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Isotachophoresis for Electrokinetic Preconcentration of Extracellular Vesicles by Capillary Electrophoresis

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Summary

Extracellular vesicles (EVs) are membrane-enclosed nanoparticles (30-1000nm) secreted by cells that exhibit an intrinsic heterogeneity in size and molecular composition (Intravesicular or membrane) and can be found in body fluids. They have gained high interest in recent years as disease/prognostic biomarkers or biotherapeutic agents and drug nanocarriers [1]. The heterogeneity and complexity of EVs along with limitations in current techniques for their isolation and characterization have hindered their full exploitation [2]. Thus, emerging approaches that bring information on their size, charge and morphology are needed. Capillary zone electrophoresis (CZE) using laser-induced fluorescent (LIF) or UV with or without large volume sample stacking for on-line preconcentration have been recently proposed for EVs separation/characterization [3,4]. However, insufficient detection sensitivity regardless of the detection modes is still the main limitation of CE. Here, we report the development of an in-line electrokinetic preconcentration method based on capillary isotachophoresis (ITP) under extremely high ionic strength (IS) to increase the detection sensitivity of CZE-UV of intact EVs. Isolated EVs are composed of a wide range of vesicles exhibiting different electrophoretic mobilities, while being very slow anions. In addition, they are prone to lysis or aggregation. Because of this, the development of ITP to preconcentrate EVs is very challenging. We focused on selecting the appropriate BGE allowing low UV background signal and current generation, decreased EOF, high stacking effect and high compatibility with the subsequent ITP method. Then, a special attention was given to the electrolyte composition, co-ions and EVs sample matrix. Our developed ITP-UV method demonstrated a detection limit down to 8.3× 108 EV/mL, allowing an enrichment of 25 folds, compared to CZE-UV. The best enrichment factor obtained so far with the few attempts reported for electrokinetic preconcentration of EVs is only 10 folds [4]. ITP-LIF could also be used to provide more specific profiling of EVs.

References

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