

(a) a composition comprising the protein, and (b) a composition comprising a virus-inactivation reagent, to obtain (c) a treatment composition having a predetermined property for inactivation of a virus; (2) transferring the treatment composition to a treatment vessel; (3) incubating the treatment composition in the treatment vessel at predetermined conditions; and (4) subjecting the treatment composition to a post-treatment processing operation; wherein: (i) the single predetermined volumetric ratio has been selected to ensure that the predetermined property of the treatment composition is maintained across a range of concentrations predicted for the protein in the composition comprising the protein; and (ii) steps (1) to (3) are carried out (a) continuously and (b) without feedback control being exerted by either adjustment of the volumetric ratio or diversion of the treatment composition, wherein the predetermined property comprises at least one of (a) a pH between 3.0 to 3.8, wherein the composition comprising the virus-inactivation reagent has a pH that is determined by equation 1 and supporting equations 2 and 3:

Equation 1:

$$\text{Viral Inactivation pH} = \text{Weak Acid pKA} + \text{Log}\left(\frac{M [\text{Sum of Base Species in System}]}{M [\text{Sum of Acid Species in System}]}\right)$$

Equation 2:

$$\text{Sum of Base Species in System} = \left(\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective Mab Charge delta} \right) + \text{Conjugate Base Buffer Species}$$

Equation 3:

$$\begin{aligned} &V_{\text{Viral Inactivation pH}} \\ &= \text{Weak Acid pKA} \\ &+ \text{Log}\left(\frac{M [\text{Initial Weak Acid Conjugate Base} + (\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective MabCharge delta}) + \text{Background Buffer Conjugate Base}]}{M [\text{Initial Weak Acid Conjuage Acid} - (\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective MabCharge delta}) - \text{Background Buffer Conjuage Base}]} \right) \end{aligned}$$

or

(b) a detergent concentration between 0.05% and 10% (v/v) .

[0009] Without wishing to be bound by theory, it is believed that by selecting the single predetermined volumetric ratio to ensure that the predetermined property of the treatment

composition is maintained across a range of concentrations predicted for the protein in the composition including the protein, and by carrying out steps (1) to (3) continuously and without feedback control being exerted by either adjustment of the volumetric ratio or diversion of the treatment composition, inactivation of virus during manufacture of a protein can be carried out in a stable manner without need for confirming that the treatment composition exhibits the predetermined property as inactivation of the virus is being carried out, allowing elimination of a control burden associated with feedback control during the inactivation of the virus.

[0010] There is also provided a method for inactivating one or more viruses in a sample. The method includes continuously mixing the sample with one or more virus-inactivating reagents as the sample flows from a first unit operation to a second unit operation during a process for purifying a target molecule. In accordance with the method, the ratio of the one or more virus-inactivating reagents to the sample is determined independently of, or without, a feedback control mechanism. Accordingly, in another aspect, there is provided a method for inactivating one or more viruses in a sample, wherein the method comprises continuously mixing the sample with one or more virus inactivating reagents as the sample flows from a first unit operation to a second unit operation during a process for purifying a target molecule, wherein the ratio of the one or more virus inactivating reagents to the sample is determined independently of, or without, a feedback control mechanism, wherein the ratio of the one or more virus inactivating reagents to the sample is determined as % v/v by equation 1 and supporting equations 2 and 3:

Equation 1:

$$\text{Viral Inactivation pH} = \text{Weak Acid pKA} + \text{Log}\left(\frac{M [\text{Sum of Base Species in System}]}{M [\text{Sum of Acid Species in System}]}\right)$$

Equation 2:

$$\text{Sum of Base Species in System} = \left(\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective Mab Charge delta} \right) + \text{Conjugate Base Buffer Species}$$

Equation 3:

$$\text{Viral Inactivation pH} = \text{Weak Acid pKA} + \text{Log}\left(\frac{M [\text{Initial Weak Acid Conjugate Base} + (\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective Mab Charge delta}) + \text{Background Buffer Conjugate Base}]}{M [\text{Initial Weak Acid Conjugate Acid} - (\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective Mab Charge delta}) - \text{Background Buffer Conjugate Base}]}\right)$$

[0011] Without wishing to be bound by theory, it also is believed that by continuously mixing a sample with one or more virus-inactivating reagents as the sample flows from a first unit operation to a second unit operation during a process for purifying a target

molecule, in accordance with the principles and approaches disclosed herein, including that the ratio of the one or more virus-inactivating reagents to the sample is determined independently of, or without, a feedback control mechanism, that inactivation of the one or more viruses can be accomplished to an extent sufficient for purposes of manufacturing biopharmaceutical products, also allowing elimination of a control burden associated with feedback control during the inactivation of the one or more viruses.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] These and other features, aspects, and advantages of the claimed methods, apparatuses, and systems are better understood when the following detailed description is read with reference to the accompanying drawings, in which:

