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(54) Benævnelse: **LYOFILISERET FORMULERING AF ANTISTOFLÆGEMIDDELKONJUGAT**

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DESCRIPTION

Description

FIELD OF THE INVENTION

[0001] The present invention relates to a lyophilized formulation suitable in particular for antibody drug conjugates (ADCs), to reconstituted formulations thereof, and to methods of preparing and using such lyophilized and reconstituted formulations in e.g. cancer therapy.

BACKGROUND OF THE INVENTION

[0002] ADCs are highly potent and specific agents for the treatment of cancer and other conditions, where the antibody portion specifically binds to its antigen on a target cell, so that the drug can exert its cytotoxic or other therapeutic effect on the target cell, optionally after internalization. Several ADCs have been described, among them ADCs based on anti-tissue factor (anti-TF) antibodies (see, e.g., WO 2011157741 A2 (D1), which describe the ADCs of the present invention).

[0003] Like other protein pharmaceuticals, however, antibodies are prone to degradation such as oxidation, deamidation and fragmentation as well as particle and aggregate formation. To provide for an ADC pharmaceutical that is stable during transport and storage, the carriers, excipients, and/or stabilizers in the pharmaceutical formulation must therefore be carefully selected. The long-term stability of an antibody or ADC can also be improved by preparing a lyophilized or freeze-dried formulation, using excipients optimized for this purpose. Many such formulations for antibodies or ADC preparations have been described in patent literature, see, *e.g.* WO9704801, WO9856418, WO02011753, WO02096457, WO03009817, WO03039485, US8372396, WO2004004639, WO2004016286, WO2004055164, WO 2004071439, WO2006014965, WO2006044908 and WO2007019232.

[0004] For ADCs, there is an additional challenge in that the drug conjugation in itself can reduce the stability and alter the physicochemical properties of the antibody. For example, it has been reported that the conjugation of the drug moiety DM1 to the anti-HER2 antibody trastuzumab resulted in destabilization of the CH2 domain of the antibody (Wakankar et al, 2010). Further, cytotoxic drugs often being hydrophobic, the ADC conjugate as a whole can be less soluble than the unconjugated antibody, thus becoming more prone to aggregation, particle formation and surface adsorption. Typically, both antibody and ADC formulations include a surfactant, frequently polysorbate 20 or 80, to reduce aggregation and adsorption (see, e.g., patent literature cited *supra*). For example, brentuximab vedotin (trade name ADCETRIS®) is an ADC based on an anti-CD30 antibody linked to the auristatin derivative MMAE, provided as a lyophilized powder which, when reconstituted in water, contains 5 mg/mL ADC, 70 mg/mL trehalose dihydrate, 5.6 mg/mL sodium citrate dihydrate, 0.21 mg/mL citric acid monohydrate, and 0.20 mg/mL polysorbate 80, at a pH of approximately 6.6.

[0005] Accordingly, surfactants are commonly used in pharmaceutical preparations and are generally perceived as acceptable pharmaceutical ingredients. As mentioned above, surfactants are commonly used to reduce aggregate formation during antibody manufacturing and formulation (see e.g. Vásquez-Rey and Lang, 2011, Biotech, Bioeng. 108:7 p 1494). However, it is a general concern of pharmaceutical formulation to reduce the usage of non-active compounds as much as possible. This concern is both to reduce the cost of the resulting drug, but also to reduce potential unwanted effects of the excipient. For example, many surfactants are more or less toxic because of the amphiphilic nature and ability to react with biological membranes. It is not uncommon to observe LC50 of surfactants in aquatic organisms as low as 10 mg/L. Further, autooxidation or the exposure to light of polysorbates can result in the formation of hydrogen peroxide which in turn can oxidize the antibody molecule leading to a an unstable product (Kerwin, 2008; Singh et al., 2012). This not only reduces the efficacy of the ADC, but can lead to the formation of potentially harmful degradation products of the same.

[0006] Indeed, a surfactant-free formulation of a huC242-DM1 ADC containing 50 mM succinic acid, pH 6.0 and 5.0% sucrose initially described in WO2004004639 as suitable for e.g. lyophilization was later reported in WO2007019232A2 to not adequately address particle and aggregate formation.

[0007] The ADCs of the present invention are disclosed in WO2011157741 (D1) and also in Breij et al, Cancer Res 2013;73(8 Suppl): Abstract nr 1234 (D2), however, these documents do not disclose the lyophilized formulation described herein.

[0008] Carter et al. (D3) discuss cyclic AMP-enzyme conjugates and lyophilization of such to overcome stability issues of these conjugates.

[0009] Rowland et al. (D4) discloses an anti-CD19-idarubicin immunoconjugate and the stability of such. The excipients of the ADC formulation are not disclosed. D4 discloses that the ADC may be lyophilized and that it has the greatest stability as a lyophilized formulation stored at - 20°C.

[0010] Selva et al., (D5) describe the beneficial effect of trehalose as a stabilizer for the lyophilization of an ADC but does not disclose concentrations thereof or other excipients.

[0011] Roy et al., (D6) disclose the effect of formulation and moisture on the stability of a lyophilized ADC preparation and teaches a formulation of equal weight ratios of mannitol, glycine and ADC in a low ionic strength phosphate buffer.

[0012] Chen et al., (D7) is an abstract briefly discussing development of a lyophilized formulation of an anti-BR96-doxorubicin ADC and teaching a formulation comprising a phosphate buffer with 5 mg/ml maltose or sucrose as lyo-protectant. It is reported that the lyophilized formulation stored at 50 degrees for 3 months maintained the potency but had a slight increase in aggregates and free doxorubicin.

[0013] There still remains a need for surfactant free pharmaceutical formulations for ADCs that are stable during transport and storage and substantially free of particles, aggregates and degradation products.

SUMMARY OF THE INVENTION

[0014] The present inventors have discovered lyophilized formulations of anti-TF ADCs in which the anti-TF ADCs remain stable and do not form aggregates or particles when reconstituted. Very surprisingly, these can be prepared without the inclusion of surfactants such as polysorbate 20 or 80, and/or without inorganic salts. The disclosure thus provides for stable, surfactant free lyophilized formulations of anti-TF ADCs with pharmaceutically acceptable excipients comprising: buffer components which limit pH shifts during the lyophilizing step, at least one stabilizing agent, typically a non-reducing sugar which forms an amorphous phase with the anti-TF ADC in solid state, and at least one bulking agent, optionally wherein the lyophilized formulation can be essentially free of any salt.

[0015] Accordingly, in a main aspect the invention relates to a lyophilized formulation of an anti-tissue factor (TF) antibody-drug conjugate (ADC), the lyophilized formulation obtainable or obtained by lyophilizing an aqueous formulation comprising from about 9 to about 11 g/L of said anti-TF ADC and pharmaceutically acceptable excipients comprising about 29 to about 31 mM histidine buffer having a pH of about 5.5 to about 6.5; about 84 to about 92 mM sucrose and about 158 to about 172 mM mannitol, and wherein:

1. a. the formulation is free of surfactant,
2. b. the anti-TF antibody portion of the ADC comprises a variable heavy (VH) region comprising a CDR1 region having the amino acid sequence set forth in SEQ ID NO: 6, a CDR2 region having the amino acid sequence set forth in SEQ ID NO: 7, and a CDR3 region having the amino acid sequence set forth in SEQ ID NO: 8, and a variable light (VL) region comprising a CDR1 region having the amino acid sequence set forth in SEQ ID NO:46, a CDR2 region having the amino acid sequence set forth in SEQ ID NO: 47, and a CDR3 region having the amino acid sequence set forth in SEQ ID NO: 48, or a variant which has at most 1, 2, or 3 amino-acid modifications, more preferably amino-acid substitutions such as conservative amino acid substitutions in said sequences,
3. c. the drug portion of the ADC is vcMMAE.

[0016] The invention further relates to a pharmaceutically acceptable liquid formulation obtained by reconstituting the lyophilized formulation of the invention in a sterile aqueous diluent and to a method for preparing the lyophilized formulation.

[0017] These and other aspects and embodiments are described in more detail in the following sections.

LEGENDS TO THE FIGURES

[0018]

Figure 1 shows the percentage HMW (A), main (B) and LMW (C) species obtained using SEC analysis for the HuMax-TF DOE samples stored for four weeks. For these bar graphs, the formulations were arranged in order of increasing pH from left to right for each buffer plus excipient sub-group. For each pair of bars, the left bar represents 2-8°C and the right bar 45°C. See Example 2 for details.

Figure 2 shows the effects of various formulations on the percentage peak area for acidic (A), main (B) and basic (C) species observed using cIEF for the HuMax-TF DOE samples stored for one week. For these bar graphs, the formulations are arranged in order of increasing pH from left to right for each buffer plus excipient sub-group. For each pair of bars, the left bar represents 2-8°C and the right bar 45°C. See Example 4 for details.

Figure 3 shows the effect of pH on the percent HMW for HuMax TF ADC as determined by SEC when stored at 40°C for 2 weeks. See Example 5 for details.

Figure 4 shows the effect of pH on the percent acidic species as determined by iCE for HuMax TF ADC solutions stored at 40°C for 2 weeks. See Example 5 for details.

Figure 5A shows the effect of sorbitol and PS80 (polysorbate 80) on percent charge main peak(by iCE) of HuMax TF ADC solutions prepared at pH 6.0 and stored at 40°C for 2 weeks. See Example 5 for details.

Figure 5B shows the effect of sorbitol and PS80 (polysorbate 80) on main peak (by SEC) of HuMax TF ADC solutions prepared at pH 6.0 and stored at 40°C for 2 weeks. See Example 5 for details.

Figure 6 shows a comparison between liquid and lyophilized formulations on main charge isoform percentage of HuMax-TF-ADC stored at 40°C for 2 weeks. See Example 6 for details.

Figure 7 shows exemplary results from accelerated stability data for different lyophilized formulations for HuMax-TF-ADC. (A) Aggregates increase at 50°C by SEC. (B) Main charge isoform decrease at 40°C by icIEF. See Example 8 for details.

Figure 8 shows DLS particle size distributions for lyophilized formulations A, B and C after storage at 40°C for 2 months. See Example 8 for details.

Figure 9 shows second derivative FTIR spectra for formulations A, B, and C after storage at 50°C for 2 weeks. The number distribution shows the number of particles in the different size bins. See Example 8 for details.

Figure 10 shows a DSC heat flow thermogram for Formulation B. See Example 11 for details.

Figure 11 shows the VH and VL sequences of exemplary anti-TF antibodies for use in the ADC formulations of the present invention. CDR1, CDR2 and CDR3 sequences according to Kabat are highlighted: sequences in italics represent the CDR1 region, underlined sequences represent the CDR2 region and bold sequences represent the CDR3 region.

Fig 12. shows SEC average Percent Main Peak for the 5 mg/mL and 30 mg/mL formulations After Storage at 40°C for up to 2 Months. See Example 12.

Figure 13. Shows SEC average percent High Molecular Weight species for the 5 mg/mL and 30 mg/mL formulations after storage at 40°C for up to 2 Months. See Example 12.

Figure 14. Shows iCE Percent Main Peak for 5 mg/mL and 30 mg/mL HuMax-TF-ADC formulations after storage at 40°C for 2 Months. See Example 12.

Figure 15. shows iCE Percent Acidic Species for 5 mg/mL and 30 mg/mL HuMax-TF-ADC formulations after storage at 40°C for 2 Months. See Example 12.

Figure 16. Shows iCE percent Basic species for 5 mg/ml and 30 mg/ml HuMax -TF-ADC formulations after storage at 40°C for 2 months. See Example 12.

Figure 17. Shows SEC average % main peak for HuMax-TF-ADC for solution samples stored at 25°C for up to 48 hours.

Figure 18. Shows SEC Average % High Molecular Weight species for solution samples stored at 25°C for up to 48 hours.

Figure 19. Shows iCE average % Main Peak for solution samples stored at 25°C for up to 48 hours.

Figure 20. Shows iCE average % Acidic species for solution samples stored at 25°C for up to 48 hours.

Figure 21. Shows iCE average % Basic species for solution samples stored at 25°C for up to 48 hours.

Figure 22. Shows SEC average Percent Main Peak for lyophilized glycine and citrate formulations stored at 40°C for up to 2 months.

Figure 23. Shows SEC Average Percent High Molecular Weight species for lyophilized glycine and citrate formulations stored at 40°C for up to 2 months.

Figure 24. Shows iCE Percent Main Peak for HuMax-TF-ADC formulations prepared with glycine or citrate after storage at 40°C for 2 months.

Figure 25. Shows iCE Percent Acidic species for HuMax-TF-ADC formulations prepared with glycine or citrate after storage at 40°C for 2 months.

Figure 26. Shows iCE Percent Basic species for HuMax-TF-ADC formulations prepared with glycine or citrate after storage at 40°C for 2 months.

Figure 27. Shows iCE Percent Main Peak for the 10 mg/mL HuMax-TF-ADC lyophilized formulation prepared with 20 mm or 50 mm histidine at pH 5, or 6, or 7 and stored at 40°C for 2 months.

Figure 28. Shows iCE Percent Acidic species for the 10 mg/mL HuMax-TF-ADC formulation prepared with 20 mM or 50 mM histidine at pH 5, or 6, or 7 and stored at 40°C for 2 months.

Figure 29. Shows iCE Percent Basic species for the 10 mg/mL HuMax-TF-ADC formulation prepared with 20 mM or 50 mM histidine at pH 5, or 6, or 7 and stored at 40°C for 2 months.

Figure 30. Shows SEC Percent Main Peak for the 10 mg/mL HuMax-TF-ADC formulation prepared with 20 mM or 50 mM histidine at pH 5, or 6, or 7 and stored at 40°C for 2 months.

Figure 31. Shows SEC Average Percent High Molecular Weight species for the 10 mg/mL HuMax-TF-ADC formulation prepared with 20 mM or 50 mM histidine at pH 5, or 6, or 7 and stored at 40°C for 2 months.

DETAILED DISCLOSURE OF THE INVENTION

Definitions

[0019] The terms "lyophilized" and "freeze-dried" are used interchangeably herein and refer to a material that is dehydrated by first freezing and then reducing the surrounding pressure to allow the frozen water in the material to sublimate.

[0020] The term "buffer" as used herein denotes a pharmaceutically acceptable buffer. The term "buffer" encompasses those agents which maintain the pH value of a solution, *e.g.*, in an acceptable range and includes, but is not limited to, histidine, TRIS® (tris (hydroxymethyl) aminomethane), citrate, succinate, glycolate and the like, as described herein. Generally, the "buffer" as used herein has a pKa and buffering capacity suitable for the pH range of about 5 to about 7, preferably of about 5.5 to 6.5, such as about pH 6 or about pH 6.0.

[0021] The term "bulking agent" includes agents that can provide additional structure to a freeze-dried product (*e.g.*, to provide a pharmaceutically acceptable cake). Commonly used bulking agents include mannitol, glycine, sucrose, and the like. In addition to providing a pharmaceutically acceptable cake, bulking agents also typically impart useful qualities to the lyophilized composition such as modifying the collapse temperature, providing freeze-thaw protection, further enhancing the protein stability over long-term storage, and the like. These agents can also serve as tonicity modifiers.

[0022] The term "stabilizer" as used herein includes agents that provide stability to a protein, *e.g.*, serving as a cryoprotectant during freezing and/or a lyoprotectant during a (freeze-) drying or 'dehydration' process. Suitable stabilizers include non-reducing sugars or saccharides and sugar alcohols such as sucrose, trehalose, mannitol, xylitol and the like, as well as amino acids such as glycine, alanine and lysine. Stabilizers can also serve as bulking agents, tonicity-modifying and/or viscosity-increasing agents. The abbreviations "cIEF", "icIEF" and "iCE" are used interchangeably herein and all mean "capillary isoelectric focusing"

[0023] A "surfactant" as used herein is a compound that is typically used in pharmaceutical formulations to prevent drug adsorption to surfaces and or aggregation. Furthermore, surfactants lower the surface tension (or interfacial tension) between two liquids or between a liquid and a solid. For example, an exemplary surfactant can significantly lower the surface tension when present at very low concentrations (*e.g.*, 5% w/w or less, such as 3% w/w or less, such as 1% w/w or less). Surfactants are amphiphilic, which means they are usually composed of both hydrophilic and hydrophobic or lipophilic groups, thus being capable of forming micelles or similar self-assembled structures in aqueous solutions. Known surfactants for pharmaceutical use include glycerol monooleat, benzethonium chloride, sodium docusate, phospholipids, polyethylene alkyl ethers, sodium lauryl sulfate and tricaprylin (anionic surfactants); benzalkonium chloride, citrimide, cetylpyridinium chloride and phospholipids (cationic surfactants); and alpha tocopherol, glycerol monooleate, myristyl alcohol, phospholipids, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbintan fatty acid esters, polyoxyethylene sterarates, polyoxyl 15 hydroxystearate, polyoxylglycerides, polysorbates, propylene glycol dilaurate, propylene glycol monolaurate, sorbitan esters sucrose palmitate, sucrose stearate, tricaprylin and TPGS (Nonionic and zwitterionic surfactants).

[0024] A "diluent" of interest herein is one which is pharmaceutically acceptable (safe and non-toxic for administration to a human) and is useful for the preparation of a reconstituted formulation. Exemplary diluents include sterile water, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), sterile saline solution, Ringer's solution or dextrose solution.

[0025] As used herein, a "therapeutic moiety" means a compound which exerts a therapeutic or preventive effect when administered to a subject, particularly when delivered as an ADC as described herein. A "cytotoxic" or "cytostatic" moiety is a compound that is detrimental to (*e.g.,* kills) cells. Some cytotoxic or cytostatic moieties for use in ADCs are hydrophobic, meaning that they have no or only a limited solubility in water, *e.g.*, 1 g/L or less (very slightly soluble), such as 0.8 g/L or less, such as 0.6 g/L or less, such as 0.4 g/L or less, such as 0.3 g/L or less, such as 0.2 g/L or less, such as 0.1 g/L or less (practically insoluble). Exemplary hydrophobic cytotoxic or cytostatic moieties include, but are not limited to, certain microtubulin inhibitors such as auristatin and its derivatives, *e.g.*, MMAF and MMAE.

[0026] The term "immunoglobulin" refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) chains and one pair of heavy (H) chains, all four inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable region (abbreviated herein as VH or V_H) and a heavy chain constant region (CH or C_H). The heavy chain constant region typically is comprised of three domains, CH1, CH2, and CH3. Each light chain typically is comprised of a light chain variable region (abbreviated herein as VL or V_L) and a light chain constant region (CL or C_L). The light chain constant region typically is comprised of one domain, CL. The VH and VL regions may be further subdivided into regions of hypervariability (or hypervariable regions, which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity-determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk J. Mol. Biol. 196, 901 917 (1987)). Typically, the numbering of amino acid residues in this region is performed by the method described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991) (phrases such as variable domain residue numbering as in Kabat or according to Kabat herein refer to this numbering system for heavy chain variable domains or light chain variable domains). Using this numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of VH CDR2 and inserted residues (for instance residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

[0027] The term "antibody" (Ab) in the context of the present invention refers to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions with a half life of significant periods of time, such as at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen and/or time sufficient for the antibody to recruit an effector activity). The variable regions of the heavy and light chains of the immunoglobulin molecule contain a binding domain that interacts with an antigen. The constant regions of the antibodies (Abs) may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation. As indicated above, the term antibody herein, unless otherwise stated or clearly contradicted by context, includes fragments of an antibody that retain the ability to specifically bind to the antigen. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antibody" include (i) a Fab' or Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains, or a monovalent antibody as described in WO2007059782 (Genmab A/S); (ii) F(ab')2 fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the VH and CH1 domains; (iv) a Fv fragment consisting essentially of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward et al., Nature 341, 544 546 (1989)), which consists essentially of a VH domain and also called domain antibodies (Holt et al; Trends Biotechnol. 2003 Nov;21(11):484-90); (vi) camelid or nanobodies (Revets et al; Expert Opin Biol Ther. 2005 Jan;5(1):111-24) and (vii) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird et al., Science 242, 423 426 (1988) and Huston et al., PNAS USA 85, 5879 5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Although such fragments are generally included within the meaning of antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility. These and other useful antibody fragments in the context of the present invention are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), bi-specific antibodies, antibody-like polypeptides, such as chimeric antibodies and humanized antibodies, and antibody fragments retaining the ability to specifically bind to the antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype.

[0028] In the context of the present invention the term "ADC" refers to an antibody drug conjugate, which in the context of the present invention refers to an anti-TF antibody which is coupled to another moiety as described in the present application. It may e.g. be coupled with a linker to e.g. cysteine or with other conjugation methods to other amino acids. The moiety may e.g. be a drug or a toxin or the like.

[0029] An "anti-TF antibody" is an antibody as described above, which binds specifically to the antigen tissue factor or tissue factor antigen. The terms "tissue factor", "TF", "CD142", "tissue factor antigen", "TF antigen" and "CD142 antigen" are used interchangeably herein, and, unless specified otherwise, include any variants, isoforms and species homologs of human tissue factor which are naturally expressed by cells or are expressed on cells transfected with the tissue factor gene. In one embodiment, the tissue factor amino acid sequence comprises the mature form of the Genbank accession NP_001984.1 sequence. Anti-TF antibodies, in particular human anti-TF antibodies, can be produced and characterized according to the methods described in WO2011/157741.

[0030] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo).

[0031] In a preferred embodiment, the antibody of the ADC or the ADC of the invention is isolated. An "isolated antibody" or "isolated ADC" as used herein, is intended to refer to an antibody or ADC which is substantially free of other antibodies having different antigenic specificities (for instance an isolated antibody that specifically binds to TF is substantially free of antibodies that specifically bind antigens other than TF). An isolated antibody drug conjugate as used herein is intended to refer to an antibody drug conjugate which is also substantially free of "free toxin", wherein "free toxin" is intended to mean toxin which is not conjugated to the antibody. The term "substantially free of" as used in relation to the toxin may in particular mean that less than 5%, such as less than 4%, or less than 3%, or less than 2%, or less than 1.5%, or less than 1%, or less than 0.5% unconjugated drug is present when determined as described in Example 16 in WO2011157741. An isolated antibody or isolated antibody drug conjugate that specifically binds to an epitope, isoform or variant of human TF may, however, have cross-reactivity to other related antigens, for instance from other species (such as tissue factor species homologs). Moreover, an isolated antibody or ADC may be substantially free of other cellular material and/or chemicals. In one embodiment of the present invention, two or more "isolated" monoclonal antibodies or ADCs having different antigen-binding specificities are combined in a well-defined composition. In one embodiment, the two or more isolated monoclonal antibodies or ADC's bind TF at two or more different epitopes. In another embodiment it may be a mix of mAbs or ADC's with binding specificity for TF and one or more mAb or ADC's with a second binding specificity which is not TF.

[0032] When used herein in the context of two or more antibodies, the term "competes with" or "cross-competes with" indicates that the antibody competes with another antibody, *e.g.*, a "reference" antibody in binding to an antigen. For example, two or more antibodies competing for binding to TF can be analysed using the assay as described in Example 12 of WO10066803, in which antibody cross-competition studies are made using sandwich ELISA. Briefly, plate wells are coated overnight with an anti-TF antibody to be tested (e.g at +4 degrees Celsius with an anti-TF antibody to be tested using 100 microliter per well of 0.5 or 2 microgram/ml antibody in PBS buffer. The ELISA wells are washed with PBS, blocked for one hour at room temperature with 2% (v/v) serum (*e.g.*, chicken serum,) in PBS and washed again with PBS. Subsequently, 50 microliter anti-TF reference antibody (10 microgram/mL) followed by 50 microliter His-tagged extracellular domain TF (TFECDHis) (0.5 or 1 microgram/ml) is added, and incubated for 1 hour at RT (while shaking). Plates are washed 3 times with PBST (PBS+0.05%tween), and incubated with 1 :2000 diluted an anti-his-biotinylated antibody (*e.g.*, anti-his biotin BAM050) for one hour at RT (while shaking). Plates are washed and incubated with streptavidin conjugated to a directly or indirectly detectable compound (*e.g.*, Streptavidin-poly-HRP (Sanquin, Amsterdam, The Netherlands)) for 20 minutes at RT, and washed again. Then, the amount of bound streptavidin is detected and/or quantified. For example, if the indirectly detectable compound is HRP, the reaction is further developed with ABTS (Roche Diagnostics) at RT in the dark, stopped after 15 minutes by adding 2% (w/v) oxalic acid and the absorbance at 405 nm measured. The assay can also be reversed, in that the plate wells can be coated with reference antibody, to which the test antibody is then added in conjunction with the TF. For some pairs of antibodies, competition as in the assay of Example 12 of WO10066803 is only observed when one antibody is coated on the plate and the other is used to compete, and not vice versa. The term "competes with" when used herein is also intended to cover such combinations of antibodies.

[0033] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. The monoclonal antibody or composition thereof may be drug conjugated antibodies according to the present invention. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. The human monoclonal antibodies may be generated by a hybridoma which includes a B cell obtained from a transgenic or trans-chromosomal non-human animal, such as a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene, fused to an immortalized cell. Using molecular biology well-known in the art, the cDNA and/or amino acid sequences of a human monoclonal antibody can then be determined so that the antibody, optionally with another isotype can be recombinantly produced.

[0034] As used herein, the terms "binding" or "specifically binds" in the context of the binding of an antibody to a pre-determined antigen typically is a binding with an affinity corresponding to a KD, the dissociation equilibrium constant of a particular antibody-antigen interaction, of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less, about 10^{-10} M or less, or about 10^{-11} M or even less when determined by for instance surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody as the analyte, and binds to the predetermined antigen with an affinity corresponding to a KD that is at least ten-fold lower, such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the pre-determined antigen or a closely-related antigen. The amount with which the affinity is lower is dependent on the KD of the antibody, so that when the KD of the antibody is very low (that is, the antibody is highly specific), then the amount with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000 fold.

[0035] The present invention also provides, in one embodiment, formulations of antibodies comprising functional variants of the VL region, VH region, or one or more CDRs of the antibodies described herein. A functional variant of a VL, VH, or CDR used in the context of an anti-TF antibody still allows the antibody to retain at least a substantial proportion (at least about 50%, 60%, 70%, 80%, 90%, 95% or more) of the affinity/avidity and/or the specificity/selectivity of the parent antibody and in some cases such an anti-TF antibody may be associated with greater affinity, selectivity and/or specificity than the parent antibody.

[0036] Such functional variants typically retain significant sequence identity to the parent antibody. The percent identity between two sequences is a function of the number of identical positions shared by the sequences (*i.e.*, % identity = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences may be accomplished using a mathematical algorithm, as described below.

[0037] For purposes of the present invention, the sequence identity between two amino acid sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:
(Identical Residues x 100)/(Length of Alignment - Total Number of Gaps in Alignment).

[0038] For purposes of the present invention, the sequence identity between two deoxyribonucleotide sequences is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, supra) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et a/., 2000, supra), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EDNAFULL (EMBOSS version of NCBI NUC4.4) substitution matrix. The output of Needle labeled "longest identity" (obtained using the -nobrief option) is used as the percent identity and is calculated as follows:

[0039] The sequence of CDR variants may differ from the sequence of the CDR of the parent antibody sequences through mostly conservative substitutions; for instance at least about 35%, about 50% or more, about 60% or more, about 70% or more, about 75% or more, about 80% or more, about 85% or more, about 90% or more, about 95% or more such as about 96%, 97% or 98% or 99% of the substitutions in the variant are conservative amino acid residue replacements.

[0040] The sequence of CDR variants may differ from the sequence of the CDR of the parent antibody sequences through mostly conservative substitutions; for instance at least 10, such as at least 9, 8, 7, 6, 5, 4, 3, 2 or 1 of the substitutions in the variant are conservative amino acid residue replacements.

