[0006] The spike addition approach entails diluting a sample with a PS20 stock solution at the formulation target concentration. This sample preparation dilutes the protein concentration while maintaining an approximately unchanged PS20 concentration. The amount of PS20 spiked into the sample is then subtracted during data analysis. Because the relationship between ELSD response and the mass analyzed in the detector follows a power-law, spiking in PS20 disproportionally decreases the contribution of protein to the ELSD signal. The spike addition approach has been shown to improve the accuracy of PS20 quantitation in some cases, but in cases where this is not a viable solution, protein precipitation must be used. While it is effective in removing protein interference, the HPLC-ELSD precipitation method is not ideal due to overnight sample preparation time, large sample volumes, and sample preparation variability. By contrast, removal of protein uses the same HPLC-ELSD conditions, but without significant sample preparation procedures. What is needed is a more robust solution to eliminate protein interference and yield consistent PS20 quantitation across all chromatography conditions.

[0007] All references cited herein, including patent applications and publications, are incorporated by reference in their entirety.

BRIEF SUMMARY

[0008] In some aspects, the disclosure provides a method for quantifying a non-ionic surfactant in a composition comprising the non-ionic surfactant and a polypeptide, wherein interference between the non-ionic surfactant and the polypeptide during quantification is reduced, wherein the method comprises the steps of a) applying the composition to a mixed mode anion exchange chromatography material, wherein the composition is loaded onto the chromatography material in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises acid in water and mobile phase B comprises acid in methanol, wherein the polypeptide binds to the chromatography material specifically and non-specifically; b) eluting the specifically bound polypeptide from the mixed mode anion exchange chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a); c) eluting the non-ionic surfactant and the non-specifically bound polypeptide from the chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step b); d) quantifying the non-ionic surfactant, wherein interference between the non-ionic surfactant and the polypeptide during quantitation is

reduced. In some examples, the ratio of mobile phase B to mobile phase A in step a) is about 10:90. In some examples, the ratio of mobile phase B to mobile phase A is increased to about 40:60 in step b). In some examples, the ratio of mobile phase B to mobile phase A is increased to about 100:0 in step c). In some examples, mobile phase A comprises about 2% acid in water. In some examples, mobile phase B comprises about 2% acid in methanol. In some examples, the acid is formic acid. In some examples, the acid is acetic acid. In some examples, the flow rate of the chromatography is about 1.25 mL/minute. In some examples, step b) starts at about 1 min after the chromatography is initiated and ends at about 3.4 min after the chromatography is initiated. In some examples, step c) starts at about 3.5 min after the chromatography is initiated and ends at about 4.6 min after the chromatography is initiated. In some examples, the non-ionic surfactant is poloxamer (P188) or a polysorbate. In some examples, the polysorbate is polysorbate 20 or polysorbate 80. In some examples, the concentration of non-ionic surfactant in the composition is in the range of about 0.001% to 1.0% (w/v). In some examples, the protein concentration in the composition is about 1 mg/mL to about 250 mg/mL. In some examples, the formulation has a pH of about 4.5 to about 7.5. In some examples, the composition further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, and a tonicity agent. In some examples, the composition is a pharmaceutical formulation suitable for administration to a subject. In some examples, the polypeptide is a therapeutic polypeptide. In some examples, the therapeutic polypeptide is a fusion protein, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, a glycoengineered antibody, an antibody fragment, an antibody drug conjugate, a THIOMAB™ or a THIOMAB™ drug conjugate. In some examples, the mixed mode anion exchange chromatography material comprises a reversed phase, strong anion exchange polymer. In some examples, the mixed mode anion exchange chromatography material comprises a quaternary amine moiety. In some examples, the mixed mode anion exchange chromatography material comprises a solid support. In some examples, the mixed mode anion exchange chromatography material is contained in a column. In some examples, the mixed mode anion exchange chromatography material is a high performance liquid chromatography (HPLC) material. In some examples, the mixed mode anion exchange chromatography material is an Oasis® MAX chromatography material. In some

examples, the non-ionic detergent is quantified by Evaporative Light Scattering (ELSD) or by using a Charged Aerosol Detector (CAD).