[0013] **FIG. 1** is a schematic view of an example treatment vessel **210** for use in a method for continuously inactivating a virus during manufacture of a protein, in which the example treatment vessel **210** has a linear shape;

CLAIMS

1. A method for continuously inactivating a virus during manufacture of a protein, the method comprising the steps of:

- (1) combining, at a single predetermined volumetric ratio,
 - (a) a composition comprising the protein, and
 - (b) a composition comprising a virus-inactivation reagent, to obtain
 - (c) a treatment composition having a predetermined property for inactivation of a virus;

- (2) transferring the treatment composition to a treatment vessel;

- (3) incubating the treatment composition in the treatment vessel at predetermined conditions; and

- (4) subjecting the treatment composition to a post-treatment processing operation; wherein:

- (i) the single predetermined volumetric ratio has been selected to ensure that the predetermined property of the treatment composition is maintained across a range of concentrations predicted for the protein in the composition comprising the protein; and

- (ii) steps (1) to (3) are carried out (a) continuously and (b) without feedback control being exerted by either adjustment of the volumetric ratio or diversion of the treatment composition, wherein the predetermined property comprises at least one of (a) a pH between 3.0 to 3.8, wherein the composition comprising the virus-inactivation reagent has a pH that is determined by equation 1 and supporting equations 2 and 3:

Equation 1:

$$\text{Viral Inactivation pH} = \text{Weak Acid pKA} + \text{Log}\left(\frac{M [\text{Sum of Base Species in System}]}{M [\text{Sum of Acid Species in System}]}\right)$$

Equation 2:

$$\text{Sum of Base Species in System} = \left(\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective Mab Charge delta} \right) + \text{Conjugate Base Buffer Species}$$

Equation 3:

Viral Inactivation pH
= *Weak Acid pKa*

$$+ \text{Log}\left(\frac{M [\text{Initial Weak Acid Conjugate Base} + (\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective MabCharge delta}) + \text{Background Buffer Conjugate Base}]}{M [\text{Initial Weak Acid Conjugate Acid} - (\text{Mab} \frac{\text{mol}}{\text{kg}} * \text{effective MabCharge delta}) - \text{Background Buffer Conjugate Base}]}\right)$$

or

(b) a detergent concentration between 0.05% and 10% (v/v).

2. The method of claim 1, wherein a constant % v/v addition of the composition comprising the virus-inactivation reagent to the composition comprising the protein is banded within a pH range of a target pH with upper and lower bands of +/- 0.1 pH.

3. The method of claims 1 or 2, wherein the virus-inactivation reagent comprises at least one of:

(a) an acid having a titratable group having a pKa between 2.3 to 4.2 and not having another titratable group having a pKa between 4.2 and 8.5;

(b) acetic acid; or

(c) a non-ionic detergent having a chromophoric group having an absorption peak between 230 nm and 600 nm.

4. The method of any one of claims 1-3, wherein:

the acid having a titratable group having a pKa between 2.3 to 4.2 and not having another titratable group having a pKa between 4.2 and 8.5 selected from the group consisting of an organic acid having a titratable group having a pKa between 2.3 to 4.2 and not having another titratable group having a pKa between 4.2 and 8.5, lactic acid, formic acid, ascorbic acid, an amino acid having a titratable group having a pKa between 2.3 to 4.2 and not having another titratable group having a pKa between 4.2 and 8.5, glycine, or a combination thereof; and the predetermined property comprises a pH between 3.0 to 3.8.

5. The method of claim 4, wherein the predetermined property comprises a pH between 3.3 to 3.7.

6. The method of claim 4, wherein the predetermined property comprises a pH between 3.4 to 3.6.
7. The method of claims 1 or 2, wherein:
the virus-inactivation reagent is acetic acid; and
the predetermined property comprises a pH between 3.0 to 3.8.
8. The method of claim 7, wherein the predetermined property comprises a pH between 3.3 to 3.7.
9. The method of claim 7, wherein the predetermined property comprises a pH between 3.4 to 3.6.
10. The method of claims 1 or 2, wherein:
the virus-inactivation reagent is a non-ionic detergent having a chromophoric group having an absorption peak between 230 nm and 600 nm selected from the group consisting of a polyethylene oxide detergent having an aromatic group, Triton-X 100 detergent, and combinations thereof; and
the predetermined property comprises a detergent concentration between 0.05% and 10% (v/v).
11. The method of any one of claims 1-10, wherein the predetermined conditions are sufficient to cause inactivation of the virus in the treatment composition by the virus-inactivation reagent during step (3) by a factor of at least 1×10^1 .
12. The method of any one of claims 1-11, wherein the predetermined conditions comprise a predetermined temperature between 17 and 40 °C and a predetermined rate of flow of the treatment composition through the treatment vessel that is 0.3 to 3 times an internal volume of the treatment vessel per hour.