[0041] In the context of the present invention, conservative substitutions may be defined by substitutions within the classes of amino acids reflected in one or more of the following three tables:

Amino acid residue classes for conservative substitutions

[0042]

Acidic Residues	Asp (D) and Glu (E)
Basic Residues	Lys (K), Arg (R), and His (H)
Hydrophilic Uncharged Residues	Ser (S), Thr (T), Asn (N), and Gln (Q)
Aliphatic Uncharged Residues	Gly (G), Ala (A), Val (V), Leu (L), and Ile (I)
Non-polar Uncharged Residues	Cys (C), Met (M), and Pro (P)
Aromatic Residues	Phe (F), Tyr (Y), and Trp (W)

Alternative conservative amino acid residue substitution classes

[0043]

1	A	S	T
2	D	E	
3	N	Q	
4	R	K	
5	I	L	M
6	F	Y	W

Alternative Physical and Functional Classifications of Amino Acid Residues

[0044]

Alcohol group-containing residues	S and T
Aliphatic residues	I, L, V, and M

Cycloalkenyl-associated residues	F, H, W, and Y
Hydrophobic residues	A, C, F, G, H, I, L, M, R, T, V, W, and Y
Negatively charged residues	D and E
Polar residues	C, D, E, H, K, N, Q, R, S, and T
Positively charged residues	H, K, and R
Small residues	A, C, D, G, N, P, S, T, and V
Very small residues	A, G, and S
Residues involved in turn formation	A, C, D, E, G, H, K, N, Q, R, S, P, and T
Flexible residues	Q, T, K, S, G, P, D, E, and R

[0045] More conservative substitution groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine. Additional groups of amino acids may also be designed using the principles described in, *e.g.*, Creighton (1984) Proteins: Structure and Molecular Properties (2d Ed. 1993), W.H. Freeman and Company.

[0046] In one embodiment of the present invention, conservation in terms of hydropathic/hydrophilic properties and residue weight/size also is substantially retained in a variant CDR as compared to a CDR of an antibody of the examples (*e.g.*, the weight class, hydropathic score, or both of the sequences are at least about 50%, at least about 60%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or more (*e.g.*, about 99%) retained). For example, conservative residue substitutions may also or alternatively be based on the replacement of strong or weak based weight based conservation groups, which are known in the art.

[0047] The retention of similar residues may also or alternatively be measured by a similarity score, as determined by use of a BLAST program (*e.g.*, BLAST 2.2.8 available through the NCBI using standard settings BLOSUM62, Open Gap=11 and Extended Gap=1). Suitable variants typically exhibit at least about 45%, such as at least about 55%, at least about 65%, at least about 75%, at least about 85%, at least about 90%, at least about 95%, or more (e.g., about 99%) similarity to the parent peptide.

[0048] As used herein, "isotype" refers to the immunoglobulin class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) that is encoded by heavy chain constant region genes.

[0049] The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide (in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide).

[0050] "Treatment" refers to the administration of an effective amount of a therapeutically active compound of the present invention with the purpose of easing, ameliorating, arresting or eradicating (curing) symptoms or disease states.

[0051] An "effective amount" or "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of an anti-TF antibody drug conjugate may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the anti-TF antibody drug conjugate to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

Specific embodiments of the invention

[0052] The present invention is based, at least in part, on the discovery of certain aqueous compositions of anti-TF ADCs which, when lyophilized, provide for stable lyophilized formulations suitable for pharmaceutical purposes and for therapeutic applications of the anti-TF ADCs. The formulations disclosed herein also provide for the option of excluding surfactants such as, *e.g.*, polysorbate 20 and 80, inorganic salts such as, *e.g.*, NaCl.

[0053] In order to ensure efficacy and safety during the time course of the shelf life of pharmaceutical compositions, the composition is stability tested. Typically, the stability tests include but are not limited to tests regarding identity, purity and potency of the composition. The stability is tested both at the intended storage temperature and at elevated temperature or temperatures. Purity tests may include but are not limited to SDS-PAGE, CE-SDS, isoelectrofocusing, immunoelectrophoresis, Western blot, reversed-phase chromatography, Size-exclusion chromatograhy (SEC), ion exchange and affinity chromatography. Other tests may include, but are not limited to: visual appearance such as colour and transparency, particulates, pH, moisture and reconstitution time.

[0054] The degradation profile regarding, in particular, purity and potency, during the stability time course is intimately coupled to the composition and/or the formulation of the pharmaceutical product. In particular, proper choice of formulation may significantly change the degradation profile. Surfactants such as for example polysorbate 20 are often added to pharmaceutical compositions to reduce the formation of shelf life limiting degradation products. Typical degradation profiles of monoclonal antibodies and conjugated drug products derived from monoclonal antibodies incudes the formation of covalent and non-covalent high molecular weight aggregates, fragments, deamidation and oxidation products. Particularly, deamidation and oxidation products as well as other acidic species usually develop during the time course of the stability testing. In some cases the acidic species limits the acceptable shelf life of the pharmaceutical composition. The formation of acidic species due to, for exampe, deamidation, can be tested by, *e.g.*, imaged capillary isoelectrofocusing (icIEF). In other cases the formation of high molecular weight aggregates limits the acceptable shelf life of the pharmaceutical composition. The formation of aggregates may be tested by for example SEC (size exclusion chromatography), DLS, MFI, SDS-PAGE or CE-SDS.

[0055] For example, an anti-TF ADC formulation of the invention of pharmaceutically acceptable stability can be one wherein, when stored at a temperature of about 5 ± 3°C or 25 ± 2°C for a period of least about 3 months, preferably about 6 months, and more preferably about 12 months or longer, such as 18 months or longer, such as for at least 24 or even 36 months, the percentage of aggregates is less than about 10%, preferably less than about 5%, more preferably less than about 2%, when determined using SEC analysis e.g. according to Example 10. Additionally or alternatively, a stable anti-TF ADC formulation of the invention can be one wherein, when stored at a temperature of about 5 ± 3°C or 25 ± 2°C for a period of least about 3 months, preferably about 6 months, and more preferably about 12 months or longer, the changes of main isoform are less than 15%, preferably less than 10%, more preferably less than 8%, most preferably less than 5%, when determined using icIEF analysis, *e.g.*, according to Example 10.

[0056] Disclosed herein is a lyophilized formulation of an anti-tissue factor (TF) antibody-drug conjugate (ADC), the lyophilized formulation obtainable or obtained by lyophilizing an aqueous formulation comprising said anti-TF ADC and pharmaceutically acceptable excipients, wherein the formulation is free of surfactant.

[0057] The invention provides for a lyophilized formulation of an anti-tissue factor (TF) antibody-drug conjugate (ADC), the lyophilized formulation obtainable or obtained by lyophilizing an aqueous formulation comprising from about 9 to about 11 g/L of said anti-TF ADC and pharmaceutically acceptable excipients comprising about 29 to about 31 mM histidine buffer having a pH of about 5.5 to about 6.5; about 84 to about 92 mM sucrose and about 158 to about 172 mM mannitol, and wherein:

d. the formulation is free of surfactant,

e. the anti-TF antibody portion of the ADC comprises a variable heavy (VH) region comprising a CDR1 region having the amino acid sequence set forth in SEQ ID NO: 6, a CDR2 region having the amino acid sequence set forth in SEQ ID NO: 7, and a CDR3 region having the amino acid sequence set forth in SEQ ID NO: 8, and a variable light (VL) region comprising a CDR1 region having the amino acid sequence set forth in SEQ ID NO:46, a CDR2 region having the amino acid sequence set forth in SEQ ID NO: 47, and a CDR3 region having the amino acid sequence set forth in SEQ ID NO: 48, or a variant which has at most 1, 2, or 3 amino-acid modifications, more preferably amino-acid substitutions such as conservative amino acid substitutions in said sequences,

f. the drug portion of the ADC is vcMMAE.

[0058] In another aspect of the disclosure the pharmaceutically acceptable excipients comprises:

1. a) a buffer which limits pH shifts during the lyophilizing step so that pH is kept between 5 and 7,
2. b) at least one non-reducing sugar which forms an amorphous phase with the anti-TF ADC in solid state; and
3. c) at least one bulking agent.

[0059] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH of about 5 to about 7, optionally between about 5.5 and about 6.5; about 10 to about 250 mM sucrose or trehalose; and about 50 mM to about 300 mM mannitol or glycine.

[0060] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 7 g/L to about 20 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH of about 5 to about 7, optionally between about 5.5 and about 6.5; about 10 to about 250 mM sucrose or trehalose; and about 50 mM to about 300 mM mannitol or glycine.

[0061] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 25 to about 40 mM histidine buffer, such as about 29 to about 31 mM, having a pH of about 5 to about 7, optionally between about 5.5 and about 6.5; about 10 to about 250 mM sucrose or trehalose; and about 50 mM to about 300 mM mannitol or glycine.

[0062] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH of about 5 to about 7, optionally between about 5.5 and about 6.5; about 50 to 225 mM sucrose or trehalose, such as about 84 to about 165 mM sucrose or trehalose; and about 50 mM to about 300 mM mannitol or glycine.

[0063] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH of about 5 to about 7, optionally between about 5.5 and about 6.5; about 10 to about 250 mM sucrose or trehalose; and about 100 mM to about 274 mM, such as about 158 to about 274, such as about 158 to about 172 mM mannitol or glycine.

[0064] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH between about 5.5 and about 6.5; about 50 to about 225 mM sucrose or trehalose; and about 100 mM to about 274 mM mannitol or glycine.

[0065] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH of about 5 to about 7, optionally between about 5.5 and about 6.5; about 84 to about 165 mM sucrose or trehalose; and about 100 to about 274 mM mannitol or glycine.

[0066] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 20 to about 50 mM histidine buffer having a pH of between about 5.5 and about 6.5; about 84 to about 146 mM sucrose; and about 158 mM to about 274 mM mannitol.

[0067] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 5 g/L to about 30 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 25 to about 40 mM histidine buffer having a pH between about 5.5 and about 6.5; about 84 to about 92 mM sucrose; and about 158 to about 274 mM mannitol.

[0068] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 7 g/L to about 20 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 25 to about 40 mM histidine buffer having a pH between about 5.5 and about 6.5; about 84 to about 92 mM sucrose; and about 100 mM to about 274 mM mannitol or glycine.

[0069] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 7 g/L to about 20 g/L, such as about 9 to about 11 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 25 to about 40 mM histidine buffer having a pH of about 5.5 to about 6.5; about 84 to about 92 mM sucrose or trehalose; and about 158 to about 172 mM mannitol or glycine.

[0070] In one embodiment, the invention relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 9 to about 11 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising about 29 to about 31 mM histidine buffer having a pH of about 5.5 to about 6.5; about 84 to about 92 mM sucrose; and about 158 to about 172 mM mannitol or glycine.

[0071] In another embodiment, the invention relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 9 to about 11 g/L anti-TF ADC, such as about 10 mg/mL anti-TF ADC, and pharmaceutically acceptable excipients comprising about 30 mM histidine buffer having a pH of about 6; about 88 mM sucrose; and about 165 mM mannitol or glycine.

[0072] In another aspect, the disclosure relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 10 mg/mL anti-TF ADC, and pharmaceutically acceptable excipients comprising about 25 to about 40 mM to histidine buffer, such as 30-35 mM histidine buffer, such as about 30 mM, and having a pH of about 6; about 88 mM sucrose; and about 165 mM mannitol.

[0073] In another embodiment, the invention relates to a lyophilized formulation prepared by lyophilizing an aqueous formulation comprising about 9 to about 11 g/L anti-TF ADC, such as about 10 mg/mL anti-TF ADC wherein the anti-TF-ADC is HuMax TF ADC (IgG1, vcMMAE), which is an ADC composed of a human monoclonal IgG1,κ antibody 011 against TF conjugated via a protease cleavable valine citrulline linker to the drug monomethyl auristatin E (vcMMAE), and pharmaceutically acceptable excipients comprising about 30 mM histidine buffer having a pH of about 6; about 88 mM sucrose; and about 165 mM mannitol or glycine.

[0074] In separate and specific disclosure, the formulations are essentially free of surfactant.

[0075] The formulations of the invention are free of surfactant.

[0076] In another aspect, the disclosure relates to a lyophilized formulation of an anti-TF ADC, the lyophilized formulation prepared by lyophilizing an aqueous formulation comprising pharmaceutically acceptable excipients comprising: a buffer which limits pH shifts during the lyophilizing step, at least one non-reducing sugar which forms an amorphous phase with the anti-TF ADC in solid state and at least one bulking agent, wherein the anti-TF ADC comprises a drug-linker which is selected from mcMMAF, mcMMAE, vcMMAF and vcMMAE and an anti-TF antibody comprising VH and VL regions selected from the group consisting of: a VH region comprising an amino acid sequence of SEQ ID NO: 5 and a VL region comprising an amino acid sequence of SEQ ID NO: 45; a VH region comprising an amino acid sequence of SEQ ID NO: 33 and a VL region comprising an amino acid sequence of SEQ ID NO: 73; a VH region comprising an amino acid sequence of SEQ ID NO: 37 and a VL region comprising an amino acid sequence of SEQ ID NO: 77; or a VH region comprising an amino acid sequence of SEQ ID NO: 1 and a VL region comprising an amino acid sequence of SEQ ID NO: 41; optionally wherein the lyophilized formulation is essentially free of any surfactant.

[0077] In an aspect of the disclosure, the antibody-moiety of the ADC of any one of the preceding embodiments comprises the VH and VL CDRs, optionally the VH (SEQ ID NO:5) and VL (SEQ ID NO:45) sequences, of human anti-TF antibody 011, and a drug-linker which is mcMMAE, vcMMAE, vcMMAF or mcMMAF.

[0078] In an aspect of the disclosure, the antibody-moiety of the ADC of any one of the preceding embodiments comprises the VH and VL CDRs, optionally the VH (SEQ ID NO:33) and VL (SEQ ID NO:73) sequences, of human anti-TF antibody 098, and a drug-linker which is mcMMAE, vcMMAE, vcMMAF or mcMMAF.

[0079] In an aspect of the disclosure, the antibody-moiety of the ADC of any one of the preceding embodiments comprises the VH and VL CDRs, optionally the VH (SEQ ID NO:37) and VL (SEQ ID NO:77) sequences, of human anti-TF antibody 111, and a drug-linker which is mcMMAE, vcMMAE, vcMMAF or mcMMAF.

[0080] In other aspects, the disclosure provides for a lyophilized formulation of any one of the preceding paragraphs, which is essentially free of any polysorbate, optionally of any surfactant.

[0081] The disclosure also provides for a lyophilized formulation consisting essentially of an anti-TF antibody drug conjugate; a buffering agent selected from histidine, citrate and tris; a non-reducing sugar selected from sucrose, trehalose and a combination thereof, and a bulking agent selected from mannitol and glycine.

[0082] In one embodiment of the invention, the anti-TF antibody comprises the VH and VL CDR regions, optionally the VH and VL sequences, of the VH region comprising an amino acid sequence of SEQ ID NO: 5 and a VL region comprising an amino acid sequence of SEQ ID NO: 45.

[0083] Also disclosed herein are: a VH region comprising an amino acid sequence of SEQ ID NO: 33 and a VL region comprising an amino acid sequence of SEQ ID NO: 73; a VH region comprising an amino acid sequence of SEQ ID NO: 37 and a VL region comprising an amino acid sequence of SEQ ID NO: 77; or a VH region comprising an amino acid sequence of SEQ ID NO: 1 and a VL region comprising an amino acid sequence of SEQ ID NO: 41. In one embodiment, the anti-TF ADC comprises the VH and VL CDRs of SEQ ID NOS:5 and 45, respectively. Also disclosed are the anti-TF ADC comprising the VH and VL CDRs of SEQ ID NOS:33 and 73, respectively and the anti-TF ADC comprising the VH and VL CDRs of SEQ ID NOS:37 and 77, respectively.

[0084] Also disclosed herein is a lyophilized formulation comprising mannitol and sucrose, wherein the weight ratio of mannitol to sucrose is at least about 1, such as between about 1 and about 30, such as between 1 and about 10, such as between about 1 and about 2, such as about 1.

[0085] Also disclosed herein is a lyophilized formulation may compriseing mannitol and trehalose, wherein the weight ratio of mannitol to trehalose is at least about 1, such as between about 1 and about 30, such as between 1 and about 10, such as between about 1 and about 2, such as about 1.

[0086] Also disclosed herein is a lyophilized formulation comprising mannitol and sucrose, wherein the weight ratio of mannitol to sucrose is between about 1 and about 10 and the weight ratio of mannitol to ADC is at least about 3, such as between 3 and 30.

[0087] The invention also provides for a lyophilized formulation obtainable by lyophilizing an aqueous formulation comprising, optionally consisting of, from about 9 to about 11 g/L anti-TF ADC and about 30 mM histidine; about 88 mM sucrose; and about 165 mM mannitol.

[0088] The disclosure also provides for an aqueous solution suitable for preparing a lyophilized formulation of an anti-TF ADC, comprising

1. a. from about 7 to about 20 g/L anti-TF ADC, optionally comprising the VH and VL CDRs or the VH and VL sequences, of anti-TF antibody 011,
2. b. about 28 to 34 mM histidine;
3. c. about 84 to about 146 mM sucrose;
4. d. about 158 to about 274 mM mannitol; or
5. e. a combination of a) and any two, three or all of (b) to (d).

[0089] The disclosure also provides for an aqueous solution suitable for preparing a lyophilized formulation of an anti-TF ADC wherein said aqueous solution does not contain a surfactant, said solution comprising:

1. a. from about 7 to about 20 g/L anti-TF ADC, optionally comprising the VH and VL CDRs or the VH and VL sequences, of anti-TF antibody 011,
2. b. about 28 to 34 mM histidine;
3. c. about 84 to about 146 mM sucrose;
4. d. about 158 to about 274 mM mannitol; or
5. e. a combination of (a) and any two, three or all of (b) to (d).

[0090] The disclosure also provides for an aqueous solution suitable for preparing a lyophilized formulation of an anti-TF ADC, consisting of:

1. a. from about 7 to about 20 g/L anti-TF ADC, optionally comprising the VH and VL CDRs or the VH and VL sequences, of anti-TF antibody 011,
2. b. about 28 to 34 mM histidine;
3. c. about 84 to about 146 mM sucrose;
4. d. about 158 to about 274 mM mannitol; or
5. e. a combination of (a) and any two, three or all of (b) to (d).

[0091] The invention also provides for a pharmaceutically acceptable liquid formulation obtained by reconstituting the lyophilized formulation of any one of the preceding embodiments in a sterile aqueous diluent. For example it is disclosed that, such a liquid formulation may comprise or essentially consist of about 5 g/L to about 30 g/L anti-TF ADC, about 20 to about 50 mM histidine having a pH of about 5 to about 7; about 10 to about 250 mM sucrose or trehalose; and about 50 mM to about 300 mM mannitol or glycine. In one embodiment of the invention, the liquid formulation comprises or essentially consists of about 9 to about 11 mg/mL anti-TF ADC, about 28 to about 34 mM histidine, about 84 to about 92 mM sucrose and about 158 to about 274 mM mannitol.

[0092] A lyophilized formulation of an anti-TF ADC can be prepared by lyophilizing an aqueous formulation comprising about 9 g/L to about 11 g/L anti-TF ADC and pharmaceutically acceptable excipients comprising: about 30 mM histidine buffer having a pH of about 5.5 to about 6.5; about 88 mM sucrose; and about 165 mM mannitol; wherein the antibody comprises a VH region comprising an amino acid sequence of SEQ ID NO: 5 and a VL region comprising an amino acid sequence of SEQ ID NO: 45; a VH region comprising an amino acid sequence of SEQ ID NO: 33 and a VL region comprising an amino acid sequence of SEQ ID NO: 73; a VH region comprising an amino acid sequence of SEQ ID NO: 37 and a VL region comprising an amino acid sequence of SEQ ID NO: 77; or a VH region comprising an amino acid sequence of SEQ ID NO: 1 and a VL region comprising an amino acid sequence of SEQ ID NO: 41, and wherein the drug is MMAF or MMAE, *e.g.*, a linker-drug which is vcMMAE.

[0093] In another embodiment, the lyophilized formulation of the invention contains less than 3.0 wt. % moisture. In another embodiment, the lyophilized formulation of the invention contains less than 2.0 wt. % moisture. In another embodiment, the lyophilized formulation of the invention contains less than 1.0 wt. % moisture. In another embodiment, the lyophilized formulation of the invention contains less than 0.5 wt. % moisture.

[0094] In another preferred aspect, any one of the preferred formulations as above comprises an exact quantity or exact quantities of one or more components as comprised therein and/or an exact pH value. In other words, one or more of the terms "about" are deleted in this other preferred aspect of the invention.

Antibody drug-conjugate

[0095] As described herein, the formulations of the invention are suitable for, e.g., anti-TF ADCs.

[0096] In one aspect, the lyophilized formulations of the disclosure comprise an anti-TF ADC conjugated to a therapeutic moiety selected from the group consisting of taxol; cytochalasin B; gramicidin D; ethidium bromide; emetine; mitomycin; etoposide; tenoposide; vincristine; vinblastine; colchicin; doxorubicin; daunorubicin; dihydroxy anthracin dione; a tubulin-inhibitor such as maytansine or an analog or derivative thereof; mitoxantrone; mithramycin; actinomycin D; 1-dehydrotestosterone; a glucocorticoid; procaine; tetracaine; lidocaine; propranolol; puromycin; calicheamicin or an analog or derivative thereof an antimetabolite such as methotrexate, 6 mercaptopurine, 6 thioguanine, cytarabine, fludarabin, 5 fluorouracil, decarbazine, hydroxyurea, asparaginase, gemcitabine, or cladribine; an alkylating agent such as mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin, carboplatin, duocarmycin A, duocarmycin SA, rachelmycin (CC-1065), or an analog or derivative thereof; pyrrolo[2,1-c][1,4] benzodiazepines (PDBs) or analogues thereof; an antibiotic such as dactinomycin, bleomycin, daunorubicin, doxorubicin, idarubicin, mithramycin, mitomycin, mitoxantrone, plicamycin, anthramycin (AMC)); diphtheria toxin and related molecules such as diphtheria A chain and active fragments thereof and hybrid molecules, ricin toxin such as ricin A or a deglycosylated ricin A chain toxin, cholera toxin, a Shiga-like toxin such as SLT I, SLT II, SLT IIV, LT toxin, C3 toxin, Shiga toxin, pertussis toxin, tetanus toxin, soybean Bowman-Birk protease inhibitor, Pseudomonas exotoxin, alorin, saporin, modeccin, gelanin, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins such as PAPI, PAPII, and PAP S, momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, and enomycin toxins; ribonuclease (RNase); DNase I, Staphylococcal enterotoxin A; pokeweed antiviral protein; diphtherin toxin; and Pseudomonas endotoxin.

[0097] In one aspect of the disclosure, the antibody is conjugated to a cytotoxic or cytostatic moiety which is a drug selected from the group consisting of dolastatin, maytansine, calicheamicin, duocarmycin, rachelmycin (CC-1065), or an analog, derivative, or prodrug of any thereof.

[0098] In one aspect of the disclosure, the antibody is conjugated to a therapeutic, cytostatic, and/or cytotoxic moiety which is a tubulin inhibitor, DNA interactive compound and/or a kinase inhibitor. In one aspect of the disclosure, the antibody is conjugated to a hydrophobic compound, such as a hydrophobic tubulin inhibitor, preferably an auristatin, more preferred MMAE or MMAF. According to the invention the antibody is conjugated to MMAE.

[0099] The drug-loading (or average number of cytostatic or cytotoxic drugs per antibody molecule), is typically 1 to about 8, *e.g.* p may be from 3-6, such as from 4-6 or from 3-5, or p may be 1, 2, 3, 4, 5, 6, 7 or 8, such as 3, 4 or 5, such as 4.

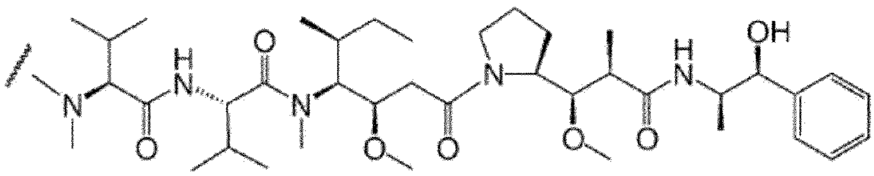
[0100] The ADCs for use in the formulations of the invention typically comprise a linker unit between the cytostatic or cytotoxic drug unit and the antibody unit.

[0101] In some aspects of the disclosure, the linker is cleavable under intracellular conditions, such that the cleavage of the linker releases the drug unit from the antibody in the intracellular environment. In yet another aspect, the linker unit is not cleavable, and the drug is for instance released by antibody degradation. In some aspects, the linker is cleavable by a cleavable agent that is present in the intracellular environment (e. g. within a lysosome or endosome or caveola). The linker can be, e. g. a peptidyl linker that is cleaved by an intracellular peptidase or protease enzyme, including but not limited to, a lysosomal or endosomal protease. In some aspects, the peptidyl linker is at least two amino acids long or at least three amino acids long. Cleaving agents can include cathepsins B and D and plasmin, all of which are known to hydrolyze dipeptide drug derivatives resulting in the release of active drug inside the target cells (see e. g. Dubowchik and Walker, 1999, Pharm. Therapeutics 83:67-123). In a specific embodiment, the peptidyl linker cleavable by an intracellular protease is a Val-Cit (valine-citrulline) linker or a Phe-Lys (phenylalanine-lysine) linker (see e.g. US6214345, which describes the synthesis of doxorubicin with the Val-Cit linker and different examples of Phe-Lys linkers). Examples of the structures of a Val-Cit and a Phe-Lys linker include but are not limited to MC-vc-PAB described below, MC-vc-GABA, MC-Phe-Lys-PAB or MC-Phe-Lys-GABA, wherein MC is an abbreviation for maleimido caproyl, vc is an abbreviation for Val-Cit, PAB is an abbreviation for p-aminobenzylcarbamate and GABA is an abbreviation for γ-aminobutyric acid. An advantage of using intracellular proteolytic release of the therapeutic agent is that the agent is typically attenuated when conjugated and the serum stabilities of the conjugates are typically high.

[0102] In some aspects of the disclosure, the linker unit is not cleavable and the drug is released by antibody degradation (see US 2005/0238649). Typically, such a linker is not substantially sensitive to the extracellular environment.

[0103] In a preferred aspect, the antibody is conjugated to a dolastatin derivative such as an auristatin. Auristatins or auristatin peptide analogs and derivates have been shown to interfere with microtubule dynamics, GTP hydrolysis and nuclear and cellular division and have anticancer and anti-fungal activity are described in, e.g., US5635483; US5780588; US5663149. The auristatin drug moiety is typically attached to the antibody via a linker, through the N (amino) terminus or the C (terminus) of the peptidic drug moiety. Exemplary auristatin examples include the N-terminus-linked monomethyl auristatin drug moieties DE and DF, disclosed in Senter et al., Proceedings of the American Association for Cancer Research. Volume 45, abstract number 623, presented March 28, 2004 and described in US 2005/0238649).

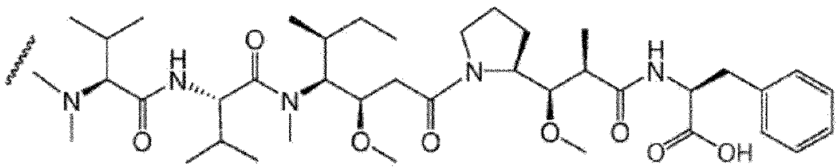
[0104] The auristatin of the invention is monomethyl auristatin E (MMAE):



MMAE

wherein the wavy line indicates the attachment site for the linker.

[0105] In another aspect of the disclosure the auristatin is monomethyl auristatin F (MMAF):

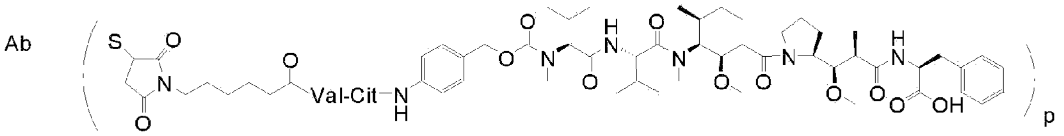


MMAF

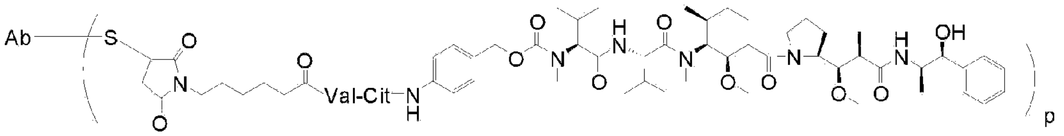
wherein the wavy line indicates the attachment site for the linker.

[0106] In one embodiment the linker is attached to sulphydryl residues of the antibody, e.g., an anti-TF antibody, obtained by (partial) reduction of the antibody.

