robust, *i.e.* can withstand fluctuations in temperature and pH, and can sufficiently resolve the charge heterogeneity is resource intensive. Methods to develop an optimal buffer system that allows development of robust assays to determine the presence of contaminants in multiple polypeptide products are desirable. The present invention provides methods to predict optimal conditions for ion exchange based on mathematical modeling of both the polypeptide and the buffering system.

[0005] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY

[0006] In one aspect, there is provided a method for identifying an optimal ion exchange chromatography separation condition to analyze a plurality of compositions, wherein each composition comprises a polypeptide and one or more contaminants, the method comprising a) plotting a net charge versus pH curve at a selected temperature based on the amino acid composition of the polypeptides of two or more of the compositions, and b) determining the inflection point of the net charge versus pH curve at or near neutral pH by determining a second derivative of the plots of step a); wherein the optimal ion exchange chromatography separation condition is a pH at about a common inflection point for the polypeptide(s) of one or more of the compositions. In some embodiments, the methods further comprise c) determining the change in the inflection point pH of the net charge versus pH curve with a change in the temperature (dIP/dT) for the polypeptides of two or more of the compositions, d) selecting a buffer for use in the chromatography, wherein a change in the acid dissociation constant of the buffer with change in temperature (dpKa/dT) is essentially the same as the dIP/dT of the polypeptides.

[0007] In another aspect, there is provided a method for identifying an optimal ion exchange chromatography separation condition to analyze a composition comprising a polypeptide and one or more contaminants, the method comprising a) plotting a net charge versus pH curve at a selected temperature based on the amino acid composition of the polypeptide, and b) determining the inflection point of the net charge versus pH curve at or near neutral pH by determining a second derivative of the plot of step a); wherein the optimal ion exchange chromatography separation condition is a pH at about the inflection point for the polypeptide.

inflection points. In some embodiments, the buffer provides an effective buffer capacity at the inflection point pH.

[0015] Provided is a method for analyzing a composition comprising a polypeptide and one or more contaminants, wherein the method effectively separates the polypeptide from the contaminants, the method comprising a) binding the polypeptide and one of more contaminants to an ion-exchange chromatography material using a loading buffer, wherein the loading buffer comprises a buffer, and wherein the pH and temperature of the chromatography has been optimized for a plurality of target polypeptides; b) eluting the polypeptide and one or more contaminants from the ion-exchange chromatography material using a gradient of an elution buffer, wherein the elution buffer comprises the buffer and a salt, wherein the polypeptide and the one or more contaminants are separated by the gradient; and c) detecting the polypeptide and the one or more contaminants; wherein the contaminant is a degradation product of the polypeptide and/or a charge variant of the polypeptide. In some embodiments, the buffer is N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES). In further embodiments, the concentration of the buffer ranges from about 5 mM to about 20 mM. In some embodiments, the change in temperature is from about 20 °C to about 70 °C. In further embodiments, the change in temperature is from about 20 °C to about 50 °C. In some embodiments, $dpKa/dT = dIP/dT \pm 50\%$. In some embodiments, the net charge of the polypeptide in the buffer changes by less than 0.5 over 30 °C. In some embodiments, the buffer is used in the chromatography at a concentration ranging from about 5 mM to about 250 mM.

[0016] In some embodiments of the above embodiments, the buffer compositions further comprise a salt. In further embodiments, the salt is NaCl, KCl, $(NH_4)_2SO_4$, or Na_2SO_4. In some embodiments, the concentration of the salt ranges from about 1 mM to about 1M. In some embodiments, the salt concentration increases from about 0 mM to about 100 mM in about 100 minutes. In other embodiments, the salt concentration increases from about 0 mM to about 80 mM in about 40 minutes.

[0017] In some embodiments of the methods of the disclosure, the polypeptide is an antibody or immunoadhesin or fragment thereof. In some embodiments, the polypeptide is a monoclonal antibody or fragment thereof. In some embodiments, the antibody is a human antibody. In other embodiments, the antibody is a humanized antibody. In yet other embodiments, the antibody is a chimeric antibody. In some embodiments, the antibody is an antibody fragment.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for identifying an optimal ion exchange chromatography separation condition to analyze a plurality of compositions, wherein each composition comprises a polypeptide and one or more contaminants, the method comprising

a) plotting a net charge versus pH curve at a selected temperature based on the amino acid composition of the polypeptides of two or more of the compositions, and

b) determining the inflection point of the net charge versus pH curve at or near neutral pH by determining a second derivative of the plots of step a);

wherein the optimal ion exchange chromatography separation condition is a pH at about a common inflection point for the polypeptide(s) of one or more of the compositions.

2. The method of claim 1, wherein if the net charge at the inflection point is positive, a cation exchange material is used for the ion exchange chromatography.

3. The method of claim 2, wherein the cation exchange chromatography material is a sulfonated chromatography material or a carboxylated chromatography material.

4. The method of claim 1, wherein if the net charge at the inflection point is negative, an anion exchange material is used for the chromatography.

5. The method of claim 4, wherein the anion exchange chromatography material is a quarternary amine chromatography material or a tertiary amine chromatography material.