[0009] In some aspects, the disclosure provides a method for quantifying a non-ionic surfactant in a composition comprising the non-ionic surfactant and a polypeptide, wherein the method comprises the steps of a) applying the composition to a mixed mode cation exchange chromatography material, wherein the composition is loaded onto the chromatography material in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises ammonium hydroxide in water and mobile phase B comprises ammonium hydroxide in an organic solvent; b) eluting the polypeptide from the mixed mode cation exchange chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a); c) eluting the non-ionic surfactant from the chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step b); d) quantifying the non-ionic surfactant. In some examples, the organic solvent of mobile phase B is methanol. In some examples, the ratio of mobile phase B to mobile phase A in step a) is about 10:90. In some examples, the ratio of mobile phase B to mobile phase A is increased to about 45:55 in step b). In some examples, the ratio of mobile phase B to mobile phase A is increased to about 100:0 in step c). In some examples, mobile phase A comprises about 2% ammonium hydroxide in water. In some examples, mobile phase B comprises about 2% ammonium hydroxide in methanol. In some examples, the flow rate of the chromatography is about 1.4 mL/minute. In some examples, step b) starts at about 1 min after the chromatography is initiated and ends at about 4.4 min after the chromatography is initiated. In some examples, step c) starts at about 4.5 min after the chromatography is initiated and ends at about 7.6 min after the chromatography is initiated. In some examples, the non-ionic surfactant is a polysorbate. In some examples, the polysorbate is polysorbate 20 or polysorbate 80. In some examples, the concentration of polysorbate in the composition is in the range of about 0.001% to 1.0% (w/v). In some examples, the organic solvent of mobile phase B is acetonitrile. In some examples, the ratio of mobile phase B to mobile phase A in step a) is about 10:90. In some examples, the ratio of mobile phase B to mobile phase A is increased to about 40:60 is step b). In some examples, the ratio of mobile phase B to mobile phase A is increased to 100:0 is step c). In some examples, mobile phase A comprises about 2% ammonium hydroxide in

water or in 43% methanol. In some examples, mobile phase B comprises about 2% ammonium hydroxide in acetonitrile. In some examples, the non-ionic surfactant is a poloxamer. In some examples, the poloxamer is poloxamer P188. In some examples, the concentration of poloxamer in the composition is in the range of about 0.001% to 1.0% (w/v). In some examples, the composition further comprises N-acetyl tryptophan and/or methionine. In some examples, the concentration of N-acetyl tryptophan in the composition ranges from about 0.1 mM to about 10 mM. In some examples, the concentration of methionine in the composition ranges from about 0.1 mM to about 100 mM. In some examples, the polypeptide concentration in the composition is about 1 mg/mL to about 250 mg/mL. In some examples, the formulation has a pH of about 4.5 to about 7.5. In some examples, the composition further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, and a tonicity agent. In some examples, the composition is a pharmaceutical formulation suitable for administration to a subject. In some examples, the polypeptide is a therapeutic polypeptide. In some examples, the therapeutic polypeptide is a fusion protein, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, glycoengineered antibody, antibody fragment, an antibody drug conjugate, a THIOMAB™, a THIOMAB™ drug conjugate. In some examples, the mixed mode cation exchange chromatography material comprises a reversed phase, strong cation exchange polymer. In some examples, the mixed mode cation exchange chromatography material comprises a sulfonic acid moiety. In some examples, the mixed mode cation exchange chromatography material comprises a solid support. In some examples, the mixed mode cation exchange chromatography material is contained in a column. In some examples, the mixed mode cation exchange chromatography material is a high performance liquid chromatography (HPLC) material. In some examples, the mixed mode cation exchange chromatography material is an Oasis® MCX chromatography material. In some examples, the non-ionic detergent is quantified by Evaporative Light Scattering (ELSD) or by using a Charged Aerosol Detector (CAD).

BRIEF DESCRIPTION OF THE DRAWINGS

[0010] FIG. 1 is an overlay of PS20-free A1 ADC and a 0.6 mg/ml PS20 standard using the multi-step gradient of increasing 5% methanol. Elution solvent contains 2% formic acid.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for quantifying a non-ionic surfactant in a composition comprising the non-ionic surfactant and a polypeptide, wherein interference between the non-ionic surfactant and the polypeptide during quantification is reduced, wherein the method comprises the steps of

a) applying the composition to a mixed mode anion exchange chromatography material, wherein the composition is loaded onto the chromatography material in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises acid in water and mobile phase B comprises acid in methanol, wherein the polypeptide binds to the chromatography material specifically and non-specifically;

b) eluting the specifically bound polypeptide from the mixed mode anion exchange chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a);

c) eluting the non-ionic surfactant and the non-specifically bound polypeptide from the chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step b);

d) quantifying the non-ionic surfactant, wherein interference between the non-ionic surfactant and the polypeptide during quantitation is reduced.