[0107] In one aspect of the disclosure the linker-auristatin is MC-vc-PAB-MMAF (also designated as vcMMAF). In an embodiment of the invention the linker-auristatin is MC-vc-PAB-MMAE (also designated as vcMMAE)



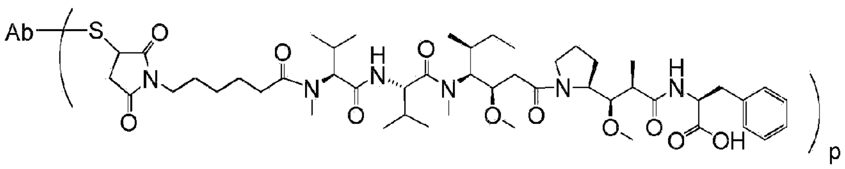
Ab-MC-vc-PAB-MMAF (Ab-vcMMAF)



Ab-MC-vc-PAB-MMAE (Ab-vcMMAE)

wherein p denotes a number of from 1 to 8, *e.g.* p may be from 3-5, S represents a sulphydryl residue of the antibody, and Ab designates the antibody. In one embodiment the linker-auristatin is vcMMAE.

[0108] In another aspect of the disclosure the linker-conjugate is mcMMAF (where mc/MC is an abbreviation of maleimido caproyl):



Ab-MC-MMAF (Ab-mcMMAF)

wherein p denotes a number of from 1 to 8, *e.g.* p may be from 3-5, S represents a sulphydryl residue of the anti-TF antibody, and Ab designates the antibody.

[0109] Although generally applicable to any anti-TF antibody, antibodies particularly suitable for the ADC formulations of the invention are those that share one or more physicochemical and/or antigen-binding binding properties with any one or more of the anti-TF antibodies for which VH and VL sequences are provided herein (see Table 1 and Figure 11), such as, *e.g.,* antibody 011, 098, 114, 017-D12, 042, 092-A09, 101, 025, 109 or 111, such as, *e.g.,* antibody 011, 098 or 111, such as 011. Accordingly, in one embodiment, when conjugated to the drug in question, the resulting ADC can have a pI in the range of about 5 to about 12, such as about 7 to about 10, such as about 8.5 to about 9.5, such as about 8.5 to about 9.0.

[0110] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 011, optionally in an IgG1,κ format.

[0111] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 098, optionally in an IgG1,κ format.

[0112] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 114, optionally in an IgG1,κ format.

[0113] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 017-D12 optionally in an IgG1,κ format.

[0114] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 042, optionally in an IgG1,κ format.

[0115] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 092-A09, optionally in an IgG1,κ format.

[0116] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 101, optionally in an IgG1,κ format.

[0117] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 025, optionally in an IgG1,κ format.

[0118] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 109 optionally in an IgG1,κ format.

[0119] In one aspect of the disclosure, the ADC can bind to the same epitope as and/or comprise one or more CDR sequence of, human antibody 111, optionally in an IgG1,κ format.

[0120] In one aspect of the disclosure the antibody is an anti-TF antibody competing for tissue factor binding with a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:33 and a VL region comprising the sequence of SEQ ID NO:73, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO: 1 and a VL region comprising the sequence of SEQ ID NO:41, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:5 and a VL region comprising the sequence of SEQ ID NO:45, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:9 and a VL region comprising the sequence of SEQ ID NO:49, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:13 and a VL region comprising the sequence of SEQ ID NO:53, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:17 and a VL region comprising the sequence of SEQ ID NO:57, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:21 and a VL region comprising the sequence of SEQ ID NO:61, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:25 and a VL region comprising the sequence of SEQ ID NO:65, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:69, or with an antibody comprising a VH region comprising the sequence of SEQ ID NO:37 and a VL region comprising the sequence of SEQ ID NO:77. In one aspect the antibody is an anti-TF antibody competing for tissue factor binding with a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:5

and a VL region comprising the sequence of SEQ ID NO:45.

[0121] In one aspect of the disclosure the anti-TF antibody comprises: a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:34, 35 and 36 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:74, 75 and 76; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:2, 3 and 4 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:42, 43 and 44; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:6, 7 and 8 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:46, 47 and 48; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:10, 11 and 12 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:50, 51 and 52, or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:14, 15 and 16 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:54, 55 and 56; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:18, 19 and 20 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:58, 59 and 60; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:22, 23 and 24 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:62, 63 and 64, or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:26, 27 and 28 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:66, 67 and 68; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:30, 31 and 32 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:70, 71 and 72; or a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:38, 39 and 40 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:78, 79 and 80; or a variant of any of said antibodies, wherein said variant preferably has at most 1, 2 or 3 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino-acid substitutions in said sequences.

[0122] In one embodiment of the invention, the anti-TF antibody comprises: a VH region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:6, 7 and 8 and a VL region comprising the CDR1, 2 and 3 sequences of SEQ ID NO:46, 47 and 48, or a variant which has at most 1, 2 or 3 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino-acid substitutions in said sequences.

[0123] In one aspect of the disclosure the anti-TF antibody comprises a VH having at least 80% identity, such as at least 90%, at least 95%, or at least 98% or 100% identity to a VH region sequence selected from the group consisting of: SEQ ID NO:33, 1, 5, 9, 13, 17, 21, 25, 37 and 29; or at most 20, such as 15, or 10, or 5, 4, 3, 2 or 1 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino-acid substitutions as compared to a VH region sequence selected from the group consisting of: SEQ ID NO: 1, 5, 9, 13, 17, 21, 25, 33, 37 and 29.

[0124] In one aspect of the disclosure, the anti-TF antibody comprises a VL having at least 80% identity, such as at least 90%, at least 95%, or at least 98% or 100% identity to a VL region sequence selected from the group consisting of: SEQ ID NO:41, 45, 49, 53, 57, 61, 65, 73, 77 and 69; or at most 20, such as 15, or 10, or 5, 4, 3, 2 or 1 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino-acid substitutions as compared to a VH region sequence selected from the group consisting of: SEQ ID NO:41, 45, 49, 53, 57, 61, 65, 73, 77 and 69.

[0125] In another aspect of the disclosure, the anti-TF antibody is the full-length, fully human monoclonal IgG1,κ antibody Anti-TF HuMab 092-A09, Anti-TF HuMab 101, Anti-TF HuMab 025, Anti-TF HuMab 109, Anti-TF HuMab 017-D12, Anti-TF HuMab 114, Anti-TF HuMab 042, Anti-TF HuMab 011, Anti-TF HuMab 098 or Anti-TF HuMab 111 or an antibody comprising the VH and VL CDRs of any thereof, or an antibody comprising the VH and VL sequence thereof. In one particular embodiment, the anti-TF antibody is anti-TF HuMab 011, or an antibody comprising the VH CDR1, 2, 3 and VL CDR 1, 2, 3 sequences thereof, or an antibody comprising the VH and VL sequence thereof. In another aspect, the anti-TF antibody is anti-TF HuMab 098, or an antibody comprising the VH CDR1, 2, 3 and VL CDR 1, 2, 3 sequences thereof, or an antibody comprising the VH and VL sequence thereof. In another aspect, the anti-TF antibody is anti-TF HuMab 111, or an antibody comprising the VH CDR1, 2, 3 and VL CDR 1, 2, 3 sequences thereof, or an antibody comprising the VH and VL sequence thereof.

[0126] Table 1 sets out the specific sequence identifiers (SEQ ID No) relating to the VH (1A) and VL (1B) sequences of these antibodies.

Table 1A

VH-region	
SEQ ID No: 1	VH 114
SEQ ID No: 2	VH 114, CDR1
SEQ ID No: 3	VH 114, CDR2
SEQ ID No: 4	VH 114, CDR3
SEQ ID No: 5	VH 011
SEQ ID No: 6	VH 011, CDR1
SEQ ID No: 7	VH 011, CDR2
SEQ ID No: 8	VH 011, CDR3
SEQ ID No: 9	VH 017-D12
SEQ ID No: 10	VH 017-D12, CDR1
SEQ ID No: 11	VH 017-D12, CDR2
SEQ ID No: 12	VH 017-D12, CDR3
SEQ ID No: 13	VH 042
SEQ ID No: 14	VH 042, CDR1
SEQ ID No: 15	VH 042, CDR2
SEQ ID No: 16	VH 042, CDR3
SEQ ID No: 17	VH 092-A09
SEQ ID No: 18	VH 092-A09, CDR1

VH-region	
SEQ ID No: 19	VH 092-A09, CDR2
SEQ ID No: 20	VH 092-A09, CDR3
SEQ ID No: 21	VH 101
SEQ ID No: 22	VH 101, CDR1
SEQ ID No: 23	VH 101, CDR2
SEQ ID No: 24	VH 101, CDR3
SEQ ID No: 25	VH 025
SEQ ID No: 26	VH 025, CDR1
SEQ ID No: 27	VH 025, CDR2
SEQ ID No: 28	VH 025, CDR3
SEQ ID No: 29	VH 109
SEQ ID No: 30	VH 109, CDR1
SEQ ID No: 31	VH 109, CDR2
SEQ ID No: 32	VH 109, CDR3
SEQ ID No: 33	VH 098
SEQ ID No: 34	VH 098, CDR1
SEQ ID No: 35	VH 098, CDR2
SEQ ID No: 36	VH 098, CDR3
SEQ ID No: 37	VH 111
SEQ ID No: 38	VH 111, CDR1
SEQ ID No: 39	VH 111, CDR2
SEQ ID No: 40	VH 111, CDR3

Table 1B

VL-region	
SEQ ID No: 41	VL 114
SEQ ID No: 42	VL 114, CDR1
SEQ ID No: 43	VL 114, CDR2
SEQ ID No: 44	VL 114, CDR3
SEQ ID No: 45	VL 011
SEQ ID No: 46	VL 011, CDR1
SEQ ID No: 47	VL 011, CDR2
SEQ ID No: 48	VL 011, CDR3
SEQ ID No: 49	VL 017-D12
SEQ ID No: 50	VL 017-D12, CDR1
SEQ ID No: 51	VL 017-D12, CDR2

VL-region	
SEQ ID No: 52	VL 017-D12, CDR3
SEQ ID No: 53	VL 042
SEQ ID No: 54	VL 042, CDR1
SEQ ID No: 55	VL 042, CDR2
SEQ ID No: 56	VL 042, CDR3
SEQ ID No: 57	VL 092-A09
SEQ ID No: 58	VL 092-A09, CDR1
SEQ ID No: 59	VL 092-A09, CDR2
SEQ ID No: 60	VL 092-A09, CDR3
SEQ ID No: 61	VL 101
SEQ ID No: 62	VL 101, CDR1
SEQ ID No: 63	VL 101, CDR2
SEQ ID No: 64	VL 101, CDR3
SEQ ID No: 65	VL 025
SEQ ID No: 66	VL 025, CDR1
SEQ ID No: 67	VL 025, CDR2
SEQ ID No: 68	VL 025, CDR3
SEQ ID No: 69	VL 109
SEQ ID No: 70	VL 109, CDR1
SEQ ID No: 71	VL 109, CDR2
SEQ ID No: 72	VL 109, CDR3
SEQ ID No: 73	VL 098
SEQ ID No: 74	VL 098, CDR1
SEQ ID No: 75	VL 098, CDR2
SEQ ID No: 76	VL 098, CDR3
SEQ ID No: 77	VL 111
SEQ ID No: 78	VL 111, CDR1
SEQ ID No: 79	VL 111, CDR2
SEQ ID No: 80	VL 111, CDR3

[0127] In a particularly preferred embodiment, the ADC is HuMax TF ADC (IgG1, vcMMAE), which is an ADC composed of a human monoclonal IgG1,κ antibody 011 against TF conjugated via a protease cleavable valine citrulline linker to the drug monomethyl auristatin E (vcMMAE). The identification and production of this antibody is described in WO 2011157741. Each monoclonal antibody (mAb) molecule carries an average of 4 drug molecules. The antibody portion has an approximate molecular weight of 147 kDa. On average, four molecules of vcMMAE (molecular weight 1.3 kDa) are attached to each mAb molecule yielding a total average molecular weight of HuMax TF ADC of 152 kDa. The isoelectric point of HuMax-TF-ADC is approximately 8.7.

Formulation

[0128] Therapeutic formulations of the antibodies used in accordance with the present invention are prepared for storage by mixing an ADC having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed.

[0129] Generally, the lyophilized and reconstituted formulations according to the disclosure comprise an anti-TF ADC, a buffering agent, a stabilizing agent (typically a non-reducing sugar or a sugar alcohol or an amino acid), and a bulking agent. Preferred stabilizing agents are sucrose, trehalose and combinations thereof. Preferred bulking agents are mannitol, glycine and combinations thereof.

[0130] The term "buffer" as used herein denotes a pharmaceutically acceptable buffer. Generally, the buffer has a pKa and buffering capacity suitable for the pH range of about 5 to about 7, preferably of about 5.5 to 6.5, such as about pH 6 or about pH 6.0. For lyophilized formulations, the buffer components should not crystallize at sub-ambient temperatures at the concentration used. Buffers having a higher collapse temperature are preferred, since it will enable a faster and more robust lyophilization cycle. Suitable pharmaceutically acceptable buffers include, but are not limited to, histidine-buffers, citrate- buffers, succinate-buffers, carbonic acid-buffers, phosphate buffers, glycolate-buffers, TRIS® (tris (hydroxymethyl) aminomethane) buffers and mixtures thereof. Preferred buffers are based on L-histidine, citrate, phosphate, carbonic acid, succinate and/or glycolate, such as histidine and/or citrate, and include also mixtures, e.g., of L-histidine with L-histidine hydrochloride or with TRIS® (tris (hydroxymethyl) aminomethane). Potentially, pH adjustment with an acid or a base known in the art may be needed. The above-mentioned L-histidine, citrate, phosphate, carbonic acid, succinate and/or glycolate buffers are generally used in an amount of about 1 mM to about 100 mM, such as from about 5 to about 80 mM, preferably of about 20 mM to about 50 mM, more preferably of about 10 to about 30 mM, and still more preferably of about 30 mM. The concentration of a phosphate buffer is preferably in the range of about 1 to about 30 mM. Independently from the buffer used, the pH can be adjusted at a value comprising about 5 to about 7 and preferably about 5.5 to about 6.5 and most preferably about 6.0 by adjustment with an acid or base known in the art or by using adequate mixtures of buffer components or both. Preferably, the buffer comprises a histidine and/or citrate buffer at a concentration of about 10 to about 30 mM, such as a histidine buffer at a concentration of about 30 mM.

[0131] The formulation of the invention can further comprise one or more pharmaceutically acceptable stabilizers as defined hereinabove and ingredients also known in the art as "lyoprotectants" such as sugars, sugar alcohols, amino sugars, amino acids and dextrans as known in the art. Typically, pharmaceutically acceptable stabilizer can be used in an amount of about 1 mM to about 500 mM. Suitable sugars comprise but are not limited to monosaccharides and disaccharides. Non-limiting examples of sugars and sugar alcohols for use according to the invention include trehalose, sucrose, mannitol, sorbitol, mannose, maltose, galactose, fructose, sorbose, raffinose, glucosamine, N-methylglucosamine (also referred to as "meglumine"), galactosamine and neuraminic acid and combinations thereof. Preferred are non-reducing sugars and sugar alcohols, such as sucrose or trehalose, at concentrations of about 10 to about 250 mM, such as about 50 to 225 mM, such as about 84 to 146, such as about 84 to 92 mM. Most preferred is sucrose.

[0132] In one embodiment the formulation comprises 84 to 92 mM sucrose, such as 85, 86, 87, 88, 89, 90, 91 or 92 mM sucrose. Most preferred the formulation comprises 88 mM sucrose.

[0133] Particularly, sugar alcohols such as mannitol may also be used as bulking agent to produce a homogeneous and stable lyophilization cake, which can be reconstituted within an acceptable time, more specifically within 0 - 600 seconds. In general, a "bulking agent" is used when the total amount of API is too small to provide adequate structure to the cake. Bulking agents should provide an inert matrix which gives a pharmaceutically elegant cake. The bulking agent also modifies the thermal characteristics of a formulation. The concentration of the active drug is often so low that the freeze-drying characteristics of the system are due solely to the bulking agent. Common bulking agents are including mannitol, glycine as crystalline bulking agents; sucrose, trehalose, gelatin, dextran as amorphous bulking agents. Preferred bulking agents are mannitol and glycine.

[0134] In one embodiment, the formulation comprises from about 158 to about 274 mM mannitol, such as 160 mM, or 162 mM, or 165 mM, or 170 mM, or 180 mM, or 200 mM mannitol. Most preferred it comprises about 165 mM mannitol, or 165 mM mannitol.

[0135] Lyophilized formulations according to the invention are designed so that it is possible to exclude surfactants. However, as a person skilled in the art can appreciate, for some purposes it may nonetheless be desirable to include a surfactant. Suitable pharmaceutically acceptable surfactants comprise but are not limited to polyethylen-sorbitan-fatty acid esters, polyethylene-polypropylene glycols, polyoxyethylene- stearates and sodium dodecyl sulphates. Polyethylen-sorbitan-fatty acid esters include polyethylen(20)- sorbitan-esters (synonym to polysorbate 20, sold under the trademark (TM) Tween 20(TM) and polyoxyethylene(20)sorbitanmonooleate (synonym to polysorbate 80 sold under the trademark Tween 80(TM)). Polyethylene-polypropylene glycols are those sold under the names Pluronic(R) F68 or Poloxamer 188(TM). Polyoxyethylene- stearates are sold under the trademark Myrj(TM). Polyoxyethylene monolauryl ether are those sold under the trademark Brij(TM). When desirable, polyethylen-sorbitan-polyethylen(20)-sorbitan-esters (Tween 20(TM)) and polyoxyethylene(20)sorbitanmonooleate (Tween 80(TM)) can be used, *e.g.*, in an amount of about 0.01% to about 0.06%, such as about 0.02% to about 0.04%.

[0136] Certain lyophilized formulations according to the invention are designed so that it is possible to exclude inorganic salts such as sodium chloride (NaCl), often used as isotonicity agent, from the pre-lyophilization liquid and lyophilized formulation. Other examples of salts include salts of any combinations of the cations sodium potassium, calcium or magnesium with anions chloride, phosphate, citrate, succinate, sulphate or mixtures thereof. However, as a person of skill in the art can appreciate, for some purposes it may nonetheless be desirable to include an inorganic salts, *e.g.*, for reconstitution of the lyophilized formulation, *i.e.*, as a diluent as described below.

[0137] The formulation of the invention can further comprise one or more of the following ingredients: antioxidants, ascorbic acid, glutathione, preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); cyclodextrin, *e.g.* hydroxypropyl- -cyclodextrin, sulfobutylethyl- -cyclodextrin, [beta]-cyclodextrin, polyethyleneglycol, e.g. PEG 3000, 3350, 4000, or 6000; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; chelating agents such as EDTA; salt-forming counter-ions such as sodium; and metal complexes (e.g. Zn-protein complexes).

Process

[0138] Freeze drying generally contains three main steps: freezing, primary drying, and secondary drying. The first stage is for the product to be frozen at a temperature lower than eutectic or glass transition temperature of the product. Rate of freezing affects size of water crystals and subsequent rate of drying. The second stage is primary drying, which removes the solvent (ice) water. It is important that product temperature remains below the collapse temperature and all ice/water is sublimed. The third stage is secondary drying which removes "bound" water or water from solute, during which the shelf temperature often raised higher than 40°C to accelerate desorption process. Lyophilization methods suitable for antibody- and other protein or protein conjugate formulations are well-known by a person the skilled in the art and are described in, *e.g.* "Lyophilization of Biopharmaceuticals" by Henry R. Costantino and Michael J. Pikal; "Freeze Drying / Lyophilization of Pharmaceuticals and Biological Products" by Louis Rey and Joan C. May.

[0139] In one aspect of the disclosure, the lyophilization of the aqueous solution comprises the steps of:

1. a. cooling the aqueous solution at 0.3°C/min to 3°C/min to between -40°C and - 60;

2. b. holding isothermally for at least 120 min;
3. c. warming to between -20°C and -15°C at a rate of 0.3°C/min to 6°C/min;
4. d. holding isothermally for at least 180 min;
5. e. applying vacuum using a pressure between 30 mTorr and 300 mTorr at a temperature between -40°C and -10°C;
6. f. increasing the temperature to between 35°C and 50°C at between 0.3°C/min and 3°C/min and
7. g. holding isothermally for at least 10 hours or until the residual moisture is not more than 2%.

[0140] In an embodiment of the invention the lyophilization of the aqueous solution comprises the steps of:

1. a. cooling the aqueous solution at a rate of from 0.5°C/min to 1°C/min to -40°C or less;
2. b. holding isothermally for at least 120 min;
3. c. warming to between -20°C and -15°C at a rate of from 0.5°C/min to 3°C/min;
4. d. holding isothermally for at least 180 min;
5. e. applying vacuum using a pressure between 50 mTorr and 200 mTorr at a temperature between -30°C and -10°C;
6. f. increasing the temperature to between 35°C and 50°C at a rate of from 0.5°C/min and 1°C/min and
7. g. holding isothermally for at least 10 hours.

[0141] In one embodiment the cooling step a) is performed by cooling the aqueous solution with at least 0.3°C/min such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0°C/min to a temperature of -40°C.

[0142] In another embodiment the cooling step a) is performed by cooling the aqueous solution with at least 0.3°C/min such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0°C/min to a temperature of - 50°C.

[0143] In another embodiment the cooling step a) is performed by cooling the aqueous solution with at least 0.3°C/min such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0°C/min to a temperature of - 60°C.

[0144] In another embodiment the warming step c) is performed by warming the material with at least 0.3°C/min such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.5, 3.8, 3.9, 4.0, 4.5, 5.0 or 6.0°C/min to a temperature of -15°C.

[0145] In another embodiment the warming step c) is performed by warming the material with at least 0.3°C/min such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.5, 3.8, 3.9, 4.0, 4.5, 5.0 or 6.0°C/min to a temperature of -20°C.

[0146] In another embodiment the temperature increase of step f) is performed with at least 0.3°C/min, such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0°C/min to a temperature of 35°C.

[0147] In another embodiment the temperature increase of step f) is performed with at least 0.3°C/min, such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0°C/min to a temperature of 40°C.

[0148] In another embodiment the temperature increase of step f) is performed with at least 0.3°C/min, such as 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0, 1.1, 1.2, 1.3, 1.4, 1.5, 1.6, 1.7, 1.8, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9 or 3.0°C/min to a temperature of 50°C.

Use

[0149] In another aspect the invention provides the ADC formulation as defined in any of aspects or embodiments herein for use in the treatment of cancer. Exemplary cancers include, but are not limited to, those caused by tumors of the central nervous system, head and neck cancer, lung cancer, such as NSCLC, breast cancer, specifically triple-negative breast cancer, esophageal cancer, gastric or stomach cancer, liver and biliary cancer, pancreatic cancer, colorectal cancer, bladder cancer, kidney cancer, prostate cancer, endometrial cancer, ovarian cancer, malignant melanoma, sarcoma, tumors of unknown primary origin, bone marrow cancer, acute lymphoblastic leukemia, chronic lymphoblastic leukemia and non-Hodgkin lymphoma, skin cancer, glioma, cancer of the brain, uterus, acute myeloid leukemia and rectum. In one embodiment, the antibody part of the ADC is an anti-TF antibody and the formulation of the invention is administered to a subject suffering from pancreatic cancer, colorectal cancer, breast cancer, bladder cancer, prostate cancer or ovarian cancer.

[0150] Prior to administration to a subject, a lyophilized formulation of the invention comprising a therapeutically effective amount of ADC is dissolved, *i.e.*, reconstituted, into a pharmaceutically acceptable diluent. Exemplary, non-limiting diluents include sterile pharmaceutical grade water (water for injection, WFI) or saline, bacteriostatic water for injection (BWFI), a pH buffered solution (*e.g.* phosphate-buffered saline), Ringer's solution and dextrose solution. For example, a lyophilized formulation of the invention can be reconstituted in water, pH buffered solution (*e.g.* phosphate-buffered saline), Ringer's solution and dextrose solution. For example, a lyophilized formulation of the invention can be reconstituted in sterile water for injection (WFI) to a concentration of about 5 to about 30 mg/mL ADC, such as about 7 to about 20 mg/mL ADC, such as about 8 to 15 mg/mL ADC, such as about 9 to about 11 mg/mL ADC, such as about 10 mg/mL ADC. The concentrate may optionally be further diluted for, *e.g.*, infusion into a pH buffered solution (*e.g.* phosphate-buffered saline, Ringer's solution and/or dextrose solution) to a concentration of about 0.05 mg/mL to 30 mg/mL ADC, such as, *e.g.*, 0.12 mg/mL to 2.40 mg/mL ADC.

[0151] Typically, the reconstituted formulation of the present invention is suitable for parenteral administration. The phrases "parenteral administration" and "administered parentally" as used herein means modes of administration other than enteral and topical administration, usually by injection or infusion, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion. In one embodiment the pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion, e.g., by reconstituting the lyophilized formulation in sterile water or saline and held in IV bags or syringes before administration to a subject.

[0152] The invention also provides for a kit comprising the lyophilized formulation of an anti-TF ADC according to the invention, typically in a hermetically sealed container such as a vial, an ampoule or sachette, indicating the quantity of the active agent. Where the pharmaceutical is administered by injection, an ampoule of sterile water for injection or saline can be, for example, provided, optionally as part of the kit, so that the ingredients can be mixed prior to administration. Such kits can further include, if desired, one or more of various conventional pharmaceutical kit components, such as, for example, containers with one or more pharmaceutically acceptable carriers, additional containers, etc., as will be readily apparent to those skilled in the art. Printed instructions, either as inserts or as labels, indicating quantities of the components to be administered, guidelines for administration, and/or guidelines for mixing the components, can also be included in the kit.

EXAMPLE 1

[0153] This example shows the effect of certain buffers and pH values on the thermal stability of Anti-TF HuMab 098 (see WO2011157741 A2), herein referred to as HuMax-TF. An accelerated stability study for selected excipients, buffers and pH conditions was designed using the histidine and acetate buffer systems at pH 4.5-6.5.

[0154] The DOE (Design of Experiments) used was a response surface, linear design with one numerical (pH) and two categorical factors (buffer and excipient types). The DOE included duplicate samples for each formulation composition to assess the experimental variability associated with the various biophysical and analytical methods. Additional off-DOE samples at 50 mg/mL in the center point formulations and at 20 mg/mL with combined sorbitol and sodium chloride excipients were included in the study. Table 2 shows the preformulation design of experiment (DOE) samples for HuMax-TF.

Table 2

	pH	Buffer	Excipient	Protein Conc (mg/ml)
A	4.5	30mM Acetate	140mM NaCl	20
B	4.5	30mM Acetate	140mM NaCl	20
C	5.0	30mM Acetate	140mM NaCl	20
D	6.0	30mM Histidine	140mM NaCl	20
E	6.5	30mM Histidine	140mM NaCl	20
F	5.5	30mM Histidine	140mM NaCl	20
G	5.5	30mM Acetate	140mM NaCl	20
H	4.5	30mM Acetate	225mM sorbitol	20
I	5.5	30mM Histidine	225mM sorbitol	20
J	5.5	30mM Acetate	140mM NaCl	20
K	6.0	30mM Histidine	225mM sorbitol	20
L	6.5	30mM Histidine	140mM NaCl	20
M	6.0	30mM Histidine	140mM NaCl	20
N	6.5	30mM Histidine	225mM sorbitol	20
O	5.0	30mM Acetate	225mM sorbitol	20
P	5.5	30mM Acetate	225mM sorbitol	20
Q	5.5	30mM Histidine	225mM sorbitol	20
R	5.0	30mM Acetate	140mM NaCl	20
S	5.5	30mM Acetate	225mM sorbitol	20
T	6.5	30mM Histidine	225mM sorbitol	20
U	5.0	30mM Acetate	225mM sorbitol	20
V	4.5	30mM Acetate	225mM sorbitol	20
W	5.5	30mM Histidine	140mM NaCl	20
X	6.0	30mM Histidine	225mM sorbitol	20
Off-DOE Compositions for Higher Protein Concentration				
Y	5.0	30mM Acetate	225mM sorbitol	50
Z	5.0	30mM Acetate	140mM NaCl	50
AA	6.0	30mM Histidine	225mM sorbitol	50
BB	6.0	30mM Histidine	140mM NaCl	50
Off-DOE Compositions for Excipient Mixtures				
CC	5.0	30mM Acetate	110mM sorbitol + 70mM NaCl	20
DD	6.0	30mM Histidine	110mM sorbitol + 70mM NaCl	20

[0155] The accelerated stability samples were tested by a variety of analytical methods. Selected results are discussed in Examples 2, 3 and 4.

