GAS-PHASE ELECTROPHORESIS AND MASS SPECTROMETRY OF LIPOSOMES - TWO TECHNIQUES THAT PERFECTLY MATCH FOR VESICLE CHARACTERIZATION

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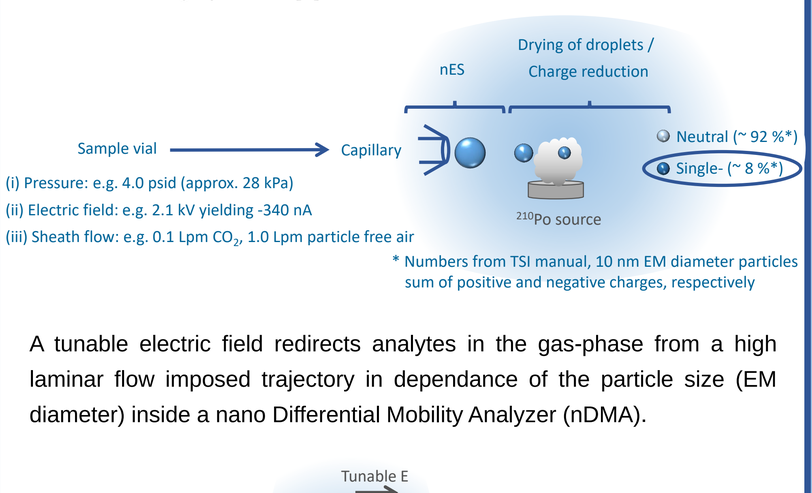
AIM OF RESEARCH

- Off-line hyphenation of gas-phase electrophoresis with MALDI MS
- Size selection of liposomes from mixtures via gas-phase electrophoresis followed by MALDI MS
- Opening avenue to size-selection of nanoparticles prior to MALDI MS
- Size-dependent composition of liposomes can be investigated in the future

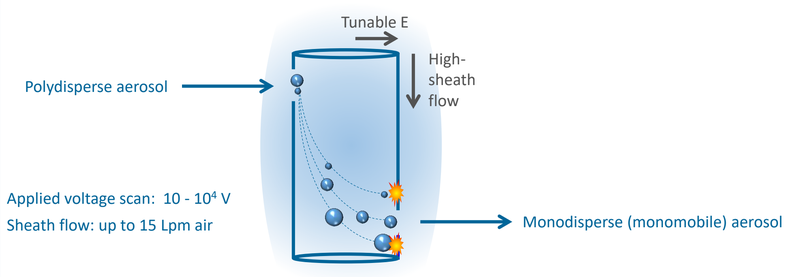
GAS-PHASE ELECTROPHORESIS

Nano Electrospray Gas-phase electrophoretic mobility molecular analyzer (nES GEMMA) also known as MacroIMS (Macro Ion Mobility Spectrometer), LiquiScan-ES (Electrospray), ES-DMA (Electrospray- Differential Mobility Analyzer), ES-SMPS (Scanning Mobility Particle Sizer))

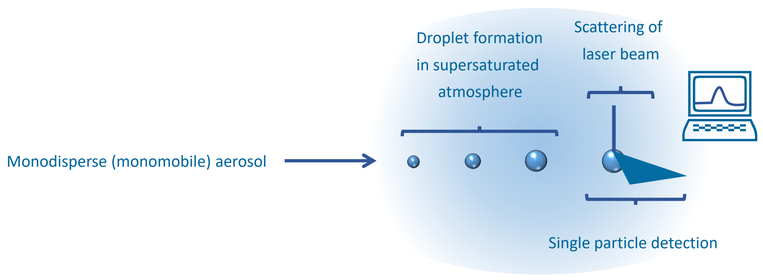
Single charged particles are obtained after electrospraying analytes from a volatile electrolyte solution (nES) and charge conditioning in a bipolar atmosphere induced by e.g. a ^{210}Po α-particle source or an alternating corona discharge process [3].



