Summary ~~of the Invention~~

The disclosure relates to~~One aspect as reported herein is~~ an ultrafiltration method for concentrating an immunoglobulin solution comprising the following steps:

a) providing an immunoglobulin solution with a pH value and with a first concentration S^+ or S^- of a buffer substance,
b) adjusting the first concentration of the buffer substance to a second concentration S' and maintaining the pH value, whereby the second concentration S' is calculated with equation 2 if the buffer substance is a cation/neutral pair or with equation 3 if the buffer substance is a neutral/anion pair,
c) concentrating the solution of b) by a tangential flow filtration,

wherein equation 2 is

$$S^+ = \frac{-zP + \sqrt{(zP)^2 + 4\left(\frac{S'}{\rho'}\left(\rho - \frac{PM_P}{1000}\right)\right)^2}}{2}$$

and equation 3 is

$$S^- = \frac{zP + \sqrt{(zP)^2 + 4\left(\frac{S'}{\rho'}\left(\rho - \frac{PM_P}{1000}\right)\right)^2}}{2}$$

with

the molar concentration in the retentate of positively/negatively charged solutes (S^+/S^-), the charge of the protein (z), the molar concentration (P) and the molecular weight (Mp) of the protein, the density of the solution in the retentate (ρ) and the permeate (ρ'), and the theoretical molar concentration of the diffusible solute (S'),

whereby the theoretical diffusible solute concentration S' is corrected by a correction factor that is considering the pH value wherein the ratio between buffer anion/buffer cation and buffer acid is calculated for each pH value determined in the retentate by using the Henderson-Hasselbach-Equation and the relative increase at each pH value is used as the respective correction factor.

One aspect as reported herein is a method for concentrating an immunoglobulin solution comprising the following steps:

a) providing an immunoglobulin solution with a pH value, with a first immunoglobulin protein concentration, and with a first concentration S^+ or S^- of a buffer substance,

b) adjusting the first concentration of the buffer substance to a second concentration S' and maintaining the pH value, whereby the second concentration S' is calculated with equation 2 if the buffer substance is a cation/neutral pair or with equation 3 if the buffer substance is a neutral/anion pair,

c) concentrating the solution of b) with a tangential flow filtration to a second immunoglobulin protein concentration,

wherein equation 2 is

$$S^+ = \frac{-zP + \sqrt{(zP)^2 + 4\left(\frac{S'}{\rho'}\left(\rho - \frac{PM_P}{1000}\right)\right)^2}}{2}$$

and equation 3 is

$$S^- = \frac{zP + \sqrt{(zP)^2 + 4\left(\frac{S'}{\rho'}\left(\rho - \frac{PM_P}{1000}\right)\right)^2}}{2}$$

with

the molar concentration in the retentate of positively/negatively charged solutes (S^+/S^-), the charge of the protein (z), the molar concentration (P) and the molecular weight (M_P) of the protein, the density of the solution in the

retentate (ρ) and the permeate (ρ'), and the theoretical molar concentration of the diffusible solute (S'),

whereby the theoretical diffusible solute concentration S' is corrected by a correction factor based on the pH, wherein a ratio between the buffer anion/buffer cation and buffer acid calculated for each pH value determined in the retentate by using the Henderson-Hasselbach-Equation and the relative increase at each pH value is used as the respective correction factor.

In one embodiment the buffer substance is histidine and that the second concentration is calculated with equation 2. In one embodiment the pH value is of from pH 5.0 to pH 6.0. In a further embodiment the pH value is pH 5.5. In another embodiment the first concentration is approximately 20 mM. In a further embodiment the second concentration is of from 24 mM to 37 mM with a protein concentration of the concentrated solution of from 100 g/l to 300 g/l, respectively. In one embodiment the protein concentration is approximately 200 g/l and the second concentration is of from 28 mM to 31 mM. In another embodiment the first concentration is approximately 46 mM. In a further embodiment the second concentration is of from 52 mM to 72 mM with a protein concentration of the concentrated solution of from 100 to 300 g/l, respectively. In one embodiment the protein concentration is approximately 200 g/l and the second concentration is of from 59 mM to 62 mM.

In another embodiment the buffer substance is acetate and the concentration is calculated with equation 3. In one embodiment the pH value is of from pH 4.5 to pH 6.0. In a further embodiment the pH value is pH 5.5. In another embodiment the first concentration is approximately 20 mM. In a further embodiment the second concentration is of from 8 mM to 19 mM with a protein concentration of the concentrated solution of from 300 g/l to 100 g/l, respectively. In one embodiment the protein concentration is approximately 200 g/l and the second concentration is of from 12 mM to 17 mM. In another embodiment the first concentration is approximately 45 mM. In a further embodiment the second concentration is of from 41 mM to 48 mM with a protein concentration of the concentrated solution of from 300 to 100 g/l, respectively. In one embodiment the protein concentration is approximately 200 g/l and the second concentration is of from 43 mM to 47 mM.

In one embodiment the immunoglobulin is an anti-P-selectin antibody or an anti-Aβ antibody.

Another aspect as reported herein is a method for producing an immunoglobulin in vitro comprising