protein instability due to alteration of the protein's tertiary structure and protein aggregation (Davies, J. Biol. Chem. 262: 9895-901 (1987)). Oxidation of protein pharmaceuticals have been reviewed by Nguyen (Chapter 4 in Formulation and Delivery of Protein and Peptides (1994)), Hovorka, (J. Pharm Sci. 90:25369 (2001)) and Li (Biotech Bioengineering 48:490-500 (1995)).

Given the above, it is evident that there is a need for the identification of compositions useful for enhancing the stability and preventing the aggregation and/or oxidation of proteins in protein-containing formulations.

SUMMARY

The present disclosure is based upon the novel finding that certain cholate surfactants are useful for stabilizing and/or reducing aggregation of antibodies or other proteins in therapeutically useful formulations and also for reducing the degradation of polysorbate surfactants in such formulations. Furthermore, the cholate surfactants herein may be useful in stabilizing protein-containing therapeutic formulations at concentrations below their critical micelle concentration (CMC) values of at least about 2.0 mM or at least about 0.2% (weight volume, w/v) as protein stabilizing, or aggregation-reducing, agents. In certain examples, a cholate-based surfactant may also protect a therapeutic protein formulation more effectively than an alkylglycoside surfactant at concentrations below the CMC value. Accordingly, in one example, the present disclosure relates to formulations of proteins, such as proteins intended for therapeutic use that comprise at least one cholate surfactant at a concentration below its CMC value measured in water at 25ºC. In certain examples, the protein present in the composition of matter is an antibody, which may optionally be a monoclonal antibody. The present disclosure also relates to containers holding such formulations, articles of matter comprising such containers, and methods of preparing the formulations.

In some examples, the formulations may be aqueous, may be stable at a temperature of about 2-8°C for at least one year, and/or may be stable at a temperature of about 30°C for at least one month. In some examples, the formulation comprises no polysorbate or poloxamer. In other examples, the formulation comprises polysorbate and/or poloxamer. In some examples, the formulation comprises no alkylglycosides. In other examples, the formulation comprises alkylglycosides. In some examples, the formulation comprises no other surfactants other than cholates. In other examples, the formulation comprises other surfactants.

The present disclosure comprises, *inter alia*, protein formulations comprising a protein and at least one cholate surfactant having a critical micelle concentration (CMC) value of 2.0 mM or greater or of 0.2% (w/v) or greater in water at 25ºC. In one aspect, the present disclosure refers to a protein formulation comprising a protein and at least one cholate surfactant having a critical micelle concentration (CMC) value of 2.0 mM or greater or of 0.2% (w/v) or greater in water at 25ºC. In some examples, the protein is an antibody, such as a monoclonal antibody. In some examples, the cholate surfactant is zwitterionic, nonionic, anionic, or is selected from CHAPS (3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate), SGH (sodium glycocholate hydrate), sodium taurocholate hydrate (STH), sodium cholate hydrate (SCH), SdTH, SdCH, ScdCH, and BigCHAP (N,N'-bis-(3-D-gluconamidopropyl) cholamide). In some examples, the formulation comprises CHAPS at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%. In some examples, the formulation comprises CHAPS at a concentration of 0.025% to 0.05% (w/v). In some examples, the formulation comprises BigCHAP at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%. In some examples, the formulation comprises BigCHAP at a concentration of 0.025% to 0.05% (w/v). In some examples, the formulation comprises SGH, STH, or SCH at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%. In some examples, the formulation comprises SGH, STH, or SCH at a concentration of 0.025% to 0.05%. In some examples, the at least one cholate surfactant is present at a concentration that is lower than its CMC value in water at 25ºC.

In some examples, the formulation comprises a zwitterionic or nonionic cholate surfactant and is a low ionic strength formulation. In some such cases, the formulation contains less than 50 mM salt, less than 40 mM salt, less than 30 mM salt, or less than 25 mM salt, such as sodium, arginine, or histidine salt.

In some examples, the formulation comprises an anionic cholate surfactant and is a high ionic strength formulation. In some such cases, the formulation comprises at least 175 mM salt, at least 200 mM salt, at least 225 mM salt, or at least 250 mM salt, such as sodium, arginine, or histidine salt.

