Summary

[0004] Provided herein are methods for reducing aggregation of bispecific antibodies, in particular, bispecific T cell engager antibodies (BiTEs), that results from storage under frozen conditions. The present application is directed to the surprising findings that holding the bispecific antibodies at certain temperatures for a period of time after thaw reduces aggregates formed during storage under frozen conditions.

[0005] In one aspect, disclosed herein is a method for reducing aggregates of a bispecific antibody comprising holding a thawed bispecific antibody at a temperature of from about 5 ºC to about 45 ºC for at least 4 hours, wherein the bispecific antibody has been stored at a temperature of from about -20 ºC to about -40 ºC before thaw, and wherein the bispecific antibody is in (scFv)2 format. In one example, the thawed bispecific antibody is held at the temperature for a period of from about 4 hours to about 96 hours. In one example, the thawed bispecific antibody is held at a temperature of from about 10 ºC to about 30 ºC for a period of from about 10 hours to about 48 hours. In one example, the thawed bispecific antibody is held at a temperature of from about 10 ºC to about 30 ºC for a period of from 8 hours to 48 hours.

[0006] In one example, the bispecific antibody is thawed at a temperature of from about 5 ºC to about 45 ºC. In one example, the bispecific antibody has been stored at a temperature of from about -20 ºC to about -35 ºC before thaw, in another example, the bispecific antibody has been stored at about -30 ºC before thaw. In one example, the aggregates comprise high molecule weight (HMW) aggregates. In one example, the HMW aggregates comprise dimers of the bispecific antibody. In one example, the bispecific antibody comprises less than about 1% of the HMW aggregates after the holding period, in another example, the bispecific antibody comprises less than about 0.5% of the HMW aggregates after the holding period.

[0007] In one example~~aspect~~, disclosed herein is a method for preparing a composition comprising a bispecific antibody, the method comprises thawing a drug substance comprising a bispecific antibody that has been stored at a temperature of from about -20 ºC to about -40 ºC, and holding the thawed drug substance comprising the bispecific antibody at a temperature of from about 5 ºC to about 45 ºC for at least 4 hours. In one example, the drug substance is held at the temperature for a period of from about 4 hours to about 96 hours. In another example, the drug substance is held at a temperature of from about 10 °C to about 30 °C for a period of from about 10 hours to about 48 hours. In another example, the drug substance is held at a temperature of from about 10 °C to about 30 °C for a period

of from 8 hours to 48 hours. In one example, the drug substance is thawed at a temperature of from about 5 ºC to about 45 ºC. In one example, the drug substance has been stored at a temperature of from about -20 ºC to about -35 ºC, in another example, the drug substance has been stored at about -30 ºC. In one example, the method for preparing the composition further comprises filtering the drug substance, in another example, the method for preparing the composition further comprises aliquoting the composition into a drug product form. In one example, the composition is a pharmaceutical composition comprising the bispecific antibody.

[0008] In one ~~aspect~~example, disclosed herein is a method for preparing a composition comprising a bispecific antibody, the method comprises holding a thawed drug substance comprising a bispecific antibody at a temperature of from about 5 ºC to about 45 ºC for at least 4 hours, wherein the drug substance has been frozen at or above the glass transition temperature (Tg') of the drug substance before thaw. In one example, the drug substance has been frozen at a temperature of from about -10 ºC to at or above the glass transition temperature of the drug substance, in another example, the drug substance has been frozen at about -32 ºC. In one example, the drug substance is held at the temperature for a period of from about 4 hours to about 96 hours. In one example, the drug substance is thawed at a temperature of from about 5 ºC to about 45 ºC. In another example, the drug substance is held at the same temperature as the temperature at which the drug substance is thawed. In another example, the drug substance is thawed and held at the same temperature of from about 15 °C to about 30 °C for a period of from 30 hours to 50 hours. In another example, the method for preparing the composition further comprises aliquoting the composition into a drug product form. In another example, the method for preparing the composition further comprises lyophilizing the composition. In yet another example, the method for preparing the composition further comprises spray drying the composition.

