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 ~~some~~ one aspect~~s~~, there ~~invention~~ is provide~~ds~~ a method~~s~~ for identifying an optimal ion exchange chromatography separation condition to analyze a plurality of compositions, wherein each composition comprises a polypeptide with 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 the 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~~s~~, there is ~~invention~~ provide~~ds~~ a method for identifying an optimal ion exchange chromatography separation condition to analyze a composition comprising a polypeptide with 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 the second derivative of the plot~~s~~ of step a); wherein the optimal ion exchange chromatography separation condition is a pH at about the inflection point for the polypeptide.

[0008] In some embodiments, if the net charge at the inflection point is positive, a cation exchange material is used for the ion exchange chromatography. In some embodiment, the cation exchange chromatography material is a sulfonated chromatography material or a carboxylated chromatography material. In other embodiments, if the net charge at the inflection point is negative, an anion exchange material is used for the chromatography. In some embodiments, the anion exchange chromatography material is a quarternary amine chromatography material or a tertiary amine chromatography material. In yet other embodiments, a mixed mode chormatography material is used for the chromatography. In some embodiments, 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.

[0009] In some embodiments, the buffer provides an effective buffer capacity at the inflection point pH. In some embodiments, the dIP/dT of the polypeptides of one or more of the compositions is about -0.02 pH units. 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 ± 50%. In some embodiments, the net charge of the polypeptide in the buffer selected in step d) changes by less than 0.5 over 30 °C. In some embodiments, the buffer selected in step d) is used in the chromatography at a concentration ranging from about 5 mM to about 250 mM.

[0010] 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.

[0011] In some embodiments of the methods of the ~~invention~~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.

[0012] In some embodiments of the methods of the ~~invention~~disclosure, the contaminant is a variant of the polypeptide. In some embodiments, the contaminant is a degradation product of the polypeptide. In some embodiments, the contaminant is a charge variant of the polypeptide.

[0013] In ~~some~~ one aspects, there ~~invention~~ is provided a methods for analyzing a composition, wherein the composition comprises a polypeptide and one or more contaminants, wherein the method

effectively separates polypeptides from the contaminants, the method comprising a) determining the optimal pH and temperature ion exchange separation conditions for a plurality of compositions, each composition comprising a target polypeptide and one or more contaminants according to the methods ~~of the invention~~described herein, b) binding the polypeptide and one of more contaminants from the composition to an ion-exchange chromatography material using a loading buffer, wherein the loading buffer comprises a buffer identified by the method ~~of the invention~~described herein; c) 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 concentration of the salt increases in a gradient over time, wherein the polypeptide and the one or more contaminants are separated by the gradient; and d) detecting the polypeptide and the one or more contaminants.

[0014] In ~~some~~ one aspects~~,~~ there ~~invention~~ is provide~~d~~s a method~~s~~ 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 by i) plotting a net charge versus pH curve at a selected temperature, wherein the curve is based on the amino acid composition of the polypeptide of two or more target polypeptides, and ii) determining the inflection point of the net charge versus pH curve by determining the second derivative of the plots of step i); wherein the optimal ion exchange chromatography condition is a pH at a common inflection point for two or more 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. In some embodiments, the selected temperature is ambient temperature. In some embodiments, 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. In some embodiments, the buffer provides an effective buffer capacity at the inflection point pH.

[0015] In ~~some~~ another aspects, there is ~~invention~~ provideds a methods 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. 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 ± 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 ~~invention~~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.

[0018] In some embodiments of the methods of the ~~invention~~disclosure, the contaminant is a variant of the polypeptide. In some embodiments, the contaminant is a degradation product of the polypeptide. In some embodiments, the contaminant is a charge variant of the polypeptide.

[0019] In some emodiments, the chromatography material is a cation exchange chromatography material. In further embodiments, the cation exchange chromatography material is a sulfonated chromatography material or a carboxylated chromatography material.

[0020] In ~~some~~ one aspect~~s~~, there is ~~invention~~ provide~~ds~~ a method~~s~~ 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. In some embodiments, the plurality of polypeptide compositions comprises different polypeptides. In some embodiments, the plurality of polypeptide compositions comprises polypeptides with different pIs. In some embodiments, the polypeptide compositions are antibody compositions.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] Figure 1 is a graph plotting the calculated net charge versus pH for monoclonal antibody mAb1 (solid blank line) and its two charge variants. The dashed lines represents an acidic variant with two negative charges and a basic variant with two positive charges as indicated. The curves were created using the amino acid sequence composition of mAb1 and its variants. The star denotes the inflection point of the curve. A platform IEC method run at the inflection point pH will provide optimal resolution and robustness with respect to pH.

[0022] Figure 2A shows a graph of the percentage of protonated histidines (positively charged) in a population (*e.g.* a polypeptide solution) over the pH scale.