2. The method of claim 1, wherein the ratio of mobile phase B to mobile phase A in step a) is 10:90.

3. The method of claim 1 or 2, wherein the ratio of mobile phase B to mobile phase A is increased to 40:60 in step b).

4. The method of any one of claims 1-3, wherein the ratio of mobile phase B to mobile phase A is increased to 100:0 in step c).

5. The method of any one of claims 1-4, wherein mobile phase A comprises about 2% acid in water.

6. The method of any one of claims 1-5, wherein mobile phase B comprises about 2% acid in methanol.

7. The method of any one of claims 1-6, wherein the acid is formic acid.

8. The method of any one of claims 1-6, wherein the acid is acetic acid.

9. The method of any one of claims 1-8, wherein the flow rate of the chromatography is about 1.25 mL/minute.

10. The method of claim 9, wherein step b) starts at about 1 min after the chromatography is initiated and ends at about 3.4 min after the chromatography is initiated.

11. The method of claim 9 or 10, wherein step c) starts at about 3.5 min after the chromatography is initiated and ends at about 4.6 min after the chromatography is initiated.

12. The method of any one of claims 1-11, wherein the non-ionic surfactant is poloxamer (P188) or a polysorbate.

13. The method of claim 12, wherein the polysorbate is polysorbate 20 or polysorbate 80.

14. The method of any one of claims 1-13, wherein the concentration of non-ionic surfactant in the composition is in the range of 0.001% to 1.0% (w/v).

15. The method of any one of claims 1-14, wherein the protein concentration in the composition is 1 mg/mL to 250 mg/mL.

16. The method of any one of claims 1-15, wherein the formulation has a pH of 4.5 to 7.5.

17. The method of any one of claims 1-16, wherein the composition further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, and a tonicity agent.

18. The method of any one of claims 1-17, wherein the composition is a pharmaceutical formulation suitable for administration to a subject.

19. The method of any one of claims 1-18, wherein the polypeptide is a therapeutic polypeptide.

20. The method of claim 16, wherein the therapeutic polypeptide is a fusion protein, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a chimeric antibody, a multispecific antibody, a glycoengineered antibody, an antibody fragment, or an antibody drug conjugate.

21. The method of any one of claims 1-20, wherein the mixed mode anion exchange chromatography material comprises a reversed phase, strong anion exchange polymer.

22. The method of any one of claims 1-21, wherein the mixed mode anion exchange chromatography material comprises a quaternary amine moiety.

23. The method of any one of claims 1-22, wherein the mixed mode anion exchange chromatography material comprises a solid support.

24. The method of any one of claims 1-23, wherein the mixed mode anion exchange chromatography material is contained in a column.

25. The method of any one of claims 1-24, wherein the mixed mode anion exchange chromatography material is a high performance liquid chromatography (HPLC) material.

26. The method of any one of claims 1-25, wherein the non-ionic surfactant is quantified by Evaporative Light Scattering (ELSD) or by using a Charged Aerosol Detector (CAD).

27. A method for quantifying a non-ionic surfactant in a composition comprising the non-ionic surfactant and a polypeptide, wherein the method comprises the steps of

a) applying the composition to a mixed mode cation exchange chromatography material, wherein the composition is loaded onto the chromatography material in a solution comprising a mobile phase A and a mobile phase B, wherein mobile phase A comprises ammonium hydroxide in water and mobile phase B comprises ammonium hydroxide in an organic solvent;

b) eluting the polypeptide from the mixed mode cation exchange chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step a);

c) eluting the non-ionic surfactant from the chromatography material with a solution comprising mobile phase A and mobile phase B wherein the ratio of mobile phase B to mobile phase A is increased compared to step b);

d) quantifying the non-ionic surfactant.