[0009] In one example, the drug substance comprises less than about 1% of HMW aggregates after the holding period, in another example, the drug substance comprises less than about 0.5% of the HMW aggregates. In one example, the HMW aggregates comprise dimers of the bispecific antibody.

[0010] In one example, the drug substance comprising the bispecific antibody at a concentration of from about 0.05 mg/mL to about 20 mg/mL.

[0011] In one example, the bispecific antibody is a bispecific T cell engager antibody (BiTE). In one example, the bispecific antibody comprises a first binding domain that binds to a target cell surface antigen, and a second binding domain that binds to human CD3, and wherein the bispecific antibody is in (scFv)2 format. In one example, the bispecific antibody comprises a first binding domain that binds to a target cell surface antigen selected from CD19, CD33 or BCMA, and a second

What is claimed is:

1. A method for reducing aggregates of a bispecific antibody comprising:
holding a thawed bispecific antibody at a temperature of from 5 ºC to 45 ºC for at least 4 hours, wherein the bispecific antibody has been stored at a temperature of from -20 ºC to -40 ºC before thaw, and wherein the bispecific antibody is in (scFv)2 format.

2. The method of claim 1, wherein the bispecific antibody is a drug substance.

3. The method of claim 1 or 2, wherein the bispecific antibody is held at the temperature for a period of from 4 hours to 96 hours.

4. The method of any one of claims 1-3, wherein the bispecific antibody is held at a temperature of from 10 ºC to 30 ºC for a period of from 8 hours to 48 hours.

5. The method of any one of claims 1-4, wherein the bispecific antibody is thawed at a temperature of from 5 ºC to 45 ºC.

6. The method of any one of claims 1-5, wherein the bispecific antibody has been stored at a temperature of from -20 ºC to -35 ºC.

7. The method of any one of claims 1-6, wherein the bispecific antibody has been stored at about -30 ºC.

8. The method of any one of claims 1-7, wherein the aggregates comprise high molecule weight (HMW) aggregates.

9. The method of any one of claims 1-8, wherein the bispecific antibody comprises less than ~~about~~ 1% of the HMW aggregates after the holding period.

10. The method of claim 9, wherein the bispecific antibody comprises less than ~~about~~ 0.5% of the HMW aggregates.

11. The method of any one of claims 1-10, wherein the HMW aggregates comprise dimers of the bispecific antibody.

12. ~~A~~ The method of any one of claims 2-11, wherein ~~for preparing a composition comprising a bispecific antibody,~~ the method comprises

thawing ~~a~~ the drug substance comprising a bispecific antibody ~~that has been stored at a temperature of from -20 ºC to -40 ºC, and~~

~~holding the thawed drug substance comprising the bispecific antibody at a temperature of from 5 ºC to 45 ºC for at least 4 hours~~.

~~13. The method of claim 12, wherein the composition is a pharmaceutical composition comprising the bispecific antibody.~~

~~14.~~13. The method of claim 12 ~~or 13~~ further comprising filtering the drug substance.

~~15.~~14. The method of ~~any one of claims 12-14~~claim 12 or claim 13 further comprising aliquoting the composition into a drug product form.

~~16.~~15. The method of any one of claims 12-~~15~~14, wherein the drug substance is held at the temperature for a period of from 4 hours to 96 hours.

~~17.~~16. The method of any one of claims 12-~~16~~15, wherein the drug substance is held at a temperature of from 10 °C to 30 °C for a period of from 8 hours to 48 hours.

~~18.~~17. The method of any one of claims 12-~~17~~16, wherein the drug substance is thawed at a temperature of from 5 ºC to 45 ºC.

~~19.~~18. The method of any one of claims 12-~~18~~17, wherein the drug substance has been stored at a temperature of from -20 ºC to -35 ºC.

~~20.~~19. The method of any one of claims 12-~~19~~18, wherein the drug substance has been stored at about -30 ºC.

