CLAIMS

WHAT IS CLAIMED IS:

1. A method for maintaining an electrospray ionization (ESI) tip at a constant voltage relative to ground while performing a separation reaction, the method comprising:

a) applying a first voltage to a proximal end of a separation channel, wherein a distal end of the separation channel is in fluid and electrical communication with the ESI tip;

b) applying a second voltage to a proximal end of an auxiliary fluid channel, wherein a distal end of the auxiliary fluid channel is in fluid and electrical communication with the distal end of the separation channel;

c) performing the separation reaction to separate a mixture of analytes, wherein the separation reaction takes place within the separation channel; and

d) monitoring a change in resistance of the separation channel or a change in voltage at the ESI tip in a feedback loop that adjusts the first and second voltages to maintain a constant voltage drop across the separation channel and a constant voltage at the ESI tip.

2. The method of claim 1, wherein the separation channel is a lumen of a capillary.

3. The method of claim 2, wherein the capillary comprises a microvial spray tip.

4. The method of claim 1, wherein the separation channel is a fluid channel within a microfluidic device.

5. The method of any one of claims 1 to 4, wherein the separation reaction comprises an isoelectric focusing reaction.

6. The method of any one of claims 1 to 4, wherein the separation reaction comprises an electrophoretic separation reaction.

7. The method of any one of claims 1 to 6, wherein the first voltage is applied at a cathode and the second voltage is applied at an anode.

8. The method of any one of claims 1 to 7, wherein the voltage at the ESI tip is held at ground.

9. The method of any one of claims 1 to 7, wherein the voltage at the ESI tip is held at the second voltage.

10. The method of any one of claims 1 to 9, wherein the adjustment to the first and second voltages comprises subtracting a transient voltage change measured at the ESI tip from the first voltage and second voltage.

11. The method of any one of claims 1 to 10, wherein the voltage at the ESI tip is measured using a power supply that provides the first or second voltage.

12. The method of any one of claims 1 to 11, wherein the feedback loop operates at a frequency of at least 0.1 Hz.

13. The method of any one of claims 1 to 11, wherein the feedback loop operates at a frequency of at least 10 Hz.

14. The method of any one of claims 1 to 13, wherein the feedback loop maintains the voltage at the ESI tip to within ± 10% of a pre-set value.

15. The method of any one of claims 1 to 13, wherein the feedback loop maintains the voltage at the ESI tip to within ± 1% of a pre-set value.

16. The method of any one of claims 1 to 15, wherein the feedback loop maintains the voltage drop across the separation channel to within ± 10% of a pre-set value.

17. The method of any one of claims 1 to 15, wherein the feedback loop maintains the voltage drop across the separation channel to within ± 1% of a pre-set value.

18. A method comprising:

a) providing a sample comprising a mixture of two or more analytes;

b) performing a separation within a fluid channel containing the sample to resolve individual analyte peaks from the mixture of two or more analytes;

c) calculating a velocity of an analyte peak upon mobilization of the fluid channel's contents towards a fluid channel exit; and

d) using the velocity of the analyte peak to determine a time at which the analyte peak reaches the fluid channel exit.

19. The method of claim 18, wherein the fluid channel is a lumen of a capillary.

20. The method of claim 18, wherein the fluid channel is part of a microfluidic device.

21. The method of any one of claims 18 to 20, wherein the separation is based on isoelectric focusing (IEF), capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), or micellar electrokinetic chromatography (MEKC).

22. The method of any one of claims 18 to 21, wherein the velocity of the analyte peak is calculated from a time interval required for the analyte peak to move from a first position to a second position.

23. The method of claim 22, wherein the first position, second position, and time interval are determined from a series of two or more images of the fluid channel.

24. The method of claim 23, wherein the series of two or more images comprise ultraviolet light absorbance images, visible light absorbance images, or fluorescence images.

25. The method of any one of claims 18 to 24, wherein the fluid channel exit comprises an electrospray interface with a mass spectrometer.

26. The method of any one of claims 18 to 25, wherein the time at which the analyte peak reaches the fluid channel exit is used to correlate mass spectrometer data with the analyte peak.

