[0003] Surfactants are generally used in pharmaceutical compositions comprising therapeutic proteins, e.g., to prevent protein aggregation and stabilize proteins. It is unclear whether surfactants can be used to effectively prevent protein loss due to surface adsorption when proteins are present at low concentrations in pharmaceutical compositions (e.g., 0.1 mg/mL or less).

[0004] There is a need for protein compositions and methods that minimize protein loss due to adsorption to solid surfaces, especially when the compositions comprising protein at low protein concentrations.

SUMMARY

[0005] Disclosed herein are compositions comprising proteins at low protein concentrations as well as methods of administering the compositions to a subject in need thereof. The compositions and methods disclosed herein have the advantage of minimizing or eliminating protein loss due to protein adsorption to solid surfaces and ensure accurate dosing of therapeutic proteins to patients.

[0005a] In one aspect, the present disclosure provides an aqueous composition comprising a bispecific antibody construct at a concentration of between about 0.001 μg/ml and about 100 μg/ml and a surfactant at a concentration of at least about 0.25 x of the critical micelle concentration (CMC) of the surfactant, wherein the bispecific antibody construct comprises a first binding domain that binds to a target cell surface antigen, a second binding domain that binds to human CD3 on the surface of a T cell, and optionally, a third domain comprising, in an amino to carboxyl order, hinge-CH2 domain-CH3 domain-linker-hinge-CH2 domain-CH3 domain, wherein the second binding domain comprises a polypeptide having the sequence of SEQ ID NO: 201.

[0005b] In another aspect, the present disclosure provides a pharmaceutical preparation comprising an aqueous pharmaceutical composition contained inside a container, wherein the aqueous pharmaceutical composition comprising: a) a bispecific antibody construct at a concentration of between about 0.001 μg/ml and about 100 μg/ml, and b) a surfactant at a concentration of at least about 0.25 x of CMC of the surfactant, wherein the surfactant has an HLB value of at least 20.

[0005c] In yet another aspect, the present disclosure provides method of administering a bispecific antibody construct to a patient comprising: a) preparing an aqueous pharmaceutical

composition in a container, wherein the aqueous pharmaceutical composition comprises the bispecific antibody construct at a concentration of between about 0.001 μg/ml and about 100 μg/ml and a surfactant at a concentration of at least about 0.25 x of CMC of the surfactant, and b) administering the aqueous pharmaceutical composition to the patient, wherein the bispecific antibody construct comprises a polypeptide having the amino acid sequence selected from SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 176, SEQ ID NO: 178, and SEQ ID NO: 192.

[0006] In certain examples, disclosed herein is an aqueous composition comprising a protein and a surfactant, wherein the protein is present in the composition at a concentration of between about 0.001 μg/ml and about 100 μg/ml, and the surfactant is present in the composition at a concentration of at least about 0.25 x of the critical micelle concentration (CMC) of the surfactant.

[0007] In certain examples, the protein is a bispecific antibody construct comprising a first binding domain that binds to a target cell surface antigen, a second binding domain that binds to human CD3 on the surface of a T cell, and optionally, a third domain comprising, in an amino to carboxyl order, hinge-CH2 domain-CH3 domain-linker-hinge-CH2 domain-CH3 domain. In certain examples, the second binding domain comprises a polypeptide having the sequence of SEQ ID NO: 201. In certain examples, the bispecific antibody construct is present at a concentration of between about 0.001 μg/ml and about 50 μg/ml, or between about 0.01 μg/ml to about 50 μg/ml, or between 0.1 μg/ml to about 50 μg/ml, or 0.1 μg/ml to about 10 μg/ml, or 1 μg/ml to about 10 μg/ml.

[0008] In certain examples, the surfactant is a polysorbate, a poloxamer or triton x-100. In certain examples, the surfactant is polysorbate 80, polysorbate 60, polysorbate 40, polysorbate 20, or Triton X-100. In certain examples, the surfactant is poloxamer 188 or poloxamer 407. In certain examples, the surfactant is present at a concentration of between about 0.25x and about 20x of the CMC, or between about 0.25x and about 10x of the CMC of the surfactant.