~~21.~~20. ~~A~~ The method of any one of claims 2-19, ~~for preparing a composition comprising a bispecific antibody, the method comprises holding a thawed drug substance comprising a bispecific antibody at a temperature of from 5 ºC to 45 ºC for at least 4 hours,~~ wherein the drug substance has been frozen at or above the glass transition temperature (Tg') of the drug substance before thaw.

~~22.~~21. The method of claim ~~21~~20, wherein the drug substance has been frozen at a temperature of from -10 ºC to at or above the Tg' of the drug substance before thaw.

~~23.~~22. The method of claim ~~21~~ 20 or ~~22~~21, wherein the drug substance has been frozen at about -32 ºC.

~~24.~~23. The method of any one of claims ~~21~~20-~~23~~22, wherein the drug substance is held at the temperature for a period of from 4 hours to 96 hours.

~~25.~~24. The method of any one of claims ~~21~~20-~~24~~23, wherein the drug substance is thawed at a temperature of from 5 ºC to 45 ºC.

~~26.~~25. The method of any one of claims ~~21~~20-~~25~~24, wherein the drug substance is held at the same temperature as the temperature at which the drug substance is thawed.

~~27.~~26. The method of claim ~~26~~25, wherein the drug substance is thawed and held at a temperature of from 15 °C to 30 °C for a period of from 30 hours to 50 hours.

~~28.~~27. The method of any one of claims ~~21~~20-~~27~~ 26 further comprising aliquoting the composition into a drug product form.

~~29.~~28. The method of any one of claims 12-~~28~~27, wherein the drug substance comprises less than ~~about~~ 1% of HMW aggregates after the holding period.

~~30.~~29. The method of claim ~~29~~28, wherein the drug substance comprises less than ~~about~~ 0.5% of the HMW aggregates.

~~31.~~30. The method of claim ~~29~~ 28 or ~~30~~29, wherein the HMW aggregates comprise dimers of the bispecific antibody.

~~32.~~31. The method of any one of claims 1-~~31~~30, wherein ~~the drug substance comprising~~ the bispecific antibody is at a concentration of from ~~about~~ 0.05 mg/mL to ~~about~~ 20 mg/mL.

~~33.~~32. The method of any one of claims 12-~~32~~ 31 further comprising lyophilizing the composition after the hold step.

~~34.~~33. The method of any one of claims 12-~~32~~ 31 further comprising spray drying the composition after the hold step.

~~35.~~34. The method of any one of claims 1-~~34~~33, wherein the bispecific antibody comprises

a first binding domain that binds to a target cell surface antigen, and

a second binding domain that binds to human CD3~~, and wherein the bispecific antibody is in $(scFv)_2$ format~~.

~~36.~~35. The method of claim ~~35~~34, wherein the target cell surface antigen is CD19, CD33 or BCMA.

~~37.~~36. The method of claim ~~36~~35, wherein the first binding domain comprises a VH region and a VL region, wherein:

the VH comprises the amino acid sequence of SEQ ID NO: 77 and the VL comprises the amino acid sequence of SEQ ID NO: 78;

the VH comprises the amino acid sequence of SEQ ID NO: 28 and the VL comprises the amino acid sequence of SEQ ID NO: 32 or 33; or

the VH comprises the amino acid sequence of SEQ ID NO: 132 and the VL comprises the amino acid sequence of SEQ ID NO: 133.

~~38.~~37. The method of claim ~~37~~36, wherein the bispecific antibody comprises the amino acid sequence of SEQ ID NO: 17, 40 or 135.

~~39.~~38. The method of any one of claims 1-~~38~~37, wherein the bispecific antibody further comprises a third domain comprising two polypeptide monomers, each comprises a hinge, a CH2 and a CH3 domain, wherein the two polypeptide monomers are linked to each other via a peptide linker.

40.39. The method of claim 3938, wherein the third domain comprises in an amino to carboxyl order hinge-CH2-CH3-linker-hinge-CH2-CH3.

