

Differential mobility analysis (nES GEMMA instrumentation) applied for virus-like particle research

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Gas-phase electrophoresis applying a nano Electrospray Gas-phase Electrophoretic Mobility Molecular Analyzer (nES GEMMA) instrument is based on an electrospray process. Analytes are transferred from a volatile electrolyte solution to the gas-phase followed by particle drying and charge-equilibration. This latter step results in mostly neutral yet also a considerable amount of single-charged, dry particles. Subsequently, analytes are separated in the nano Differential Mobility Analyzer (nDMA) part of the nES GEMMA by voltage scanning in a tunable electric field and an orthogonal high sheath flow of particle-free air. As particles are single-charged (neutral species are not regarded), separation of analytes is solely based on their dry particle electrophoretic mobility (EM) diameter. Furthermore, in good accordance with a recommendation of the European Commission for nanoparticle research (2022/C 229/01, June 10th, 2022), particle detection is based on the number of particles – analytes are counted after gas-phase electrophoretic separation yielding a corresponding spectrum comparing EM diameter and particle count values [1].

nES GEMMA has yielded valuable insights regarding sample purity, analyte stability and aggregational behavior as well as dry particle sizes. Also, given that a correlation based on molecular weight and EM diameter of well-defined standards can be set up, even the molecular weight of an analyte can be assessed from its EM diameter as first demonstrated for proteins [2] and recently even for intact viruses and virus-like particles (VLPs) [3]. Orthogonal analytical techniques like atomic force microscopy (AFM) and mass spectrometry corroborated results obtained from gas-phase electrophoresis. Exemplary results of several VLPs e.g. in development of gene therapy applications will be discussed.

1. Kaufman S.L., et al., Macromolecule analysis based on electrophoretic mobility in air: globular proteins. *Anal Chem*, 1996. 68(11): pp. 1895-904.
2. Bacher G., et al., Charge-reduced nano electrospray ionization combined with differential mobility analysis of peptides, proteins, glycoproteins, noncovalent protein complexes and viruses. *J Mass Spectrom*, 2001. 36(9): pp. 1038-52.
3. Weiss V.U., et al., Virus-like particle size and molecular weight/mass determination applying gas-phase electrophoresis (native nES GEMMA). *Anal Bioanal Chem*, 2019. 411(23): pp. 5951-62.

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