reduced protein production. When culturing is performed on an industrial scale, this reduced production can result in serious financial losses.

[0006] Therefore, a need exists for a simple, inexpensive solution to reduce poloxamer variability and to improve poloxamer performance, particularly for poorly performing lots.

[0007] All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

SUMMARY ~~OF THE INVENTION~~

[0008] The ~~invention~~ disclosure provided herein discloses, *inter alia*, methods of preparing a poloxamer (e.g., for use in a cell culture medium). Also provided are poloxamers prepared by the methods described herein. Further disclosed herein are cell culture media compositions containing a poloxamer prepared by the methods described herein. Further disclosed herein are methods of producing a polypeptide in a cell culture by culturing a cell that produces the polypeptide in a cell culture medium containing a poloxamer prepared by the methods described herein.

[0009] Accordingly, in one aspect, provided herein are methods of preparing a poloxamer for use in a cell culture medium, comprising the steps of: (a) heating a solid poloxamer to at least about 60°C to form a liquid poloxamer; and (b) cooling the liquid poloxamer to a temperature below about 50°C to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device, and wherein the poloxamer comprises a copolymer of ethylene oxide and propylene oxide. In some embodiments, cell viability in a cell culture medium comprising the heat-treated poloxamer is increased as compared to cell viability in a cell culture medium comprising the poloxamer before step (a). In some embodiments, the poloxamer in step (a) is heated to between about 60°C and about 185°C. In some embodiments, the poloxamer in step (a) is heated to between about 157°C and about 185°C. In some embodiments, the poloxamer is heated to between about 157°C and about 185°C for at least 1 minute. In some embodiments, the poloxamer is heated to between about 157°C and about 185°C for between 1 minute and about 250 minutes. In some embodiments, the poloxamer in step (a) is heated to between about 134°C and about 157°C. In some embodiments, the poloxamer is heated to between about 134°C and about 157°C for at least 1 minute. In some

[0013] Accordingly, ~~in one aspect,~~ provided herein are methods of preparing a poloxamer (e.g., for use in a cell culture medium), comprising the steps of (a) heating a purified poloxamer to about 80°C or above (e.g., about 80°C to about 100°C) to form a liquid poloxamer, and (b) cooling the liquid poloxamer to a temperature at about 50°C or below to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device. In some embodiments, the poloxamer comprises a copolymer of ethylene oxide and propylene oxide.

[0013a] In one aspect, provided herein are methods of preparing a poloxamer for use in a cell culture medium, comprising the steps of: (a) heating a poloxamer to about 80°C to about 100°C to form a liquid poloxamer; and (b) cooling the liquid poloxamer to a temperature below about 50°C to form a solid heat-treated poloxamer, wherein the cooling is not conducted in a prilling or milling device; wherein the poloxamer comprises a copolymer of ethylene oxide and propylene oxide.

[0013b] In some embodiments, the poloxamer comprises a copolymer of ethylene oxide and propylene oxide having a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$, wherein n is from about 60 to about 150 and m is from about 25 to about 60. In some embodiments, cell viability in a cell culture medium comprising the heat-treated poloxamer is increased as compared to cell viability in a cell culture medium comprising the poloxamer before step (a). In some embodiments, the poloxamer is a purified poloxamer. In some embodiments, a purified poloxamer is a poloxamer composition that does not contain another therapeutic or pharmaceutical compound. In some embodiments, the poloxamer in step (a) does not contain another therapeutic or pharmaceutical compound. In some embodiments, the poloxamer in step (a) is heated to about 85°C to about 91°C. In some embodiments herein, the poloxamer is heated from about 10 to about 15 minutes. In some embodiments herein, the liquid poloxamer in step (b) is cooled at ambient temperature, about 2°C to about 8°C, or below 0°C. In some embodiments herein, the liquid poloxamer is cooled for at least about 20 minutes. In some embodiments herein, the heated-treated poloxamer produced in step (b) is added into a cell culture medium. In some embodiments herein, steps (a) and (b) are repeated at least once before adding the heated-treated poloxamer into the cell culture medium. In some embodiments herein, the cell viability is increased by at least about 10%. In some embodiments herein, the cell viability is increased by at least about 30%. In some embodiments herein, the cell viability in a cell culture medium comprising the poloxamer before step (a) is below about 80%. In some embodiments herein, the poloxamer has been treated by a prilling process before step (a). In some embodiments herein, the poloxamer has a melting temperature in the range of about 45°C to about 60°C. In some embodiments herein, the poloxamer has an average molecular weight of from about 6,000 to about 18,000 Daltons. In some embodiments herein, the poloxamer is poloxamer 188. In some embodiments, the poloxamer comprises a copolymer having a formula of $HO(C_2H_4O)_n(C_3H_6O)_m(C_2H_4O)_nH$ with n having a value of about 80, with m having a value

about 10 g/L. In some embodiments, the polypeptide produced by the cell is an antibody or antigen-binding fragment thereof.

[0016] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the present ~~invention~~disclosure. These and other aspects and embodiments of the ~~invention~~ disclosure will become apparent to one of skill in the art.

BRIEF DESCRIPTION OF THE DRAWINGS

[0017] **FIG. 1** shows that heat treatment of poloxamer improves its effect on cell viability in culture. The diamond plot indicates the mean (center line) and upper/lower 95% confidence intervals (points of diamonds), as well as the cell viability values (dots) for each experimental condition. Each experiment compares untreated and heat-treated (HT) poloxamer from a good or bad ("suspect") poloxamer lot, as indicated on the x-axis. Arrows indicate improvement in cell viability upon heat treatment of bad lots. Note that heat treatment also improved the cell viability of the good lot, from an average of approximately 85% to a value of approximately 95%.

[0018] **FIG. 2** shows cell viability (%) in a high shear shake flask model (HSSF) test for untreated and heat treated (_HT) poloxamer samples A, B, and C (see also Table 2).

[0019] **FIG. 3** shows the response surface design of conditions tested in ovens. Each condition is labeled in the format "time, temperature." Conditions marked with '♦' were tested in HSSF. Conditions marked with '◊' were prepared in ovens but not tested in HSSF. Temperature (in °C) represents the set temperature of the oven, while time (in min) represents the time the poloxamer sample was in the oven.

[0020] **FIG. 4** shows the improvement in cell viability (%)($Vf_{(treated)} - Vf_{(untreated)}$) for samples of C poloxamer heat treated in ovens under the indicated conditions, compared to positive ("Good lot") and negative ("Untreated bad lot") controls (D and C, respectively). Error bars depict one standard deviation.

[0021] **FIG. 5** shows a contour plot from a full design of experiments (DOE) exploring effects of different heat treatment conditions on poloxamer performance in cell culture.