

multispecific antibody is constructed as a single-chain Fab (scFab), a 5/4 antibody by-product (comprising an additional heavy or light chain variable domain) may be observed. Such corresponding product-specific impurities would not arise in standard antibody production.

[0006] Conventional purification techniques designed to remove process-related impurities such as HCPs, DNA, endotoxins, and other materials that have very different characteristics and properties from the antibodies can be inadequate when implemented to remove impurities that are more similar to the multispecific antibodies. As such, there is a need to develop manufacturing and purification schemes that effectively remove product-specific impurities and yield sufficient amount of the correct and complete multispecific antibody.

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

SUMMARY

[0008] As described and exemplified herein, Applicants have discovered that the use of at least two mixed mode (also referred to herein as multi-modal or multimodal) chromatography steps after an initial capture chromatography step results in greater removal of product-specific impurities and an improved process for purifying multispecific antibodies. Thus, in one aspect, provided is a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and an impurity, wherein the multispecific antibody comprises multiple arms, each arm comprising a VH/VL unit, the method comprising the sequential steps of: a) subjecting the composition to a capture chromatography to produce a capture chromatography eluate; b) subjecting the capture chromatography eluate to a first mixed mode chromatography to generate a first mixed mode eluate; c) subjecting the first mixed mode eluate to a second mixed mode chromatography to generate a second mixed mode eluate; and d) collecting a fraction comprising the multispecific antibody, wherein the method reduces the amount of a product-specific impurity from the composition. In some embodiments according to (or as applied to) any of the embodiments above, the capture chromatography eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography prior to the first mixed mode chromatography. In some embodiments according to (or as applied to) any of the embodiments above, the second mixed mode eluate is subjected to an ion exchange chromatography (*e.g.*, anion exchange) or to hydrophobic interaction chromatography.

[0009] In another aspect, provided is a method for purifying a multispecific antibody from a composition comprising the multispecific antibody and an impurity, wherein the multispecific antibody comprises multiple arms, each arm comprising a VH/VL unit, wherein each arm of the multispecific antibody is produced separately, the method comprising the sequential steps of a) subjecting each arm of the multispecific antibody to capture chromatography to produce capture eluates for each arm of the multispecific antibody, b) forming a mixture comprising capture eluates of each arm of the multispecific antibody under conditions sufficient to produce a composition comprising the multispecific antibody, c) subjecting the composition comprising the multispecific antibody to a first mixed mode chromatography to generate a first mixed mode eluate, and d) subjecting the first mixed mode eluate to a second mixed

bispecific antibody. In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody is a CrossMab bispecific antibody.

[0026] In some embodiments according to (or as applied to) any of the methods above, a fraction is collected after the last chromatography step, comprising at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% multispecific antibody. In some embodiments according to (or as applied to) any of the embodiments above, a fraction is collected after the last chromatography step, comprising a reduced amount of a product-specific impurity, wherein the product-specific impurity is one or more of: non-paired antibody arms, antibody homodimers, high molecular weight species (HMWS), low molecular weight species (LMWS), or $\frac{3}{4}$ antibodies. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% non-paired antibody arms. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains less than about 5%, less than about 4%, less than about 3%, less than about 2%, or less than about 1% antibody homodimers. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains no more than about 1% or no more than about 2% HMWS. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains no more than about 2% or no more than about 1% LMWS. In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains no more than about 5%, no more than about 4%, no more than about 3%, no more than about 2%, or no more than about 1% of $\frac{3}{4}$ antibodies.

[0027] In some embodiments according to (or as applied to) any of the embodiments above, the fraction contains

- a) at least about 95% - 100% multispecific antibody;
- b) less than about 1%- 5% non-paired antibody arms;
- c) less than about 1%- 5% antibody homodimers;
- d) no more than about 1% or 2% HMWS;
- e) no more than about 1% or 2% LMWS; and/or
- f) no more than about 5% of $\frac{3}{4}$ antibodies.

[0028] In one aspect, provided is a composition comprising a multispecific antibody purified by the method of any one of the methods described above.

