

CLAIMS

WHAT IS CLAIMED IS:

1. A method for introducing a mobilization electrolyte into a separation channel comprising a plurality of separated analytes, the method comprising:
using data derived from images of the separated analytes to automatically initiate introduction of the mobilization electrolyte into the separation channel.
2. The method of claim 1, wherein the separation channel is a microchannel in a microfluidic device.
3. The method of claim 1, wherein the separation channel is a capillary.
4. The method of claim 1, further comprising separating the plurality of analytes by isoelectric focusing.
5. The method of claim 1, further comprising mobilizing the separated analytes towards an electrospray ionization interface with a mass spectrometer.
6. The method of claim 1, wherein the mobilization electrolyte comprises a zwitterionic buffer.
7. The method of claim 1, wherein the mobilization electrolyte comprises acetic acid, formic acid, carbonic acid, or any combination thereof.
8. The method of claim 1, further comprising acquiring images of all or a portion of the separation channel.
9. The method of claim 8, wherein the images are acquired using light transmitted through the separation channel.
10. The method of claim 9, wherein the images are UV absorbance images or fluorescence images.
11. The method of claim 1, wherein the image-derived data comprises separated analyte peak information selected from the group consisting of peak position, peak width, and peak velocity.
12. The method of claim 1, wherein introduction of the mobilization electrolyte is performed electrophoretically.
13. The method of claim 12, wherein:
 - a) a first end of the separation channel device is electrically-coupled to a first electrode, a second end of the separation channel is electrically-coupled to a second electrode, and a

mobilization channel that intersects the second end of the separation channel is electrically-coupled to a third electrode; and

b) the electrophoretic introduction of the mobilization electrolyte is performed by switching the electrical-coupling of the second or third electrodes with their respective channels between on and off states.

14. The method of claim 13, wherein the first electrode is an anode and the second and third electrodes are cathodes.

15. The method of claim 13, wherein an absence of change or a reduction in a rate of change in peak position or peak width derived from image data for one or more separated analyte peaks over a time period of at least 20 seconds triggers the switching of the second and third electrodes between on and off states.

16. The method of claim 15, wherein the time period is at least 30 seconds, 40 seconds, 50 seconds, or 30 seconds.

17. The method of claim 13, wherein the electrophoretic introduction of mobilization electrolyte enables more accurate pI determinations for one or more separated analytes.

18. The method of claim 13, wherein the electrophoretic introduction of the mobilization electrolyte leads to improved separation resolution after mobilization compared that attained at the completion of an isoelectric focusing separation.

19. A system comprising:

a) a separation channel for performing isoelectric focusing to separate a mixture of analytes contained therein;

b) a mobilization channel that intersects with a distal end of the separation channel for delivery of a mobilization electrolyte to the separation channel; and

c) three electrodes comprising a first electrode that is electrically-coupled to a proximal end of the separation channel, a second electrode that is electrically-coupled to the distal end of the separation channel, and a third electrode that is electrically-coupled with the mobilization channel;

wherein the electrical-coupling of the second or third electrodes with their respective channels is switchable between on and off states.

20. The system of claim 19, wherein the first electrode is an anode and the second and third electrodes are cathodes.

21. The system of claim 19, wherein the switching of the second or third electrodes between on and off states initiates an electrophoretic introduction of the mobilization electrolyte into the separation channel.
22. The method of claim 21, wherein the electrophoretic introduction of mobilization electrolyte enables more accurate pI determinations for one or more separated analytes.
23. The system of claim 21, wherein the electrophoretic introduction of the mobilization electrolyte leads to improved separation resolution after mobilization compared that attained at the completion of an isoelectric focusing separation.
24. The system of claim 19, wherein the system is configured to switch the second or third electrodes between on and off states at a user-specified time following an initiation of an isoelectric focusing separation.
25. The system of claim 24, wherein the user-specified time is at least 30 second following the initiation of the isoelectric focusing separation.
26. The system of claim 24, wherein the user-specified time is at most 20 minutes following the initiation of the isoelectric focusing separation.
27. The system of claim 19, wherein the system is configured to monitor a current flowing through the separation channel during an isoelectric focusing separation reaction and switch the second or third electrodes between on and off states when the current drops below a specified current threshold.
28. The system of claim 27, wherein the specified threshold current ranges in value from about 1 microampere (μA) to about 10 microamperes (μA).
29. The system of claim 19, further comprising: (i) an imaging unit, and (ii) a processor unit, wherein a series of images captured by the imaging unit as the separation is performed are further processed by the processor unit to generate a trigger signal that triggers the switching of the second and third electrodes between on and off states in an asymmetric manner.
30. The system of claim 29, wherein the imaging unit is configured to capture images of all or a portion of the separation channel.
31. The system of claim 30, wherein the imaging unit is configured to capture images using light transmitted through a separation channel window.
32. The system of claim 31, wherein the imaging unit is configured to capture UV absorbance images or fluorescence images.

33. The system of claim 29, wherein the series of images are acquired at a rate of at least 1 image every 15 seconds.
34. The system of claim 29, wherein the processor unit is configured to perform an image processing algorithm used to monitor the presence or absence of an analyte peak in the separation channel.
35. The system of claim 34, wherein the system is configured to maintain the third electrode in an off state if no analyte peak is detected in the separation channel.
36. The system of claim 29, wherein the processor unit is configured to perform an image processing algorithm used to monitor changes in position of separated analyte peaks over time or changes in the width of separated analyte peaks over time.
37. The system of claim 36, wherein an absence of change or a reduction in a rate of change in peak position or peak width for one or more separated analyte peaks over a time period of at least 20 seconds triggers the switching of the second and third electrodes between on and off states.
38. The system of claim 37, wherein the time period is at least 30 seconds, 40 seconds, 50 seconds, or 30 seconds.
39. The system of claim 19, further comprising an orifice in fluid communication with the distal end of the separation channel, wherein the orifice is configured to function as an electrospray ionization interface with a mass spectrometer.
40. The system of claim 39, wherein the system is configured to correlate mass spectrometer data with pI data for analytes separated by isoelectric focusing.