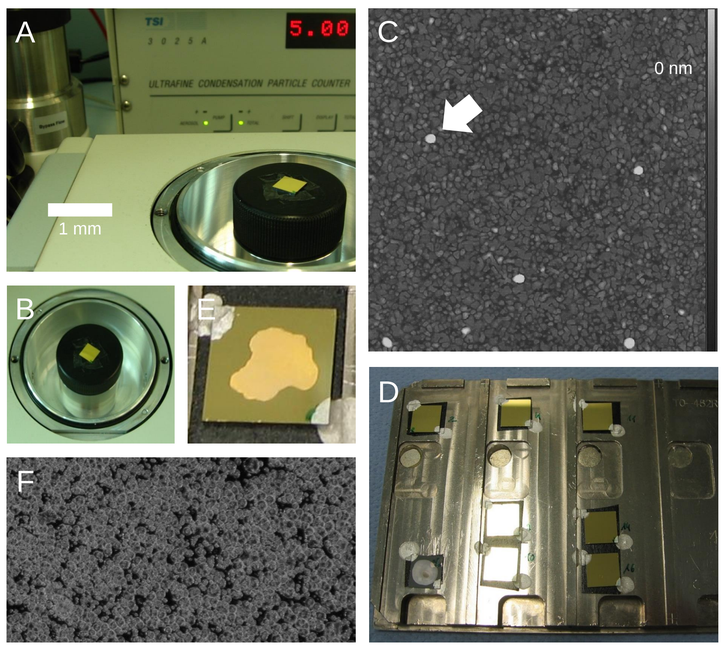
A tunable electric field redirects analytes in the gas-phase from a high laminar flow imposed trajectory in dependance of the particle size (EM diameter) inside a nano Differential Mobility Analyzer (nDMA).



Monodisperse aerosol particles act as condensation nuclei. Subsequently, single-particle, number-based detection of analytes via scattering of a focused laser beam is possible. Spectra relate particle counts and EM diameters of surface dry particles.



Instead of scanning a size range (determined by variation of the voltage applied in the nDMA), nanoparticles of a fixed EM diameter can be collected on supporting materials in a electrostatic nanoparticle aerosol sampler (ENAS) by keeping the nDMA voltage constant.



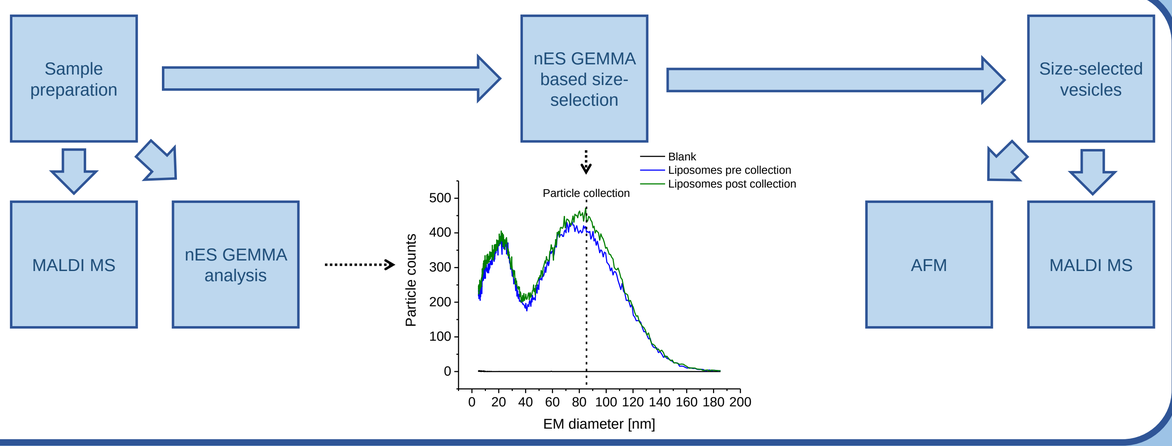
Positioning of an approx. 1 cm² gold-coated silicon wafer as supporting material in the ENAS prior to particle collection (A, B). After collection, liposomes (arrow) were visualized via atomic force microscopy (AFM, C) and wafers attached to a Shimadzu MALDI MS target support (D). Subsequently, MALDI MS matrix (THAP) was applied (E), leading to the formation of even crystals as observed via a light microscope (F).