27. The method of any one of claims 18 to 26, wherein the mobilization of the fluid channel's contents comprises the use of an electroosmotic mobilization technique, a chemical mobilization technique, a hydrodynamic mobilization technique, or any combination thereof.

28. The method of any one of claims 18 to 27, wherein the two or more analytes comprise proteins, protein-drug conjugates, peptides, nucleic acid molecules, carbohydrate molecules, lipid molecules, metabolite molecules, small organic compounds, or any combination thereof.

29. The method of any one of claims 26 to 28, wherein a comparison of mass spectrometer data collected for samples of a biologic drug candidate and a reference drug is used to make a determination of biosimilarity.

30. The method of any one of claims 18 to 29, wherein the velocity of the analyte peak is used in a feedback loop to adjust a control parameter for the separation or mobilization of the analyte peaks.

31. The method of claim 30, wherein the control parameter is a voltage.

32. The method of claim 30 or claim 31, wherein the feedback loop operates at a frequency of at least 0.1 Hz.

33. A method comprising:

a) providing a sample comprising a mixture of two or more analytes;

b) performing a separation within a fluid channel containing the sample to resolve individual analyte peaks from the mixture of two or more analytes; and

c) collecting mass spectrometer data for the two or more individual analyte peaks emitted from the fluid channel via an electrospray interface with a mass spectrometer; wherein a data collection mode for the mass spectrometer is alternated between a high mass scan and a low mass scan.

34. The method of claim 33, wherein the mass spectrometer is switched between the high mass scan and low mass scan data collection modes at a frequency of at least 0.5 Hz.

35. The method of claim 33 or claim 34, wherein the fluid channel is a lumen of a capillary.

36. The method of claim 33 or claim 34, wherein the fluid channel is part of a microfluidic device.

37. The method of any one of claims 33 to 36, wherein the separation is based on isoelectric focusing (IEF), capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), or micellar electrokinetic chromatography (MEKC).

38. The method of any one of claims 33 to 37, wherein the high mass scan captures mass spectral data for biological macromolecules.

39. The method of claim 39, wherein the biological macromolecules comprise proteins, protein-drug conjugates, nucleic acid molecules, reduced proteins, fusion proteins, protein complexes, or any combination thereof.

40. The method of any one of claims 33 to 39, wherein the m/z ratio for the high mass scan ranges from 1500 to 6000.

41. The method of any one of claims 33 to 40, wherein the low mass scan captures mass spectral data for solution-phase ampholytes used in performing an isoelectric focusing separation.

42. The method of any one of claims 33 to 42, wherein the m/z ratio for the low mass scan ranges from 150 to 1500.

43. The method of any one of claims 41 to 42, wherein the mass spectra of one or more solution-phase ampholytes are used to calibrate the isoelectric points (pIs) for biological macromolecules identified in the high mass scans.

44. A method comprising:

a) performing a separation within a fluid channel containing a sample, wherein the sample comprises a mixture of two or more analytes, and wherein the separation resolves individual analyte peaks from the mixture of two or more analytes;

b) mobilizing the fluid channel's contents towards a fluid channel exit, wherein the fluid channel exit comprises an electrospray interface with a mass spectrometer; and

c) simultaneously or alternately imaging: (i) at least a portion of the fluid channel to monitor a position of an analyte peak during (a) and (b), and (ii) a Taylor cone existing

between the fluid channel exit and an inlet to the mass spectrometer to monitor electrospray performance.

45. The method of claim 44, wherein the positions of the analyte peak in two or more images of at least a portion of the fluid channel are used to calculate velocity for the analyte peak.

46. The method of claim 45, wherein the velocity of the analyte peak is used to determine a time at which the analyte peak will reach the fluid channel exit.

47. The method of claim 46, wherein the time at which the analyte peak reaches the fluid channel exit is used to correlate mass spectrometer data with the analyte peak.

48. The method of any one of claims 44 to 47, wherein data derived from the imaging of the Taylor cone is used in a feedback loop to adjust electrospray performance.

49. The method of claim 48, wherein the feedback loop operates at a frequency of at least 0.1 Hz.

50. The method of any one of claims 44 to 49, wherein the fluid channel is a lumen of a capillary.

51. The method of any one of claims 44 to 49, wherein the fluid channel is part of a microfluidic device.

52. The method of any one of claims 44 to 51, wherein the separation is based on isoelectric focusing (IEF), capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), or micellar electrokinetic chromatography (MEKC).