13. The method of claim 12, wherein the internal volume of the treatment vessel is sufficiently large to ensure that not more than one part per million of the treatment composition has a residence time in the treatment vessel of a shorter duration than that required to cause inactivation of the virus in the treatment composition by the virus-inactivation reagent during step (3) by a factor of at least 1×10^1 .
14. The method of any one of claims 1-13, wherein steps (1) to (3) are carried out continuously for at least one hour.
15. The method of any one of claims 1-14, wherein step (4) is carried out continuously with steps (1) to (3).
16. The method of any one of claims 1-15, wherein the composition comprising the protein is obtained from a pre-treatment processing operation.
17. The method of claim 16, wherein the pre-treatment processing operation comprises immunoglobulin-binding protein affinity chromatography.
18. The method of any one of claims 1-17, wherein the post-treatment processing operation comprises one or more of anion exchange chromatography, cation exchange chromatography, or passage through viral filtration media.
19. The method of claim 18, wherein the post-treatment processing operation comprises anion exchange chromatography.
20. The method of any one of claims 1-19, wherein the protein comprises an antibody, antibody fragment, or antibody derivative.
21. The method according to claim 20, wherein the antibody, antibody fragment, or antibody derivative is selected from the group consisting of an antibody, a monoclonal antibody, a polyclonal antibody, a mammalian antibody, a murine antibody, a primate antibody, a human

antibody, a chimeric antibody, a primatized antibody, a humanized antibody, an immunoglobulin light chain, an immunoglobulin heavy chain, an immunoglobulin light chain and an immunoglobulin heavy chain, an antibody fragment, an antibody derivative, an Fab fragment, an F(ab')₂ fragment, an Fc fragment, an Fc-Fc fusion protein, an Fv fragment, a single chain Fv fragment, a single domain Fv fragment, a tetravalent single chain Fv fragment, a disulfide-linked Fv fragment, a diabody, a triabody, a tetrabody, a pentabody, a minibody, a miniantibody, an immunoglobulin single variable domain, an immunoglobulin single variable heavy domain, an immunoglobulin single variable light domain, a VHH domain, a humanized VHH domain, a single-domain antibody, a protein comprising an immunoglobulin single variable domain linked together in a modular format with another immunoglobulin single variable domain or a functional domain, a multivalent protein comprising two or more of the same immunoglobulin single variable domain linked together in a modular format, a biparatopic protein comprising two different immunoglobulin single variable domains linked together in a modular format, a bispecific protein comprising two different immunoglobulin single variable domains linked together in a modular format, a bi-functional protein comprising an immunoglobulin single variable domain and a functional domain linked together in a modular format, a domain-deleted antibody, a fusion polypeptide of an antibody fragment with another peptide or polypeptide, an Fc-peptide fusion, an Fc-toxin fusion, and a fusion of an antibody fragment and a scaffold protein.

22. The method of any one of claims 1-21, wherein concentration of the protein in the composition comprising the protein is 10 to 70 mg/mL.

23. A method for inactivating one or more viruses in a sample, wherein the method comprises continuously mixing the sample with one or more virus inactivating reagents as the sample flows from a first unit operation to a second unit operation during a process for purifying a protein, wherein the ratio of the one or more virus inactivating reagents to the sample is determined independently of, or without, a feedback control mechanism, wherein the ratio of the one or more virus inactivating reagents to the sample is determined as % v/v by equation 1 and supporting equations 2 and 3:

Equation 1:

$$\text{Viral Inactivation pH} = \text{Weak Acid pKA} + \text{Log}\left(\frac{M [\text{Sum of Base Species in System}]}{M [\text{Sum of Acid Species in System}]}\right)$$

Equation 2:

$$\text{Sum of Base Species in System} = \left(Mab \frac{\text{mol}}{\text{kg}} * \text{effective Mab Charge delta}\right) + \text{Conjugate Base Buffer Species}$$

Equation 3:

$$\begin{aligned} &\text{Viral Inactivation pH} \\ &= \text{Weak Acid pKA} \\ &+ \text{Log}\left(\frac{M [\text{Initial Weak Acid Conjugate Base} + (Mab \frac{\text{mol}}{\text{kg}} * \text{effective MabCharge delta}) + \text{Background Buffer Conjugate Base}]}{M [\text{Initial Weak Acid Conjuage Acid} - (Mab \frac{\text{mol}}{\text{kg}} * \text{effective MabCharge delta}) - \text{Background Buffer Conjuage Base}]}\right) \end{aligned}$$

24. The method of claim 23, wherein the feedback control mechanism that the ratio of the one or more virus inactivating reagents to the sample is determined independently of, or without, is a pH feedback loop.