In some examples, the formulation is suitable for therapeutic use. In some examples, the formulation has not been subjected to lyophilization, such as a ready-

to-use, liquid formulation. Alternatively, the formulation is a reconstituted, lyophilized formulation.

In some examples, the formulation does not comprise any polysorbate, poloxamer, pluronic, Brij, or alkylglycoside surfactant. In some examples, the formulation does not comprise any non-cholate surfactant. In some examples, the formulation consists essentially of at least one cholate surfactant, at least one protein species, at least one buffer species, and at least one non-surfactant stabilizer (e.g., a sugar, sugar alcohol, amino acid, peptide, salt, or other protein). In some examples, the formulation further comprises at least one polysorbate or poloxamer, such as polysorbate 20 or polysorbate 80. In some examples, the formulation comprises 1.0% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, or 0.01% or less of polysorbate 20 or 80. In other examples, the formulation does not comprise any surfactant other than the cholate surfactant and the polysorbate 20 or 80.

In another aspect, the present disclosure refers to a therapeutic protein formulation, comprising at least one therapeutic protein species, and a surfactant consisting essentially of CHAPS at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%, and optionally further comprising one or more of a buffer, a salt, a lyoprotectant, or stabilizer comprising one or more of a sugar, sugar alcohol, amino acid, or other protein species, optionally wherein: (a) the formulation is low ionic strength; (b) the at least one therapeutic protein is an antibody; and/or (c) the formulation is a liquid formulation that is not lyophilized prior to use. In some examples, the surfactant consists essentially of 0.01 to 0.05% or 0.025% to 0.05% (w/v) CHAPS. In some examples, at least one therapeutic protein species, and a surfactant consisting essentially of BigCHAP at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%, and optionally further comprising one or more of a buffer, a salt, a lyoprotectant, or stabilizer comprising one or more of a sugar, sugar alcohol, amino acid, or other protein species, optionally wherein: (a) the formulation is low ionic strength; (b) the at least one therapeutic protein is an antibody; and/or (c) the formulation is a liquid formulation that is not lyophilized prior to use. In some examples, the surfactant consists essentially of 0.01 to 0.05% or 0.025% to 0.05% (w/v) BigCHAP.

In yet another aspect, the present disclosure refers to a therapeutic protein formulation, comprising at least one therapeutic protein species, and a surfactant consisting essentially of BigCHAP at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%, and optionally further comprising one or more of a buffer, a salt, a lyoprotectant, or stabilizer comprising one or more of a sugar, sugar alcohol, amino acid, or other protein species, optionally wherein: (a) the formulation is low ionic strength; (b) the at least one therapeutic protein is an antibody; and/or (c) the formulation is a liquid formulation that is not lyophilized prior to use.

In one aspect, the present disclosure refers to a therapeutic protein formulation, comprising at least one therapeutic protein species, and a surfactant consisting essentially of STH, SGH, or SCH at a concentration (w/v) of 0.5% or less, 0.4% or less, 0.3% or less, 0.1% or less, 0.05% or less, 0.04% or less, 0.025% or less, 0.02% or less, 0.01 to 0.5%, 0.01 to 0.1%, 0.01 to 0.05%, or 0.025% to 0.05%, wherein the formulation is a high ionic strength formulation, optionally further comprising one or more of a buffer, a salt, a lyoprotectant, or stabilizer comprising one or more of a sugar, sugar alcohol, amino acid, or other protein species, and optionally wherein: (a) the at least one therapeutic protein is an antibody; and/or (b) the formulation is a liquid formulation that is not lyophilized prior to use. In some examples, the surfactant consists essentially of 0.01 to 0.05% or 0.025% to 0.05% (w/v) STH, SGH, or SCH.