41.40. The method of claim 4039, wherein the third domain is a HLE domain.

42.41. The method of any one of claims 3938-4140, wherein the first binding domain binds to at least one target cell surface antigen selected from CD19, CD33, EGFRvIII, MSLN, CDH19, FLT3, DLL3, CDH3, CD70, BCMA or PSMA.

43.42. The method of claim 4241, wherein the first binding domain comprises a VH region and a VL region, wherein

the VH comprises the amino acid sequence of SEQ ID NO: 108 and the VL comprises the amino acid sequence of SEQ ID NO: 109;

the VH comprises the amino acid sequence of SEQ ID NO: 27 and the VL comprises the amino acid sequence of SEQ ID NO: 32;

the VH comprises the amino acid sequence of SEQ ID NO: 48 and the VL comprises the amino acid sequence of SEQ ID NO: 49;

the VH comprises the amino acid sequence of SEQ ID NO: 59 and the VL comprises the amino acid sequence of SEQ ID NO: 60;

the VH comprises the amino acid sequence of SEQ ID NO: 77 and the VL comprises the amino acid sequence of SEQ ID NO: 78;

the VH comprises the amino acid sequence of SEQ ID NO: 108 and the VL comprises the amino acid sequence of SEQ ID NO: 112;

the VH comprises the amino acid sequence of SEQ ID NO: 89 and the VL comprises the amino acid sequence of SEQ ID NO: 90;

the VH comprises the amino acid sequence of SEQ ID NO: 100 and the VL comprises the amino acid sequence of SEQ ID NO: 101;

the VH comprises the amino acid sequence of SEQ ID NO: 121 and the VL comprises the amino acid sequence of SEQ ID NO: 122;

the VH comprises the amino acid sequence of SEQ ID NO: 188 and the VL comprises the amino acid sequence of SEQ ID NO: 189;

the VH comprises the amino acid sequence of SEQ ID NO: 132 and the VL comprises the amino acid sequence of SEQ ID NO: 133; or

the VH comprises the amino acid sequence of SEQ ID NO: 173 and the VL comprises the amino acid sequence of SEQ ID NO: 174.

~~44.~~43. The method of claim ~~43~~42, wherein the bispecific antibody comprises the amino acid sequence of SEQ ID NO: 63, 114, 41, 82, 136, 104, 93, 177, 125, 190 or 52.

~~45.~~44. Then method of claim ~~44~~43, wherein the bispecific antibody consisting of the amino acid sequence of SEQ ID NO: 63, 114, 41, 82, 136, 104, 93, 177, 125, 190 or 52.

~~46.~~45. The method of any one of claims 1-~~34~~33, wherein the bispecific antibody is a bispecific masked antigen binding protein.

~~47.~~46. The method of claim ~~46~~45, wherein the bispecific masked antigen binding protein comprises:

(a) a first antibody or antigen binding fragment thereof (AB1) that binds to a first antigen and a masking domain (MD1) coupled to AB1, wherein the MD1 comprises

(1) a first masking peptide (MP1) that inhibits or reduces the binding of AB1 to its antigen, and
(2) a protein recognition site (PR1), wherein binding to or cleavage of the PR1 by a protein or a protease increases AB1 binding to its antigen;

(b) a second antibody or antigen binding fragment there of (AB2) that binds to a second antigen, and a second masking domain (MD2) coupled to AB2, wherein the MD2 comprises:

(1) a second masking peptide (MPs) that inhibits or reduces the binding of AB2 to its antigen, and
(2) a second protein recognition site (PR2), wherein binding to or cleavage of the PR2 by a protein or a protease increases AB2 binding to its antigen.

~~48.~~47. The method of claim ~~46~~ 45 or ~~47~~46, wherein the PR1 and PR2 comprise the same protein recognition sequence.

~~49.~~48. The method of any one of claims ~~46~~45-~~48~~47, wherein the AB1 binds to human CD3 and the AB2 binds to human EGFR.