CLAIMS

WHAT IS CLAIMED IS:

1. A system comprising:

a) a separation channel configured to perform isoelectric focusing to separate a mixture of analytes contained therein;

b) a mobilization channel comprising a mobilization electrolyte, wherein the mobilization channel is configured to deliver the mobilization electrolyte to the separation channel, wherein the mobilization channel is distinct from the separation channel, and wherein a distal end of the mobilization channel intersects the separation channel at a single point at a distal end of the separation channel;

c) three electrodes comprising a first electrode that is in electrical contact with a proximal end of the separation channel, a second electrode that is in electrical contact with the distal end of the separation channel, and a third electrode that is in electrical contact with a proximal end of the mobilization channel; and

d) a controller configured to automatically switch the second and third electrodes between “on” or “off” states to initiate mobilization, switch the first electrode to an “on” state while both isoelectric focusing and mobilization of separated analytes are performed, switch the second electrode to an “on” state and the third electrode to an “off” state while isoelectric focusing is performed, and switch the second electrode to an “off” state and the third electrode to an “on” state while mobilization of separated analytes is performed.

2. The system of claim 1, wherein the first electrode is an anode and the second and third electrodes are cathodes.

3. The system of claim 1, 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.

4. The system of claim 3, wherein the electrophoretic introduction of the mobilization electrolyte leads to improved separation resolution after mobilization compared to that attained at the completion of an isoelectric focusing separation.

5. The system of claim 3, wherein the electrophoretic introduction of the mobilization electrolyte enables more accurate pI determinations for one or more separated analytes.

6. The system of claim 1, wherein the system is configured to automatically switch the second and third electrodes between “on” and “off” states at a user-specified time following an initiation of an isoelectric focusing separation.

7. The system of claim 6, wherein the user-specified time is at least 30 seconds following the initiation of the isoelectric focusing separation.

8. The system of claim 6, wherein the user-specified time is at most 20 minutes following the initiation of the isoelectric focusing separation.

9. The system of claim 1, 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 automatic switching of the second and third electrodes between “on” and “off” states.

10. The system of claim 9, wherein the imaging unit is configured to capture images of all or a portion of the separation channel.

11. The system of claim 10, wherein the imaging unit is configured to capture images using light transmitted through a separation channel window.

12. The system of claim 11, wherein the imaging unit is configured to capture UV absorbance images or fluorescence images.

13. The system of claim 12, wherein the fluorescence images are native fluorescence images.

14. The system of claim 9, wherein the series of images are acquired at a rate of at least 1 image every 15 seconds.

15. The system of claim 9, 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.

16. The system of claim 15, wherein the system is configured to maintain the third electrode in an “off” state if no analyte peak is detected in the separation channel.

17. The system of claim 9, 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.

18. The system of claim 17, 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 automatic switching of the second and third electrodes between “on” and “off” states.

19. The system of claim 18, wherein the time period is at least 30 seconds, 40 seconds, 50 seconds, or 60 seconds.

20. The system of claim 9, wherein the series of images captured by the imaging unit are processed to detect pI markers in the separation channel and determine the pIs of separated analytes.

21. The system of claim 20, wherein the system is configured for characterization of biologic or biosimilar pharmaceuticals.

22. The system of claim 1, 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.

23. The system of claim 22, wherein the system is configured to correlate mass spectrometer data with pI data for analytes separated by isoelectric focusing.

24. The system of claim 23, wherein the system is configured for characterization of biologic or biosimilar pharmaceuticals.

25. The system of claim 1, wherein the separation channel and mobilization channel are channels in a microfluidic device.

26. The system of claim 1, wherein the first electrode is a cathode and the second and third electrodes are anodes.

27. The system of claim 1, wherein the mobilization electrolyte comprises acetic acid, formic acid, carbonic acid, or any combination thereof.

28. The system of claim 1, wherein the analytes are proteins.

29. The system of claim 1, wherein “on” and “off” states for the first, second, and third electrodes comprise complete connection or disconnection respectively of an electrical coupling between the first, second, and third electrodes and the proximal end of the separation channel, the distal end of the separation channel, and the proximal end of the mobilization channel respectively.

30. The system of claim 1, wherein “on” and “off” states for the first, second, and third electrodes comprise holding a current passing through the first, second, and third electrodes respectively to a non-zero value or to zero microamperes respectively.