EXAMPLE 2

[0156] The HuMax-TF samples incubated at 5 and 45°C (non-stressed and stressed, respectively) for four weeks were analyzed using SEC (size exclusion chromatography) to determine the effect of various formulations on the extent of aggregation and degradation. The SEC chromatograms of non-stressed HuMax-TF samples showed one major peak that corresponded to the monomeric antibody, and accounted for more than 98% of the total peak area. This one major SEC peak reflected the antibody's size homogeneity in each formulation. For the comparison of SEC data between various formulations, the peak area values for species that eluted before the main peak were combined and reported as percent HMW. Similarly, the peak area values for species that eluted after the main peak were combined and reported as percent degraded. The bar graphs presented in Figure 1 showed no significant trends for any of the species for any of the formulations under the non-stressed conditions. For the stressed conditions, however, increased %HMW and %LMW and decreased %Main values due to aggregation and degradation were observed. Higher %HMW and %LMW values were observed for the acetate formulations relative to the histidine formulations. For both buffer types, and more notably in acetate formulations, the %HMW and %LMW species decreased in with increasing pH and with sorbitol relative to sodium chloride.

EXAMPLE 3

[0157] SDS-PAGE analysis was performed under both reducing and non-reducing conditions for selected four-week DOE samples. Under reducing conditions, the heavy chain band appeared at approximately 54 kDa, and the light chain band appeared at approximately 26 kDa. Under non-reducing conditions, the main HuMax-TF band migrated at approximately 145 kDa. Samples were analyzed using reduced and non-reduced SDS-PAGE. Compared to the non-stressed samples, the heat stressed formulations showed an increase in the intensity of LMW bands below the intact IgG in the non-reduced gel. Similarly, increased intensities of new bands were observed between the heavy and light chain bands for the stressed samples relative to the non-stressed samples. For both the reduced and non-reduced samples, the acetate formulations showed a larger increase in intensity of these degraded species relative to the histidine formulations.

EXAMPLE 4

[0158] The one-week stressed and non-stressed HuMax-TF DOE samples were analyzed using cIEF (Imaged Capillary Isoelectric Focusing) to evaluate the effects of various formulation components on the observed peak areas. The results are shown in Figure 2. The percent peak area values for the species with pI values lower than that of the main peak were combined and reported as percent acidic, and the percent peak area values for the species with pI values higher than that of the main peak were combined and reported as percent basic.

[0159] The cIEF analysis of non-stressed HuMax-TF DOE samples presented in Figure 2 did not show any obvious trends for the dependence of cIEF results on pH, buffer type or excipient type for the non-stressed samples. The cIEF analysis of stressed DOE samples showed 5-13% decrease in the main peak percent area. The decrease in main peak area occurred concomitantly with an increase in the peak area for acidic species and, for most formulations, an increase in the percent basic peak area as well. The changes in percent acidic species depicted in the bar graphs did not show obvious pH dependency, but the acetate formulations generally showed slightly lower percent acidic peak areas than the histidine formulations. However, the percent basic species showed strong pH dependence with percent basic peak areas decreasing with increasing pH. Additionally, histidine formulations showed lower percent basic species than the acetate formulations.

EXAMPLE 5

[0160] A screening study was designed to test the effect of pH, presence of polysorbate, and presence of sorbitol on the stability of HuMax-TF-ADC in solution. HuMax-TF-ADC is an antibody-drug conjugate composed of the human monoclonal IgG1 antibody HuMax-TF 011 chemically conjugated via a protease cleavable valine citrulline (vc) linker to the microtubule-disrupting agent monomethyl auristatin E (MMAE).

[0161] Twelve different formulations were prepared using 3 different pH values. The solution formulation study design is shown in Table 3. All formulations contained HuMax-TF-ADC at 10 mg/mL with 30 mM Histidine. Tween = polysorbate 80 (PS80).

Table 3

Formulation	pH	Tween Concentration (%)	Sorbitol (mM)
1	5.5	0	0
2	5.5	0	225
3	5.5	0.02	0
4	5.5	0.02	225
5	6.0	0	0
6	6.0	0	225
7	6.0	0.02	0
8	6.0	0.02	225
9	6.5	0	0

Formulation	pH	Tween Concentration (%)	Sorbitol (mM)
10	6.5	0	225
11	6.5	0.02	0
12	6.5	0.02	225

[0162] The 12 formulations prepared for the screening study were analyzed using UV/Vis (UltraViolet-Visible Spectroscopy), icIEF, SEC, and DAR-HIC (Drug-to-Antibody Molar Ratio using Hydrophobic Interaction Chromatography). Formulation screening studies had shown that the main stability indicating parameters for HuMax-TF-ADC were aggregates measured by SEC and acidic isoforms (presumably due to deamidation) measured by icIEF.

[0163] The effect of pH on the percentage of HMW formation in solution samples stored at 40 °C for 2 weeks was measured by SEC (Figure 3 shows SEC % HMW for formulations 1, 5, and 9 in table 3). A pH of 6.0 was shown to be the most efficient on limiting aggregation of HuMax-TF-ADC in the histidine buffer.

[0164] The effect of pH on deamidation of HuMax-TF-ADC in solution samples stored at 40°C for 2 weeks was measured by acidic species increase in icIEF (Figure 4 shows results for formulations 1, 5, and 9 in table 3). A pH of 5.5 was found to be most efficient to limit deamidation, followed by pH 6.0 and pH 6.5.

[0165] The use of sorbitol or PS80 did not have any marked effect on the percent main charge peak by icIEF , as well as the main peak by SEC, in the HuMax-TF-ADC formulations stored at 40°C for 2 weeks (Figure 5A and 5B). This shows that the formulation may be free of surfactants.

EXAMPLE 6

[0166] Formulation screening had shown that the main stability indicating parameters were aggregates measured by SEC and acidic isoforms (presumably deamidation) measured by icIEF. The deamidation rate in all tested liquid formulations measured under accelerated conditions was fast, thus the tested liquid formulations of HuMax-TF-ADC were clearly unstable. A comparison between some liquid formulations and preliminary lyophilized formulations are shown in Figure 6.

EXAMPLE 7

[0167] A pilot lyophilization study was conducted with two formulations containing different concentrations of sucrose (150 mM and 250mM), 10 mg/mL HuMax-TF-ADC in a 30 mM histidine buffer at pH 6.0 to investigate the possible influence of the fill volume and vial volume/shape on the lyophilized cake. For the histidine, sucrose formulation of HuMax-TF-ADC, the lyophilized cake appearances were different by different fill volumes and vial sizes:

1. A:The first group of samples of a formulation were filled into 0.25 mL per 2 mL glass vials and lyophilized. The samples appeared pharmaceutically elegant with no obvious signs of collapse.
2. B: The second group of samples with the same formulation were concurrently being lyophilized using a 4 mL fill volume in a 10 mL vial, using the same lyophilization cycle. The samples appeared with severe shrinkage.

[0168] Accordingly, the formulation could support a pharmaceutically elegant cake for a low fill volume 0.25 ml in a 2 mL glass vial, but is less suitable for a higher fill volume of 4 mL in a 10 mL vial.

EXAMPLE 8

[0169] The objective of this example was to test the effects of sucrose and mannitol concentrations on the stability of lyophilized formulations for HuMax-TF-ADC.

[0170] Initial tests showed that sucrose may be readily substituted with trehalose, maintaining the primary properties of the formulation. Testing continued using sucrose only.

[0171] Three different lyophilized formulations containing 10 mg/mL HuMax-TF-ADC, 30 mM histidine, at pH 6.0 were prepared with different concentrations of sucrose and mannitol. The details are shown in Table 4.

Table 4

Formulation	HuMax-TF-ADC	Histidine	Sucrose	Mannitol
A	10 mg/mL	30 mM	225 mM (7.7% w/v)	274 mM (5% w/v)
B	10 mg/mL	30 mM	88 mM (3% w/v)	165 mM (3% w/v)
C	10 mg/mL	30 mM	160 mM (5.5% w/v)	274 mM (5% w/v)

[0172] Lyophilized formulations with different concentrations of sucrose and mannitol were placed on accelerated stability tests at 25°C, 40°C, and 50°C. The stressed samples were analyzed by methods such as SEC (Size Exclusion Chromatography), icIEF (Imaged Capillary Isoelectric Focusing), FTIR (Fourier transform infrared spectroscopy), DLS (Dynamic Light Scattering. The accelerated stability data for the 3 formulations showed that formulation B had an advantage over the other formulations regarding the aggregates and main charge isoform, especially when comparing the stability data at 50°C and 40°C. Exemplary results are shown in Figure 7.

[0173] Furthermore, formulation B exhibited the least growth in more particles after storage at 40°C for 2 months, when compared to formulations A and C (Figure 8).

[0174] No apparent changes in the amide I region of second derivative FTIR spectra for formulations A, B, and C were observed after storage at 50°C for 2 weeks (Figure 9). The data suggested that the secondary structure for the molecule remained the same in each formulation when stored under stressed temperature conditions.

EXAMPLE 11

[0175] This example verified the suitability of mannitol as a crystalline bulking agent in the HuMax-TF-ADC formulation, especially, the annealing temperature and time for the crystallization of mannitol in the lyophilized formulation for HuMax-TF-ADC. Differential scanning calorimetry (DSC) is a thermo analytical technique which measures energy directly and allows precise measurements of heat capacity of samples. When the sample undergoes a physical transformation such as phase transitions, the difference in heat flow between the sample and reference can be detected by DSC.

[0176] In the current example, DSC was used to determine the time required for the onset of the crystallization of mannitol in the formulation. Two different annealing temperatures(-15°C and -20°C) were tested. After the solutions were cooled to -40°C at 1°C/min, the temperature were ramped to either -15°C or -20°C at 1°C/min, and held isothermally for 120 minutes. The onset time for the crystallization of mannitol for Formulation B was approximately 10 minutes when annealed at -15°C or -20°C, as shown in Figure 10. The data demonstrated that mannitol readily crystallized during annealing, thus function well as the crystalline bulking agent in the formulation.

EXAMPLE 10

[0177] Lyophilized HuMax TF-ADC formulation samples were evaluated on long-term and accelerated stability programs. The composition of HuMax-TF-ADC formulation after reconstitution is 10 mg/mL formulated in 30 mM histidine (corresponding to 4.65 mg/mL), 88 mM sucrose (corresponding to around 30 mg/mL), 165 mM mannitol (corresponding to around 30 mg/mL), pH 6.0. The long term stability samples were analysed at time zero, 1 month, 2 month, 3 months, 6 months, 9 months, 12 months and 18 months, etc. The lyophilized formulation was, at the different time points, reconstituted with water for injection (WFI) and tested by analytical methods including SEC, HIC, CE-SDS and icIEF.

[0178] After at least 6 months storage at 5 ± 3°C and 25 ± 2°C, all samples remained stable by all test methods. The samples stored at 5 ± 3°C and 25 ± 2°C showed no significant changes by any test methods at at least 6 month time point compared to the study start. Expected minor changes in the purity profile during accelerated stability testing at 25°C were observed by icIEF, reduced CE-SDS, and SEC testing.

[0179] In particular, with respect to aggregation, SEC analysis showed that the percentage of aggregates remained at around 2.2% for samples both at 5 ± 3°C and at 25 ± 2°C over at least 6 months. IcIEF was used to determine changes to the charge profile of HuMax-TF-ADC. For samples stored at 5 ± 3°C, the changes of main charge isoform are within 0.4% and for samples stored at 25°C, the change is only 1.7%, over at least 6 months compared to time zero.

[0180] Furthermore, the average DAR (moles auristatins/moles mAb),drug load and free drug remains almost constant over at least 6 month at both 5 °C and 25 C. There are no sign of degradation over at least 6 month both at 5° C and 25°C, as shown by CE-SDS (non-reduced) and CE-SDS (reduced), as well as LMW by SEC. Last but not least, the bioassay by Cytotoxcity proved the biological functional of HuMaxTF-ADC could be preserved for at least 6 month at both 5°C and 25°C. Therefore HuMax-TF-ADC in such a formulation is acceptably stable for pharmaceutical use.

Table 5A Example data from stability program of HuMax-TF-ADC drug product at 5 ± 3°C

Assay	Time point (months)				
	0	1	2	3	6
SEC % main	97.6	97.7	97.8	97.7	97.6
SEC % HMW	2.3	2.2	2.1	2.2	2.2
SEC % LMW	0.1	0.1	0.1	0.1	0.2
Average DAR by HIC (moles auristatins/moles mAb)	4.1	4.1	4.1	4.1	4.1
Drug load or unconjugated antibody by HIC (area %)	1.9	1.8	1.9	1.8	1.8
CE-SDS (non-reduced)	CP	CR	CP	CR	CR
CE-SDS (reduced)	CP	CR	CP	CR	CR
CE-SDS (reduced) % LC0 + LC1	31.8	31.9	32.8	32.0	30.5
CE-SDS (reduced) % HC	66.2	65.9	65.3	65.9	67.2

Assay	Time point (months)				
	0	1	2	3	6
CE-SDS (reduced) LC0 + LC1 + HC	98.0	97.8	98.1	97.9	97.7
Free Drug (w/w % free drug/mAb)	<0.01	<0.01	<0.01	<0.01	<0.01
icIEF % Main	70.3	70.7	NA	69.6	69.9
icIEF % Acidic	25.2	24.2	NA	24.7	25.2
icIEF % Basic	4.5	5.2	NA	5.7	4.9
Cytotoxcity (based on reference standard)	106	102	111	122	100
Visible Particulates	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles
CR= Comparable to reference standard ; HC= Heavy chain; LC= Light chain; TQI= Total quantifiable impurities; NT= Not tested ; NA= Not analyzed					

Table 5B. Example data from stability program of HuMax-TF-ADC drug product at 25 ± 3°C

Assay	Time point (months)				
	0	1	2	3	6
SEC % main	97.6	97.6	97.7	97.7	97.6
SEC % HMW	2.3	2.3	2.2	2.2	2.2
SEC % LMW	0.1	0.1	0.1	0.1	0.2
Average DAR by HIC (moles auristatins/moles mAb)	4.1	4.1	4.1	4.1	4.1
Drug load by HIC (area %)	1.9	1.8	1.9	1.8	1.8
CE-SDS (non-reduced)	CR	CP	CP	CR	CR
CE-SDS (reduced)	CP	CR	CP	CR	CR
CE-SDS (reduced) % LC0 + LC1	31.8	32.0	32.8	32.0	30.8
CE-SDS (reduced) % HC	66.2	66.1	65.3	65.8	66.8

Assay	Time point (months)				
	0	1	2	3	6
CE-SDS (reduced) LC0 + LC1 + HC	98.0	98.1	98.1	97.8	97.6
Free Drug (w/w % free drug/mAb)	<0.01	<0.01	<0.01	<0.01	<0.01
icIEF % Main	70.3	69.8	NA	68.6	68.6
icIEF % Acidic	25.2	24.7	NA	25.1	25.6
icIEF % Basic	4.5	5.4	NA	6.3	5.9
Cytotoxcity (based on reference standard)	106	109	104	111	98
Visible Particulates	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles	Practically free from visible particles
CR= Comparable to reference standard ; HC= Heavy chain; LC= Light chain; TQI= Total quantifiable impurities; NT= Not tested ; NA= Not analyzed					

EXAMPLE 11

[0181] This study was to examine the effect of higher or lower excipient concentrations on the stability of HuMax-TF-ADC, in order to verify the acceptable range for excipient concentrations. Three formulations of HuMax-TF-ADC were prepared according to Table 6. The solutions were filled into 10 mL glass vials at 4 mL per vial and lyophilized. Samples of each formulation were placed on accelerated stability at 50°C for 4 weeks and sampled after 2, 3, and 4 weeks. The samples were tested for concentration, pH, reconstitution time, appearance, iCE, CE-SDS, SEC, DAR-HIC, Free drugDLS, and MFI, in order to obtain data to evaluate the impact of higher or lower concentration of excipients on the stability of HuMax-TF-ADC. The data showed that the formulations behaved similarly in stressed stability testing.

Table 6

Description	**Concentration of excipients**		
	Histidine	Sucrose	Mannitol
Lower Excipient Concentration	29.5 mM / 4.58 g/L	84 mM / 28.75 g/L	158mM / 28.78 g/L
Target Formulation	30 mM / 4.65 g/L	88 mM / 30.12 g/L	165 mM/ 30.06 g/L

Description	Concentration of excipients		
	Histidine	Sucrose	Mannitol
Higher Excipient Concentration	30.5 mM / 4.73 g/L	92 mM/ 31.49 g/L	172 mM/ 31.33 g/L

EXAMPLE 12

[0182] The effect of the HuMax-TF-ADC concentrations on the stability of lyophilized HuMax-TF-ADC formulations was examined, in order to verify the acceptable range for HuMax-TF-ADC concentrations in the formulation.

[0183] The formulations prepared by 30 mM histidine, 88 mM sucrose, 165 mM mannitol, and 5 mg/mL or 30 mg/mL HuMax-TF-ADC were lyophilized and stored at 40°C for 2 months or at 50°C for 2 weeks. The main peak by SEC after 2 months storage at 40°C stayed above 97% (Figure 12), only slight increases in the average percent high molecular weight species were observed for both concentrations after storage at 40 °C (Figure 13). The trends of charge profile change under stressed conditions, as shown by ICE data, are similar for both concentrations (Figure 14, 15 and 16).

[0184] This shows that the HuMax-TF-ADC may be formulated at least at concentrations in the range of 5 mg/mL and 30 mg/mL.

Example 13

[0185] The in-use stability of HuMax-TF-ADC were studied in different concentrations (up to 48 mg/ml) and different diluents, i.e. water for injection (WFI), 0.9% NaCl(saline) and dextrose 5% (D5W) solution, for at least 48 hours at room temperature.

[0186] SEC data showed that the average percent main peak remained greater than 97% for the samples stored at 25°C for 48 hours (Figure 17). The 48 mg/mL samples contained approximately 0.5% more high molecular weight species than the other samples (Figure 18). Different diluents did not influence the aggregation propensity.

[0187] The in-use solution samples stored at 25°C were also examined using iCE. The sample reconstituted with 5% dextrose had the lowest percent main peak (Figure 19) after 48 hours, and the highest percent acidic species (Figure 20) when compared to the other samples. This indicates that WFI and saline are potentially better dilute to preserve the charge profile of the HuMax-TF-ADC.

Example 14

[0188] The effect of buffer type and crystallizing excipient type on the stability of HuMax-TF-ADC formulations are demonstrated in this example.

[0189] When replacing histidine by citrate as a buffer in the lyophilized formulation, or when replacing mannitol by glycine as a crystallizing excipient, their stability was analysed by SEC and iCE, after storage at 40°C for 2 months.

[0190] In figure 22 to figure 26, the legend "Glycine" refers to the formulation containing 10 mg/mL HuMax-TF-ADC, 30 mM histidine, 88 mM sucrose, 165 mM glycine, pH 6.0; whereas the legend "Citrate" refers the formulation containing 10 mg/mL HuMax-TF-ADC, 30 mM citrate, 88 mM sucrose, 165 mM mannitol, pH 6.0.

[0191] No marked changes in the SEC average percent main peak (Figure 22) or high molecular peak (Figure 23) were observed in the citrate or glycine formulations after storage at 40°C for 2 months. No low molecular peaks were observed in any of the samples.

[0192] The decreasing rate of iCE percent main peak at 40°C for 2 months in the formulations containing glycine and citrate were (Figure 24) comparable to the formulation containing 30 mM Histindine, 88 mM (3%) Sucrose and 165 mM (3%) Mannitol (Figure 7). It is observed that under heat stressed conditions, the citrate formulation was associated with more acidic species compared to formulation with Histidine (Figure 25) and the glycine formulation was associated with more basic species compared to formulation with mannitol (Figure 26).

Example 15

[0193] The effects of pH and buffer concentrations on the stability of HuMax-TF-ADC were demonstrated in this example. HuMax-TF-ADC was prepared at 10 mg/mL concentration with 3% mannitol and 3% sucrose but with either 20 mM or 50 mM histidine with the pH of the samples adjusted to 5, 6, or 7. The lyophilized samples were stored at 40°C for up to 2 months. The formulations prepared with 20 mM histidine or 50 mM histidine at pH 5, 6 or 7 exhibited similar SEC results at 40°C regardless of buffer concentration and pH, except that the formulation with 50 mM histidine at pH 5 showed a slight increase in aggregation (Figures 30 and 31).

[0194] As shown in figure 27- 29 by iCE, it is observed that pH play a more important role than the buffer concentration regarding the charge profile change under stressed testing. The formulations prepared at pH 6 displayed the least decrease in main peak during 2 month at 40°C storage. An apparent decrease in the main peak is observed, which is potentially larger than the analytical method variation. The formulation prepared at pH 5 exhibited the largest decrease in percent main peak (Figure 27).The formulation prepared at pH 7 had the highest percentage of acidic species (Figure 28) and the formulation prepared at pH 5 had the highest percentage of basic species (Figure 29).

Example 16

[0195] The lyophilization cycle for the lyophilized formulation of HuMax-TF-ADC of the invention may be performed as described below.

[0196] Firstly, the vials may be cooled at 0.5°C/min to 1°C/min to -40°C or less and held isothermally for at least 120 min (cooling step). Subsequently the vials are warmed to between -20°C and -15°C at a rate of 0.5°C/min to 3°C/min and held isothermally for at least 180 min (annealing step). Afterwards Vacuum is initiated using a pressure between 50 mTorr and 200 mTorr with a temperature between -30°C and -10°C(primary drying). Lastly, the temperature is increased to between 35°C and 50°C at between 0.5°C/min and 1°C/min and held isothermally for at least 10 hours (secondary drying).The residual moisture should not be more than 2% by weight.

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[0197]

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KRAV

1. Lyofiliseret formulering af et antivævsfaktor-(TF)-antistoflægemiddelkonjugat (ADC), hvor den lyofiliserede formulering kan opnås eller er opnået ved lyofilisering af en vandig formulering omfattende fra ca. 9 til ca. 11 g/L af anti-TF-ADC'et og farmaceutisk acceptable excipienser omfattende ca. 29 til ca. 31 mM histidinbuffer med en pH på ca. 5,5 til ca. 6,5; ca. 84 til ca. 92 mM sukrose og ca. 158 til ca. 172 mM mannitol, og hvor:

a. formuleringen er fri for overfladeaktivt stof,
b. anti-TF-antistofdelen af ADC'et omfatter en variabel tung (VH)-region omfattende en CDR1-region med aminosyresekvensen angivet i SEQ ID NO: 6, en CDR2-region med aminosyresekvensen angivet i SEQ ID NO: 7, og en CDR3-region med aminosyresekvensen angivet i SEQ ID NO: 8, og en variabel let (VL)-region omfattende en CDR1-region med aminosyresekvensen angivet i SEQ ID NO:46, en CDR2-region med aminosyresekvensen angivet i SEQ ID NO: 47, og en CDR3-region med aminosyresekvensen angivet i SEQ ID NO: 48, eller en variant, som har højst 1, 2 eller 3 aminosyremodifikationer, mere fortrinsvis aminosyre-substitutioner såsom konservative aminosyresubstitutioner i nævnte sekvenser,
c. lægemiddeldelen af ADC'et er vcMMAE.

2. Lyofiliseret formulering ifølge et hvilket som helst af krav 1, hvor den vandige formulering omfatter histidinbufferen i en koncentration på ca. 30 mM buffer.

3. Lyofiliseret formulering ifølge et hvilket som helst af kravene 1 eller 2, hvor den vandige formulering omfatter sukrosen i en koncentration på ca. 88 mM.

4. Lyofiliseret formulering ifølge et hvilket som helst af kravene 1 til 3, hvor den vandige formulering omfatter mannitolen i en koncentration på ca. 165 mM.

5. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor den vandige formulering omfatter ca. 10 g/L anti-TF ADC.

6. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor den vandige formulering omfatter fra ca. 9 til ca. 11 g/L anti-TF-ADC, såsom ca. 10 mg/ml anti-TF ADC, ca. 30 mM histidin, ca. 88 mM sukrose og ca. 165 mM mannitol.

7. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor antistoffet omfatter en VH-region omfattende en aminosyresekvens af SEQ ID NO: 5 og en VL-region omfattende en aminosyresekvens af SEQ ID NO: 45.

8. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor det gennemsnitlige absolutte antal lægemiddeldele pr. antistofmolekyle er 1, 2, 3, 4, 5, 6, 7 eller 8, såsom 3, 4 eller 5, fortrinsvis 4.

9. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor anti-TF-antistoffet er et antistof i fuld længde.

10. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor anti-TF-ADC'et er stabilt ved 2-8 °C, såsom ved 5 °C til farmaceutisk anvendelse i mindst 6 måneder, såsom i mindst 9 måneder, såsom i mindst 15 måneder eller fortrinsvis i mindst 18 måneder, eller endnu mere foretrukket i mindst 24 måneder, eller mest foretrukket i mindst 36 måneder.

11. Lyofiliseret formulering ifølge krav 10, hvor formuleringen er stabil, når den har mindre end 3,0 % aggregater, såsom mindre end 2,0 % aggregater, når den opbevares ved 5°C i mindst 6 måneder, såsom i mindst 9 måneder, såsom i mindst 15 måneder eller fortrinsvis i mindst 18 måneder, eller endnu mere foretrukket i mindst 24 måneder, eller mest foretrukket i mindst 36 måneder.

12. Lyofiliseret formulering ifølge krav 10 eller 11, hvor stabiliteten bestemmes ved SEC-analyse ifølge eksempel 10.

13. Lyofiliseret formulering ifølge et hvilket som helst af de foregående krav, hvor formuleringen er fri for uorganiske salte.

14. Farmaceutisk acceptabel flydende formulering opnået ved rekonstituering af den lyofiliserede formulering ifølge et hvilket som helst af kravene 1-13 i et sterilt vandigt fortyndingsmiddel.

15. Flydende formulering ifølge krav 14, omfattende ca. 9 til ca. 11 mg/ml anti-TF-ADC, ca. 28 til ca. 34 mM histidin, ca. 84 til ca. 92 mM sukrose og ca. 158 til ca. 172 mM mannitol.

16. Fremgangsmåde til fremstilling af den lyofiliserede formulering ifølge et hvilket som helst af kravene 1-13, omfattende trinene:

a. køling af den vandige opløsning med en hastighed på fra 0,5°C/min til 1°C/min til en temperatur på -40°C eller mindre;

b. isotermisk opretholdelse i mindst 120 min;

c. opvarmning til mellem -20°C og -15°C med en hastighed på fra 0,5°C/min til 3°C/min;

d. isotermisk opretholdelse i mindst 180 min;

e. påføring af vakuum under anvendelse af et tryk mellem 50 mTorr og 200 mTorr ved en temperatur mellem -30°C og -10°C;

f. forøgelse af temperaturen til mellem 35°C og 50°C med en hastighed på mellem 0,5°C/min og 1°C/min og

g. isotermisk opretholdelse i mindst 10 timer.

17. Fremgangsmåde til fremstilling af en injicerbar opløsning af et anti-TF-ADC, omfattende trinnet at rekonstituere den lyofiliserede formulering ifølge et hvilket som helst af kravene 1 til 13 i et sterilt vandigt fortyndingsmiddel.

