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Electrospray differential mobility analysis for nanoscale medicinal and pharmaceutical applications

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Abstract

Nanoscale characterization tools hold the potential to overcome long-standing medicinal and pharmaceutical challenges. For example, electrospray differential mobility analysis (ES-DMA) is an emerging tool that rapidly provides label-free multimodal size distributions for proteins and particles from ~1 nm to <500 nm with subnanometer precision. Here we critically review the contributions of this tool to medicine, pharmaceutical practice, and pharmaceutical production. Our review critically evaluates, first, the use of ES-DMA for diagnostic strategies that detect and quantify lipoproteins, bacterial infections, viruses and amyloid fibrillation and then focuses on ES-DMA's contribution to treatment strategies that employ tailored virus like particles as vaccines and decorated nanoparticle vectors for gene delivery. Our review also highlights ES-DMA's contribution to viral clearance, antibody aggregation, and potential as a process analytical technology (PAT).

Key words: Nanomedicine; Diagnosis; Gas-phase electrophoretic macromolecular mobility analyzer (GEMMA); Ion mobility spectroscopy; Cholesterol levels; Hyperlipidemia

Introduction

Nanomedicine techniques have the potential to change the way that common and emerging diseases are diagnosed, classified, and treated. These changes will be enabled by novel nanoscale particles, structures, and phenomena, each of which places new demands on quantitative characterization techniques.[1–3] Indeed, new techniques are essential to meet unique design specifications and incorporate nanoscale elements into traditional pharmaceutical processes and medicinal products. One characterization technique refined over the past decade is electrospray differential mobility analysis (ES-DMA).[1,4–7] This label-free technique provides subnanometer resolution and full multimodal size distributions.[8–10] Because many proteins, protein complexes and organisms are nanoscale entities, ES-DMA is particularly well suited to medicinal and pharmaceutical applications.[3,10–14] Here we critically review the highlights. This review systematically describes recent applications of ES-DMA to characterize nanoscale biomaterials with specific relevance to medicine, pharmaceutical practice, and pharmaceutical production.

ES-DMA is a type of ion mobility analyzer or ion mobility spectrometer that operates at atmospheric pressure and can analyze materials from ~1 nm to <500 nm. Historically, ES-DMA (also called a scanning mobility particle sizer, SMPS) has been used to analyze soot and other environmental pollutants.[15–20] Although these environmental applications suggest potential human health relevance, only since 1996 has ES-DMA been applied to the analysis of biologically derived particles when Kaufman et al., first characterized globular proteins.[21] In short succession, Mouridan and Kaufman analyzed double stranded DNA, and Bacher et al., analyzed over 20 distinct proteins (see Table 1).[4,22] Since these early developments, ES-DMA has been systematically applied to medicinal and pharmaceutical applications.[3,10–14] Here we first describe ES-DMA operation, then its application to disease diagnosis, treatment, and pharmaceutical manufacturing before concluding with an outlook toward future developments.

ES-DMA

Typical ES-DMA processes follow two continuous steps (see Figure 1). First, proteins or particles in aqueous solution are

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Table 1
Proteins and glycoproteins analyzed by ES-DMA. Adapted from Bacher et al., *Journal of Mass Spectrometry*, Copyright 2001 John Wiley & Sons, Ltd

