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Bead injection for biomolecular assays: Affinity chromatography enhanced by bead injection spectroscopy{{

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Selective capture of target biomolecules by ligands immobilized on a solid support is a cornerstone of two seemingly unrelated techniques: micro-Affinity Chromatography (mAC) and micro-Bead Injection Spectroscopy (mBIS). This work shows, for the first time, how these techniques can be carried out using the same instrument and how the data obtained this way complement each other, yielding complete information on retention and elution of target biomolecules. Biomolecular association and dissociation were investigated by mAC and mBIS, using computer-controlled programmable flow and the same instrument for automated bead transport, packing of a micro-column, assay of the analyte, and bead disposal. The absorbance of the analyte was monitored within the fiber optic flow cell configured either for monitoring directly on the beads or post-column after elution. The separation, binding, and elution of immunoglobulins (human IgG, rabbit IgG, and horse IgG) on protein G-coated Sepharose beads were studied as model systems. The limit of detection of the mAC technique was determined to be 5 ng mL21 IgG, and that of the mBIS technique was 50 ng mL21 IgG.

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