

aggregated IgG using an elution buffer containing 1 M glycine in 20 mM Tris, 20 mM Hepes, 20 mM MES, 50 mM NaCl with a linear gradient from pH 9.0 to pH 4.5.

SUMMARY OF THE INVENTION

- 5 In a first aspect the present invention relates to a method for purifying or enriching a biomolecule, said method comprising the steps:
- (a) providing an aqueous solution containing said biomolecule;
 - (b) contacting said aqueous solution with a multimodal resin;
 - (c) optionally washing the multimodal resin with an aqueous washing buffer;
 - 10 (d) eluting the biomolecule from the multimodal resin with an aqueous buffer solution, wherein said aqueous buffer solution comprises, essentially consists of or consists of: at least 15 mM at least one Good's buffer; between 0 and 400 mM in total concentration of one or more of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, 15 phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; between 0 and 400 mM in total concentration of one or more of urea, guanidinium chloride, guanidinium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium caprylate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium phosphate, 20 potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, and taurine; and between 0% and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol; and
 - (e) collecting biomolecule containing fractions, thereby obtaining the biomolecule in 25 purified or enriched form.

In one embodiment, the aqueous buffer solution comprises 15 mM to 2.5 mM Good's buffer, such as at least 20 mM, 50 mM, 100 mM, 150 mM, 200 mM or more of a Good's buffer. In another embodiment, the aqueous buffer comprises between 10 mM and 400 mM of one or more of alanine, asparagine, aspartic acid, cysteine, glutamine, 30 glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; between 10 mM and 400 mM of one or more of guanidinium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium 35 phosphate, potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, and taurine; and

between 1% and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol.

In one embodiment, the Good's buffer in the aqueous buffer is MES, MOPS or Tris.

5 In a second aspect, the invention relates to a purified biomolecule, such as a protein, obtainable by the method according to the first aspect.

In a third aspect the present invention relates to a preparation comprising:

- (a) a biomolecule; and
- (b) an aqueous buffer solution comprising, essentially consisting of or consisting of at least 15 mM of at least one Good's buffer; between 0 and 400 mM in total concentration of one or more of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; between 0 and 400 mM in total concentration of one or more of urea, guanidinium chloride, guanidinium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium caprylate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium phosphate, potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, and taurine; and between 0% and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol.

In one embodiment, the aqueous buffer solution comprises 15 mM to 2.5 mM of a Good's buffer, such as at least 20 mM, 50 mM, 100 mM, 150 mM, 200 mM or more of a Good's buffer. In another embodiment, the aqueous buffer comprises between 10 mM and 400 mM of one or more of alanine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; between 10 mM and 400 mM of one or more of guanidinium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium phosphate, potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, and taurine; and between 1% and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol.

35 In one embodiment, the Good's buffer in the aqueous buffer is MES, MOPS or Tris.

In a fourth aspect, the invention relates to a use of an aqueous buffer solution for desorbing a biomolecule from a multimodal resin, wherein said aqueous buffer solution comprises, essentially consists of or consists of at least 15 mM of at least one Good's buffer; between 0 and 400 mM in total concentration of one or more of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; between 0 and 400 mM in total concentration of one or more of urea, guanidinium chloride, guanidinium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium caprylate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium phosphate, potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, and taurine; and between 0% and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol.

For example, in certain embodiments, the aqueous buffer solution comprises 15 mM to 2.5 mM of a Good's buffer, such as at least 20 mM, 50 mM, 100 mM, 150 mM, 200 mM or more of a Good's buffer. In another embodiment, the aqueous buffer comprises between 10 mM and 400 mM of one or more of alanine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine; between 10 mM and 400 mM of one or more of guanidinium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium phosphate, potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, and taurine; and between 1% and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol.

In one embodiment, the Good's buffer in the aqueous buffer is MES, MOPS or Tris.

