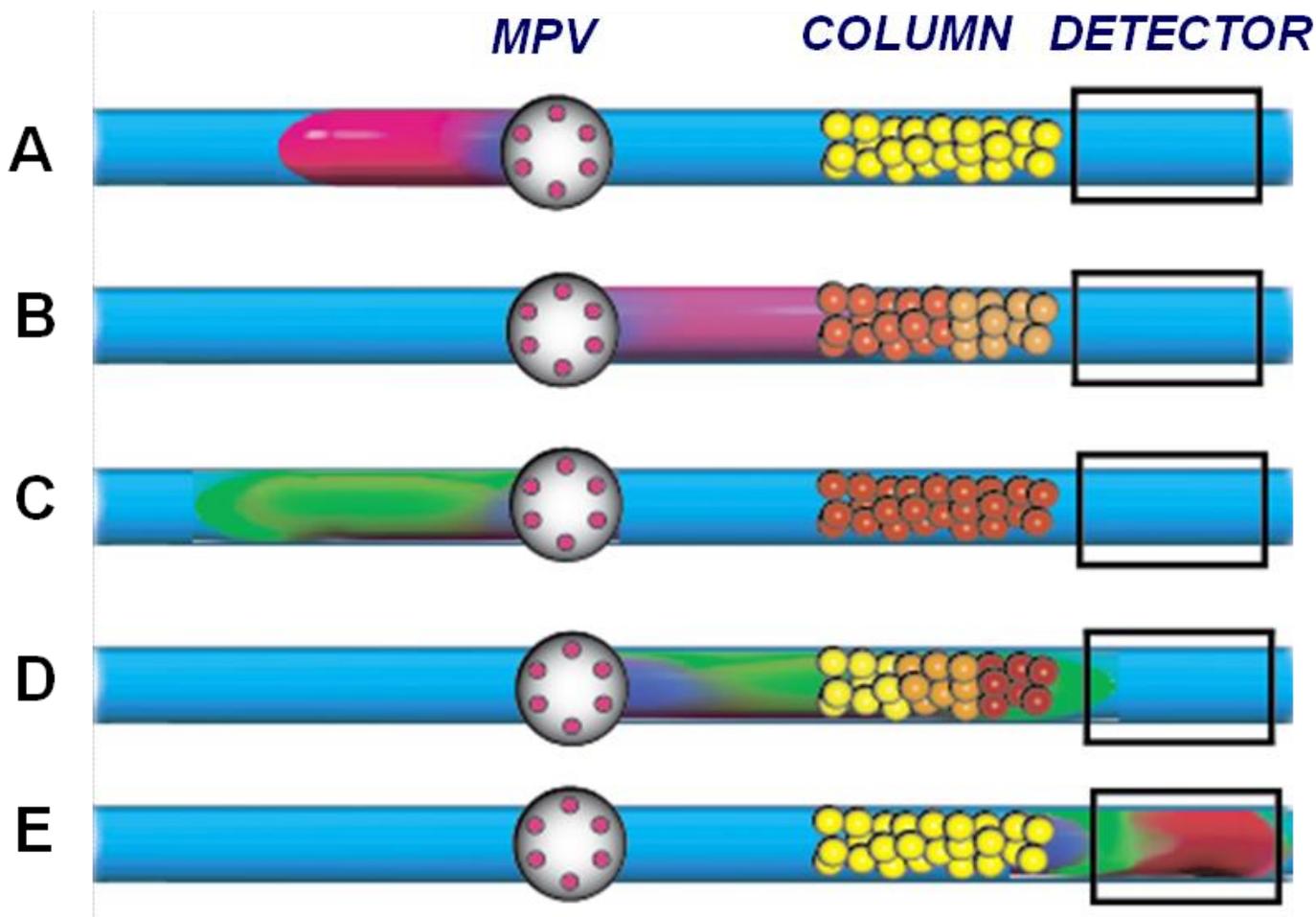


Sequential Injection Chromatography

Sequential Injection Chromatography (SIC) can be viewed as a marriage of two technologies: Liquid Chromatography (LC) and SI.

It combines the separation power of LC with versatility of sample processing inherent in SI. Sample and eluent solutions are manipulated by **programmable flow**, prior to their passage through a separation column. SIC has been performed in a reverse phase, ion-exchange or affinity chromatography format.

SIC has been carried out on monolithic columns, using a medium pressure pump, or on microcolumns using a conventional low pressure syringe pump. Since conceived only recently (Satinsky, Solich, Chocholous & Karlicek 2003), it is too soon to tell how wide a field of applications this novel technique will have. Yet, it is safe to assume that SIC will be accepted by the analytical community, since it provides numerous unexplored research opportunities.



Unlike the conventional chromatographic techniques, SIC is based on **programmable flow of the mobile phase**, which allows convenient selection of injected sample volume and a simple formation of elution gradient. The apparatus comprises a bidirectional

pump, holding coil, separation column, multiposition valve (MPV) and a detector. The assay protocol has the following steps:

- A) A precise volume of sample (red) is aspirated via MPV by flow reversal. Note that this allows change of injected sample volume without physical recombination of the injector.
- B) The MPV is switched towards the column and the flow is reversed. The injected sample zone passes through the column while analytes are retained.
- C) A precise volume of eluent is aspirated via MPV.
- D) Flow is reversed and a gradient of eluent is passed via MPV into the column.
- E) Separated analytes are measured as they flow through the detector.

