Automated particle tracking (APT) for simultaneous zeta potential-, size distribution and concentration analysis of exosomes

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Abstract

Exosomes are extracellular vesicles between 30 and 170 nm in size range and occur naturally in bodyfluids. Research about the role of exosomes as transmitters of information between cells is a fast growing field which could open up the door to new approaches in diagnosis and therapy [1]. Research into this new field has been aggravated by the lack of techniques suitable for exosome characterization in buffers of high ionic strength.

Direct 2D light scattering of liquid suspensions is an attractive instrument to assist researchers on the quantification of particle size, zeta potential and concentration. By tracking the 90° scattering light of particles while undergoing Brownian migration, the particle size is derived by applying the Stokes-Einstein equation [2]. Trajectories of several thousands of single particles of the same sample are analyzed resulting in a representative particle size distribution (PSD) of high resolution (Fig. 1). The agglomeration of exosomes in the presence of the high ionic strength of the phosphate buffer (pH 6.5) resulted in a shift of the PSD towards larger hydrodynamic particle size. The electrokinetic properties of charged particles, i.e. electrophoretic mobility and zeta potential are determined by micro-electrophoresis. In micro-electrophoresis, the migration of particles in an electrical field is recorded. Electrophoretic mobility and zeta potential are calculated from the trajectory data by an automated algorithm. Fig. 2 shows examples of zeta potential distributions of exosomes determined in different buffers.The bench-top system is equipped with an automated start-up routine. After performing of auto-focus, auto-alignment and check of cell quality, the system is ready for measurements. Multiple measurements of concentration, particle size and zeta potential can be carried out in up to 11 positions within the electro-optical cell to enhance statistical significance. As the particles are visualized, influences affecting the quality of the measurement such as agglomeration, fluctuation of concentration and background noise due to precipitation are better detectable by APT than with any other indirect characterization method. Particularly with regard to samples of low exosome concentration, the analysis of samples with particle concentrations as low as 0.5 million particles per mL can be performed.

In summary, automated measurement of size, zeta potential and concentration of particles is the outcome of one simple measurement sequence. The analysis of several thousands of single particles is completed within minutes. Results on this technique will be demonstrated on selected exosome samples.

References

[1] Ludwig, A. K., Giebel, B., Exosomes: Small Vesicles Participating in Intercellular Communication, Int J Biochem Cell Biol. (2012), 44, 11-5.

[2] Crocker, J. C., Grier, D. G., Methods of Digital Video Microscopy for Colloidal Studies, J. Colloid Interf Sci. (1996) 174, 298-310.

Figures

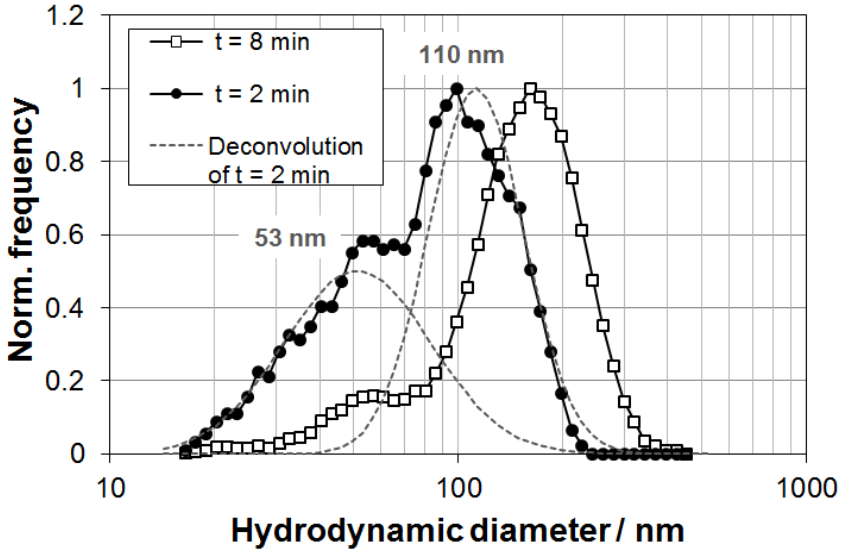

Fig 1: **Effect of agglomeration of Exosomes isolated from BMBO cell line in phosphate buffer (pH 6.5) after 2 and 8 min of preparation.**

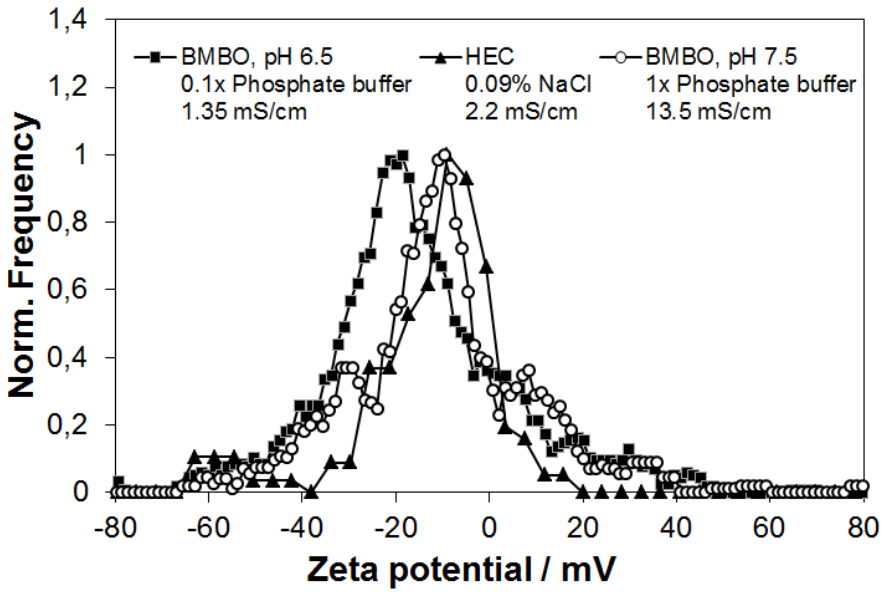

Fig 2: **Zeta potential distributions of more than 400 single particles of each sample measured by automated particle tracking. Aquisition time was less than 2 min.**