DRAWINGS

Drawing

Fig. 1A

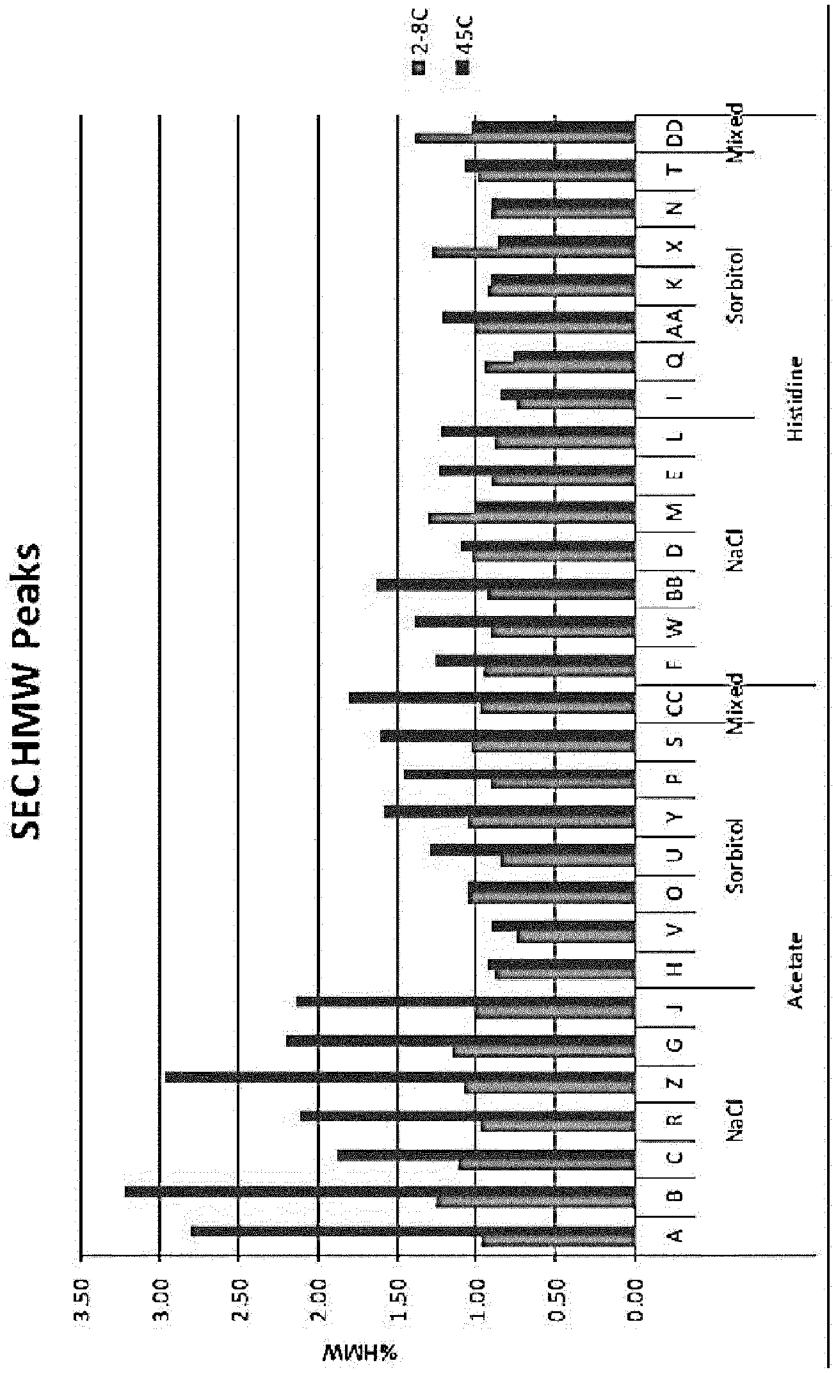


Fig. 1B

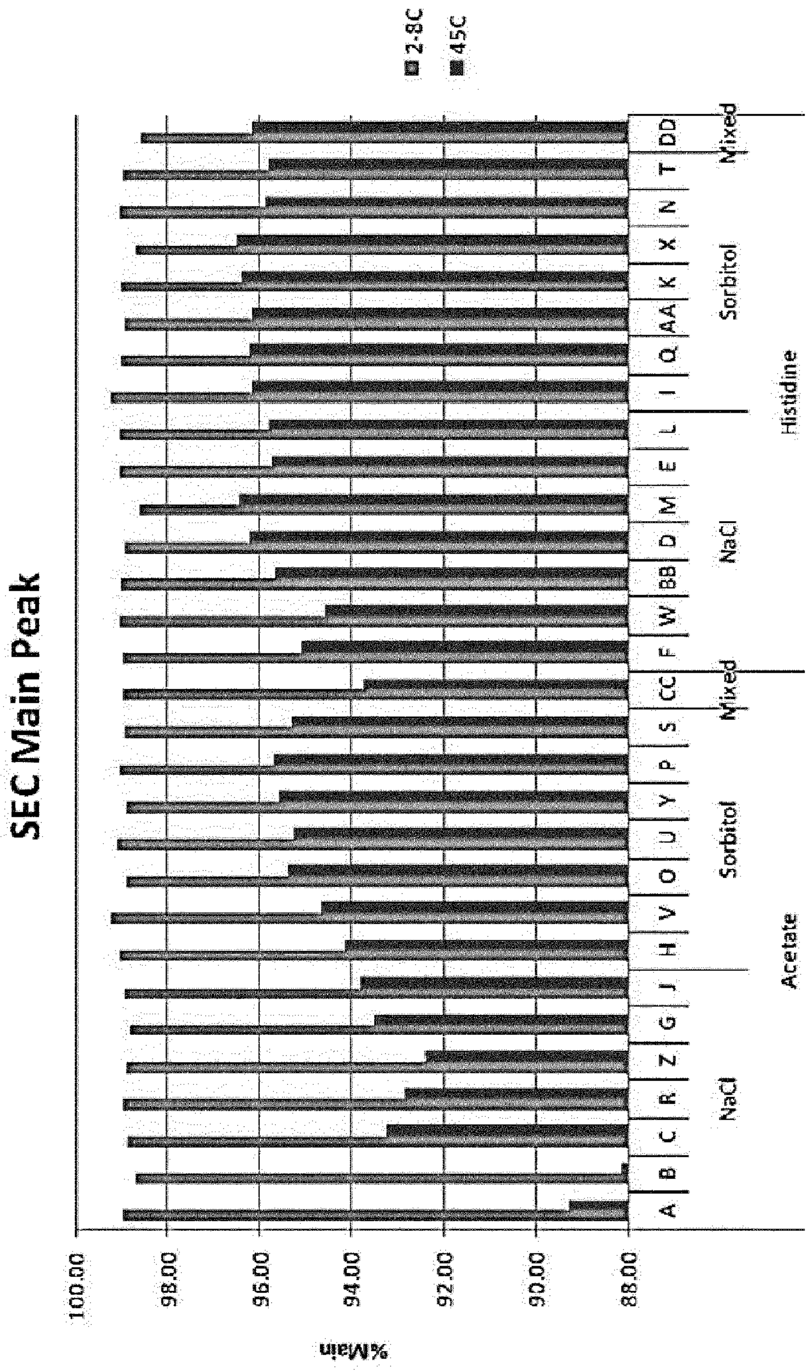

SEC Main Peak
2-8C
45C
%Main
100.00
98.00
96.00
94.00
92.00
90.00
88.00
A
B
C
R
Z
G
J
H
V
O
U
Y
P
S
CC
F
W
BB
D
M
E
L
I
Q
AA
K
X
N
T
DD
NaCl
Sorbitol
Mixed
NaCl
Sorbitol
Mixed
Acetate
Histidine

Fig. 1C

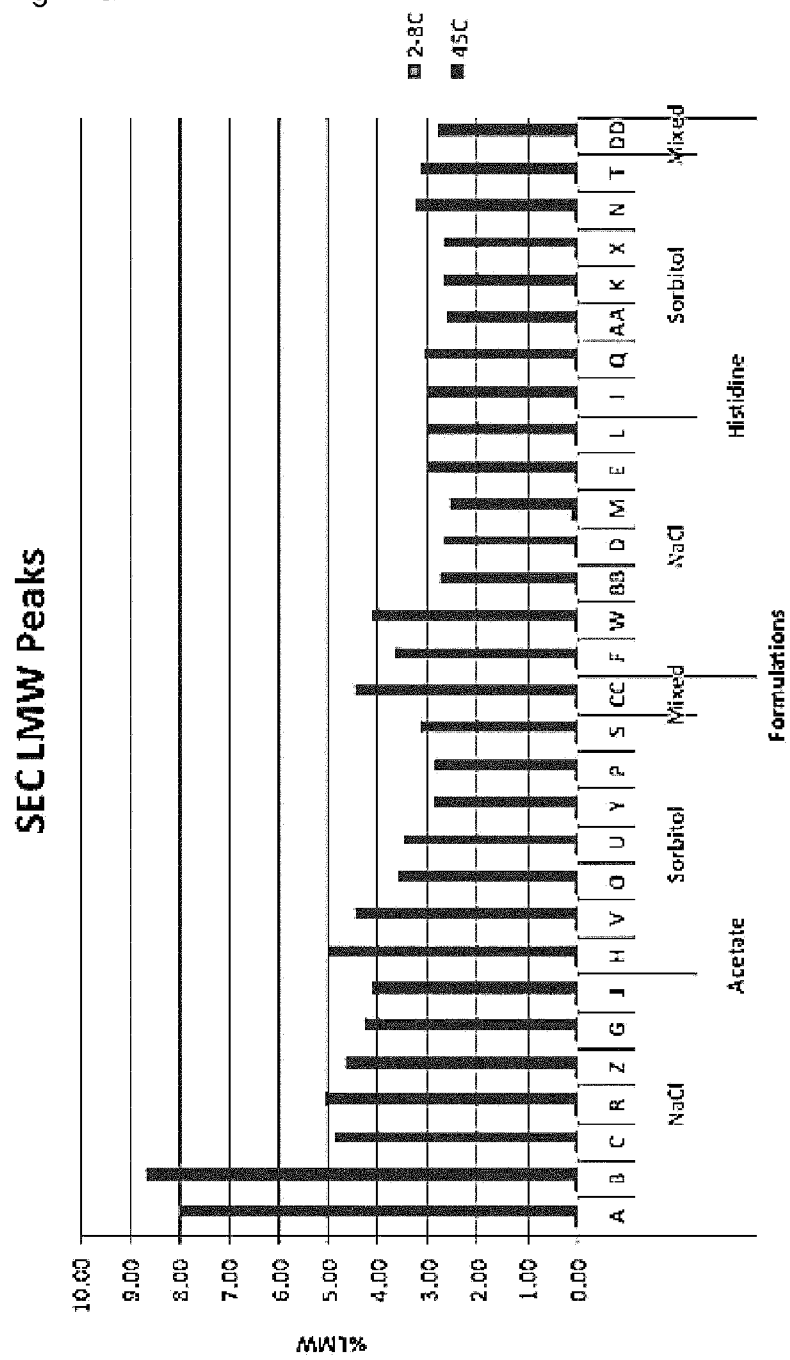

SEC LMW Peaks
2-8C
45C
%LMW
10.00
9.00
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00
A
B
C
R
Z
G
J
H
V
O
U
Y
P
S
CC
F
W
BB
D
M
E
L
I
Q
AA
K
X
N
T
DD
NaCl
Acetate
Sorbitol
Mixed
NaCl
Histidine
Sorbitol
Mixed
Formulations

Fig. 2A

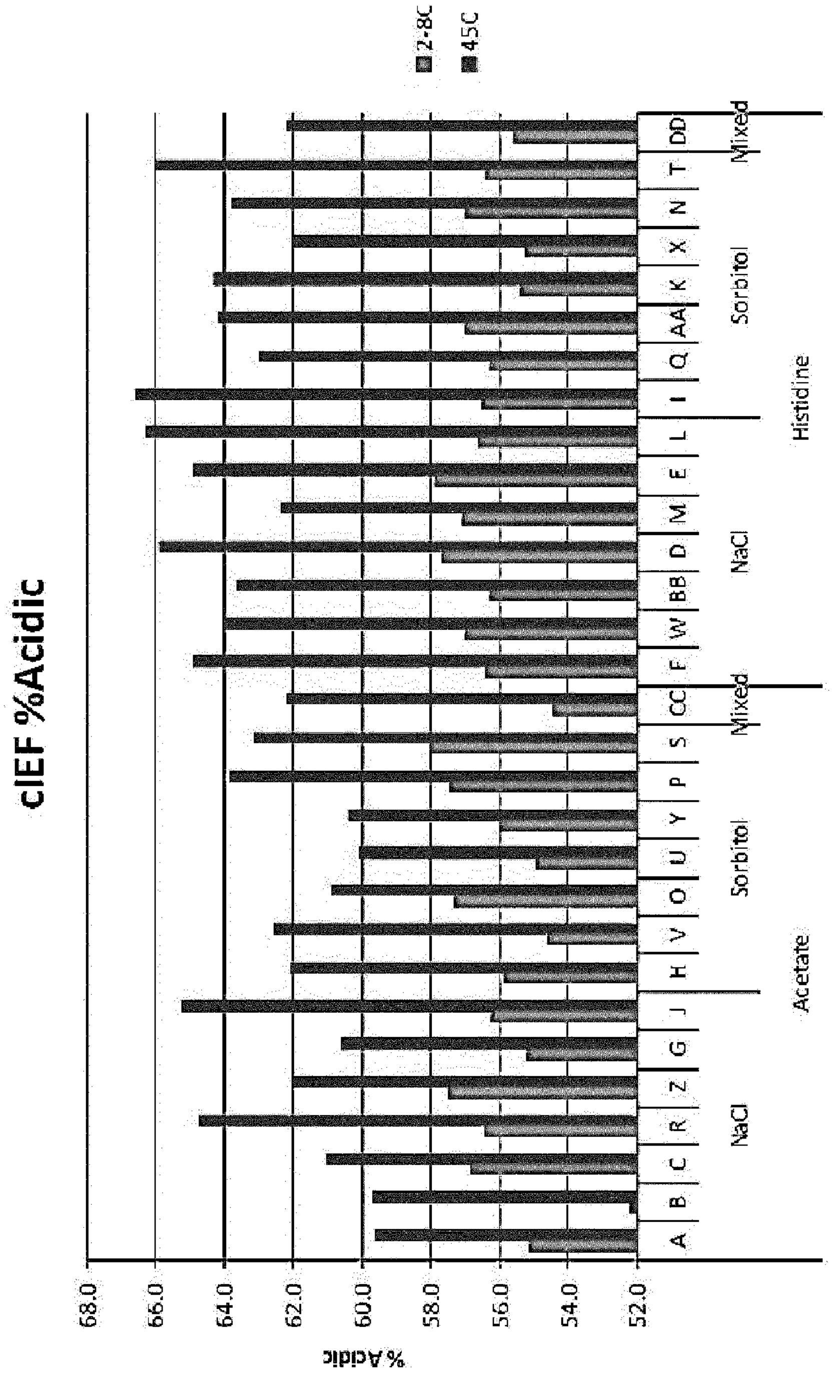

cIEF %Acidic
% Acidic
68.0
66.0
64.0
62.0
60.0
58.0
56.0
54.0
52.0
2-8C
45C
A
B
C
R
Z
G
J
H
V
O
U
Y
P
S
CC
F
W
BB
D
M
E
L
I
Q
AA
K
X
N
T
DD
NaCl
Sorbitol
Mixed
NaCl
Sorbitol
Mixed
Acetate
Histidine

Fig. 2B

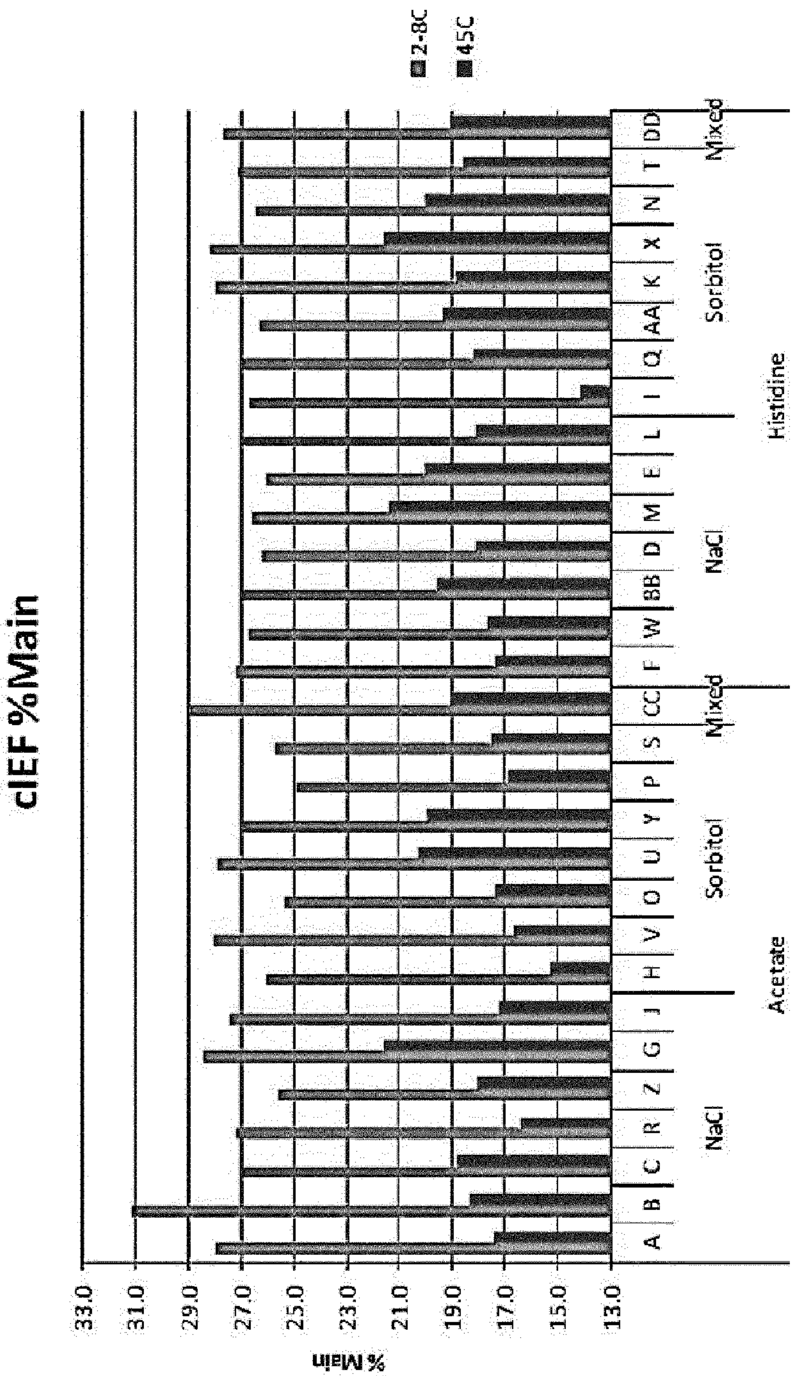

cIEF %Main
2-8C
45C
% Main
33.0
31.0
29.0
27.0
25.0
23.0
21.0
19.0
17.0
15.0
13.0
A
B
C
R
Z
G
J
H
V
O
U
Y
P
S
CC
F
W
BB
D
M
E
L
I
Q
AA
K
X
N
T
DD
NaCl
Acetate
Sorbitol
Mixed
NaCl
Histidine
Sorbitol
Mixed

Fig. 2C

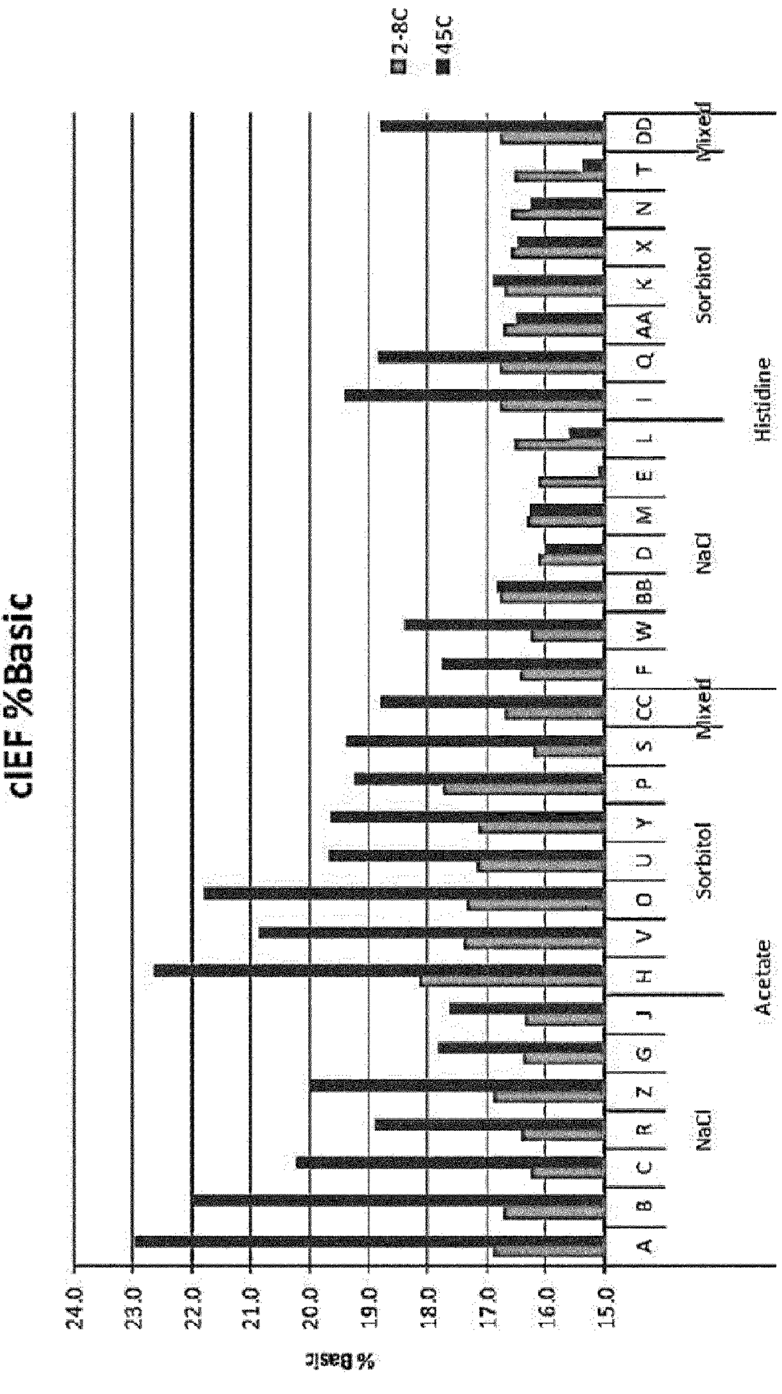

cIEF %Basic
2-8C
45C
% Basic
24.0
23.0
22.0
21.0
20.0
19.0
18.0
17.0
16.0
15.0
A
B
C
R
Z
G
J
H
V
O
U
Y
P
S
CC
F
W
BB
D
M
E
L
I
Q
AA
K
X
N
T
DD
NaCl
Acetate
Sorbitol
Mixed
NaCl
Histidine
Sorbitol
Mixed

Fig. 3

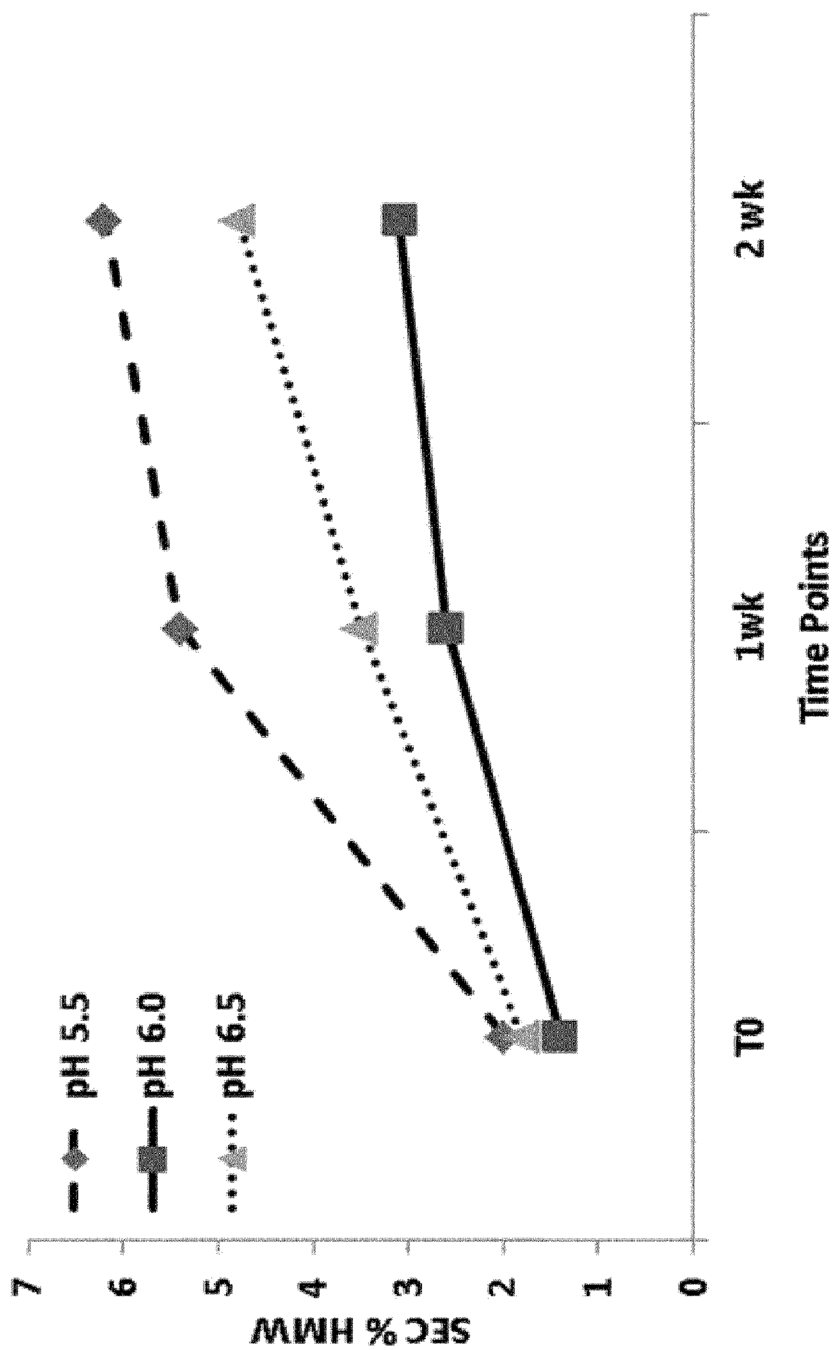


Fig. 4

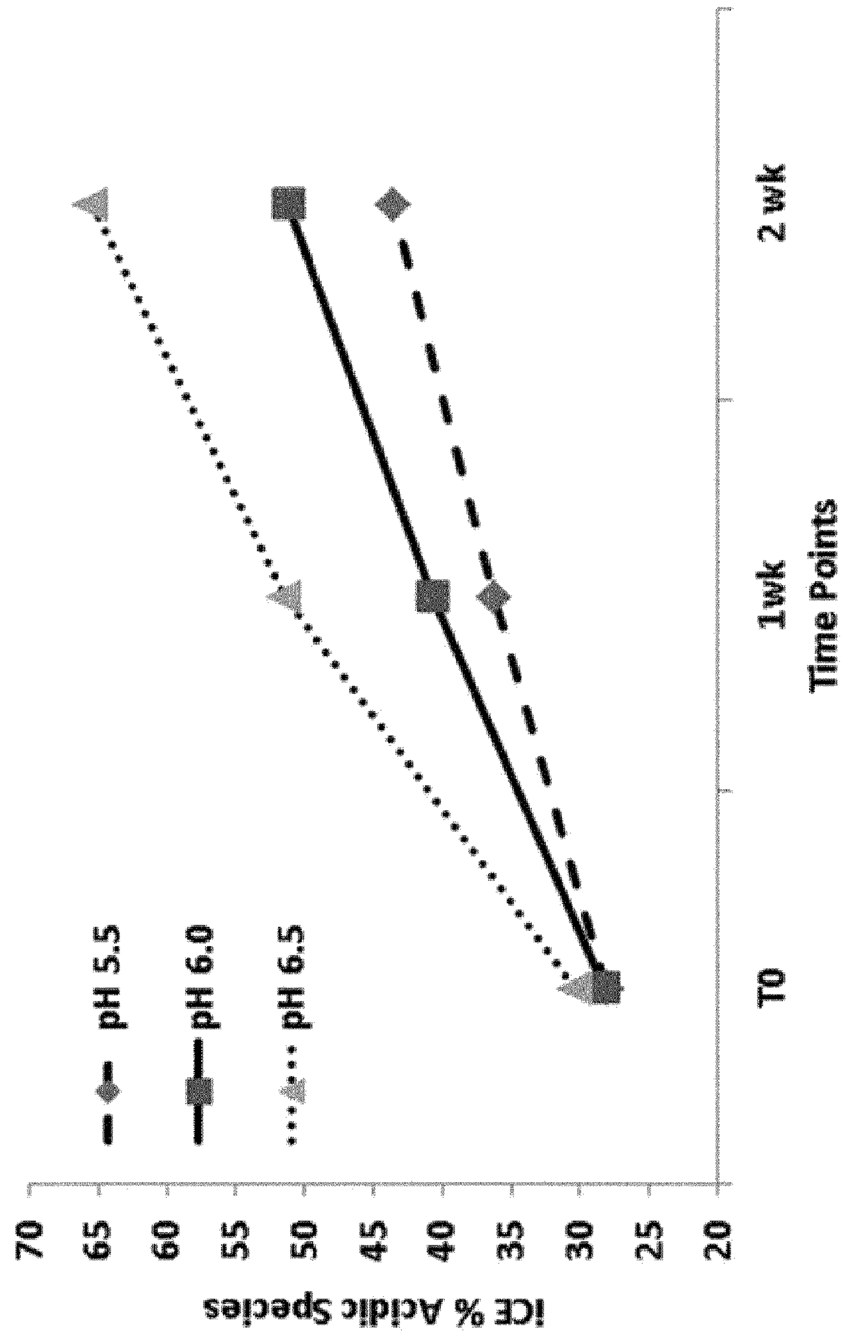

iCE % Acidic Species
70
65
60
55
50
45
40
35
30
25
20
pH 5.5
pH 6.0
pH 6.5
T0
1wk
2 wk
Time Points