53. The method of any one of claims 44 to 52, wherein the imaging comprises ultraviolet light absorbance imaging, visible light absorbance imaging, or fluorescence imaging.

54. The method of any one of claims 44 to 53, wherein the mobilization of the fluid channel's contents comprises the use of an electroosmotic mobilization technique, a chemical mobilization technique, a hydrodynamic mobilization technique, or any combination thereof.

55. The method of any one of claims 44 to 54, wherein the two or more analytes comprise proteins, protein-drug conjugates, peptides, nucleic acid molecules, carbohydrate molecules, lipid molecules, metabolite molecules, small organic compounds, or any combination thereof.

56. A computer-implemented method for maintaining an electrospray ionization (ESI) tip at a constant voltage relative to ground while performing a separation reaction, the method comprising:

a) receiving, using a processor, a first measurement of a voltage at the ESI tip, wherein a distal end of the separation channel is in fluid and electrical communication with the ESI tip;

b) receiving, using the processor, a second measurement of the voltage at the ESI tip;

c) comparing the second measurement to the first measurement using the processor, wherein if the second measurement differs from the first measurement, the processor causes a voltage at a proximal end of the separation channel and a voltage at a proximal end of an auxiliary fluid channel comprising a distal end that is in fluid and electrical communication with the distal end of the separation channel, to be adjusted such that the voltage at the ESI tip is returned to the first measurement value; and

d) repeating steps (a) through (c) at a specified frequency.

57. The computer-implemented method of claim 56, wherein the separation channel comprises a lumen of a capillary or a fluid channel within a microfluidic device.

58. The computer-implemented method of claim 56 or claim 57, wherein the separation reaction comprises an isoelectric focusing reaction.

59. The computer-implemented method of claim 56 or claim 57, wherein the separation reaction comprises an electrophoretic separation reaction.

60. The computer-implemented method of any one of claims 56 to 59, wherein the voltage at the ESI tip is held at ground.

61. The computer-implemented method of any one of claims 56 to 60, wherein the specified frequency is at least 1 Hz.

62. The computer-implemented method of any one of claims 56 to 61, wherein the voltage at the ESI tip is maintained to within ± 5% of a specified value.

63. A computer-implemented method comprising:

a) receiving, using a processor, image data comprising two or more images acquired using a detector configured to image all or a portion of a separation channel in a capillary or a microfluidic device;

b) processing the image data using the same or a different processor to determine a position of an analyte peak within the separation channel in the two or more images;

c) calculating, using the same or a different processor, a velocity of the analyte peak based on the position of the analyte peak in the two or more images and a known time interval between acquisition of the two or more images; and

d) determining, using the same or a different processor, a time at which the analyte peak will reach a separation channel outlet.

64. The computer-implemented method of claim 63, wherein a separation reaction performed within the separation channel comprises isoelectric focusing (IEF), capillary zone electrophoresis (CZE), capillary gel electrophoresis (CGE), capillary isotachophoresis (CITP), or micellar electrokinetic chromatography (MEKC).

65. The computer-implemented method of claim 63 or claim 64, wherein the two or more images comprise ultraviolet light absorbance images, visible light absorbance images, or fluorescence images.

66. The computer-implemented method of any one of claims 63 to 65, wherein the separation channel outlet is in fluid communication with or comprises an electrospray interface with a mass spectrometer.

67. The computer-implemented method of any one of claims 63 to 66, wherein the time at which the analyte peak reaches the separation channel outlet is used to correlate mass spectrometer data with the analyte peak.

68. The computer-implemented method of any one of claims 63 to 67, wherein the analyte is separated from a mixture and comprises a protein, a protein-drug conjugate, a peptide, a nucleic acid molecule, a carbohydrate molecule, a lipid molecule, a metabolite molecule, or a small organic compound.

69. The computer-implemented method of any one of claims 63 to 68, wherein a comparison of mass spectrometer data collected for samples of a biologic drug candidate and a reference drug is used to make a determination of biosimilarity.