[0029] In some embodiments according to (or as applied to) any of the methods above, provided is a composition comprising a multispecific antibody, wherein the composition comprises at least about 95%, at least about 96%, at least about 97%, at least about 98%, at least about 99%, or about 100% multispecific antibody. In some embodiments according to (or as applied to) any of the embodiments above, provided is a composition comprising a multispecific antibody, wherein the composition comprises a reduced amount of a product-specific impurity, wherein the product-specific impurity is one or more of: non-paired antibody arms, antibody homodimers, high molecular weight species (HMWS), low molecular weight species (LMWS), or $\frac{3}{4}$ antibodies. In some embodiments according to (or as

that binds ANG2 and VEGF) purified by any one of the methods described above for the treatment of cancer or eye disease. In one aspect, provided is a composition comprising a multispecific antibody purified by the method as described above for the treatment of cancer or eye disease.

[0032] In some embodiments according to (or as applied to) any of the embodiments above, provided is the use of a comprising a multispecific or bispecific antibody (such as a bispecific antibody that binds ANG2 and VEGF) purified by any one of the methods described above for the manufacture of a medicament for the treatment of cancer or eye disease.

[0033] In some embodiments according to (or as applied to) any of the embodiments above, the methods provided herein are used for the purification of an Fc-containing heterodimeric polypeptide.

[0034] In some embodiments according to (or as applied to) any of the embodiments above, provided is the use of the any of the methods provided herein for the reduction of Fc-containing heterodimeric polypeptide-related impurities in a composition.

[0035] In one aspect, provided is a method for purifying an Fc-region containing heterodimeric polypeptide with a multi-step chromatography method wherein the method comprises an affinity chromatography step followed by two different multimodal ion exchange chromatography steps, and thereby purifying the Fc-region containing heterodimeric polypeptide. In some embodiments according to (or as applied to) any of the embodiments above, the multi-step chromatography method comprises (i) an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step; or (ii) an affinity chromatography step, followed by a multimodal cation exchange chromatography step, followed by a multimodal anion exchange chromatography step.

[0036] In some embodiments according to (or as applied to) any of the embodiments above, the multi-step chromatography method comprises an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step. In some embodiments according to (or as applied to) any of the embodiments above, the multi-step chromatography method comprises exactly three chromatography steps. In some embodiments according to (or as applied to) any of the embodiments above, the multimodal anion exchange chromatography step is performed in flow-through mode. In some embodiments according to (or as applied to) any of the embodiments above, the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value of less than 7 mS/cm. In some embodiments according to (or as applied to) any of the embodiments above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value in the range of about 6 mS/cm to about 2 mS/cm. In some embodiments according to (or as applied to) any of the embodiments above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide is applied in a solution with a conductivity value of about 4.5 mS/cm. In some embodiments according to (or as applied to) any of the embodiments above, the multimodal anion exchange chromatography step is performed at a pH of about 7. In some embodiments according to (or as applied to) any of the embodiments above, in the multimodal anion exchange chromatography step the Fc-region containing heterodimeric polypeptide

(or as applied to) any of the embodiments above, the bispecific antibody is vanucizumab. In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 1, and as light chain variable domain (VL) the SEQ ID NO: 2; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 3, and as light chain variable domain (VL) the SEQ ID NO: 4. In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody comprises a first heavy chain with the amino acid sequence of SEQ ID NO: 9 and a second heavy chain with the amino acid sequence of SEQ ID NO: 10 and a first light chain with the amino acid sequence of SEQ ID NO: 11 and a second light chain with the amino acid sequence of SEQ ID NO: 12.

[0040] In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 5, and as light chain variable domain (VL) the SEQ ID NO: 6; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 7, and as light chain variable domain (VL) the SEQ ID NO: 8. In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody comprises a first heavy chain with the amino acid sequence of SEQ ID NO: 13 and a second heavy chain with the amino acid sequence of SEQ ID NO: 14 and a first light chain with the amino acid sequence of SEQ ID NO: 15 and a second light chain with the amino acid sequence of SEQ ID NO: 16.

[0041] In some embodiments according to (or as applied to) any of the embodiments above, the purified Fc-region containing heterodimeric polypeptide contains no more than about 5% of $\frac{3}{4}$ antibodies.

