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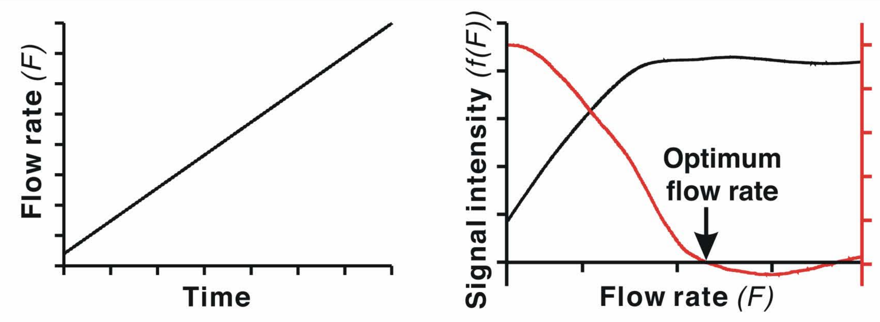
Programmable Sample Introduction and Processing Methods for Mass Spectrometric Analysis
應用於質譜分析之可程式樣品導入與樣品處理方法

5th year PhD student: Gurupur Rakesh Damodar Prabhu[a,b]
Advisors: Assoc. Prof. Pawel L. Urban[b], Prof. Henryk A. Witek[a]
[a]Department of Applied Chemistry, National Chiao Tung University
[b]Department of Chemistry, National Tsing Hua University

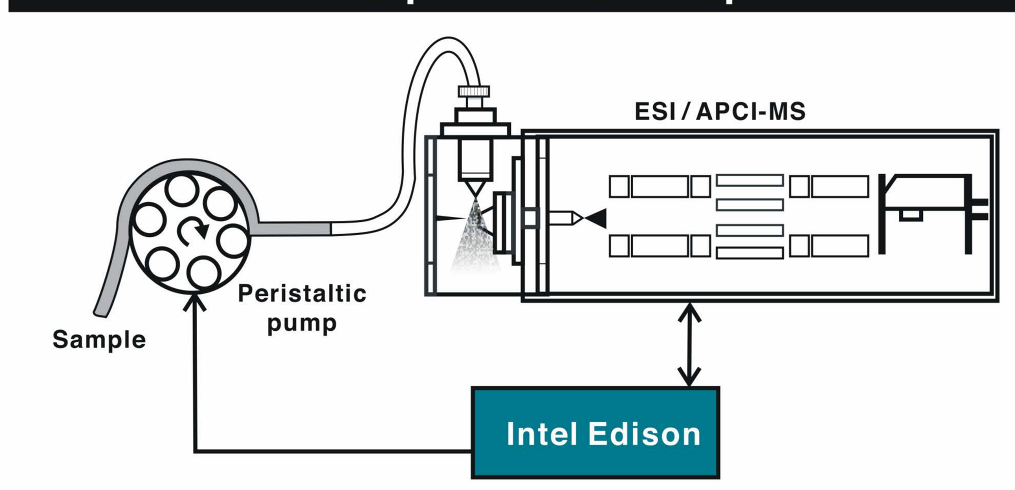
Abstract
- An automated flow rate optimization system was developed comprising a peristaltic pump and a single-board computer.
- The slope of response-*vs.*-sample flow rate determines the sensitivity regime of a detector.
- A Python script computes first derivatives on-the-fly with Savitzky-Golay algorithm.
- Tested with electrospray ionization (ESI) mass spectrometry (MS) and atmospheric pressure chemical ionization (APCI)-MS.
- Detector sensitivity regime thresholds for samples containing low-molecular-weight analytes are reported to the user within 8 minutes.
- The influence of sample flow rate on protein charge state patterns in ESI-MS in the presence of various additives was studied.
- The flow rate scan of protein samples suggests that at low flow rates the protein molecules follow charged residue model of ionization mechanism, and at high flow rates—due to structural changes in protein molecules in large ESI droplets—the charged residue and chain ejection models can possibly co-exist.

