hereditary recessive bleeding disorder hemophilia B, which can be successfully treated by administration of posttranslational modified, *i.e.* phosphorylated and sulfated FIX.

[006] Further, Factor VII (FVII) is a vitamin K-dependent serine protease which plays a significant role in the coagulation cascade, where it initiates the process of coagulation with tissue factor (TF). Upon vessel injury, TF is exposed to the blood and circulating FVII. Once bound to TF, FVII is activated to FVIIa by thrombin, Factor Xa, IXa, XIIa, and the FVIIa-TF complex whose substrates are FX and FIX. Furthermore, Annexin V is a cellular protein in the annexin group, having the ability to bind in a calcium-dependent manner to phosphatidylserine and to form a membrane-bound two dimensional crystal lattice. It may play a role in blood coagulation, apoptosis, phagocytosis and formation of plasma membrane-derived microparticles.

[007] Thus, the problem underlying the present invention is to provide an improved method for the purification of divalent cation binding proteins with a high purity. The solution to the above technical problem is achieved by the embodiments characterized in the claims.

SUMMARY ~~OF THE INVENTION~~

[008] The present disclosure~~invention~~ relates to a method for the purification of a divalent cation binding protein comprising the steps of:

(a) loading an anion exchange resin material with the divalent cation binding protein in a loading buffer in the absence of divalent cations, and optionally washing the loaded anion exchange resin material with a washing buffer in the absence of divalent cations; and

(b) eluting the divalent cation binding protein with an eluant comprising at least one divalent cation to form an eluate containing the divalent cation binding protein;

wherein the eluant in step (b) has a pH higher than the pH of the washing buffer in step (a), or, in case no washing step is carried out, of the loading buffer in step (a).

[008a] In one aspect, there is provided a method for the purification of a divalent cation binding protein comprising the steps of:

a) loading an anion exchange resin material with the divalent cation binding protein in a loading buffer in the absence of divalent cations, and optionally washing the loaded anion exchange resin material with a washing buffer in the absence of divalent cations; and

b) eluting the divalent cation binding protein with an eluant comprising at least one divalent cation to form an eluate containing the divalent cation binding protein;

wherein the eluant in step (b) has a pH higher than the pH of the washing buffer in step (a), or, in case no washing step is carried out, of the loading buffer in step (a),

wherein the pH of the eluant in step (b) is at least 0.5 pH units higher than the pH of the washing buffer in step (a), or, in case no washing step is carried out, of the loading buffer in step (a).

[009] Further, the present disclosure~~invention~~ relates to purified divalent cation binding proteins obtainable by the above method, and to a kit comprising means for carrying out the above method.

[009a] In another aspect, there is provided a kit comprising means for carrying out the method as defined herein, comprising an eluant comprising at least one divalent cation, suitable for eluting a divalent cation binding protein from an anion exchange resin material in a buffer, wherein the eluant has a pH, which is at least 0.5 units higher than the pH of said buffer.

DETAILED DESCRIPTION OF THE INVENTION

[010] In one aspect, the present invention relates to a method for the purification of a divalent cation binding protein comprising the steps of:

(a) loading an anion exchange resin material with the divalent cation binding protein in a loading buffer in the absence of divalent cations, and optionally washing the loaded anion exchange resin material with a washing buffer in the absence of divalent cations; and

(b) eluting the divalent cation binding protein with an eluant comprising at least one divalent cation to form an eluate containing the divalent cation binding protein;

wherein the eluant in step (b) has a pH higher than the pH of the washing buffer in step (a), or, in case no washing step is carried out, of the loading buffer in step (a).

AMENDED CLAIMS
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1. A method for the purification of a divalent cation binding protein comprising the steps of:
   a) loading an anion exchange resin material with the divalent cation binding protein in a loading buffer in the absence of divalent cations, and optionally washing the loaded anion exchange resin material with a washing buffer in the absence of divalent cations; and
   b) eluting the divalent cation binding protein with an eluant comprising at least one divalent cation to form an eluate containing the divalent cation binding protein;

   wherein the eluant in step (b) has a pH higher than the pH of the washing buffer in step (a), or, in case no washing step is carried out, of the loading buffer in step (a),

   wherein the pH of the eluant in step (b) is at least 0.5 pH units higher than the pH of the washing buffer in step (a), or, in case no washing step is carried out, of the loading buffer in step (a).

2. The method according to claim 1, wherein the washing buffer in step (a), or, in case no washing step is carried out, the loading buffer in step (a) has a conductivity that is equal to or lower than the conductivity of the eluant in step (b).

3. The method according to any of claims 1 or 2, wherein the at least one divalent cation in step (b) is selected from the group consisting of Ca^{2+}, Be^{2+}, Ba^{2+}, Mg^{2+}, Mn^{2+}, Sr^{2+}, Zn^{2+}, Co^{2+}, Ni^{2+}, and Cu^{2+}, or combinations thereof.

4. The method according to any one of claims 1 to 3, wherein the anion exchange resin material has a positively charged group which is selected from the group, consisting of diethylaminoethane (DEAE), dimethylaminoethane (DMAE), trimethylaminoethyl (TMAE), polyethyleneimine (PEI), quaternary aminoalkyl, quaternary aminoethane (QAE), and quaternary ammonium (Q).

5. The method according to any one of claims 1 to 4, wherein the anion exchange resin material carries a primary amine as ligand which is selected from the group, consisting of aminohexyl, benzamidine, lysine, and arginine.

6. The method according to any one of claims 1 to 5, wherein the divalent cation binding protein is a calcium binding protein.

7. The method according to any one of claims 1 to 6, wherein the divalent cation binding protein is a vitamin K-dependent protein.

8. The method according to any one of claims 1 to 7, wherein the divalent cation binding protein is selected from the group, consisting of Factor IX, Factor VII, and Annexin V.

9. A kit comprising means for carrying out the method according to any one of claims 1 to 8, comprising an eluant comprising at least one divalent cation, suitable for eluting a divalent cation binding protein from an anion exchange resin material in a buffer, wherein the eluant has a pH, which is at least 0.5 units higher than the pH of said buffer.