Fig. 5A

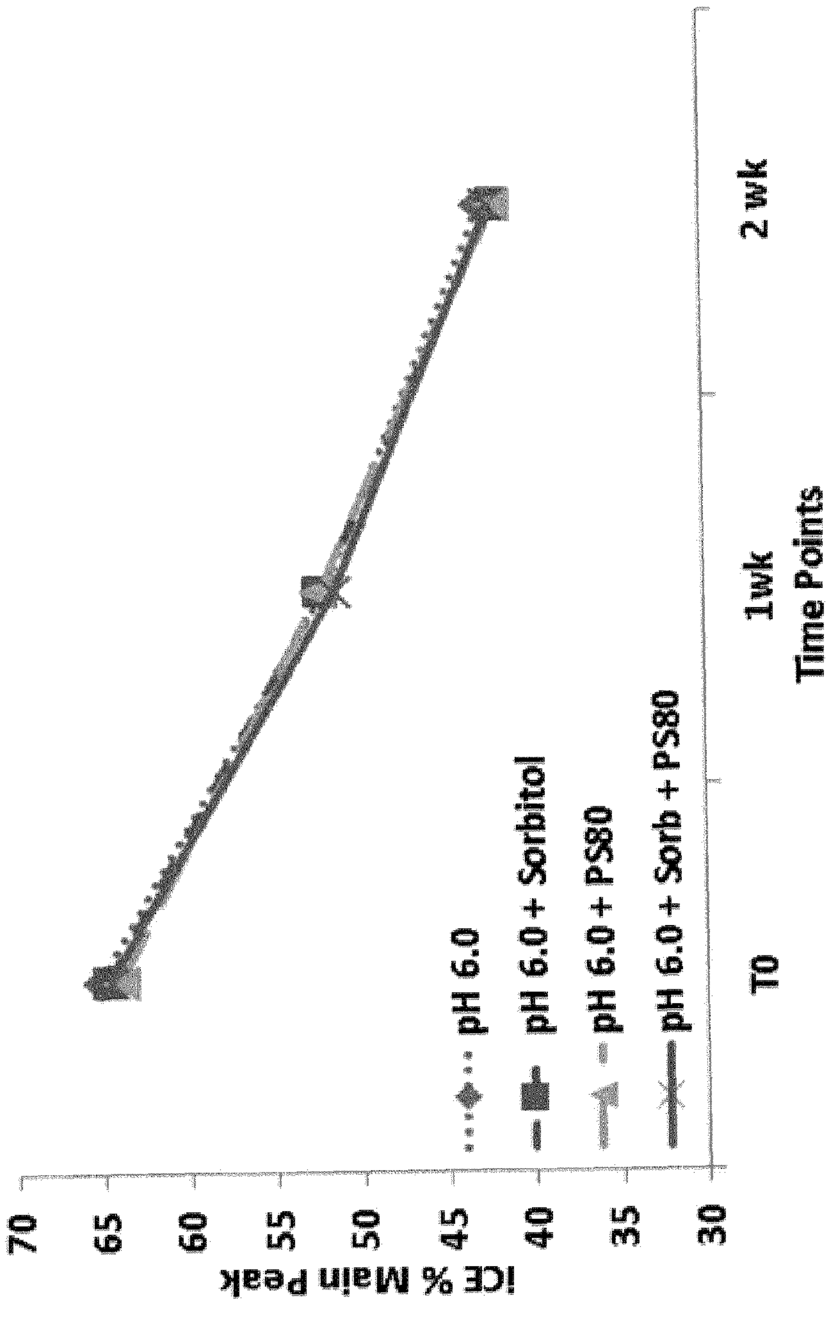

iCE % Main Peak
70
65
60
55
50
45
40
35
30
T0
1wk
2 wk
Time Points
pH 6.0
pH 6.0 + Sorbitol
pH 6.0 + PS80
pH 6.0 + Sorb + PS80

Fig. 5B

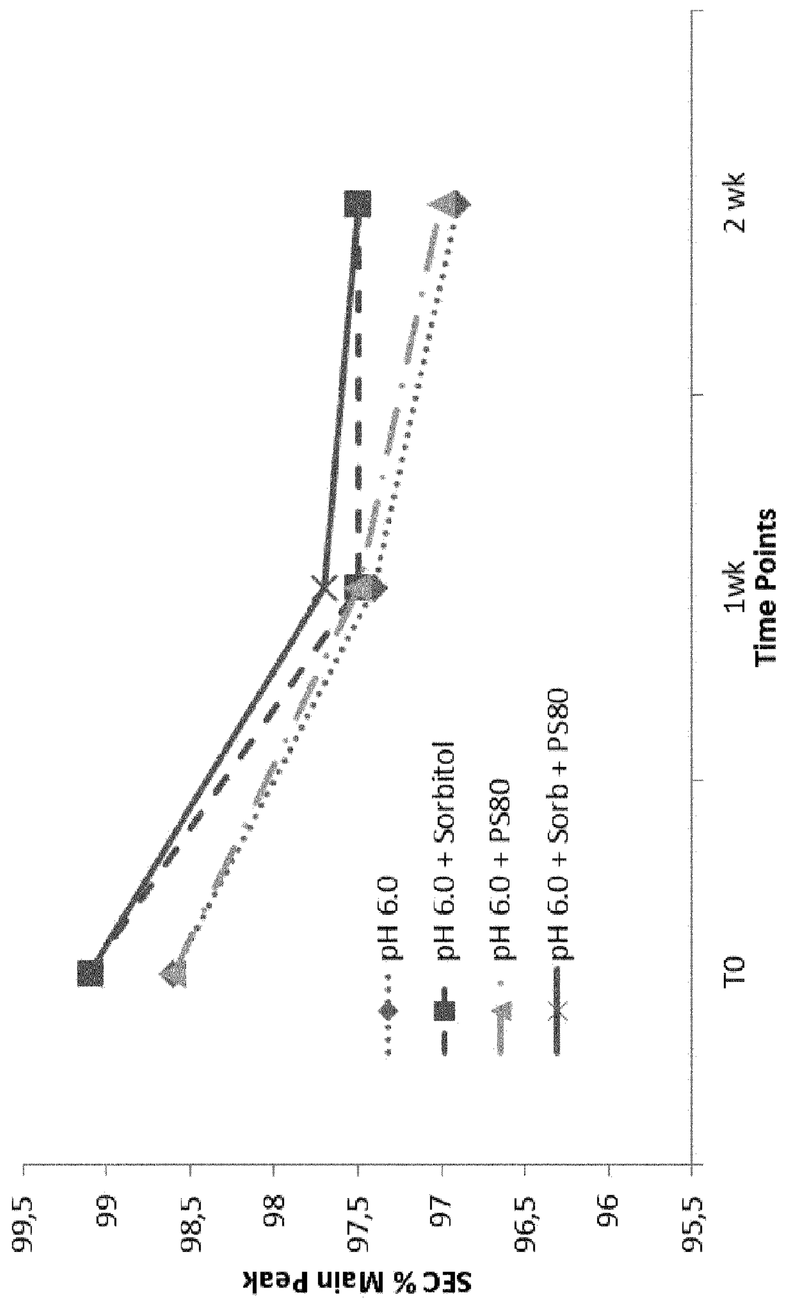
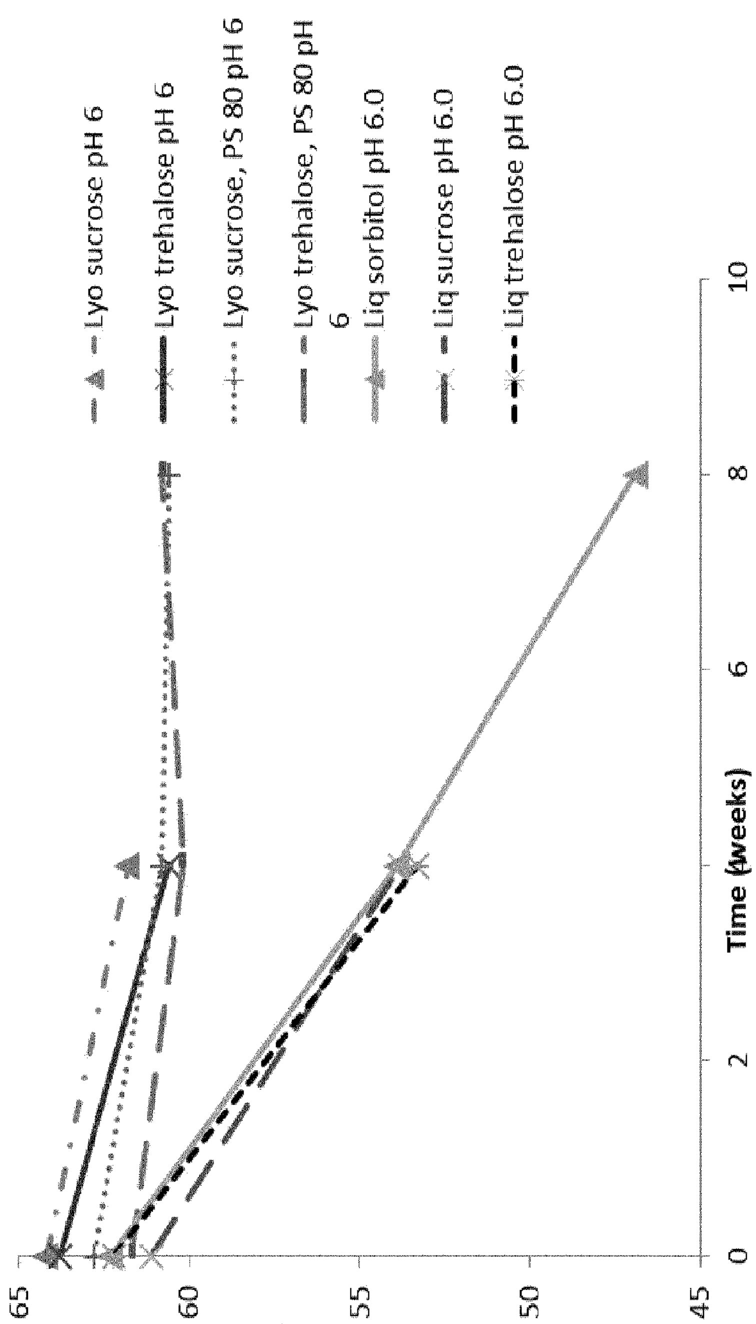

SEC % Main Peak
99,5
99
98,5
98
97,5
97
96,5
96
95,5
T0
1wk
2 wk
Time Points
pH 6.0
pH 6.0 + Sorbitol
pH 6.0 + PS80
pH 6.0 + Sorb + PS80

Fig. 6



icIEF % main peak
65
60
55
50
45
0
2
4
6
8
10
Time (weeks)
Lyo sucrose pH 6
Lyo trehalose pH 6
Lyo sucrose, PS 80 pH 6
Lyo trehalose, PS 80 pH 6
Liq sorbitol pH 6.0
Liq sucrose pH 6.0
Liq trehalose pH 6.0

Fig. 7

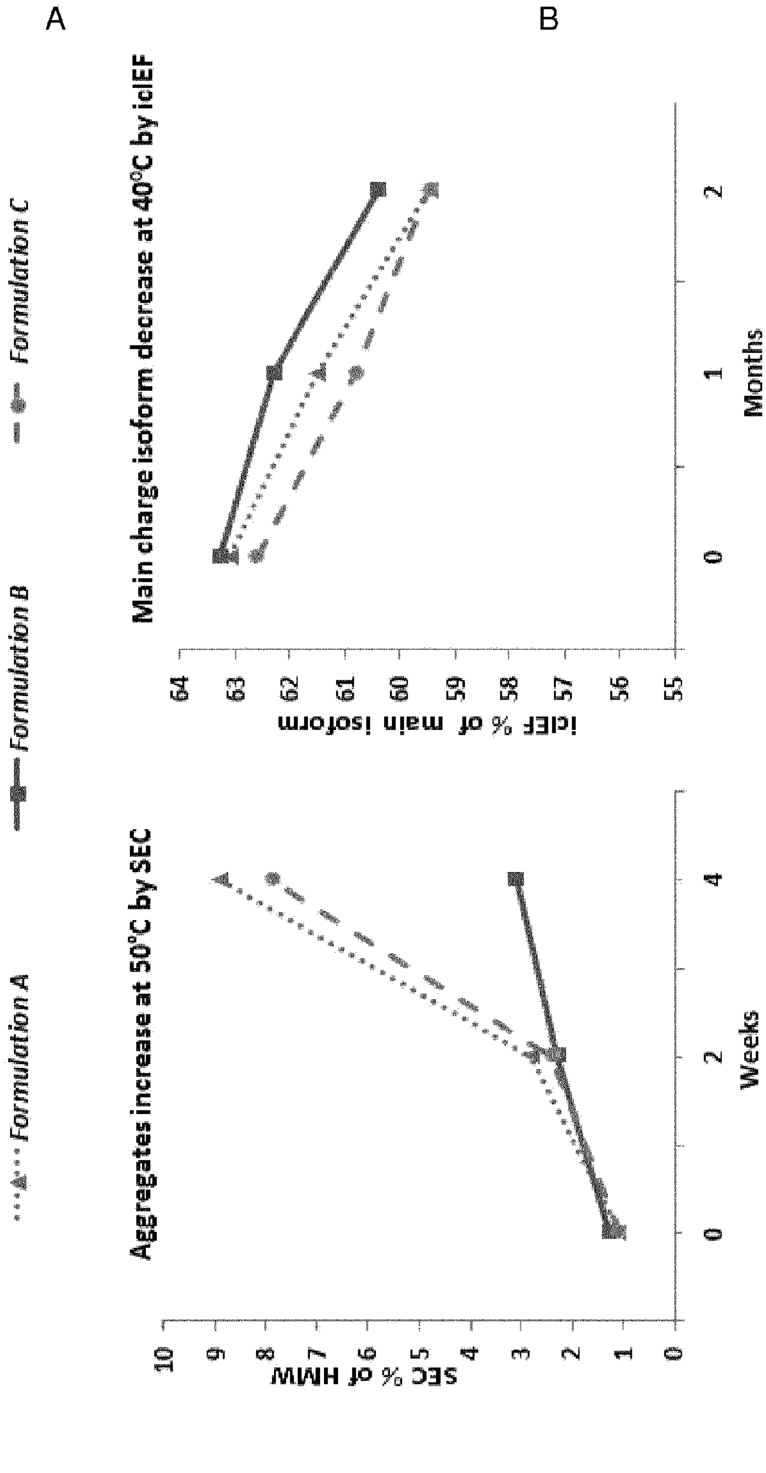

A
B
Formulation A
Formulation B
Formulation C
Aggregates increase at 50°C by SEC
SEC % of HMW
Weeks
Main charge isoform decrease at 40°C by icIEF
icIEF % of main isoform
Months

Fig. 8

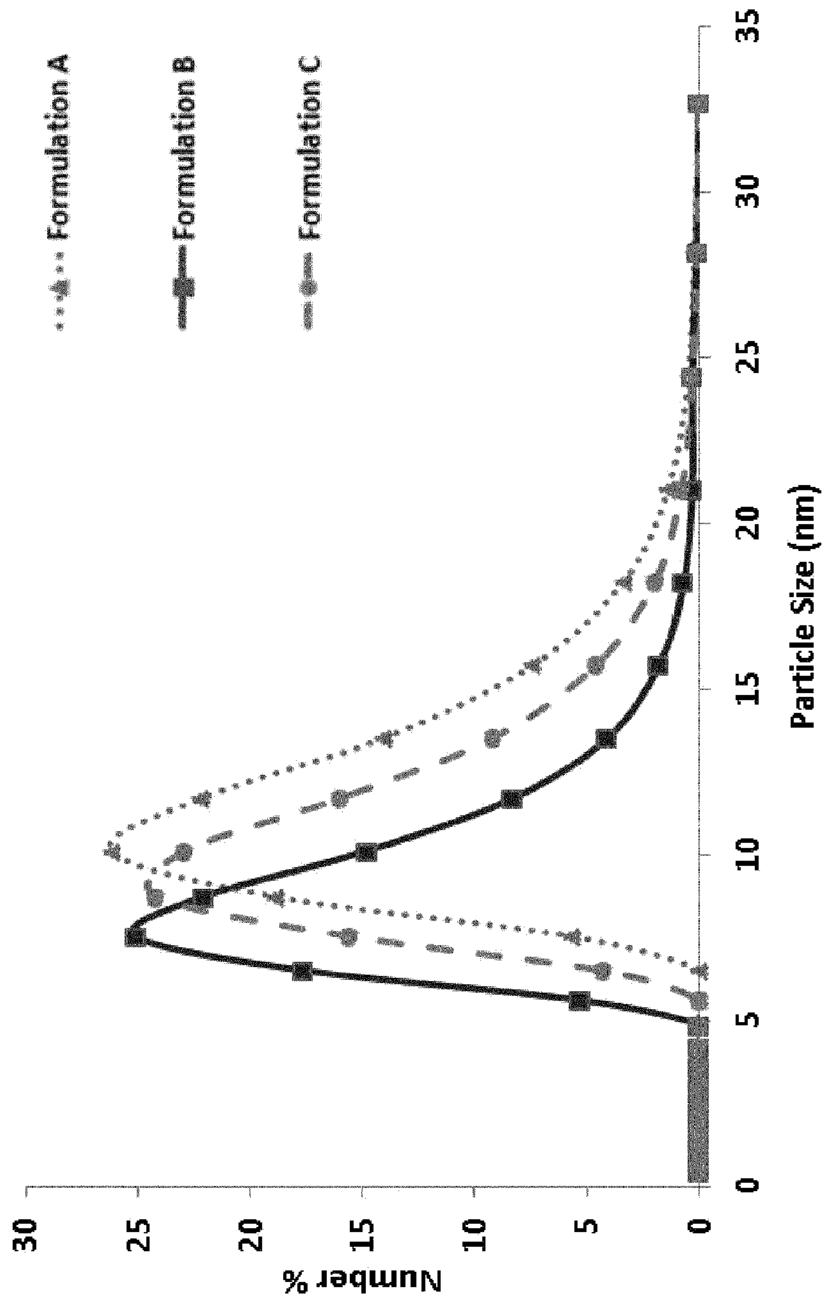

Formulation A
Formulation B
Formulation C
Number %
Particle Size (nm)
0
5
10
15
20
25
30
35

Fig. 9

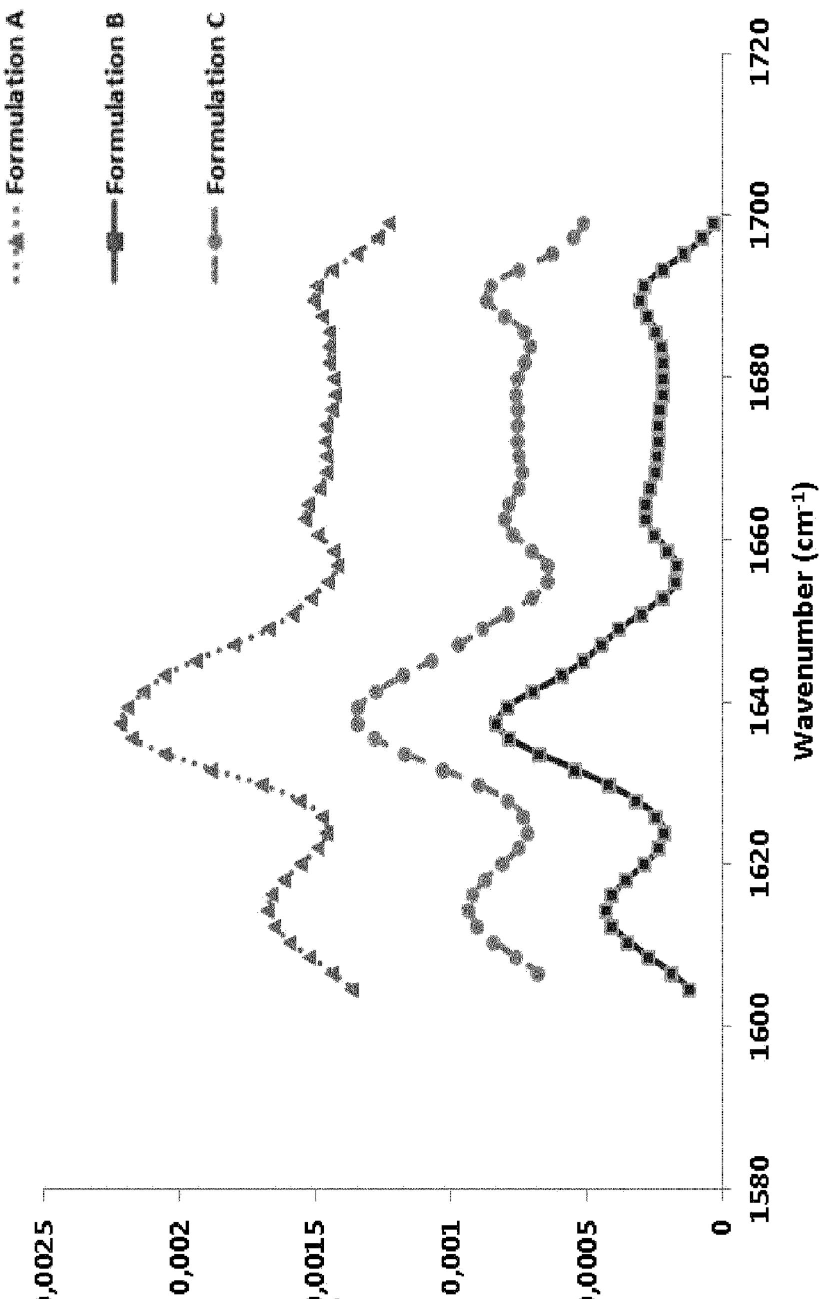

Formulation A
Formulation B
Formulation C
Intensity
0,0025
0,002
0,0015
0,001
0,0005
0
Wavenumber (cm-1)
1580
1600
1620
1640
1660
1680
1700
1720

Fig. 10

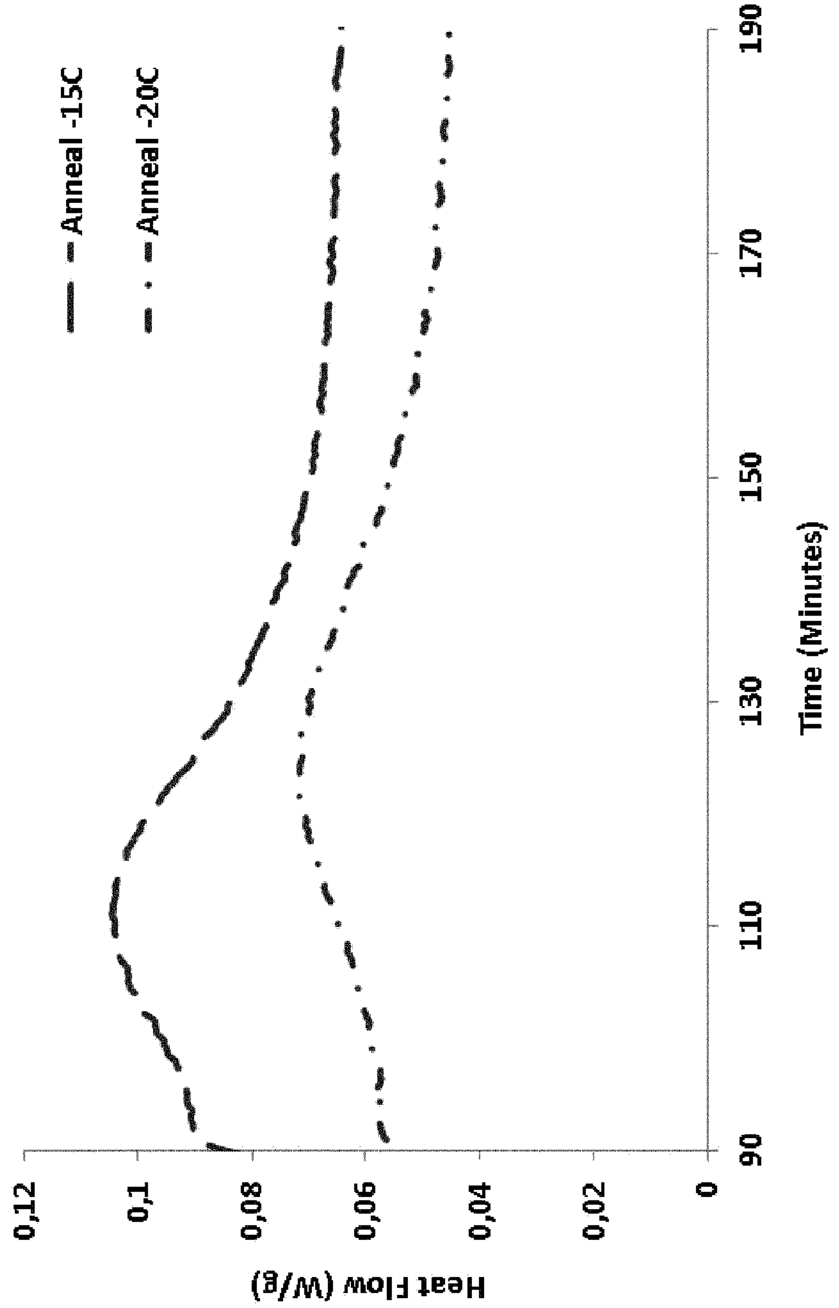

Anneal -15C
Anneal -20C
Time (Minutes)
Heat Flow (W/g)
0,12
0,1
0,08
0,06
0,04
0,02
0
90
110
130
150
170
190

Fig. 11

VH:

```
                         |--CDR1--|                 |--CDR2--|                                           |--------CDR3---------|
QVQLVESGGGVVQPGRSLRLSCVASGFTVSNDGMHWVRQAPGKGLEWVALIWYDGVNKNYADSVKGRFTISRDKSKNTLYLQMNSLRAEDTAVYYCARRPGT-------FYGLDVWGQGTTVTVSS VH1015-114 (1)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPGKGLEWVSSISGSGDYTYYTDSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARSPWG--------YYLDSWGQGTLVTVSS VH1015-C11 (5)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGDSTNYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKDGYFL------LWYFDLWGRGTLVTVSS VH1015-C17 (9)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLEWVSVISGSGGTTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKAPWT--------YYFDYWGQGTLVTVSS VH1015-C42 (13)
EVQLLESGGGLVQPGGSLRLSCAASGFTFNNYAMSWVRQAPGKGLEWVSSISGSGGRTYYADSVKGRFTISRDNSKNTLFLQMNSLRAEDTAVYYCAKTPWG--------YYFDYWGQGTLVTVSS VH1015-C92 (17)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYAMSWVRQAPAKGLDWVSGISGSGVTTYYADSVKGRFTISRDNSKDTLYLQMNSLRAEDTAVYFCAKTPWG--------YYFDYWGQGILVAVSS VH1015-101 (21)
QVQLVESGGGVVQPGRSLRLSCAASGFTFSRYAMHWVRQAPGKGLEWVAVISNDGYNDYYADSVKGRFTVSRDNSKNTLYLQMNSLRAEDTAVYYCARDGQLG------RGYFDYWGQGTLVTVSS VH1015-C25 (25)
QVQLVESGGGVVQPGRSLRLSCPASGFTFSTYAMHWVRQAPGKGLEWVAVVSNDGYNKYYADSVKGRFTISRDNSKDTLYLQMNSLRAEDTAVYYCARDGQLG------RGYFDYWGQGTLVTVSS VH1015-109 (29)
QVQLVQSGAEVRKPGSSVKVSCKASGGSFNNYFIFWVRQAPGQGFEWMGRIIPILGITAYAQKFQGRVTITADKSTSTAYMELNSLRSEDTAVYYCAGGDD------LD--AFDIWGQGTMVSVSS VH1015-C98 (33)
QVQLVESGGGVVQPGRSLRLSCAGSGFTFNRYAMYWVRQAPGKGLDWVAVISNDGINKYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDHTMV------RGAFDYWGQGTLVTVSS VH1015-111 (37)
```

