

## GRADIENT ELUTION ELECTROPHORESIS

## TECHNICAL FIELD

The present invention relates in general to electrophoretic separations, both in capillaries and in microfluidic channels, in order to preconcentrate, separate and/or purify compounds from a sample mixture. More particularly the present invention relates to the combination of electrophoresis in the presence of a variable hydrodynamic flow with continuous introduction of sample. Further the present invention relates to microfluidic devices for conducting trace analysis which have small total footprint areas.

## BACKGROUND ART

Electrophoresis is a well known analytical method for separation of ionic compounds (analytes) in a sample mixture through use of an applied voltage. The voltage is applied to either end of a separation path (U-tube, gel, capillary, microfluidic channel, etc.), and the analytes are thereby caused to move along the separation path. The separation mechanism is based mainly on differences in analytes' size and charge, although other factors can be exploited such as shape, density, affinity, etc. Early electrophoresis work utilized continuous sample introduction into a relatively large glass U-tube across which a voltage was applied; analytes were detected in free solution as discrete boundaries throughout the tube. Electrophoresis was then applied to hydrated gel supports (e.g., polyacrylamide gel electrophoresis, PAGE) and later to micron diameter capillary tubes (i.e., capillary electrophoresis or CE). Typically, CE utilizes a discrete sample injection into the capillary in the presence of buffer electrolytes where analytes are separated into discrete zones along the separation path; this mode is termed capillary zone electrophoresis (CZE). A fixed detector is placed near the capillary outlet, past which analytes of highest mobility migrate first followed by species of slower mobility; individual analytes are typically detected as bands or peaks. For example, with a negative voltage applied to the capillary inlet, positively charged analytes will be detected first, followed by neutral species, and finally species exhibiting a negative charge. The detector response can then be correlated to analyte migration speed and, through the use of standard mixtures, be used to identify and/or quantify analytes.

An alternative mode of CE utilizes a discontinuous buffer system formed by using leading and terminating electrolytes (LE and TE, respectively) which define the boundaries between which the analyte ions are compressed into adjacent zones; this mode is termed isotachopheresis (ITP), referring to the uniform velocity of the electrophoresis (iso=same and tacho=speed). The discontinuous electrolytes are associated with a discontinuous electric field across the ion zones, with the lowest field in the LE zone and the highest in the TE zone. Sharp boundaries exist between each adjacent zone. In conventional capillary ITP (cITP), a sample mixture is injected into a capillary filled with LE. Either TE is added to the sample or the sample reservoir is replaced following injection with a reservoir containing TE. Under electrophoresis conditions the sample components begin to separate into individual zones. Should an analyte begin to enter the LE zone (or next fastest analyte), it experiences a drop in field and lower velocity, hence returning it to its own zone. If the analyte enters the TE zone (or next slowest analyte), it experiences a higher electric field and higher velocity, again returning it to its own zone. These counter balancing forces lead to a steady-state of analyte concentrations in their respective zones. The concen-

tration of analyte ( $C_A$ ) in the zone is fixed based upon the Kohlrausch regulating function of the LE concentration ( $C_{LE}$ ):

$$C_A = C_{LE} \frac{\mu_A(\mu_{LE} + \mu_{CE})}{\mu_{LE}(\mu_A + \mu_{CE})}$$

where  $\mu_A$ ,  $\mu_{LE}$ , and  $\mu_{CE}$  are the electrophoretic mobilities of the analyte, LE, and counter ion electrolyte in the LE solution, respectively. The above shows that as the mobility of the analyte approaches that of the LE, they will have equivalent concentrations. When high concentrations of LE are used, dilute analyte samples become highly concentrated. For relatively high analyte concentration, the upper bound on the focused analyte zone given by the Kohlrausch regulating function is quickly reached, and the length of the zone will increase as additional analyte is injected. The entire 'train' of LE, analyte(s), and TE all move at a uniform velocity. Commonly conductivity detection is employed and a 'staircase' output is observed, with each rise in conductance indicating a zone boundary and a plateau indicating the zone width. When using other on-line detectors, such as single response optical detection (e.g., fixed wavelength absorbance or fluorescence), differentiation of the continuous zones becomes difficult. In this case, non-detectable 'spacer' ions can be introduced, either as discrete spacers between two zones or as a continuum of electrophoretic mobilities, which can be realized through ampholytic mixtures.

At very low analyte concentration, the analytes are focused on the boundary between the LE and TE and form narrow, Gaussian peaks with a maximum concentration much lower than that of the LE. In this regime of operation with cITP, all of the analytes are focused at the same boundary, and there is no resolution of analytes. Consequently, concentration and resolution of trace analytes with prior art ITP methods are typically done in two steps in a technique known as transient ITP. Another approach that has been implemented in prior art ITP methods is to use one or more species of spacer electrolytes with mobilities that lie between those of the analytes of interest. Analytes are then focused at the interfaces between the LE, TE, and spacer electrolytes and can be well resolved. An additional approach is the use of a large number of spacer electrolytes with mobilities that form a nearly continuous range, such as an isoelectric focusing ampholyte mixture. With this approach, analytes with a continuous range of mobilities can be concentrated and focused.

The two primary electromotive forces in electrophoresis in a confined separation medium (i.e., tubular, capillary, or microfluidic channel) are analyte electrophoretic mobility and electroosmotic flow (EOF). EOF arises when a surface charge is present along the separation column; in glass-based separations with solutions above pH of approximately 2 the surface silanol groups exhibit net negative charges. Positively charged ions in the solution media (typically a buffered electrolyte solution) are drawn towards the negative surface forming an electrical double layer which can be mobilized axially under an applied voltage along the length the separation media. The mobile cations additionally cause movement of the bulk solution through viscous drag, referred to as EOF.

There are two primary disadvantages to the common method of performing cITP. First, the method requires an injection to load the mixture to be analyzed into the capillary as a minimally wide plug between the TE and LE. Second, long channels are required to produce high enrichment factors for dilute solutions or to produce high-resolution separations.