Experimental section

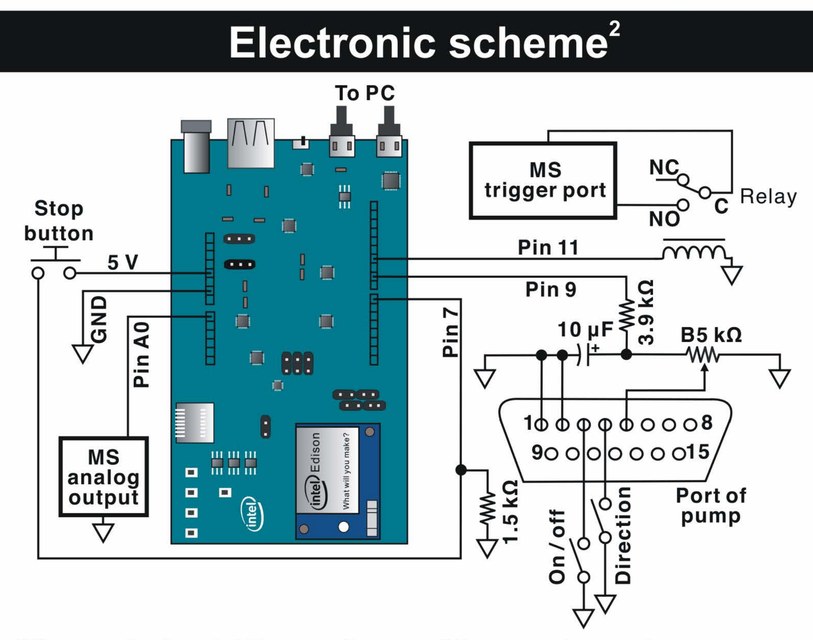
Concept[1]



Experimental setup[2]