Flow programming also facilitates precolumn derivatization, allows generation of elution gradients, speeds up column regeneration and accelerates the assay by fast column flushing.

Columns

Since the flow programmable pumps are presently available only for **moderate** (up to 500psi) or **low pressures** (up to 75 psi) the choice of columns for SIC are limited to monolithic columns for moderate pressures or microcolumns for low pressure separations.

Sol-Gel Monoliths are a novel type of HPLC columns manufactured by, e.g., [Phenomenex](#) and [Merck](#). In contrast to conventional HPLC columns, the sol-gel monoliths are formed as a single piece of porous silica, having a higher porosity than conventional particulated columns. Merck reports a 15% greater porosity for their Chromolith columns over conventional columns.

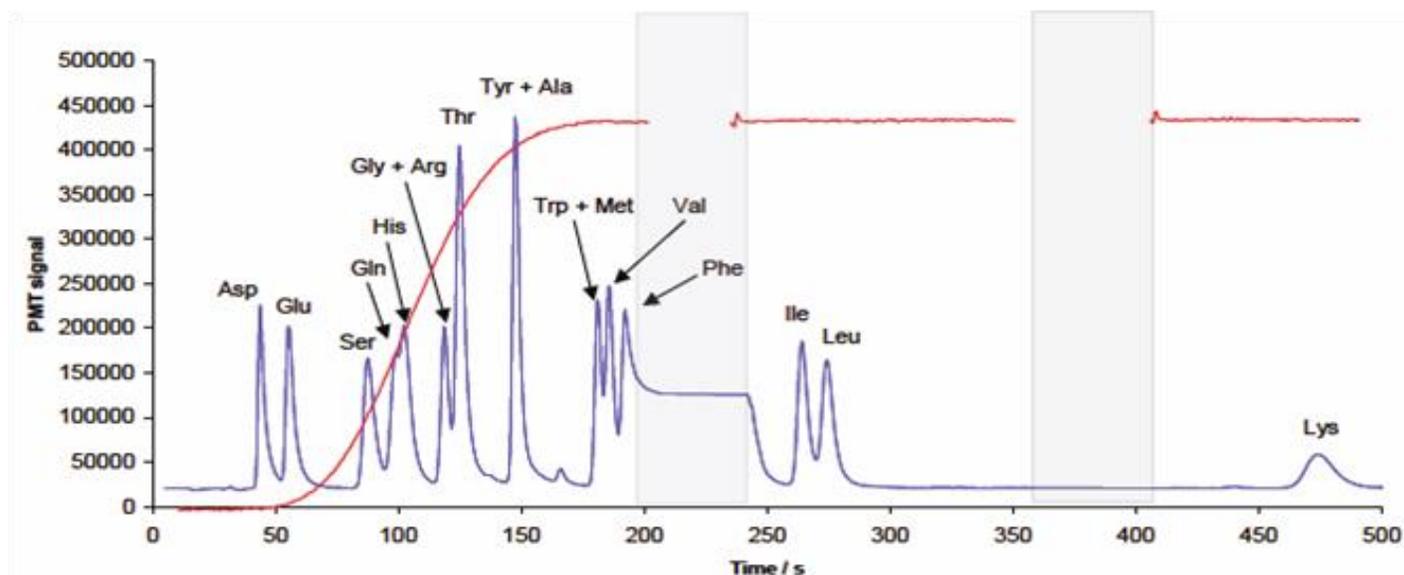
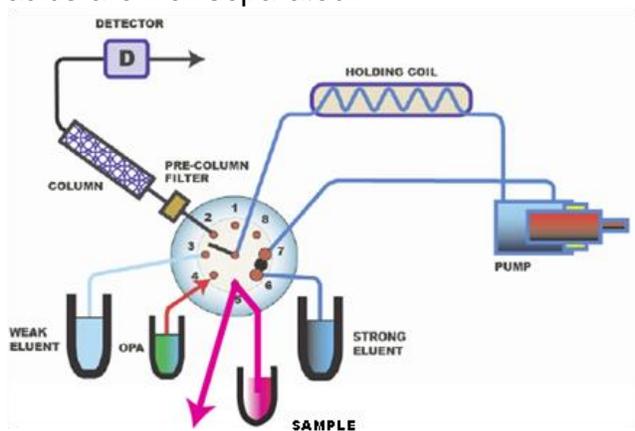


Consequently, column backpressure is substantially reduced allowing higher flow rates and faster throughputs. Monolith sol-gel columns show good separation performance at lower pressures, a critical feature that is integral to the SICrom performance.

Monolithic columns that use reverse phase are most popular and they have found many applications including separation of pharmaceuticals, proteins, oligonucleotides, organic acids, and enantiomeric compounds. Monoliths with ion exchanger sites found application in ion chromatography.

Example: Derivatization and Separation of Aminoacids

Amino acid separation was performed with an Onyx C18 4.6x25 mm monolithic column attached at the periphery of the SIC instrument valve. A pre-column filter of 2 µm porosity was used in front of the separation column to prevent clogging. The chromatogram shows that some amino acids (Gly+Arg, Tyr+Ala, Trp+Met) co-elute as one peak. Also, there is little resolution between Gln and His. The remaining amino acids are well separated.



For further details on this assay please consult [pdf file](#) or CD Tutorial Section 4.2.3. For micro SI Chromatography consult [Bead Injection](#) page.