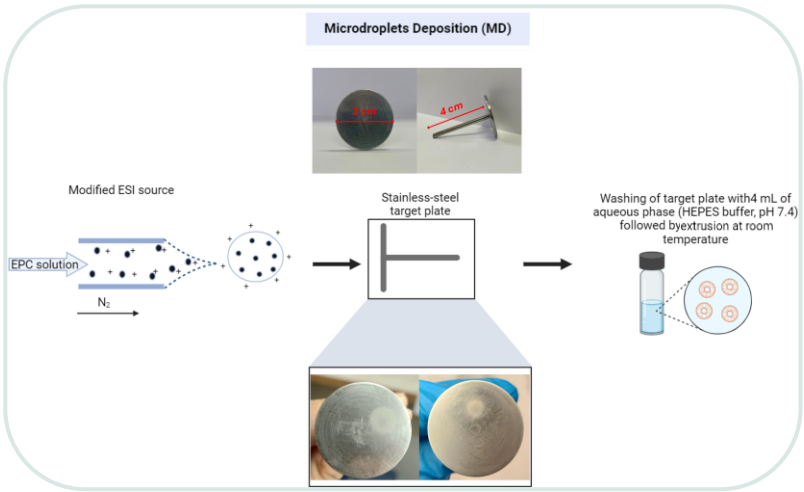
Chiara Salvitti (1), Barbara Bigi (1), Marta Managò (1), Marco Agostini (1), Marco Rossi (2), Francesco Mura (2), Maria Antonietta Casadei (1), Patrizia Paolicelli (1), Stefania Petralito (1), Anna Troiani (1), Federico Pepi (1)

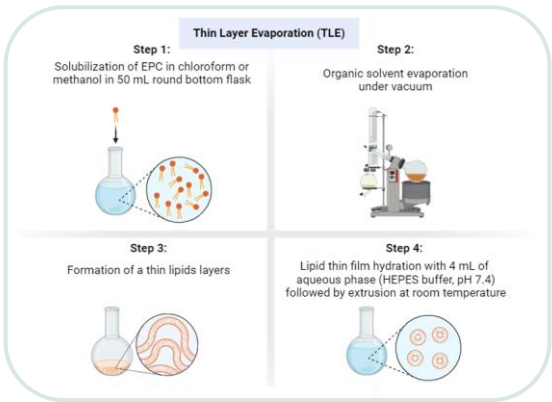
(1) Department of Chemistry and Technology of Drugs, Sapienza University of Rome, Italy
(2) Department of Basic and Applied Engineering, Sapienza University of Rome, Italy
barbara.bigi@uniroma1.it

Background & Aim

Phospholipids self-assemble into **macromolecular aggregates** in water, ranging from simple spherical and worm-like micelles to vesicles and planar lipid bilayers, to inverted micelles. The type of aggregates formed depends primarily on the **structure of the amphiphiles** according the so-called **packing parameter** (***P***) calculated from the area of the head group (*A*), the length of the alkyl chain (l_c) and its volume (*V*), $P = \frac{V}{A \cdot l_c}$. It is possible to estimate that for the **Egg-derived phosphatidylcholine** (**EPC**) the expected type of structure via *P* could be vesicles, but the specie of the aggregates is not exclusively determined by the molecular structure. The **concentration** of the phospholipid in the aqueous dispersion also play a **major role**. This research compares the type of aggregates formed by a traditional **Thin Layer Evaporation** (**TLE**) and a novel **Microdroplets Deposition** (**MD**) technique using a modified electrospray ionization source (ESI) when a low concentration of EPC was used in the HEPES buffer (pH 7.4) dispersion. By investigating the formation and morphology of EPC aggregates, this study aims to enhance liposome preparation methods, particularly for applications in nanomedicine.

Methodologies





Characterization

- Dynamic Light Scattering (**DLS**) for size distribution
- Transmission Electron Microscopy (**TEM**) for image analysis

