A tale of gas-phase electrophoresis (nES GEMMA instrumentation), extracellular vesicles, and mass spectrometry

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Introduction: Extracellular vesicles (EVs), being cell-derived membrane-enclosed vesicles, are essential for cell/cell communication in an organism, and envisioned as personalized pharmaceutical cargo transporters, with the goal to significantly reduce the side effects of the carrier. In order to reach this goal, well characterized EV preparations are a necessary prerequisite. In this context we concentrated on gas-phase electrophoresis on a nano Electrospray Gas-Phase Electrophoretic Mobility Molecular Analyzer (nES GEMMA) [1]. Such an instrument enables the characterization of (bio-)nanoparticles in terms of size distribution, particle number concentration and the occurrence of smaller sized building blocks next to large sample constituents. Also, offline hyphenation of gas-phase electrophoresis with orthogonal analysis methods, for instance atomic force microscopy (AFM) or mass spectrometry (MALDI MS) is possible.

Materials and methods: EVs from human blood were isolated via ultracentrifugation and a first vesicle characterization was carried out via nanoparticle tracking analysis (NTA, ParticleMetrix). The sample buffer (PBS) was exchanged to 40 mM ammonium acetate, pH 8.4, with centrifugal filters (10 kDa, MWCO, polyethersulfone membrane). Subsequent analyses were performed on a nES-GEMMA instrumentation (TSI Inc.).

Proteinaceous contaminants detected via gas-phase electrophoresis were verified with SDS-PAGE and tryptic in-gel digest followed by MALDI TOF/RTOF (UltrafleXtreme, Bruker Daltonics). Contaminants were depleted via size exclusion chromatography (SEC) after ultracentrifugation and Annexin V-positive fractions were pooled for further characterization.

Results and discussion: We succeeded in gas-phase electrophoretic characterization of EVs, demonstrating protein co-purification [2] and loss of EV stability upon further polishing of vesicle preparations [3]. Co-purified proteins could subsequently be identified via a SDS-PAGE and in-gel tryptic digestion MALDI MS/MS approach. Furthermore, nES GEMMA spectra of these proteins - haemoglobin, β-actin(-like-protein), and α-2-macroglobulin - perfectly fitted to nES GEMMA spectra recorded for EV samples.

In addition, we could demonstrated that size collection of vesicles from an aqueous sample is possible via gas-phase electrophoresis applying liposomes as test components. Vesicles are collected in a surface-dry form on a solid sample support enabling later MS characterization of lipid components via MALDI MS [4].

Summary/Conclusion:

- Characterization of EVs is possible via gas-phase electrophoresis on a nES GEMMA instrumentation regarding particle size distribution, particle-number distribution and co-purified proteins.
- MALDI MS/MS of in-gel digested proteins corroborates nES GEMMA findings.
- Gas-phase electrophoresis can be applied as size filter for subsequent MS-based characterization of well-defined, size-collected EV material.
- In overall, nES GEMMA and MS perfectly complement each other for characterization of EVs.

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Literature:

[1] S. L. Kaufman, Anal. Chem., 1996, 68, 11.
[2] S. Steinberger, Anal. Bioanal. Chem., 2021, 413, 30.
[3] S. Steinberger, Membranes, 2022, 12, 9.
[4] V. Weiss, J. Pharm. Biomed. Anal., 2020, 179, 112998.