INTRODUCTION

Liposomes, vesicles consisting of a lipid shell and an aqueous core, are applied in e.g.the pharmaceutical industry for cargo transport. Particle surface modifications enable targeted delivery or increased lifetime of vesicles in biological systems, decreasing the overall drug burden to an organism as sustained release of active ingredients at their immediate site of action occurs. However, for corresponding applications the thorough characterization of liposomes in terms of e.g. vesicle size, particle number-concentration, purity, lipid composition and particle heterogeneity is a necessary prerequisite. These questions we are targeting via gas-phase electrophoresis on a nano Electrospray Gas-phase Electrophoretic Mobility Molecular Analyzer (nES GEMMA) aka nES Differential Mobility Analyzer (nES DMA) [1]. Following separation, vesicles can be size-collected to allow for their subsequent characterization via MALDI MS as demonstrated with a mixture of liposomes and very-low-density lipoprotein (VLDL) [2].

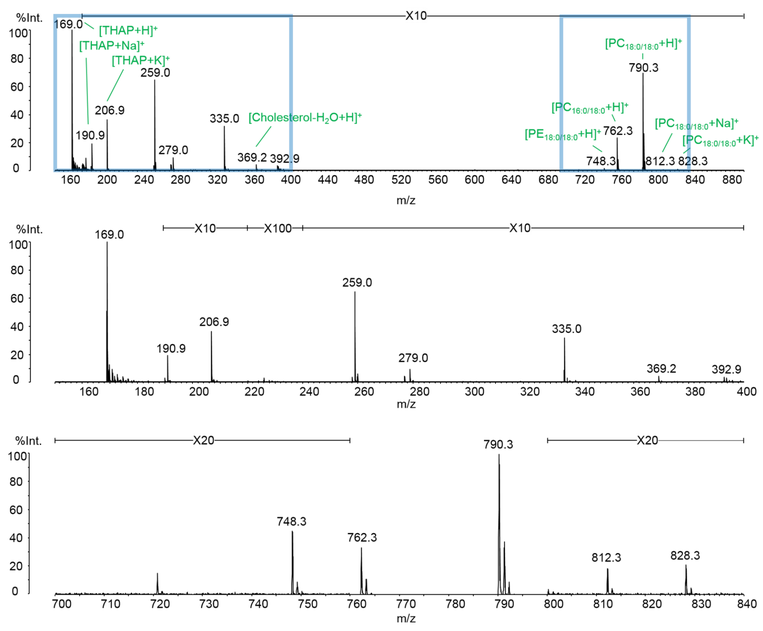
EXPERIMENTAL DESIGN

Liposomes were prepared from lipid stocks via hydration of a thin lipid film and extrusion to 100 nm diameter. Subsequently, they were analyzed via MALDI MS and gas-phase electrophoresis. After nES GEMMA based size-selection at 85 nm EM diameter, gas-phase electrophoresis demonstrated stability of the collection system. Liposomes are analyzed via AFM and MALDI MS after size-collection.

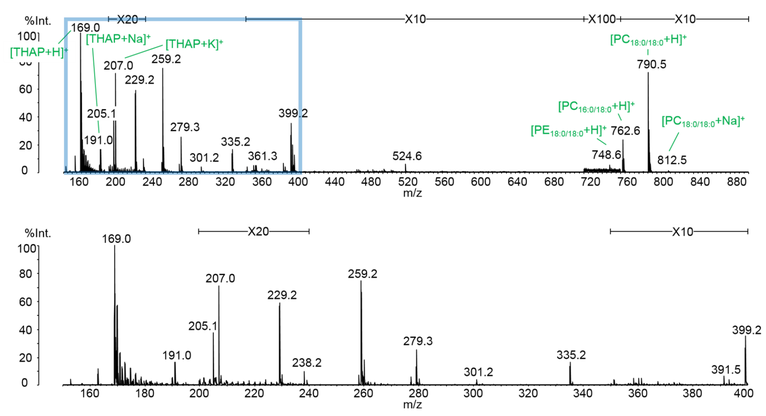


LIPOSOME ANALYSIS

Liposomes were analyzed via MALDI MS **prior to nES GEMMA** based size-collection. Signals for PC 18:0/18:0 and PC 16:0/18:0 (both components of HSPC), PE 18:0/18:0 (DSPE) and cholesterol were detected. Additional peaks correspond to protonated and sodiated matrix molecules as well as the potassium adduct of THAP. m/z regions enlarged in subjacent panels are marked in blue, respectively.



Liposomes were analyzed via MALDI MS **after nES GEMMA based size-selection** and particle collection (85 nm EM diameter, 150 min). m/z regions enlarged in subjacent panels are marked in blue, respectively.