Protein	Monomers		Dimers		Trimers	
	M_w (kDa)	d_m (nm)	M_w (kDa)	d_m (nm)	M_w (kDa)	d_m (nm)
Oxytocin	-	-	-	-	3.02	2.6
Angiotensin I	-	-	-	-	3.89	2.7
Insulin B-chain	3.50	2.9	6.99	3.4	10.49	3.9
Insulin	5.73	3.2	11.47	3.9	17.20	4.5
Ubiquitin	8.57	3.6	17.13	4.5	25.69	5.2
Ferredoxin	11.01	3.9	22.01	4.8	-	-
Cytochrome C	12.29	4.2	24.57	5.2	36.86	5.9
Ribonuclease A	13.68	4.3	27.36	5.4	-	-
Lysozyme	14.31	4.3	28.61	5.4	42.92	6.2
Ribonuclease B	14.77	4.4	29.53	5.5	-	-
Myoglobin	17.57	4.6	35.14	5.7	52.70	6.5
β-Lactoglobulin A	18.28	4.7	36.55	5.8	54.83	6.6
Trypsin	23.85	5.3	47.71	6.5	-	-
Alcohol dehydrogenase	36.82	5.8	73.63	7.3	-	-
Fetuin	43.36	6.2	86.72	7.8	-	-
Albumin hen egg	44.56	6.3	89.13	7.8	-	-
Enolase	46.70	6.4	93.39	7.9	140.1	9.2
Factor IX	53.20	6.7	-	-	-	-
Hemoglobin (bovine)	64.71	6.9	129.4	8.7	-	-
Hemoglobin (human)	65.00	6.9	130.0	8.8	-	-
Bovine serum albumin	66.40	7.1	132.8	9	199.2	10.4
Holo-Transferrin	78.38	7.6	156.8	9.5	235.1	10.9
Acylase I	90.52	8.0	-	-	-	-
β-Galactosidase	116.4	8.8	232.7	11.1	349.1	12.7
Ig G(bovine)	148.9	9.3	297.7	11.7	446.6	13.4
Ig G-peroxidase (conjugate)	188.9	10.2	-	-	-	-
Ferritin	483.2	14.3	-	-	-	-
Thyroglobulin	660.0	14.9	-	-	-	-
IgM	960.5	17.4	1921	21.7	-	-

aerosolized using electrospray ionization with charge reduction.[8,10,23] Most systems use pressure differences (typically ~4 psi = 27 kPa) to drive flow (~1-300 nL/min) through fused silica capillaries.[8,10,24,25] Commercially available systems use a single capillary, though multiple capillaries in parallel remain a viable option to enhance the flow and droplet production rates.[26–29] Some authors coat the internal surface of capillaries with bovine serum albumin, gelatin, and methyl groups to prevent adsorption, though this is often unnecessary.[30–34] Downstream, a large field (~1-4 kV) applied at the end of the capillary morphs the liquid meniscus into a Taylor cone from the tip of which droplets containing proteins or particles emerge.[35–41] Highly charged droplets 100-400 nm in diameter form depending on the capillary diameter, flow rate, pH and solution conductivity.[8,42,43] Each droplet contains one or more proteins or particles or may be empty depending on their concentration and droplet volume.[44,45] The droplets then dry as they pass through a corona discharge system or an alpha radiation source (e.g. Po-210) that neutralizes or reduces the charge on the droplets to ±1 or zero for most (≥98%) particles less than ~45 nm (the Bjeerum length in air) as described by Fuchs and Wiedensohler.[46] Larger particles support higher charge states. This process decouples the well known charge states for hydrated biomolecules from the reduced charge states observed in the gas phase.[8] The result is a dry particle with a fixed charge state.

The second step determines the size distribution by sequentially measuring both dry particle size and gas phase particle density. A differential mobility analyzer (DMA) determines the size, while a condensation particle counter (CPC) gives the gas-phase number density (in number of particles per cubic centimeter). Specifically, the DMA carries the dry proteins or particles through an annular space from entrance to exit in a high speed gas flow (typically N_2 at 10-30 L/min), while an electrical field normal to the flow attracts them toward the central electrode (0 to −10 kV). Proteins or particles with the appropriate balance of drag and electrical forces or size-to-charge ratio (the drag force depends on size) enter a collection slit near the exit.[10] Because the neutralizer resets the charge (typically ±1) and the DMA only analyzes one polarity in a given run (typically positive with a negative bias, leaving only +1), the separation is effectively based on size. This size is called the mobility size (because the ratio of the protein or particle velocity to the applied electrical field is a mobility by definition) or the aerodynamic size to differentiate it from the hydrodynamic sizes measured by dynamic light scattering (DLS) and field flow fractionation.[11] Much of the innovation over the past decade has been relating the physical particle or protein dimensions to the mobility via new drag force models.[3,6,8,10,11,42,47–49] Collected proteins or particles transit through a saturated butanol or water vapor in the CPC and heterogeneously nucleate droplets several microns in size. Heterogeneous nucleation ensures that one particle gives one droplet. These droplets are counted one by one as they