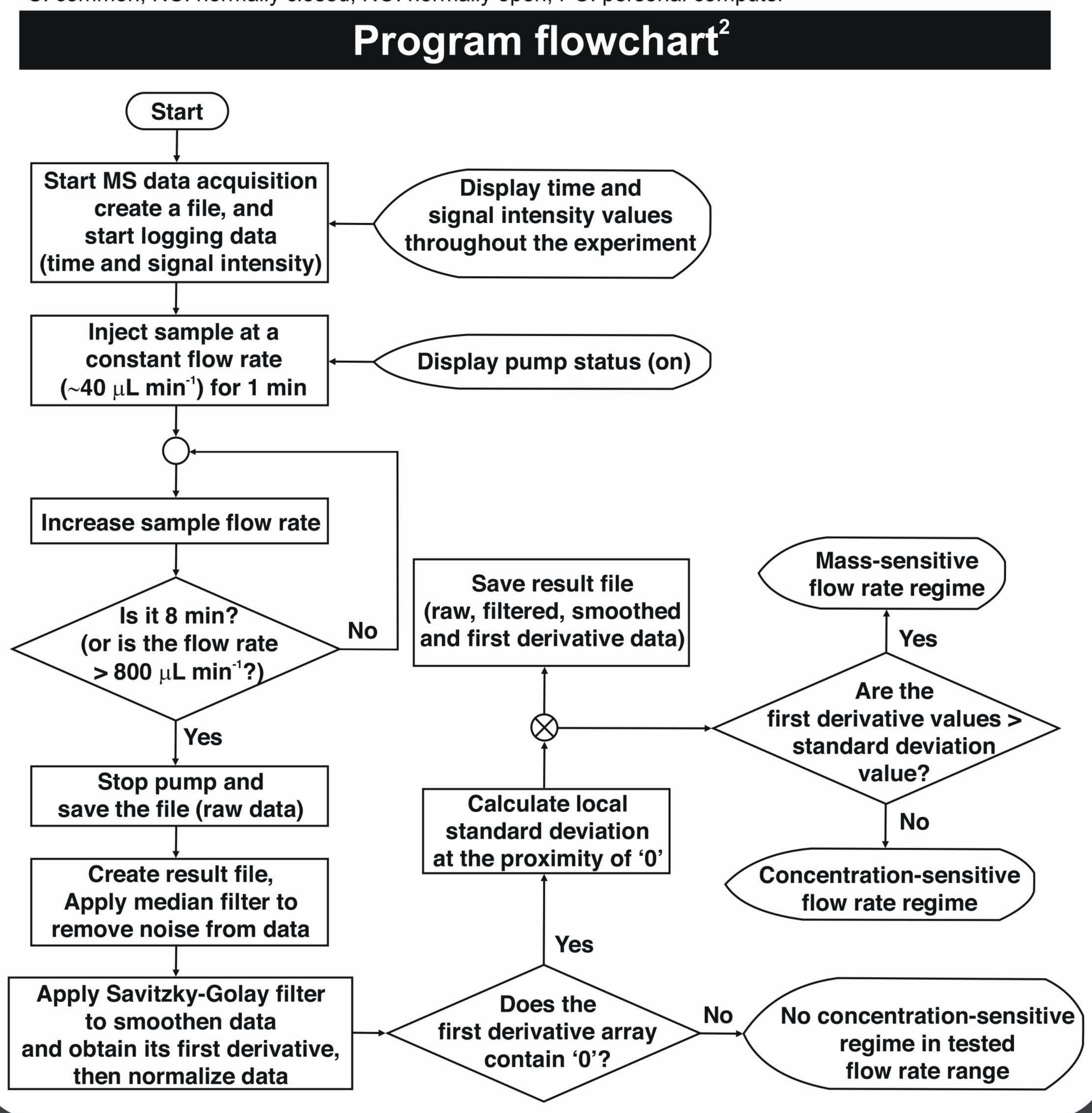


Electronic scheme[2]



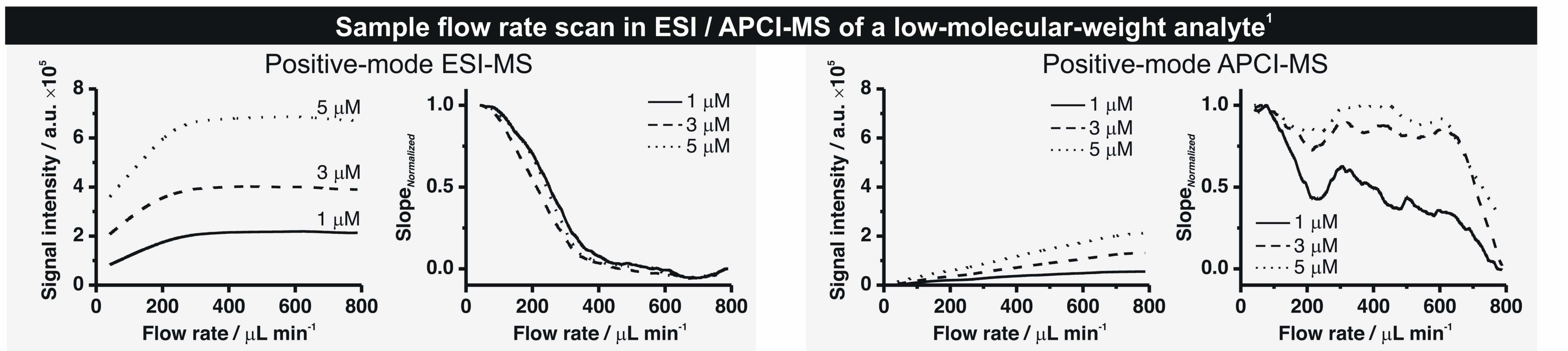
C: common; NC: normally closed; NO: normally open; PC: personal computer

Program flowchart[2]