6. The method of claim 1, wherein a mixed mode chromatography material is used for the chromatography.

7. The method of claim 6, wherein the mixed mode ion exchange material is a mixture of sequentially packed sulfonated chromatography material or carboxylated chromatography

28. A method for identifying an optimal ion exchange chromatography separation condition to analyze a composition comprising a polypeptide and one or more contaminants, the method comprising

a) plotting a net charge versus pH curve at a selected temperature based on the amino acid composition of the polypeptide, and

b) determining the inflection point of the net charge versus pH curve at or near neutral pH by determining a second derivative of the plot of step a);

wherein the optimal ion exchange chromatography separation condition is a pH at about the inflection point for the polypeptide.

29. The method of claim 28, wherein if the net charge at the inflection point is positive, a cation exchange material is used for the ion exchange chromatography.

30. The method of claim 29, wherein the cation exchange chromatography material is a sulfonated chromatography material or a carboxylated chromatography material.

31. The method of claim 28, wherein if the net charge at the inflection point is negative, an anion exchange material is used for the chromatography.

32. The method of claim 31, wherein the anion exchange chromatography material is a quarternary amine chromatography material or a tertiary amine chromatography material.

33. The method of claim 28, wherein a mixed mode chromatography material is used for the chromatography.

34. The method of claim 33, wherein the mixed mode ion exchange material is a mixture of sequentially packed sulfonated chromatography material or carboxylated chromatography material and a quarternary amine chromatography material or tertiary amine chromatography material.

35. The method of any one of claims 28-34, further comprising

58. The method of claim 56 or 57, wherein the buffer is identified by

a) determining the change in the inflection point pH of the net charge versus pH curve with a change in the temperature (dIP/dT) for the two or more target polypeptides,

b) selecting a buffer for which a change in the acid dissociation constant buffer with change in temperature (dpKa/dT) is essentially the same as the dIP/dT of the one or more target polypeptides with common inflection points.

59. The method of claim 58, wherein the buffer provides an effective buffer capacity at the inflection point pH.

60. The method of any one of claims 55-59, wherein the buffer is N-(2-Acetamido)-2-aminoethanesulfonic acid (ACES) or 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES).

61. The method of any one of claims 55-60, wherein the concentration of the buffer ranges from about 5 mM to about 20 mM.

62. The method of any one of claims 55-61, wherein the pH of the buffer ranges from about 6.5 to about 8.5 at a temperature range of about 20 °C to about 70 °C.

63. The method of any one of claims 55-62, wherein the pH of the buffer ranges from about 6.5 to about 8.5 at a temperature range of about 20 °C to about 50 °C.

64. The method of any one of claims 55-63, wherein the pH of the buffer and the polypeptide at the inflection point is about 7.8 at about 22 °C, about 7.5 at about 37 °C, or about 7.2 at about 50 °C.

65. The method of any one of claims 55-64, wherein the salt gradient is a linear gradient.

66. The method of any one of claims 55-65, wherein the salt gradient is a step gradient.

67. The method of any one of claims 55-66, wherein the salt gradient is a NaCl gradient, a KCl gradient, $(NH_4)_2SO_4$ gradient, or a Na_2SO_4 gradient.

68. The method of any one of claims 55-67 wherein the salt concentration in the gradient increases from about 0 mM to about 1M.

69. The method of claim 68, wherein the salt concentration increases from about 0 mM to about 100 mM in about 100 minutes.

70. The method of claim 68, wherein the salt concentration increases from about 0 mM to about 80 mM in about 40 minutes.

71. The method of any one of claims 55-70, wherein the polypeptide is an antibody or immunoadhesin or fragment thereof.

72. The method of any one of claims 55-71, wherein the polypeptide is a monoclonal antibody or fragment thereof.

73. The method of claim 71 or 72, wherein the antibody is a human antibody.

74. The method of claim 71 or 72, wherein the antibody is a humanized antibody.

75. The method of claim 71 or 72, wherein the antibody is a chimeric antibody.

76. The method of any one of claims 71-75, wherein the antibody is an antibody fragment.

77. The method of any one of claims 55-76, wherein the contaminant is a variant of the polypeptide.

78. The method of any one of claims 55-59, wherein the contaminant is a degradation product of the polypeptide.

79. The method of any one of claims 55-59, wherein the contaminant is a charge variant of the polypeptide.

80. The method of any one of claims 55-79, wherein the chromatography material is a cation exchange chromatography material.

81. The method of claim 80, wherein the cation exchange chromatography material is a sulfonated chromatography material or a carboxylated chromatography material.

82. A method for analyzing a plurality of polypeptide compositions, wherein each polypeptide composition comprises an polypeptide and one or more charge variants of the polypeptide, wherein the method effectively separates the polypeptide from its charge variants;

for each polypeptide composition the method comprises,

a) binding the polypeptide and one of more charge variants to an ion-exchange chromatography material using a loading buffer at a flow rate of about 1 mL/minute, wherein the loading buffer comprises 10 mM HEPES buffer at about pH 7.6 at about 40 °C;

b) eluting the polypeptide and the charge variants contaminants from the ion-exchange chromatography material using a gradient of an elution buffer, wherein the elution buffer comprises about 10 mM HEPES buffer at about pH 7.6 and a NaCl, wherein the concentration of the NaCl increases in the gradient from about 0 mM to about 80 mM in about 40 minutes, wherein the polypeptide and its charge variants are separated by the gradient; and

c) detecting the polypeptide and the one or more charge variants.

83. The method of claim 82, wherein the plurality of polypeptide compositions comprises different polypeptides.

84. The method of claim 82 or 83, wherein the plurality of polypeptide compositions comprises polypeptides with different pIs.

85. The method of any one of claims 82-84, wherein the polypeptide compositions are antibody compositions.