[0009] In certain examples, wherein the composition further comprising a salt, an amino acid, a saccharide or saccharide derivative, or combinations thereof. In certain examples, the salt is NaCl. In certain examples, the saccharide or saccharide derivative is a monosaccharide, a

disaccharide, a cyclic polysaccharide or a sugar alcohol. In certain examples, the saccharide is sucrose, trehalose, mannitol or sorbitol. In certain examples, the amino acid is lysine.

[0010] In certain examples, wherein the pH of the composition is between about 3.5 and about 7.5. In certain examples, the pH of the composition is between about 4.2 and about 7.0.

[0011] In certain examples, the composition further comprises a buffer or a preservative. In certain examples, the buffer is an acetate buffer, a glutamate buffer, a citrate buffer, a succinate buffer, a tartrate buffer, a fumarate buffer, a maleate buffer, a histidine buffer, or phosphate buffer.

[0012] In certain examples, wherein each of the first and second binding domains of the bispecific antibody construct comprises a VH region and a VL region. In certain examples, the bispecific antibody construct is a single chain antibody construct. In certain examples, the bispecific antibody construct comprises a polypeptide having the amino acid sequence selected from SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 176, SEQ ID NO: 178, and SEQ ID NO: 192. In certain examples, the bispecific antibody construct comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 48, SEQ ID NO: 67, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 178 or SEQ ID NO: 192.

[0013] In certain examples, the composition is contained in a plastic container such as an IV bag or IV tube. In certain examples, the container is made of a material comprising polyolefin, polyvinyl chloride (PVC), ethyl vinyl acetate (EVA), or polyurethane. In certain examples, the container is made of a material comprising PVC and wherein the PVC is substantially free of di-2-ethylhexyl phthalate(DEHP) or tri-2-ethylhexyltrimellitate (TOTM)

[0014] In certain examples, disclosed herein is a pharmaceutical preparation comprising an aqueous pharmaceutical composition contained inside a container, wherein the aqueous pharmaceutical composition comprising: a bispecific antibody construct at a concentration of between about 0.001 μg/ml and about 100 μg/ml, and a surfactant at a concentration of at least about 0.25 x of CMC of the surfactant, wherein the surfactant has an HLB value of at least 20. In certain examples, the surfactant is poloxamer 188 or poloxamer 407. In certain examples, the aqueous pharmaceutical composition comprises the bispecific antibody construct at a

concentration of between about 0.001 μg/ml and about 50 μg/ml. In certain examples, the aqueous pharmaceutical composition comprises the surfactant is at a concentration of between about 0.25x and about 20x of the CMC or between about 0.25x and about 10x of the CMC, of the surfactant.

[0015] In certain examples, the aqueous pharmaceutical composition further comprising a salt, a buffer, an amino acid, a saccharide or saccharide derivative, or combinations thereof. In certain examples, the aqueous pharmaceutical composition has a pH of between about 4.2 and about 7.0.

[0016] In certain examples, the container is made of a material comprising polyolefin, PVC, EVA or polyurethane (e.g., polyester and polyether).

[0017] In certain examples, the bispecific antibody construct comprises a polypeptide having the amino acid sequences selected from SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 176, SEQ ID NO: 178, and SEQ ID NO: 192. In certain examples, the bispecific antibody construct comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 48, SEQ ID NO: 67, SEQ ID NO: 78, SEQ ID NO: 88, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 178 or SEQ ID NO: 192.