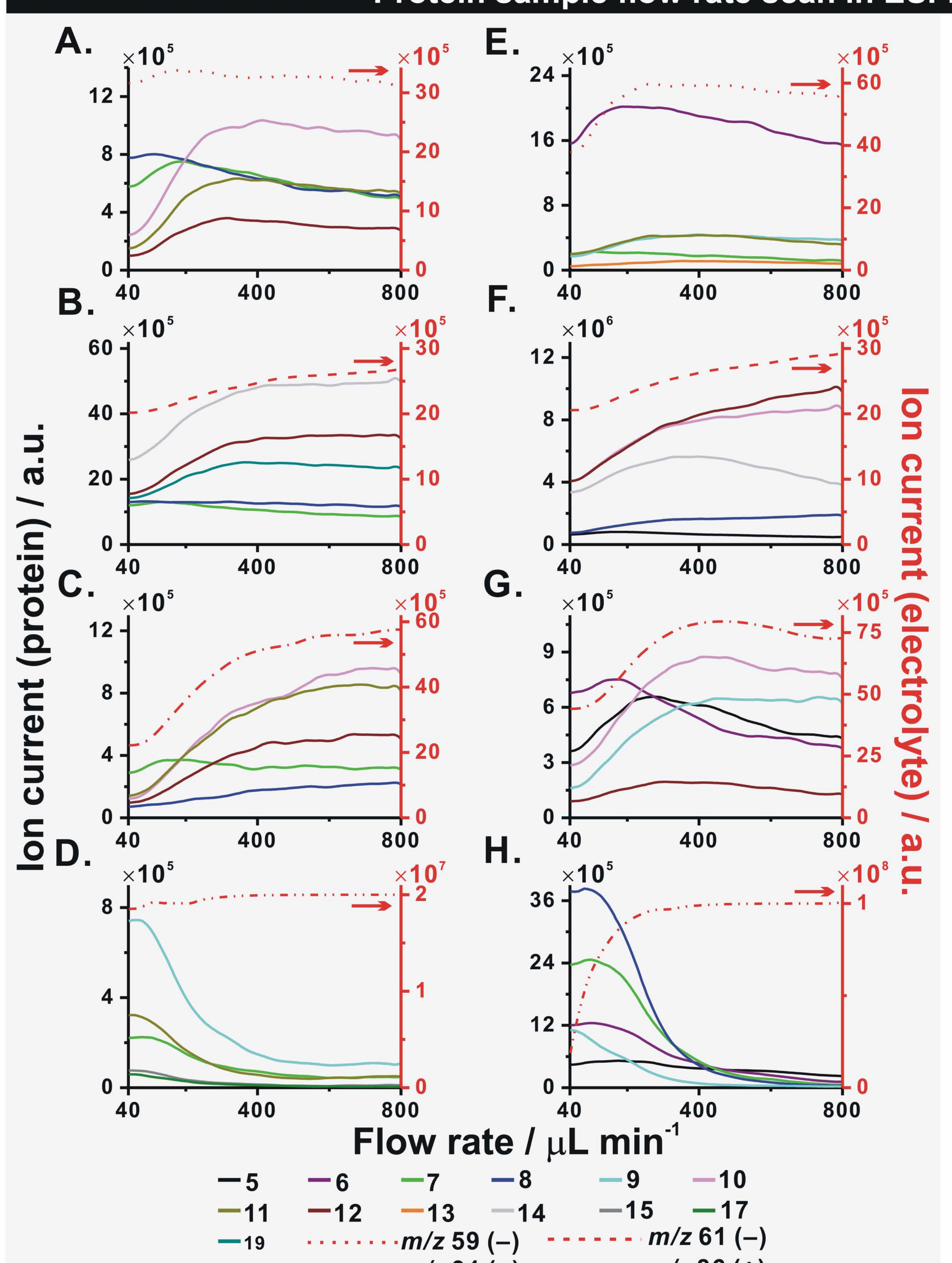
Results

Sample flow rate scan in ESI / APCI-MS of a low-molecular-weight analyte[1]

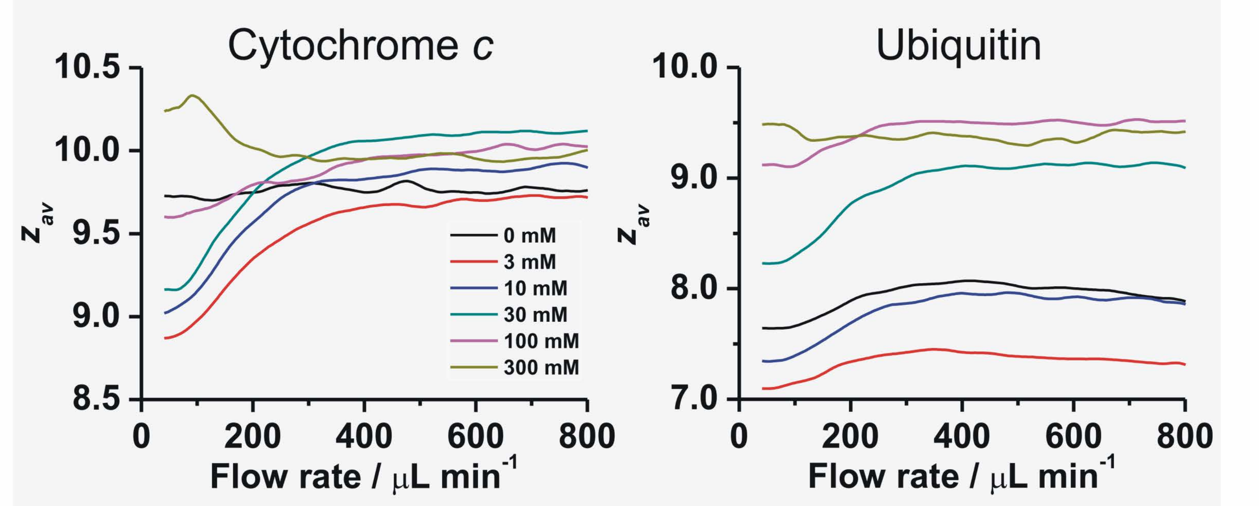


Determining mass-to-concentration sensitivity regime thresholds for a low-molecular-weight analyte based on the flow-rate scan data sets.
Sample: different concentrations of acetaminophen in 50% (v/v) ethanol in water spiked with acetic acid (0.05% (v/v). Selected reaction monitoring mode *m/z* 152→110.