In some examples, the formulation has one or more of the following properties: (a) the formulation shows no visible aggregates after 24 hours of agitation at 100 revolutions per minute (rpm) at room temperature; (b) the formulation shows no more than 2% high molecular weight protein aggregates after 24 hours of agitation at 100 rpm at room temperature; (c) the formulation shows no more than 1% high molecular weight protein aggregates after 24 hours of agitation at 100 at room temperature; (d) high molecular weight protein aggregates in the formulation do not increase by more than 0.2% after 24 hours of agitation at 100 rpm at room temperature compared to a non-agitated control; (e) if the formulation comprises polysorbate 20 or polysorbate 80, the polysorbate 20 or polysorbate 80 in the formulation remains intact to a larger degree after 2 weeks storage at 40ºC or after treatment with *Candida antarctica* lipase B (CALB, Sigma Aldrich CAS# 9001-62-1) lipase than a formulation with the same ingredients and concentrations, but without cholate.

The present disclosure refers to containers comprising the formulations disclosed herein, and articles of manufacture comprising the containers comprising the formulations.

In one aspect, the present disclosure refers to a container comprising the formulation disclosed herein.

In another aspect, the present disclosure refers to an article of manufacture comprising the container disclosed herein.

The present disclosure refers to methods of making the protein formulations herein, comprising mixing the protein with the at least one cholate surfactant to form a cholate-containing aqueous solution. In one aspect, the present disclosure refers to a method of making the protein formulation disclosed herein, comprising mixing the protein with the at least one cholate surfactant to form a cholate-containing aqueous solution. The present disclosure refers to methods of inhibiting aggregation of a protein present in an aqueous solution, said method comprising adding to the aqueous solution at least one cholate surfactant having a critical micelle concentration (CMC) value of about 2.0 mM or greater or 0.2% (w/v) in water at 25ºC, at a concentration below its CMC value in water at 25ºC, to form a

cholate-containing aqueous solution. In another aspect, the present disclosure refers to a method of inhibiting aggregation of a protein present in an aqueous solution, said method comprising adding to the aqueous solution at least one cholate surfactant having a critical micelle concentration (CMC) value of about 2 mM or greater or 0.2% (w/v) in water at 25°C, at a concentration below its CMC value in water at 25°C, to form a cholate-containing aqueous solution. In some such examples, the protein is an antibody, such as a monoclonal antibody. In some examples, the methods further comprise lyophilizing the cholate-containing aqueous solution. In other examples, the methods do not comprise lyophilizing the cholate-containing aqueous solution.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows results from mixing 0.05% (w/v) of a cholate surfactant or a control surfactant (cholates CHAPS, SGH, or STH, polysorbate 20 (PS20), or poloxamer 188 (PX188)) with an exemplary monoclonal anti-PDL1 antibody at 1 mg/mL in 20 mM histidine acetate and 240 mM sucrose at pH 5.5. Solutions were 5 mL volume in a 15 mL glass vial. Control solutions with the above ingredients but without surfactants were also prepared. The figure shows whether visible aggregates form after agitation for 24 hours at ambient temperature in an arm shaker (Glas-Col bench top arm shaker) at 100 revolutions per minute (rpm).

Figure 2 shows from mixing an exemplary monoclonal anti-Tryptase antibody at 1 mg/mL in a solution of 200 mM arginine succinate at pH 5.8. Solutions were 5 mL volume in a 15 mL glass vial. Control solutions with the above ingredients but without surfactants were also prepared. The figure shows whether visible aggregates form after agitation for 24 hours at ambient temperature in an arm shaker at 100 rpm.

Figure 3 shows that cholates can protect free fatty acids in protein solutions from precipitating. Solutions containing 5 mg/mL anti-Tryptase antibody and 200 mM arginine succinate and 0.02% PS20 at pH 5.8 were mixed with various concentrations of a cholate surfactant and then spiked with 0.04 units/mL CALB at 5°C. If cholates protect PS20 from degradation to FFAs, then visible FFA precipitate particles should not form in the protein solutions or such particles, once formed, should re-solubilize upon addition of cholate, while, if cholates provide no protection or solubilization, visible FFA precipitate particles should form to the same degree as protein solutions in which no cholate was added. Results show that addition of 0.5% SCH, SGH, or CHAPS protects against visible particulate formation in the solutions, while such particulates still form at 0.02% to 0.1% of each added surfactant.

Figure 4 shows a summary of results from incubation of cholate surfactants at different concentrations on PS20 degradation induced by added CALB lipase. The dashed line in the graph provides the PS20 concentration observed upon complete degradation, as shown by the “lipase only” control solution.