VL:

```
                          |-CDR1--|                 |CDR2|                                |---CDR3----
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSS--LTFGGGTKVEIK VL1015-114 (41)
DIQMTQSPPSLSASAGDRVTITCRASQGISS-RLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYP-YTFGQGTKLEIK VL1015-011 (45)
EIVLTQSPGTLSLSPGERATLSCRASQSVRSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP-RTFGQGTKVEIK VL1015-017 (49)
EIVLTQSPGTLSLSPGERATLSCRASQSVGSSSLAWYQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSP-RTFGQGTKVEIK VL1015-042 (53)
DIQMTQSPSSLSASVGDRVTITCRASQGISS-RLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYP-YTFGQGTKLEIK VL1015-092 (57)
DIQMTQSPSSLSASVGDRVTITCRASQGISS-WLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPLYTFGQGTKLEIK VL1015-101 (61)
EIVLTQSPATLSLSPGERATLSCRASQSVSS-YLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWP-LTFGGGTKVEIK VL1015-025 (65)
EIVLTQSPATLSLSPGERAILSCRASQSVSS-YLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWP-LTFGGGTKVEIK VL1015-109 (69)
DIQMTQSPSSLSASVGDRVTITCRASQGISS-WLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYP-YTFGQGTKLEIK VL1015-098 (73)
EIVLTQSPATLSLSPGERATLSCRASQSVSS-YLAWYQQKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWP-LTFGGGTKVEIK VL1015-111 (77)
```

Fig. 12

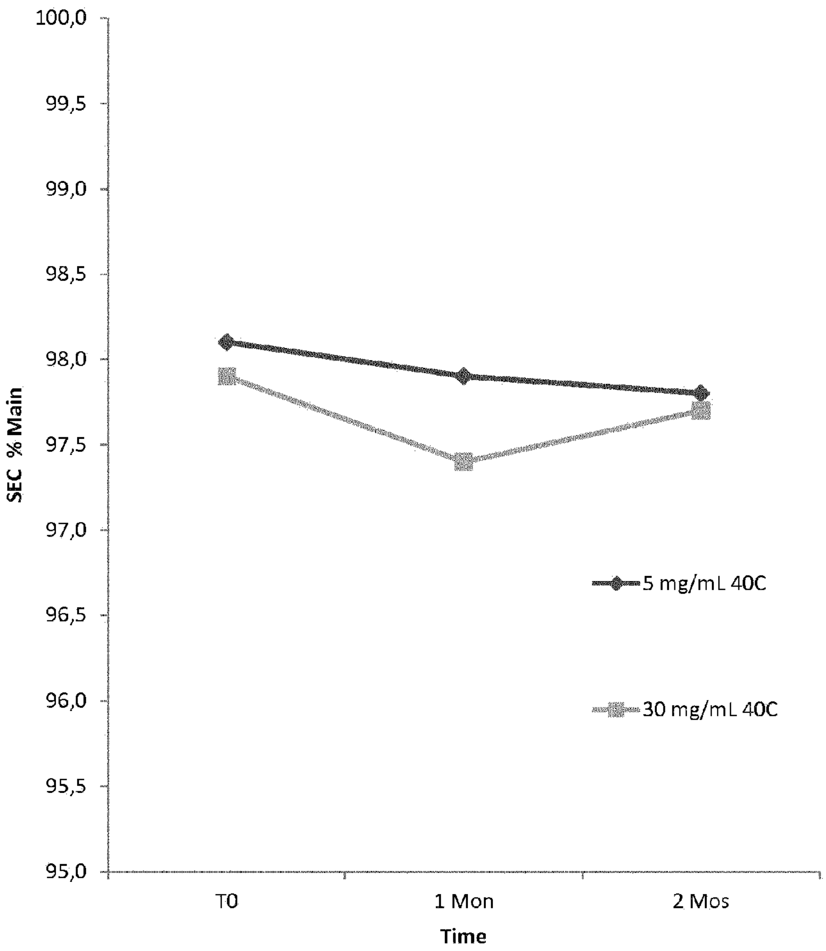

100,0
99,5
99,0
98,5
98,0
97,5
97,0
96,5
96,0
95,5
95,0
SEC % Main
T0
1 Mon
2 Mos
Time
5 mg/mL 40C
30 mg/mL 40C

Fig. 13

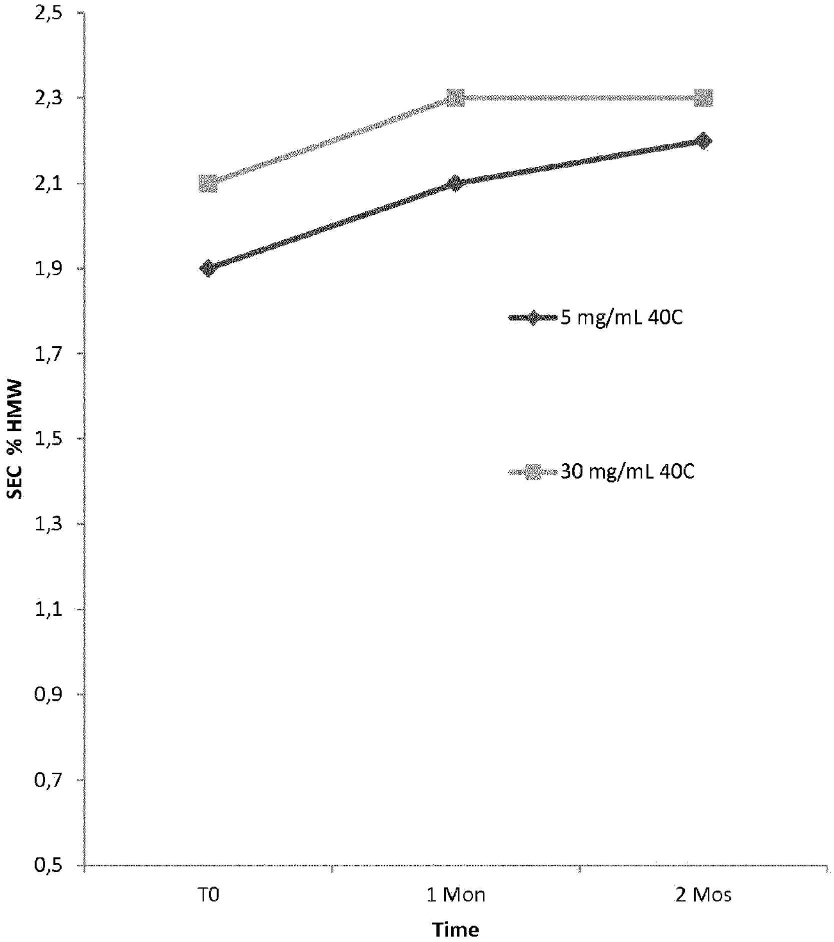

2,5
2,3
2,1
1,9
1,7
1,5
1,3
1,1
0,9
0,7
0,5
SEC % HMW
5 mg/mL 40C
30 mg/mL 40C
T0
1 Mon
2 Mos
Time

Fig. 14

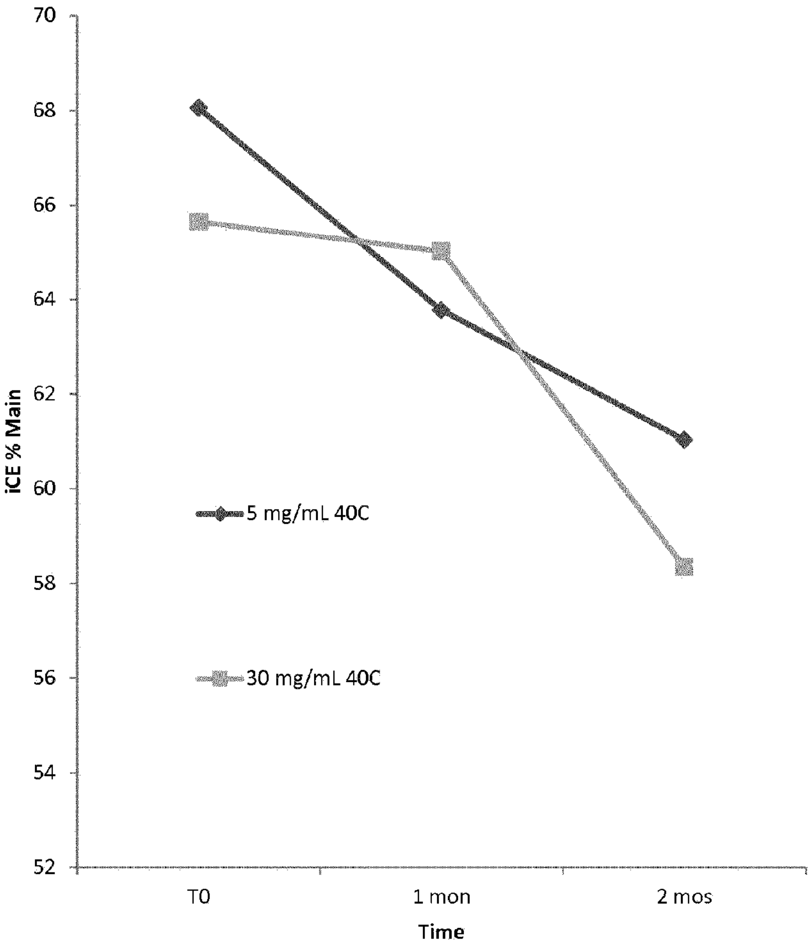

70
68
66
64
62
60
58
56
54
52
iCE % Main
5 mg/mL 40C
30 mg/mL 40C
T0
1 mon
2 mos
Time

Fig. 15

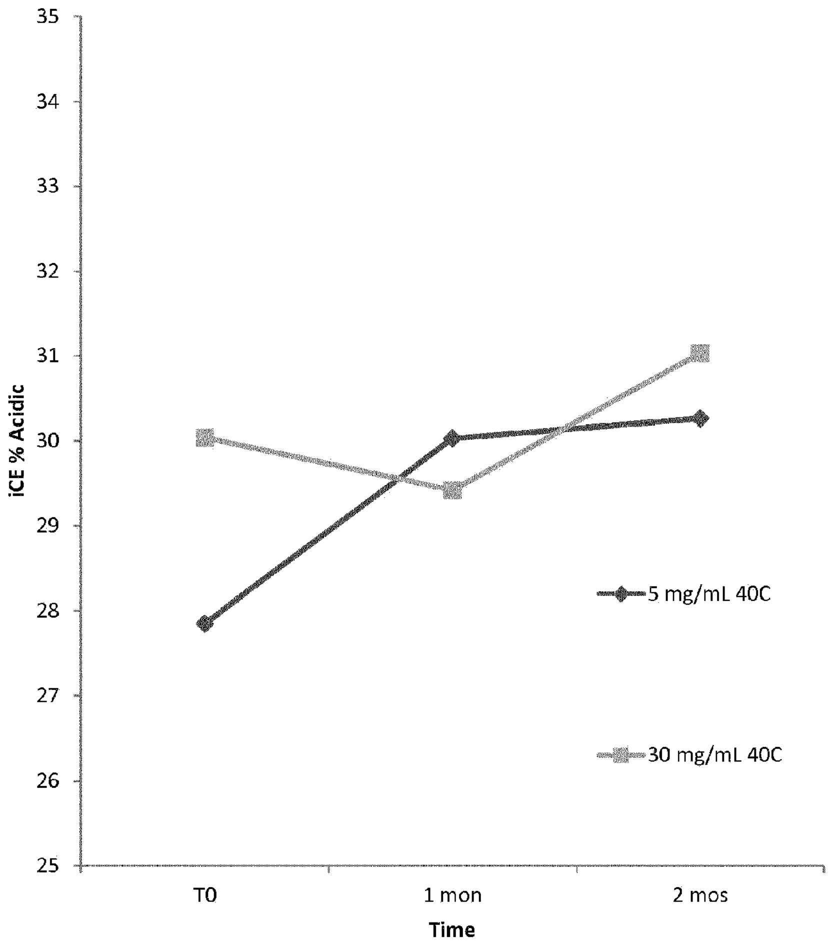

35
34
33
32
31
30
29
28
27
26
25
iCE % Acidic
5 mg/mL 40C
30 mg/mL 40C
T0
1 mon
2 mos
Time

Fig. 16

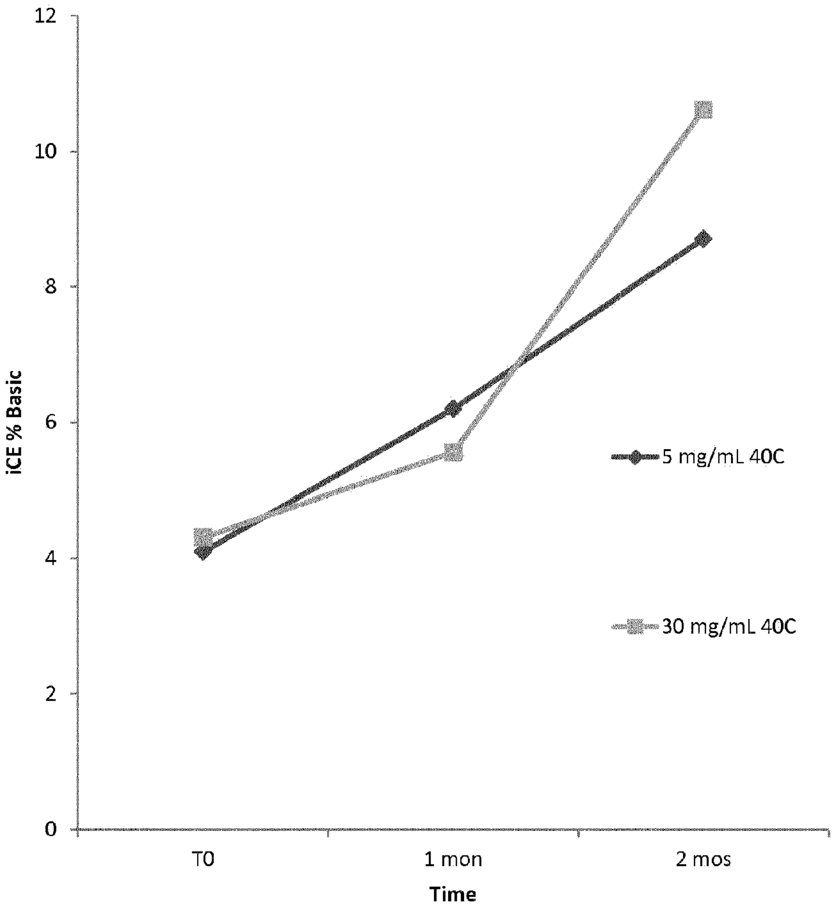


Fig. 17

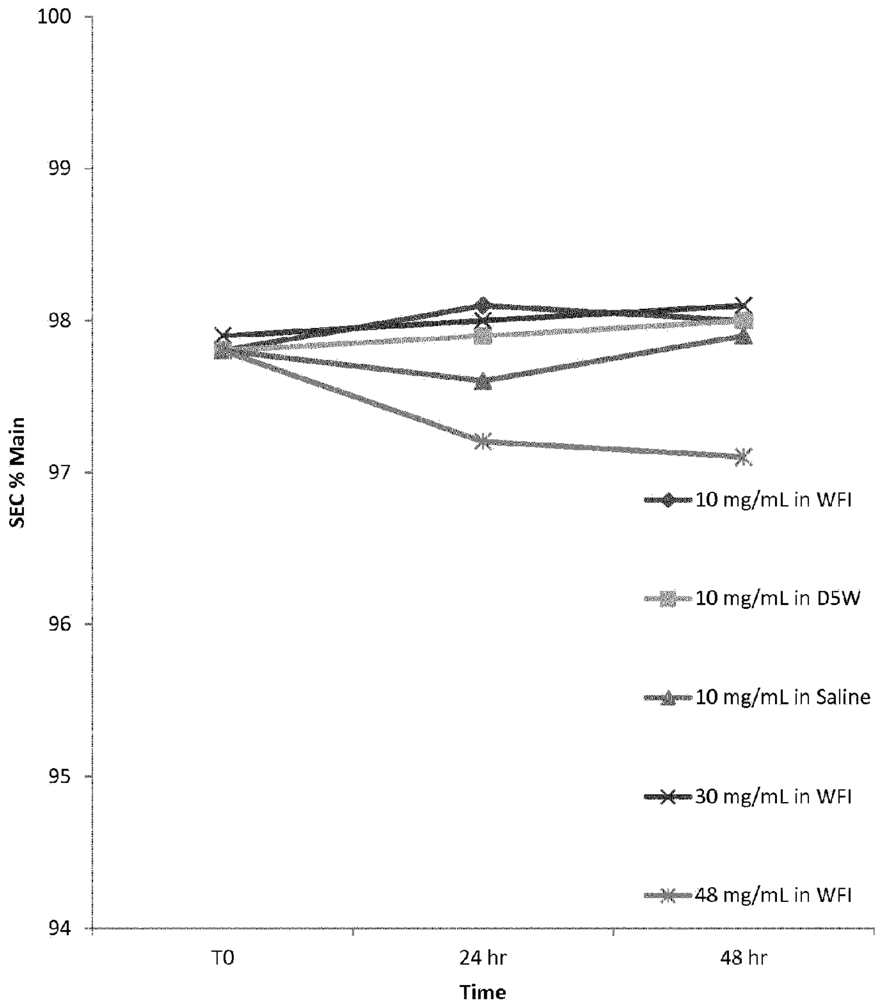

100
99
98
97
96
95
94
SEC % Main
10 mg/mL in WFI
10 mg/mL in D5W
10 mg/mL in Saline
30 mg/mL in WFI
48 mg/mL in WFI
T0
24 hr
48 hr
Time

Fig. 18

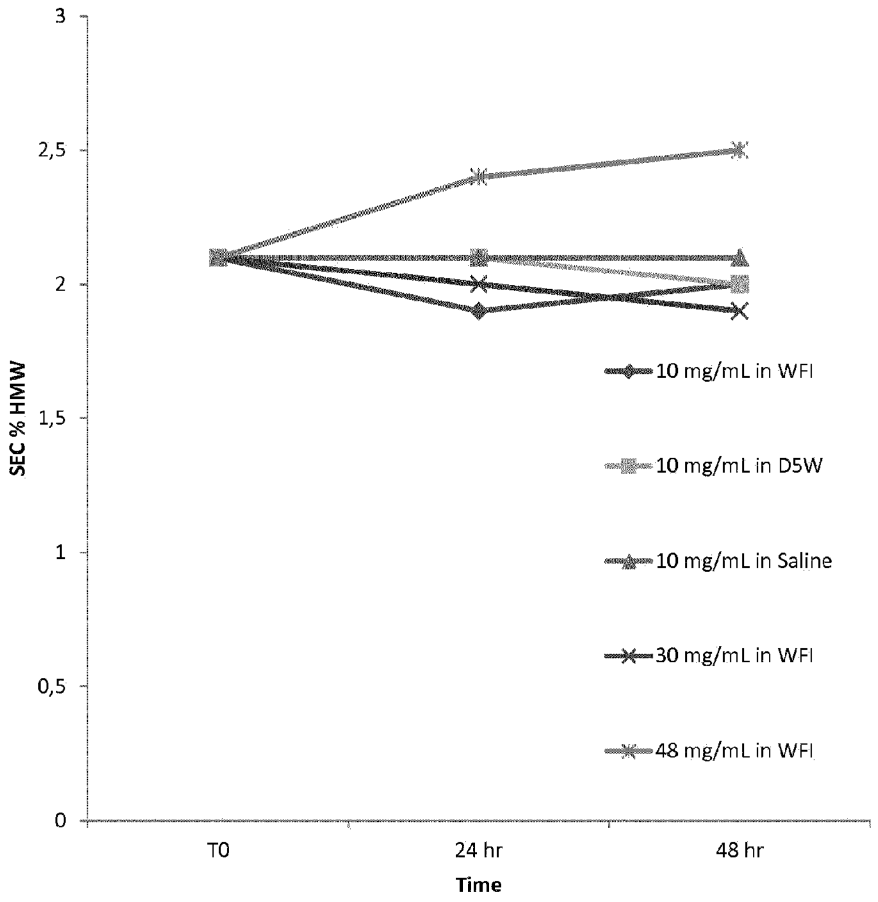

3
2,5
2
1,5
1
0,5
0
SEC % HMW
T0
24 hr
48 hr
Time
10 mg/mL in WFI
10 mg/mL in D5W
10 mg/mL in Saline
30 mg/mL in WFI
48 mg/mL in WFI

Fig. 19

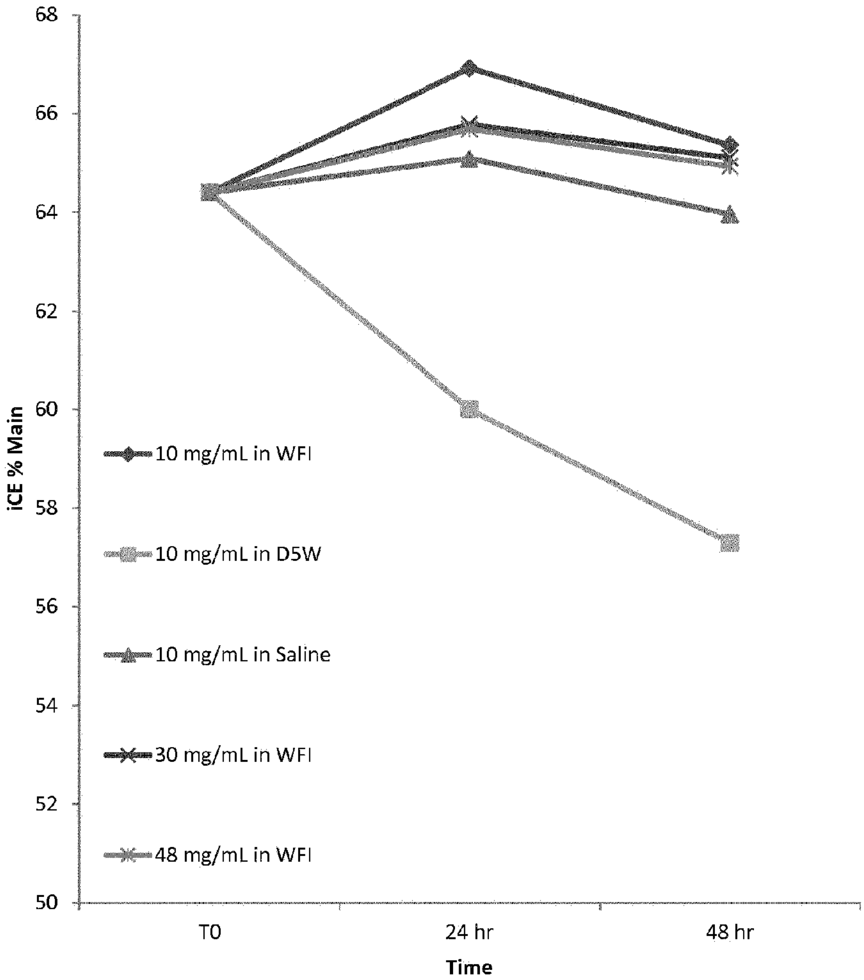

68
66
64
62
60
58
56
54
52
50
iCE % Main
10 mg/mL in WFI
10 mg/mL in D5W
10 mg/mL in Saline
30 mg/mL in WFI
48 mg/mL in WFI
T0
24 hr
48 hr
Time

Fig. 20

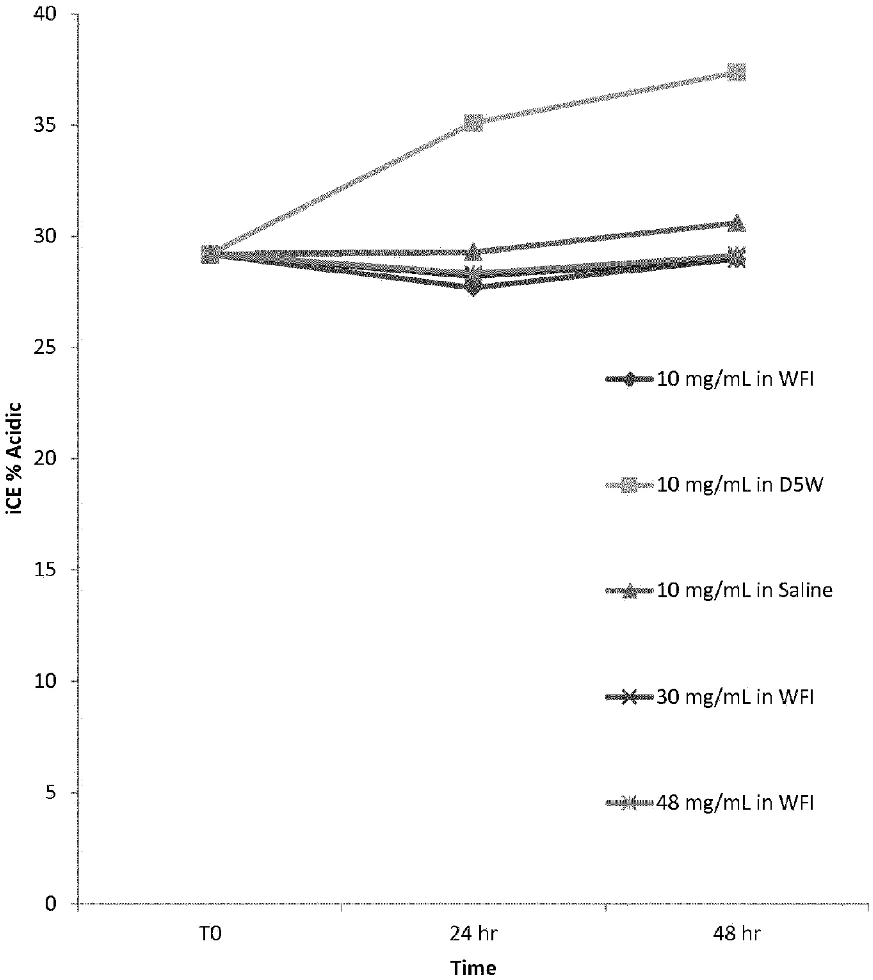

40
35
30
25
20
15
10
5
0
iCE % Acidic
T0
24 hr
48 hr
Time
10 mg/mL in WFI
10 mg/mL in D5W
10 mg/mL in Saline
30 mg/mL in WFI
48 mg/mL in WFI

Fig. 21

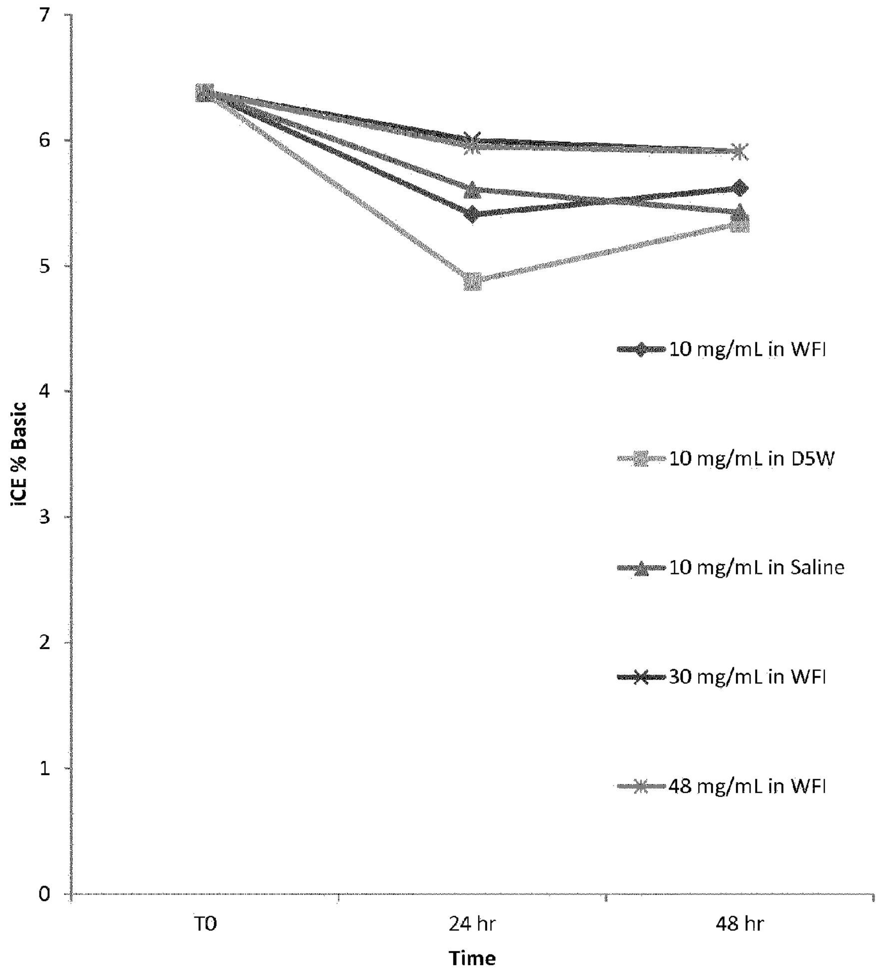

7
6
5
4
3
2
1
0
iCE % Basic
T0
24 hr
48 hr
Time
10 mg/mL in WFI
10 mg/mL in D5W
10 mg/mL in Saline
30 mg/mL in WFI
48 mg/mL in WFI