Lipid species detected in original species were **also detectable after nES GEMMA based size-selection**.

INSTRUMENTATION

Liposomes prepared from HSPC (hydro soy phosphatidylcholine), DSPE (1,2-dioctadecanoyl-sn-glycero-3-phosphoethanolamine) and cholesterol (Avanti Polar Lipids, 4:3:3 molar ratio, thin lipid film hydration method, 40 mM ammonium acetate, pH 8.4, extrusion to 100 nm diameter).

Gas-phase electrophoresis was on a nES GEMMA with an electrostatic nanoparticle sampler from TSI Inc (Shoreview, MN, USA) [4].

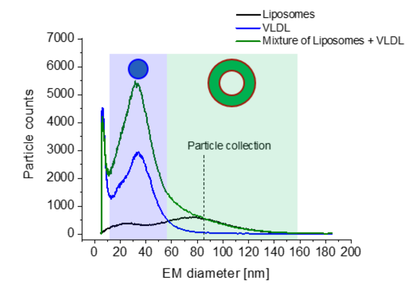
MALDI MS on an Axima TOF² (Shimadzu Kratos Analytical, Manchester, UK) in reflector positive ion mode (THAP, 2,4,6-trihydroxyacetophenon, 20 mg/mL in methanol as matrix). Liposomes were collected from a 1 mM total lipid sample.

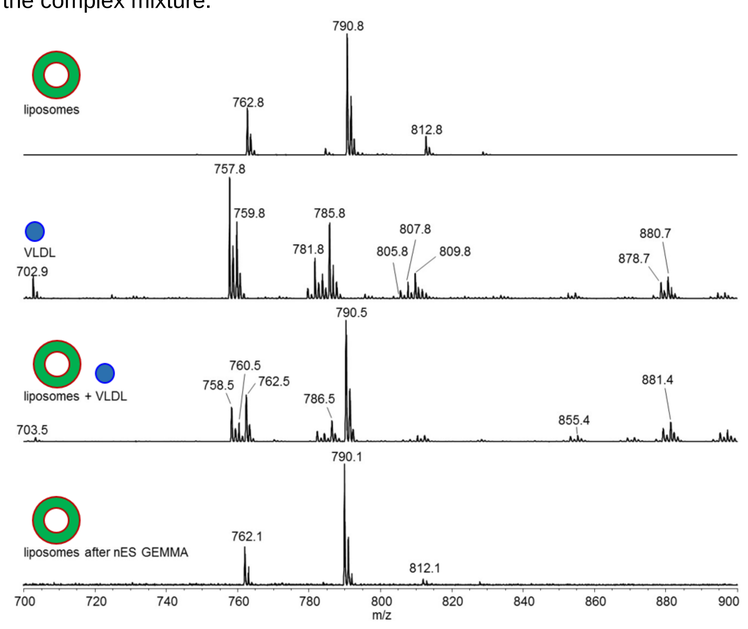
LITERATURE

1. Kaufman S. L., et al.; *Anal Chem.* **1996**; 68(11): 1895-1904.
2. Weiss V. U., et al.; *J Pharm Biomed Analysis.* **2020**; 179: 112998-113005.
3. Weiss V. U., et al.; *Anal Chem.* **2020**; 92: 8665-8669.

LIPOSOME / VLDL MIXTURES

Subsequently, liposomes were targeted in a mixture with very low density lipoproteins (VLDL). Therefore, both components were measured as single compounds with nES GEMMA and MALDI MS as well as in a corresponding mixture. For mixture samples both components contribute to obtained signals as demonstrated below. nES GEMMA based size-collection of particles (85 nm EM diameter, 150 min) enables to specifically target liposomes from the complex mixture.





After nES GEMMA based size-selection again **only signals associated with liposomes are detected via MALDI MS from the complex sample**.

SUMMARY AND DISCUSSION

- Offline hyphenation of nES GEMMA based size separation and MALDI MS is possible [2].
- Lipid patterns for liposomes and VLDL particles are comparable prior and after nanoparticle collection, i.e. monitoring of size-dependent composition is feasible.
- nES GEMMA based size separation enables MALDI MS targeting of (bio-) nanoparticles of a certain surface-dry particle size from a complex mixture.
- A similar protocol can be applied e.g. for characterization of extracellular vesicles.

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