Results

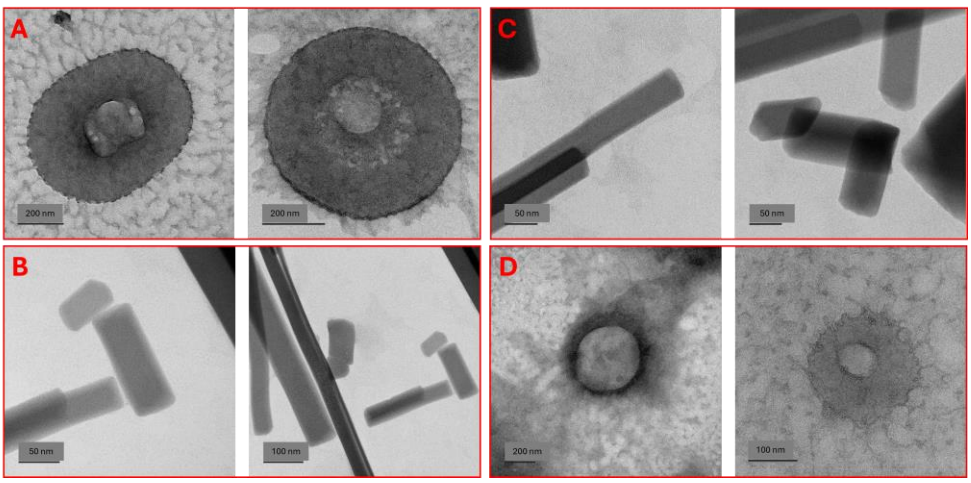


Figure 1. TEM images: A) TLE standard liposomal formulation, 50 mg/mL subsequently diluted; B) TLE formulation 1 mg/mL, $CHCl_3$ as organic solvent for lipids solubilization; C) TLE formulation 1 mg/mL, CH_3OH as organic solvent for lipids solubilization; D) MD formulation 1 mg/mL in CH_3OH.

At first, 4 or 200 mg of EPC are weighted in a pre-weighted 50 mL rotary flask, then dissolved in 2–4 mL of methanol or chloroform. The thin film is formed in both cases, but **TLE** produced **spherical bilayer liposomes**, confirmed by Transmission Electron Microscopy (TEM), **only** when **50 mg/mL** of EPC is used (**Fig.1A**). However, at **1 mg/mL**, TLE failed to form spherical vesicles generating **elongated**, **rod-like aggregates**. regardless the organic solvent used for phospholipids solubilization (**Fig.1B, 1C**).

In contrast, the MD technique successfully produced spherical bilayer vesicles at 1 mg/mL concentration of EPC (**Fig.1D**). All samples were characterized by DLS analysis (**Fig.2**, data not showed for TLE formulation 1 mg/mL in $CHCl_3$ and CH_3OH): size distribution analysis revealed dimension and polydispersity index for TLE standard formulation at 50 mg/mL and MD formulation at 1 mg/mL, respectively, of 161 nm ± 2 nm, 0.064 ± 0.038 and 153 nm ± 4 nm, 0.129 ± 0.009.

These results suggest that the MD method is more effective than TLE at forming stable liposomes at low lipid concentrations, likely due to the confined volume and localized high concentration within microdroplets, which support bilayer formation even at reduced lipid levels. This highlights a **potential threshold concentration** below which TLE is ineffective, whereas MD can still produce liposomal structures.

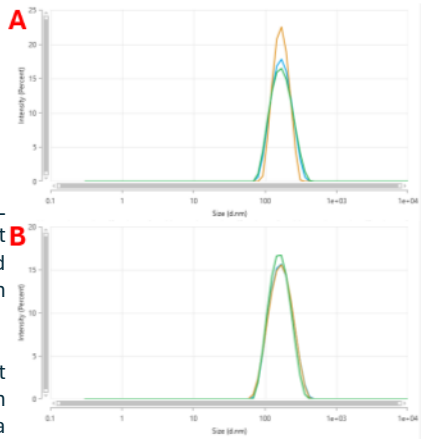


Figure 2. Size distribution by intensity by DLS analysis of A) TLE standard liposomal formulation, 50 mg/mL and B) MD formulation 1 mg/mL in CH_3OH.

Conclusions & Future Perspectives

Our findings demonstrate that the Microdroplets Deposition (**MD**) technique is **more effective** than the traditional Thin Layer Evaporation (TLE) method **at low lipid concentrations**, producing stable lipid bilayer vesicles. This highlights MD's potential for precise liposome formation, particularly in nanomedicine. Further research should investigate the mechanisms behind MD's improved performance, focusing on physicochemical parameters like local lipid concentration, solvent evaporation rates, and electrospray ionization (ESI) conditions. To advance the application of MD in drug delivery and other therapeutic areas, it is essential to assess the stability and functionality of MD-produced liposomes in biological systems. For scale-up, optimizing process parameters and ensuring reproducibility across different phospholipids will be critical, as well as integrating MD with automated systems for large-scale production and industrial applications.