In a fifth aspect, the present invention relates to use of an aqueous buffer solution for increasing the recovery rate of a biomolecule adsorbed on a multimodal resin by contacting (a) the multimodal resin onto which said biomolecule has been adsorbed with (b) the aqueous buffer solution, wherein the aqueous buffer solution comprises, essentially consists of or consists of at least 15 mM of at least one Good's buffer; between 0 and 400 mM in total concentration of one or more of alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine,

CLAIMS

1. A method of purifying or enriching a biomolecule, the method comprising:

- (a) providing an aqueous solution comprising the biomolecule;
- (b) contacting the aqueous solution with a multimodal resin;
- (c) optionally washing the multimodal resin with an aqueous washing buffer;
- (d) eluting the biomolecule from the multimodal resin using an aqueous buffer solution,

wherein the aqueous buffer solution comprises:

~~(i) at least 15 mM N-(2-acetoamido)iminodiacetic acid (ADA), N-(2-acetamido)-2-aminoethanesulfonic acid (ACES), N,N-bis(2-hydroxyethyl)-2-aminoethanesulfonic acid (BES), N,N-bis(2-hydroxyethyl)glycine (Bicine), 3-(cyclohexylamino)-1-propanesulfonic acid (CAPS), 3-(cyclohexylamino)-2-hydroxy-1-propanesulfonic acid (CAPSO), 2-(cyclohexylamino)ethanesulfonic acid (CHES), 4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), 3-(N-morpholino)propanesulfonic acid (MOPS), 1,4-piperazinediethanesulfonic acid (PIPES), N-[tris(hydroxymethyl)methyl]-3-aminopropanesulfonic acid (TAPS), or 2-[(2-hydroxyl-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid (TES); and~~

(i) at least 15 mM 2-(N-Morpholino)ethanesulfonic acid (MES); and

(ii) ~~one or both of:~~

~~between 10 mM and 400 mM alanine, arginine, asparagine, aspartic acid, cysteine, glutamic acid, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, or valine; and~~

~~between 10 mM and 400 mM guanidium chloride, guanidium thiocyanate, ammonium chloride, ammonium sulfate, ammonium phosphate, ammonium acetate, sodium chloride, sodium citrate, sodium caprylate, sodium phosphate, sodium iodide, potassium chloride, potassium citrate, potassium sulfate, potassium phosphate, potassium iodide, magnesium chloride, magnesium sulfate, magnesium phosphate, calcium chloride, calcium sulfate, calcium phosphate, or taurine; and~~

optionally, further comprising (iii) between 1% (v/v) and 60% (v/v) of ethylene glycol or propylene glycol or a mixture of ethylene glycol and propylene glycol;

(e) collecting a biomolecule-containing fraction; and

(f) applying the biomolecule-containing fraction to one or more of ultrafiltration/diafiltration, tangential flow filtration, microfiltration, and hydrophobic interaction chromatography, thereby obtaining the biomolecule in purified or enriched form.

2. The method according to claim 1, wherein the aqueous buffer solution comprises 15 mM to 2.5 M of ~~ADA, ACES, BES, Bicine, CAPS, CAPSO, CHES, HEPES, MOPS, PIPES, TAPS, or TESMES.~~

3. The method according to claim 1 ~~or claim 2, wherein the aqueous buffer solution comprises MOPS.~~

~~4. The method according to any one of claims 1 to 3, wherein the aqueous buffer solution comprises 10 mM to 400 mM arginine.~~

~~5. The method according to claim 4,~~ wherein the aqueous buffer solution comprises 150 mM to 400 mM arginine.

~~64.~~ The method according to any one of claims 1 to ~~53,~~ wherein the multimodal resin is selected from the group consisting of: Capto™ Adhere, Capto™ MMC, Pall PPA HyperCel™, Pall HEA HyperCel™, Pall MEP HyperCel™, and Eshmuno® HCX.

~~75.~~ The method according to any one of claims 1 to ~~64,~~ wherein the biomolecule is a protein.

~~86.~~ The method according to claim ~~75,~~ wherein the protein is an enzyme.

~~97.~~ The method according to claim ~~86,~~ wherein the enzyme is a glycosidase.

~~108.~~ The method according to claim ~~97,~~ wherein the glycosidase is an alpha-galactosidase.

~~119.~~ The method according to claim ~~108,~~ wherein the alpha-galactosidase is selected from the group consisting of agalsidase alpha and agalsidase beta.

~~1210.~~ The method according to claim ~~119,~~ wherein the agalsidase beta is Fabrazyme®.

~~1311.~~ The method according to claim ~~119,~~ wherein the agalsidase alpha is Replagal™.

1412. The method according to any one of claims 1-~~13~~11, wherein step (f) comprises applying the biomolecule-containing fraction to ultrafiltration/diafiltration.

1513. The method according to any one of claims 1-~~13~~11, wherein step (f) comprises applying the biomolecule-containing fraction to tangential flow filtration.

1614. The method according to any one of claims 1-~~13~~11, wherein step (f) comprises applying the biomolecule-containing fraction to microfiltration.

1715. The method according to any one of claims 1-~~13~~11, wherein step (f) comprises applying the biomolecule-containing fraction to hydrophobic interaction chromatography.