Fig. 22

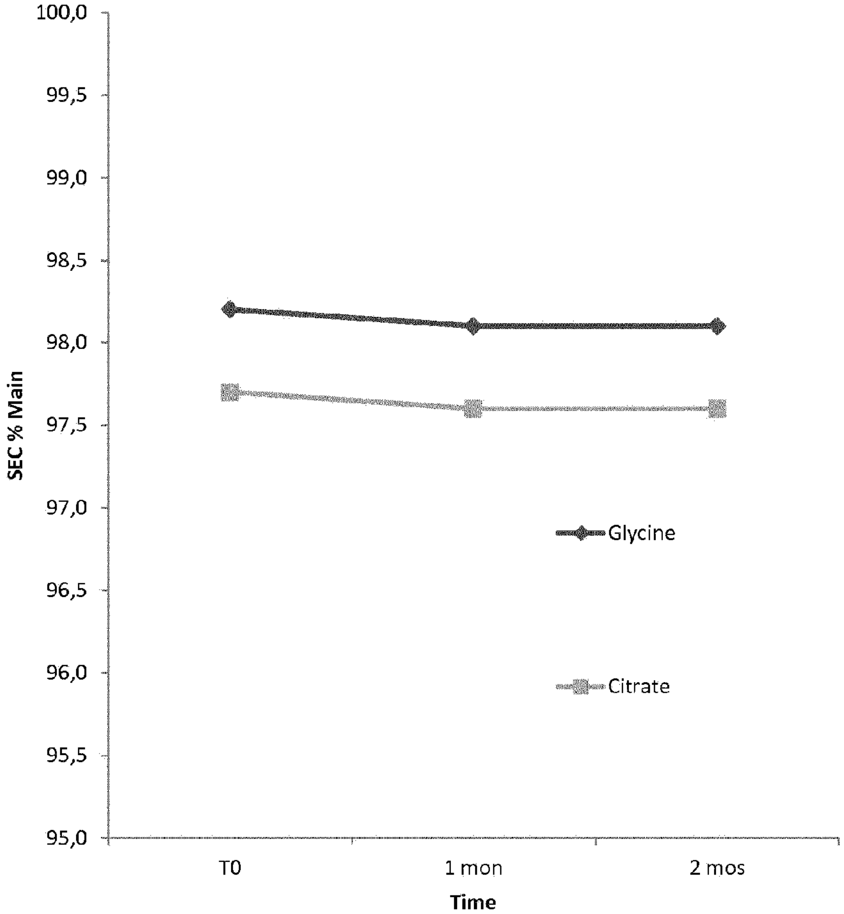

100,0
99,5
99,0
98,5
98,0
97,5
97,0
96,5
96,0
95,5
95,0
SEC % Main
Glycine
Citrate
T0
1 mon
2 mos
Time

Fig. 23

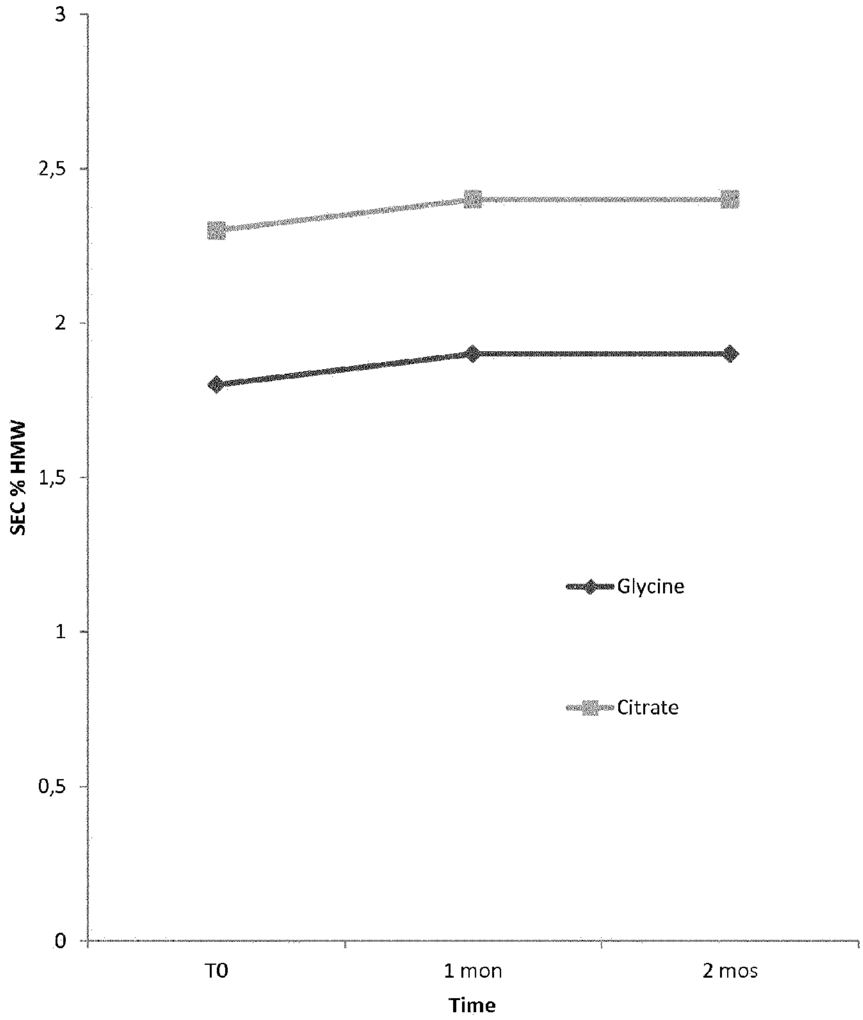


Fig. 24

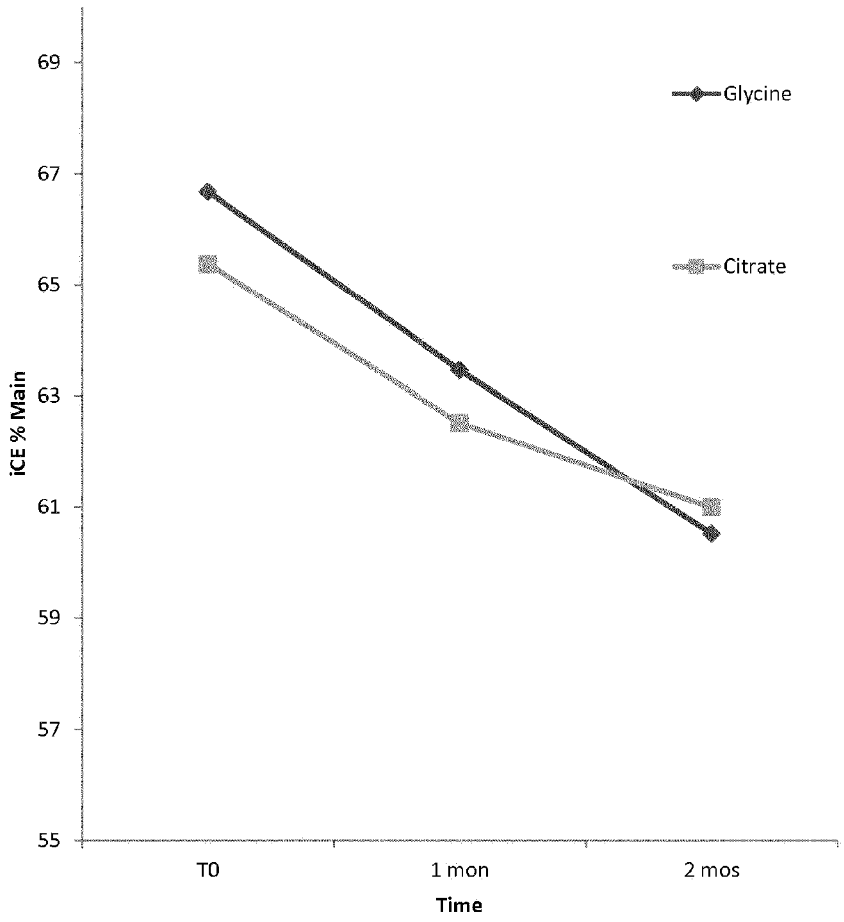

69
67
65
63
61
59
57
55
iCE % Main
Glycine
Citrate
T0
1 mon
2 mos
Time

Fig. 25

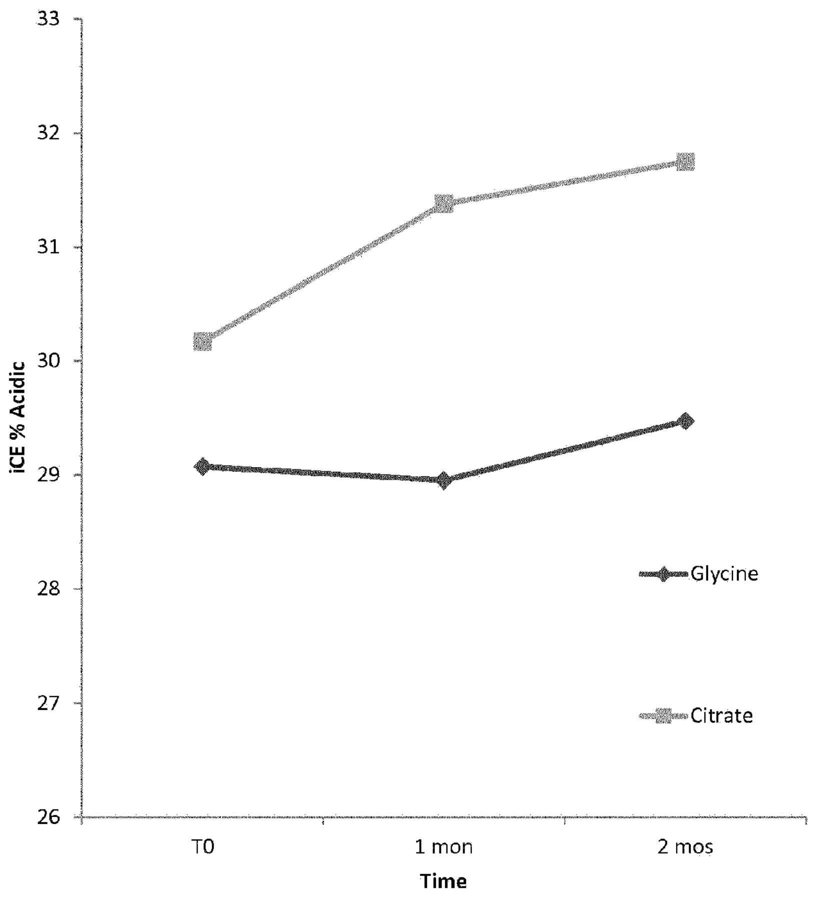

33
32
31
30
29
28
27
26
iCE % Acidic
Glycine
Citrate
T0
1 mon
2 mos
Time

Fig. 26

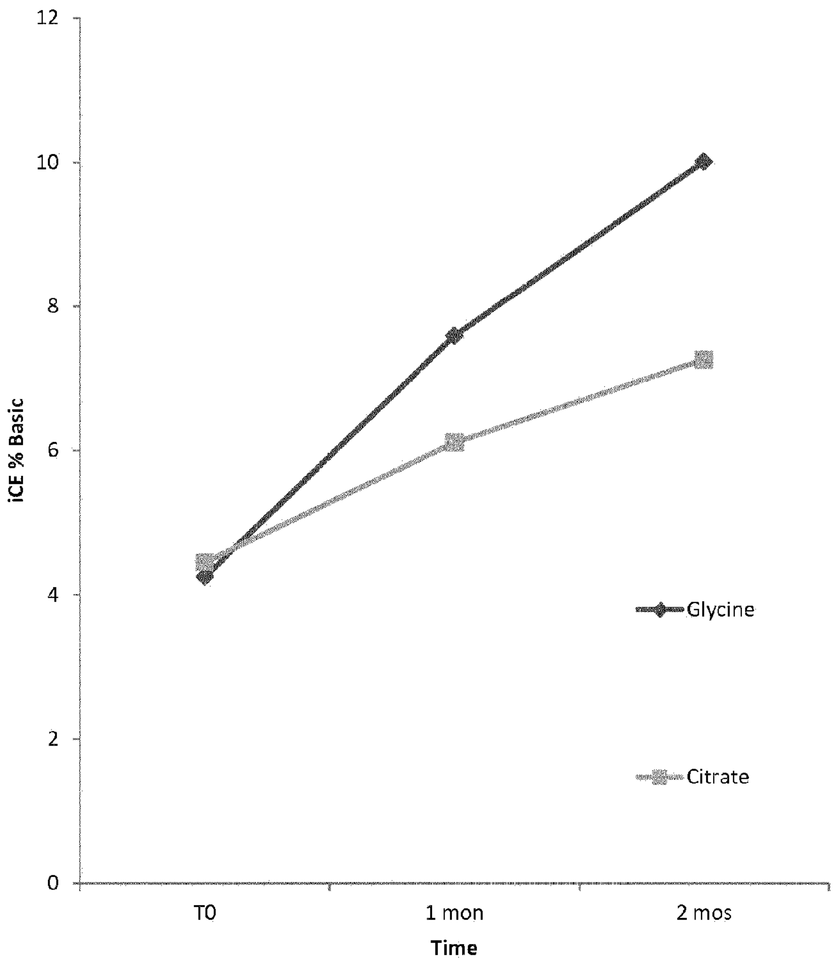


Fig. 27

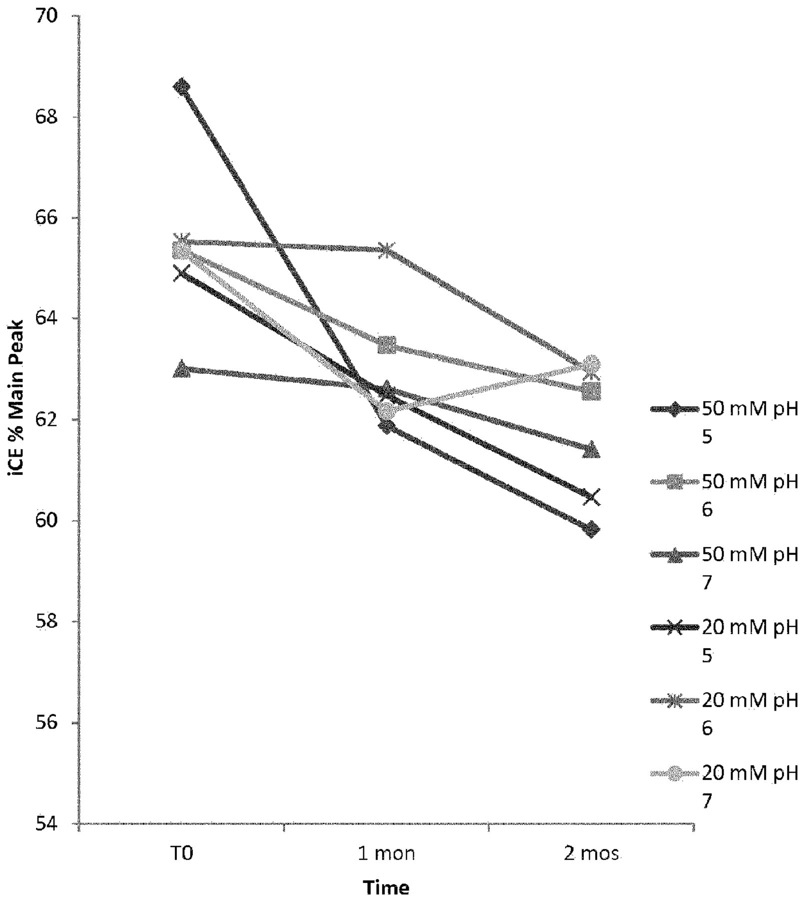

70
68
66
64
62
60
58
56
54
iCE % Main Peak
T0
1 mon
2 mos
Time
50 mM pH 5
50 mM pH 6
50 mM pH 7
20 mM pH 5
20 mM pH 6
20 mM pH 7

Fig. 28

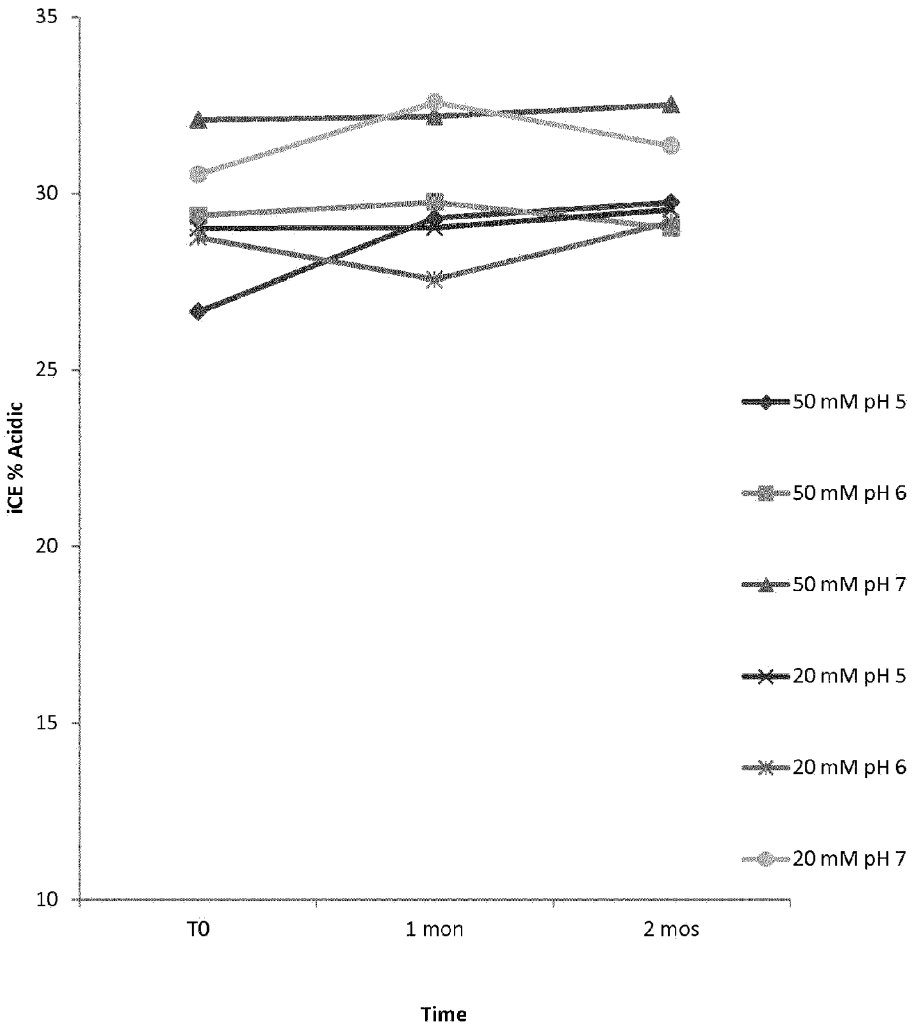

35
30
25
20
15
10
iCE % Acidic
T0
1 mon
2 mos
Time
50 mM pH 5
50 mM pH 6
50 mM pH 7
20 mM pH 5
20 mM pH 6
20 mM pH 7

Fig. 29

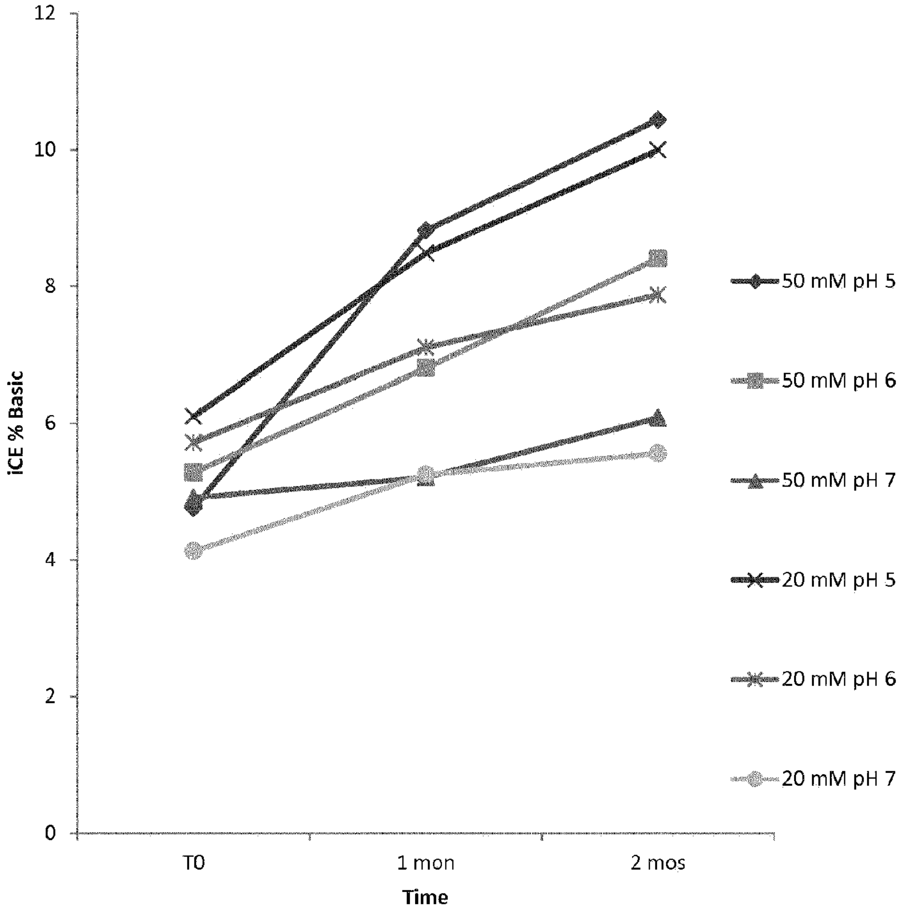

12
10
8
6
4
2
0
iCE % Basic
T0
1 mon
2 mos
Time
50 mM pH 5
50 mM pH 6
50 mM pH 7
20 mM pH 5
20 mM pH 6
20 mM pH 7

Fig. 30

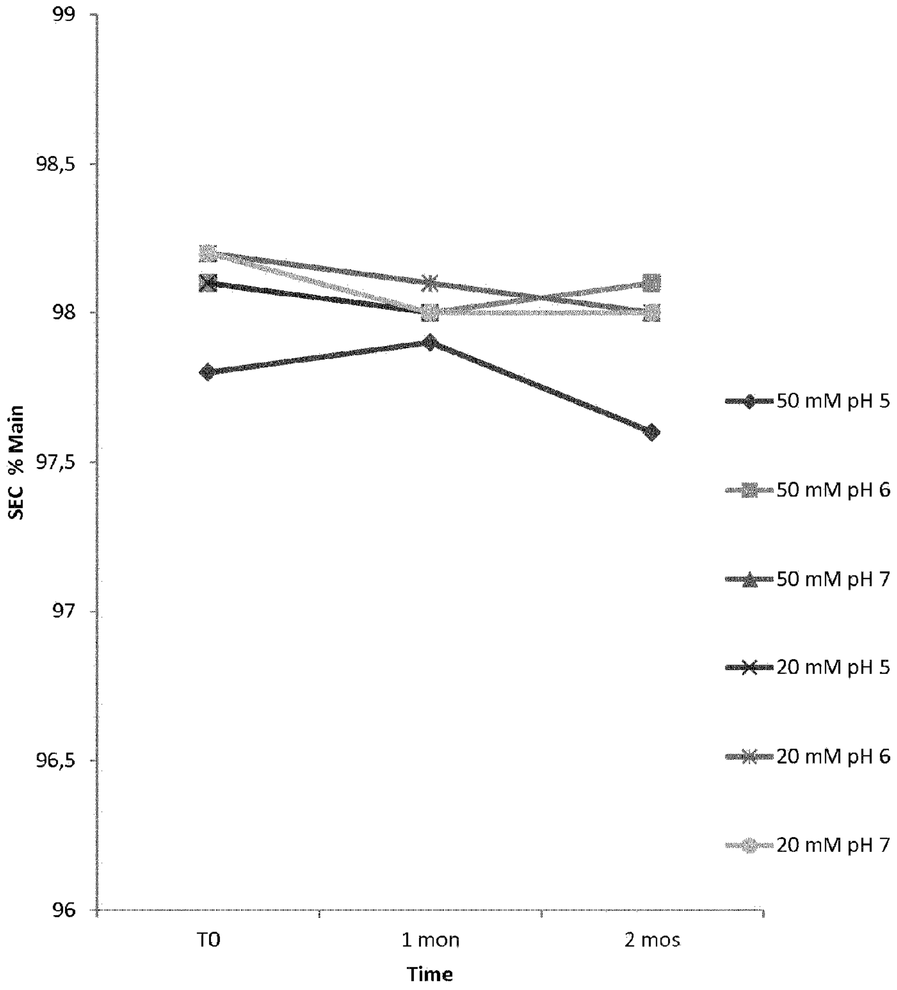
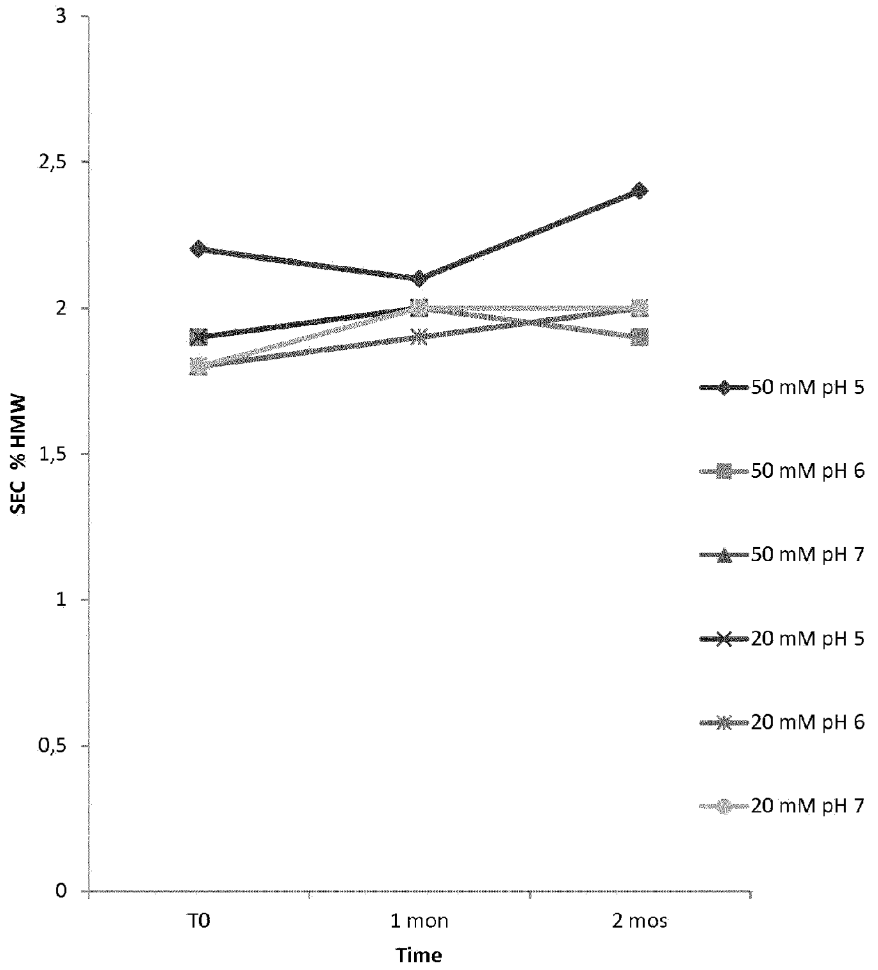

99
98,5
98
97,5
97
96,5
96
SEC % Main
T0
1 mon
2 mos
Time
50 mM pH 5
50 mM pH 6
50 mM pH 7
20 mM pH 5
20 mM pH 6
20 mM pH 7

Fig. 31


3
2,5
2
1,5
1
0,5
0
SEC % HMW
T0
1 mon
2 mos
Time
50 mM pH 5
50 mM pH 6
50 mM pH 7
20 mM pH 5
20 mM pH 6
20 mM pH 7

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.