Protein sample flow rate scan in ESI-MS in the presence of different additives[3]

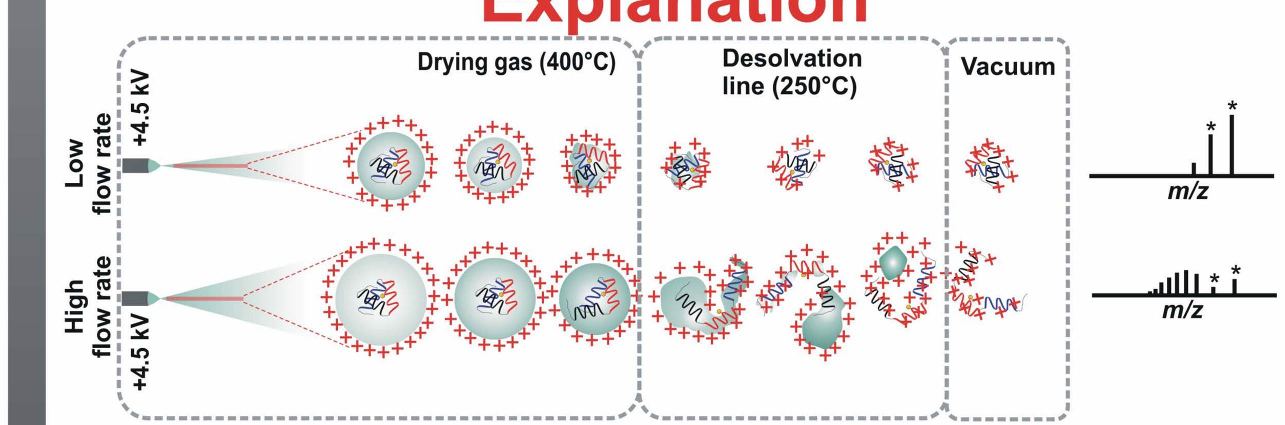


Dependencies of charge state ion currents on flow rate in the presence of four different electrolytes obtained for two proteins:(A-D) cytochrome c; (E-H) ubiquitin. Sample: 5 µM protein in 25% (v/v) methanol in water with 10 mM ammonium acetate (A,E); ammonium bicarbonate (B,F); ammonium formate (C,G); and piperidine (D,H). Solid lines represent charge states: positive mode (A-C,E-G); negative mode (D,H). Dotted lines represent *m/z* 59 (–) – $[CH_3COO]^-$; dashed lines represent *m/z* 61 (–) – $[HCO_3]^-$; dashed-dotted lines represent *m/z* 91 (–) – $[(HCOOH)_2 - H]^-$; and dashed double dotted lines represent *m/z* 86 (+) – $[(CH_2)_5NH + H]^+$.



The influence of sample flow rate on protein charge state distribution in the presence of ammonium acetate. Sample: 5 µM protein in 25% (v/v) methanol in water containing different concentrations (0 to 300 mM) of ammonium acetate. Weighted average charge state of protein, $z_{av} = \Sigma(z_q I_F)/\Sigma I_F$, where, I_F is the intensity of a given charge state z_q (q = charge state number) at sample flow rate F.

Explanation[3]



Influence of sample flow rate in protein ESI-MS. The increased concentration of additives combined with the temperature effect during the evaporation of droplets can cause structural changes to the protein molecules, that leads to the observation of altered charge state patterns.

Selected publications
1. **Prabhu G.R.D.**, Witek H.A.*, Urban P.L.* *Sensor. Actuat., B-Chem.*, **2019**, *282*, 992-998.
2. **Prabhu G.R.D.**, Yang T.-H., Hsu C.-Y., Shih C.-P., Chang C.-M., Liao P.-H., Ni H.-T., Urban P.L.* *Nat. Protoc.*, **2020**, *15*, 925-990.
3. **Prabhu G.R.D.**, Ponnusamy V.K., Witek H.A.*, Urban P.L.* *Anal. Chem.*, **2020**, *92*, 13042-13049.

Acknowledgements
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