28. The method of claim 27, wherein the organic solvent of mobile phase B is methanol.

29. The method of claim 27 or 28, wherein the ratio of mobile phase B to mobile phase A in step a) is 10:90.

30. The method of any one of claims 27-29, wherein the ratio of mobile phase B to mobile phase A is increased to 45:55 in step b).

31. The method of any one of claims 27-30, wherein the ratio of mobile phase B to mobile phase A is increased to 100:0 in step c).

32. The method of any one of claims 27-31, wherein mobile phase A comprises about 2% ammonium hydroxide in water.

33. The method of any one of claims 27-32, wherein mobile phase B comprises about 2% ammonium hydroxide in methanol.

34. The method of any one of claims 27-33, wherein the flow rate of the chromatography is about 1.4 mL/minute.

35. The method of claim 34, wherein step b) starts at about 1 min after the chromatography is initiated and ends at about 4.4 min after the chromatography is initiated.

36. The method of claim 34 or 35, wherein step c) starts at about 4.5 min after the chromatography is initiated and ends at about 7.6 min after the chromatography is initiated.

37. The method of any one of claims 27-36, wherein the non-ionic surfactant is a polysorbate.

38. The method of claim 37, wherein the polysorbate is polysorbate 20 or polysorbate 80.

39 The method of claim 37 or 38, wherein the concentration of polysorbate in the composition is in the range of 0.001% to 1.0% (w/v).

40. The method of claim 27, wherein the organic solvent of mobile phase B is acetonitrile.

41. The method of claim 40, wherein the ratio of mobile phase B to mobile phase A in step a) is 10:90.

42. The method of claim 40 or 41, wherein the ratio of mobile phase B to mobile phase A is increased to 40:60 is step b).

43. The method of any one of claims 40-42, wherein the ratio of mobile phase B to mobile phase A is increased to 100:0 is step c).

44. The method of any one of claims 40-43, wherein mobile phase A comprises about 2% ammonium hydroxide in water or in 43% methanol.

45. The method of any one of claims 40-44, wherein mobile phase B comprises about 2% ammonium hydroxide in acetonitrile.

46. The method of any one of claims 40-45, wherein the non-ionic surfactant is a poloxamer.

47. The method of claim 46, wherein the poloxamer is poloxamer P188.

48. The method of claim 46 or 47, wherein the concentration of poloxamer in the composition is in the range of 0.001% to 1.0% (w/v).

49. The method of any one of claims 27-48, wherein the composition further comprises N-acetyl tryptophan and/or methionine.

50. The method of claim 49, wherein the concentration of N-acetyl tryptophan in the composition ranges from 0.1 mM to 10 mM.

51. The method of claim 49, wherein the concentration of methionine in the composition ranges from 0.1 mM to 100 mM.

52. The method of any one of claims 27-51, wherein the polypeptide concentration in the composition is 1 mg/mL to 250 mg/mL.

53. The method of any one of claims 27-52, wherein the formulation has a pH of 4.5 to 7.5.

54. The method of any one of claims 27-53, wherein the composition further comprises one or more excipients selected from the group consisting of a stabilizer, a buffer, and a tonicity agent.

55. The method of any one of claims 27-54, wherein the composition is a pharmaceutical formulation suitable for administration to a subject.

56. The method of any one of claims 27-55, wherein the polypeptide is a therapeutic polypeptide.

57. The method of claim 56, wherein the therapeutic polypeptide is a fusion protein, a polyclonal antibody, a monoclonal antibody, a humanized antibody, a human antibody, a

chimeric antibody, a multispecific antibody, glycoengineered antibody, antibody fragment, or an antibody drug conjugate.

58. The method of any one of claims 27-57, wherein the mixed mode cation exchange chromatography material comprises a reversed phase, strong cation exchange polymer.

59. The method of any one of claims 27-59, wherein the mixed mode cation exchange chromatography material comprises a sulfonic acid moiety.

60. The method of any one of claims 27-59, wherein the mixed mode cation exchange chromatography material comprises a solid support.

61. The method of any one of claims 27-60, wherein the mixed mode cation exchange chromatography material is contained in a column.

62. The method of any one of claims 27-61, wherein the mixed mode cation exchange chromatography material is a high performance liquid chromatography (HPLC) material.

63. The method of any one of claims 27-62, wherein the non-ionic detergent is quantified by Evaporative Light Scattering (ELSD) or by using a Charged Aerosol Detector (CAD).