70. The computer-implemented method of any one of claims 63 to 69, wherein the velocity of the analyte peak is used in a feedback loop to adjust a control parameter for a separation reaction performed in the separation channel.

71. The computer-implemented method of claim 70, wherein the control parameter is a voltage.

72. The computer-implemented method of claim 70 or claim 71, wherein the feedback loop operates at a frequency of at least 0.1 Hz.

CLAIMS

What is claimed is:

1. A method for maintaining an electrospray ionization (ESI) tip at a constant voltage relative to ground while performing a separation reaction, the method comprising:

a) applying a first voltage to a proximal end of a separation channel, wherein a distal end of the separation channel is in fluid and electrical communication with the ESI tip;

b) applying a second voltage to a proximal end of an auxiliary fluid channel, wherein a distal end of the auxiliary fluid channel is in fluid and electrical communication with the distal end of the separation channel;

c) performing the separation reaction to separate a mixture of analytes, wherein the separation reaction takes place within the separation channel; and

d) monitoring a change in resistance of the separation channel or a change in voltage at the ESI tip in a feedback loop that adjusts the first and second voltages to maintain a constant voltage drop across the separation channel and a constant voltage at the ESI tip.

2. The method of claim 1, wherein the separation channel is a lumen of a capillary.

3. The method of claim 2, wherein the capillary comprises a microvial spray tip.

4. The method of claim 1, wherein the separation channel is a fluid channel within a microfluidic device.

5. The method of claim 1, wherein the separation reaction comprises an isoelectric focusing reaction.

6. The method of claim 1, wherein the separation reaction comprises an electrophoretic separation reaction.

7. The method of claim 1, wherein the first voltage is applied at a cathode and the second voltage is applied at an anode.

8. The method of claim 1, wherein the voltage at the ESI tip is held at ground.

9. The method of claim 1, wherein the voltage at the ESI tip is held at the second voltage.

10. The method of claim 1, wherein the adjustment to the first and second voltages comprises subtracting a transient voltage change measured at the ESI tip from the first voltage and second voltage.

11. The method of claim 1, wherein the voltage at the ESI tip is measured using a power supply that provides the first or second voltage.

12. The method of claim 1, wherein the feedback loop operates at a frequency of at least 0.1 Hz.

13. The method of claim 1, wherein the feedback loop operates at a frequency of at least 10 Hz.

14. The method of claim 1, wherein the feedback loop maintains the voltage at the ESI tip to within ±10% of a pre-set value.

15. The method of claim 1, wherein the feedback loop maintains the voltage at the ESI tip to within ±1% of a pre-set value.

16. The method of claim 1, wherein the feedback loop maintains the voltage drop across the separation channel to within ±10% of a pre-set value.

17. The method of claim 1, wherein the feedback loop maintains the voltage drop across the separation channel to within ±1% of a pre-set value.

18. A computer-implemented method for maintaining an electrospray ionization (ESI) tip at a constant voltage relative to ground while performing a separation reaction, the method comprising:

a) receiving, using a processor, a first measurement of a voltage at the ESI tip, wherein a distal end of the separation channel is in fluid and electrical communication with the ESI tip;

b) receiving, using the processor, a second measurement of the voltage at the ESI tip;

c) comparing the second measurement to the first measurement using the processor, wherein if the second measurement differs from the first measurement, the processor causes a voltage at a proximal end of the separation channel and a voltage at a proximal end of an auxiliary fluid channel comprising a distal end that is in fluid and electrical communication with the distal end of the separation channel, to be adjusted such that the voltage at the ESI tip is returned to the first measurement value; and

d) repeating steps (a) through (c) at a specified frequency.

19. The computer-implemented method of claim 18, wherein the separation channel comprises a lumen of a capillary or a fluid channel within a microfluidic device.

20. The computer-implemented method of claim 18, wherein the separation reaction comprises an isoelectric focusing reaction.

21. The computer-implemented method of claim 18, wherein the separation reaction comprises an electrophoretic separation reaction.

22. The computer-implemented method of claim 18, wherein the voltage at the ESI tip is held at ground.

23. The computer-implemented method of claim 18, wherein the specified frequency is at least 1 Hz.

24. The computer-implemented method of claim 18, wherein the voltage at the ESI tip is maintained to within ±5% of a specified value.