[0042] In one aspect, provided is a method for purifying a bispecific antibody that binds to ANG2 and VEGF with a multi-step chromatography method wherein the method comprises an affinity chromatography step, followed by a multimodal anion exchange chromatography step, followed by a multimodal cation exchange chromatography step, and thereby purifying the bispecific antibody that binds to ANG2 and VEGF, wherein bispecific antibody that binds to ANG2 and VEGF comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 1, and as light chain variable domain (VL) the SEQ ID NO: 2; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 3, and as light chain variable domain (VL) the SEQ ID NO: 4 or that comprises a first antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 5, and as light chain variable domain (VL) the SEQ ID NO: 6; and a second antigen-binding site that comprises as heavy chain variable domain (VH) the SEQ ID NO: 7, and as light chain variable domain (VL) the SEQ ID NO: 8. In some embodiments according to (or as applied to) any of the embodiments above, the bispecific antibody that binds to ANG2 and VEGF comprises a) the heavy chain and the light chain of a first full length antibody that comprises the first antigen-binding site; and b) the modified heavy chain and modified light chain of a full length antibody that comprises the second antigen-binding site, wherein the constant domains CL and CH1 are replaced by each other.

[0042a] In one aspect, provided is a composition comprising a bispecific antibody, wherein the composition contains at least about 95%, bispecific antibody.

[0042b] In one aspect, provided is a composition comprising a CrossMab antibody, wherein the composition contains at least 95% CrossMab antibody.

[0042c] In one aspect, provided is a composition comprising a bispecific antibody that binds to ANG-2 and VEGF, wherein the composition contains no more than about 5% , about 4%, about 3%, about 2%, or about 1% of $\frac{3}{4}$ antibodies.

[0043] In one aspect, provided is the use of any method according to (or as applied to) any of the aspects or embodiments above for the reduction of Fc-containing heterodimeric polypeptide related impurities.

[0044] In one aspect, provided is an Fc-containing heterodimeric polypeptide obtained with the method according to (or as applied to) any of the aspects or embodiments above, or a bispecific antibody that binds to ANG-2 and VEGF obtained using the method according to (or as applied to) any of the aspects or embodiments above for the manufacture of a medicament for the treatment of cancer or eye disease.

[0045] In one aspect, provided is an Fc-containing heterodimeric polypeptide obtained from the method according to (or as applied to) any of the aspects or embodiments above or a bispecific antibody that binds to ANG-2 and VEGF obtained using the method according to (or as applied to) any of the aspects or embodiments above for use in the treatment of cancer or eye disease.

[0046] In one aspect, provided is a method for producing an Fc-containing heterodimeric polypeptide comprising the steps of (i) cultivating a cell comprising a nucleic acid encoding an Fc-containing heterodimeric polypeptide; (ii) recovering the Fc-containing heterodimeric protein from the cell or the cultivation medium; (iii) purifying the Fc-containing heterodimeric polypeptide using a method according to (or as applied to) any of the aspects or embodiments above, and thereby producing the Fc-containing heterodimeric polypeptide.

[0047] In certain embodiments, provided is a method of producing a bispecific antibody that binds to ANG-2 and VEGF obtained using the method according to any of the aspects or embodiments above comprising the steps of: (i) cultivating a cell comprising a nucleic acid encoding the bispecific antibody; (ii) recovering the bispecific antibody from the cell or the cultivation medium; (iii) purifying the bispecific antibody using a method according to (or as applied to) any of the aspects or embodiments above, and thereby producing the bispecific antibody that binds to ANG-2 and VEGF.

BRIEF DESCRIPTION OF THE DRAWINGS

[0048] **FIG. 1A** depicts a first purification scheme used in Example 1.

[0049] **FIG. 1B** depicts a second purification scheme used in Example 1.

[0050] **FIG. 1C** depicts a third purification scheme used in Example 1.

[0051] **FIG. 2** depicts a purification scheme used in Example 2.

[0052] **FIG. 3A** depicts a first purification scheme used in Example 3.

[0053] **FIG. 3B** depicts a second purification scheme used in Example 3.

[0054] **FIG. 4** depicts a purification scheme used in Example 4.

[0055] **FIG. 5A** depicts a first purification scheme used in Example 6.

[0056] **FIG. 5B** depicts a second purification scheme used in Example 6.

[0057] **FIG. 6A** depicts a first purification scheme used in Example 7.

[0058] **FIG. 6B** depicts a second purification scheme used in Example 7.

[0059] **FIG. 7A** depicts a first purification scheme described in Example 8.

[0060] **FIG. 7B** depicts a second purification scheme described in Example 8.

[0061] **FIG. 7C** depicts a third purification scheme used described in Example 8.