[0018] Also disclosed herein is a method of administering a bispecific antibody construct to a patient comprising: preparing an aqueous pharmaceutical composition in a container, wherein the aqueous pharmaceutical composition comprises the bispecific antibody construct at a concentration of between about 0.001 μg/ml and about 100 μg/ml and a surfactant at a concentration of at least about 0.25 x of CMC of the surfactant, and administering the aqueous pharmaceutical composition to the patient, wherein the bispecific antibody construct comprises a polypeptide having the amino acid sequence selected from SEQ ID NO: 11, SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 47, SEQ ID NO: 48, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 76, SEQ ID NO: 78, SEQ ID NO: 87, SEQ ID NO: 88, SEQ ID NO: 108, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 176, SEQ ID NO: 178, and SEQ ID NO: 192. In certain examples, the bispecific antibody construct comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 13, SEQ ID NO: 15, SEQ ID NO: 25, SEQ ID NO: 48, SEQ ID NO: 67, SEQ ID

NO: 78, SEQ ID NO: 88, SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 178 or SEQ ID NO: 192. In certain examples, the aqueous pharmaceutical composition comprises the bispecific antibody construct is at a concentration of between about 0.001 μg/ml and about 50 μg/ml.

[0019] In certain examples, the aqueous pharmaceutical composition comprises the surfactant is at a concentration of between about 0.25x and about 20x of the CMC or between about 0.25x and about 10x of the CMC, of the surfactant. In certain examples, the surfactant is polysorbate 80, polysorbate 60, polysorbate 40, polysorbate 20, poloxamer 188, poloxamer 407, or Triton X-100.

[0020] In certain examples, the aqueous pharmaceutical composition further comprising one or more selected from a salt, a buffer, an amino acid, a saccharide or a saccharide derivative, and a preservative. In certain examples, the aqueous pharmaceutical composition has a pH of between about 4.2 and about 7.0.

[0021] In certain examples, the container is made of a material comprising polyolefin, PVC, EVA, polyurethane. In certain examples, the surfactant is polysorbate 80, polysorbate 60, polysorbate 40, or polysorbate 20, and wherein the container is made of a material comprising PVC that is substantially free of DEHP or TOTM.

[0022] In certain examples, the aqueous pharmaceutical composition is prepared by diluting a first composition comprising the bispecific antibody construct with a suitable aqueous solution. In certain examples, the first composition is a liquid composition comprising the bispecific antibody construct. In certain examples, the first composition is a liquid composition reconstituted from a lyophilized composition comprising the bispecific antibody construct. In certain examples, the suitable solution comprises the surfactant at a concentration of at least about 0.25 x of CMC of the surfactant. In certain examples, the aqueous pharmaceutical composition is prepared by adding the suitable aqueous solution into the container followed by adding an appropriate amount of the first composition into the container.

[0023] In certain examples, the patient is a cancer patient. In certain examples, the administration is IV administration.

BRIEF DESCRIPTION OF THE DRAWINGS

[0024] Figure 1 shows a diagram of the assay for measuring protein binding to a solid surface.

[0025] Figure 2 shows protein binding to a solid surface in the absence of surfactants.

[0026] Figure 3 shows different the addition of various surfactants prevented protein binding to solid surfaces.

[0027] Figure 4 shows that adding surfactants to the solid surface before adding protein prevents protein binding to surfaces more efficiently.

[0028] Figure 5 shows the impacts of different surfactants at the same concentration on the leaching of di-2-ethylhexyl phthalate (DEHP) from DEHP-containing PVC.

[0029] Figure 6 shows the impacts of different surfactants at same folds of CMC on leaching of DEHP from DEHP-containing PVC.

DETAILED DESCRIPTION

[0030] Inventions disclosed herein are based on the surprising finding that surfactants, when used at concentrations lower than their critical micelle concentration, can stabilize proteins at low concentrations in liquid compositions and effectively prevent protein loss due to adsorption to solid surfaces.

[0031] In some embodiments, disclosed herein is an aqueous composition comprising a protein and a surfactant, wherein the protein is present in the composition at a concentration of between about 0.001 μg/ml and about 100 μg/ml, and the surfactant is present in the composition at a concentration of at least about 0.25x of the critical micelle concentration (CMC) of the surfactant.

[0032] Surfactants that can be used in the composition can be any surfactant typically used in pharmaceutical compositions. In some embodiments, the surfactant is a non-ionic surfactant. In some embodiments, the surfactant is a polysorbate such as polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 65, polysorbate 80 or polysorbate 85. In some