production of these proteins contain impurities beyond host cell DNA. Some of these impurities, such as small molecule compounds, can have direct deleterious effects on the biologics and biosimilars being produced by these cells (*i.e.*, the target proteins), such as by inhibiting the transcription or translation of a target protein, inhibiting the activity of the expressed target protein, or by interfering with efforts to measure or monitor the target protein as it undergoes a purification process.

[0005] Some polyanionic compounds typically found in viable cells used in culture-based recombinant target protein expression can be found as impurities in cell cultures. Additionally, some of these polyanionic compounds found in buffers and cell culture media are known inhibitors of various enzymes. Polyvinyl Sulfonate (PVS) is a polyanionic compound that is a known inhibitor of several enzymes, including RNA enzymes and DNA polymerases. PVS is also known to be present in preparations of 2-(N-morpholino)-ethanesulfonic acid (MES), a common buffer in biotherapeutic processing and purification operations. PVS may also be (undesirably) present in other buffer systems, *e.g.*, Goods buffers, which utilize vinyl sulfonate as a starting material.

[0006] Thus, a need persists in the art for methods of accurately quantitating host cell DNA contained in protein formulations intended for administration to humans or other animals. Moreover, a need continues to exist for methods of reducing or removing such impurities from protein formulations intended for such administration.

Summary

[0007] The disclosure provides methods of assaying for polyanionic PCR inhibitors such as polyvinyl sulfonate compounds. These compounds inhibit a variety of enzymes, including nucleic acid polymerases such as DNA polymerases. Even the engineered forms of DNA polymerases that now dominate in PCR amplification methods are inhibited by these compounds, and the disclosure herein presents methods for detecting, and quantifying, these inhibitory compounds. The disclosure further provides methods for removing such compounds from buffers (*e.g.*, MES and Goods buffers) as well as from protein solutions. These methods provide a significant advance in the processing of biologics and proteins in general by providing methods for monitoring the presence of a major class of PCR inhibitors that confound efforts to monitor the purity of biologics produced in cell culture, where host DNA must be monitored to ensure purity of the protein of interest sufficient to be used as a therapeutic in humans and/or other animals.

[0008] In one aspect, the disclosure provides a method for quantification of a polyanionic PCR inhibitor in a sample comprising host cell DNA, the method comprising: a) preparing a dilution series of the sample, the dilution series comprising at

least four members; b) spiking each member of the dilution series of the sample with a constant amount of a template DNA distinguishable from host cell DNA; c) generating a polyanionic inhibitor standard curve comprising serial dilutions of the polyanionic inhibitor in the absence of any sample; d) spiking each serial dilution of the polyanionic inhibitor standard curve with the constant amount of the template DNA; e) performing a PCR assay of the template DNA on each member of the dilution series of the sample and on each serial dilution of the polyanionic inhibitor standard curve in the absence of any sample; f) comparing the PCR assay results of the dilution series of the sample to the PCR assay results of the polyanionic inhibitor standard curve in the absence of any sample; and f) identifying the concentration of polyanionic PCR inhibitor in the sample. In some examples, the concentration of polyanionic PCR inhibitor in the sample is a range defined by the concentration of polyanionic PCR inhibitor in the least diluted member of the dilution series showing complete spike recovery and the most diluted member of the dilution series not showing complete spike recovery. In some examples, the number of members in the dilution series is 5, 6, 7, 8, 9, 10, 12, 15, or 20, thereby narrowing the range of concentration of the polyanionic PCR inhibitor in the sample relative to the range provided by assaying fewer members of a dilution series. In some examples, the constant amount of a template DNA is at least 100 pg. In some examples, the polyanionic PCR inhibitor is a sulfone (sulfonate) compound, such as polyvinyl sulfonate. In some examples, polyvinyl sulfonate is the polyanionic PCR inhibitor used in generating the polyanionic inhibitor standard curve and the concentration of polyanionic PCR inhibitor in the sample is in units of polyvinyl sulfonate concentration equivalents. Some examples further comprise spiking a sample diluted sufficiently to recover amplification with one or more amounts of a polyanionic PCR inhibitor such as polyvinyl sulfonate to demonstrate that with added inhibitor, the recovery of amplification is impaired or lost.

[0009] Another example of the disclosure is drawn to a method for removing a polyanionic PCR inhibitor (a polyanionic impurity) from a buffer solution comprising: a) preparing a buffer solution of an acidic buffering species, a basic buffering species, or a combination thereof; b) contacting the buffer solution with an anion exchange medium; and c) separating the buffer solution from the polyanionic impurity, thereby removing the polyanionic impurity from the buffer solution. A related example of the disclosure provides a method for removing a polyanionic PCR inhibitor from a buffer solution comprising: a) preparing a buffer solution of an acidic buffering species, a basic buffering species, or a combination thereof; b) contacting the buffer solution with a mixed mode resin; and c) separating the buffer solution from the polyanionic impurity, thereby removing the polyanionic impurity from the buffer solution. In either of the foregoing methods for removing a polyanionic PCR inhibitor, the volume of buffer subjected to the method is not limiting and may extend from small analytical volumes in the range of milliliters to commercial scale buffer preparations involving many liters. In some examples of either removal method, the polyanionic impurity is a sulfone (sulfonate) compound, such as a polyvinyl

CLAIMS

WHAT IS CLAIMED IS:

1. A method for quantification of a polyanionic PCR inhibitor in a sample comprising host cell DNA, the method comprising:

a) Preparing a dilution series of the sample, the dilution series comprising at least four members;

b) Spiking each member of the dilution series of the sample with a constant amount of a template DNA distinguishable from host cell DNA;

c) Generating a polyanionic inhibitor standard curve comprising serial dilutions of the polyanionic inhibitor in the absence of any sample;

d) Spiking each serial dilution of the polyanionic inhibitor standard curve with the constant amount of the template DNA;

e) Performing a PCR assay of the template DNA on each member of the dilution series of the sample and on each serial dilution of the polyanionic inhibitor standard curve in the absence of any sample;

f) Comparing the PCR assay results of the dilution series of the sample to the PCR assay results of the polyanionic inhibitor standard curve in the absence of any sample; and

g) Identifying the concentration of polyanionic PCR inhibitor in the sample.

2. The method of claim 1 wherein the concentration of polyanionic PCR inhibitor in the sample is a range defined by the concentration of polyanionic PCR inhibitor in the least diluted member of the dilution series in the sample showing complete spike recovery and the most diluted member of the dilution series in the sample not showing complete spike recovery.

3. The method of claim 2 wherein the number of members in the dilution series is 5, 6, 7, 8, 9, 10, 12, 15, or 20, thereby narrowing the range of concentration of the polyanionic PCR inhibitor in the sample relative to the range provided in claim 2.

4. The method of claim 1 wherein the constant amount of a template DNA is at least 100 pg.

5. The method of claim 1 wherein the polyanionic PCR inhibitor is a sulfonate compound.

6. The method of claim 5 wherein the sulfonate compound is polyvinyl sulfonate.

7. The method of claim 1 wherein polyvinyl sulfonate is the polyanionic PCR inhibitor used in generating the polyanionic inhibitor standard curve and the concentration of polyanionic PCR inhibitor in the sample is in units of polyvinyl sulfonate concentration equivalents.