

[001]. Title

[002]. ELECTRODE AND SYSTEM FOR INDUSTRIAL SCALE MEMBRANE BASED FREE-FLOW ISOELECTRIC FOCUSING

[003]. Field

[004]. Industrial Scale Membrane based Free-Flow Isoelectric Focusing (FFIEF)

[005]. Background introduction

[006]. The production of biomolecules is a large and growing industry. As an industrial average, 90-95% of production costs of biomolecules come from the separation and purification processes. Traditionally gel based isoelectric focusing has been used in laboratories for protein separation. Such methods require casting pH gradient gels would introduce the possibility of variation and is not suitable for industrial scale separation and purification.

[007]. Another method uses ultrafiltration (UF) membrane separation technology, porous ion exchange membranes have been extensively examined owing to their possibilities for biomolecular separations [1~2]. However, it seems a laborious work for breaching the bottleneck of low flux and low selectivity based on the currently used ultrafiltration (UF) technology. Since most ultrafiltration (UF) membranes fabricated by the phase-inversion method possess a very wide pore size distribution on the selective skin. On the other hand, only when the ratio of molecular weights (MW) of two biomolecules is larger than 10, the separation selectivity based on the size-exclusive effects can reach an acceptable level [3]. Moreover, it is very difficult to realize the continuous operation for most membrane filtration methods. This is due to the fact that the processes are usually disturbed by elution, regeneration or backwash steps to get rid of the effects of adsorption, saturation and fouling [4~5]. Therefore, many investigations have primarily concentrated on the development of membrane materials with high separation performance and low fouling.

[008]. Devices of continuous operation for membrane-based biomolecule separations have not received much attention. Most of the existing membrane-based biomolecular separation devices are designed only for a binary mixture. Some multi-compartment devices for

electrophoresis where neutral ultrafiltration membranes or pH imbedded gel like membranes were applied as separation medium, have obtained high selectivity and reasonable fluxes for some specific samples. However, gel-like membranes have a short life time [6~18] and the neutral UF membranes do not have high enough selectivity [19~20]. Therefore the multi-compartments electrophoresis technology has not been widely applied in industry because of the bottle neck of membrane technology. Nevertheless, the combination of the multi-compartment electrophoresis technology and the porous ion-exchange membrane technology [21~25] provides a possibility of a high flux and high selectivity for separations of multi-component, biomolecular mixtures simultaneously. Previous research has proven that, isoelectric focusing conditions combined with highly charged ion-exchange membrane will be very effective in obtaining pure charging products, such as proteins, hormones, RNA, DNA. The difficulty with this method is designing large scale equipment for manufacturing.

[009]. In multi-compartment electrophoresis, in order to obtain a linear pH gradient along the electric field, most researchers' apply pH imbedded gel membranes to separated chambers. This will give a high resolution in short time operation. However, the imbedded pH component would deplete in few hours. For one batch of loading, this technology requires the customer to cast more than ten membranes with more than 10 different compositions correspondingly, which is tedious, inconstant and difficult to handle. These problems are escalated on an industrial scale.

[0010]. The inventor has overcome the above disadvantage through the use of homogenous membranes. By applying a series of identical membranes inside an electric field. Through the synergetic effect of the special designed pH control system and the buffer, we can maintain the linear pH gradient a longer time than currently available, which is essential for isoelectric focusing on an industrial scale. The method was more convenient and user friendly.

[0011]. When this method was done on a large scale the circulation loop had a serious short circuit within an individual chamber resulting in a lightening phenomenon within the chamber, which can destroy the whole chamber, or even may cause injury or fatality. However, compulsory circulation is very important and necessary for the process. This is due

to the fact that bio-macromolecules will precipitate at its isoelectric point which could lead to clogging the chamber.

[0012]. An object of the invention is to ameliorate some of the difficulties of the prior art to allow protein separation and or purification on an industrial scale.

[0013]. **Summary**

[0014]. An aspect of the invention includes an electrode for Free-Flow Isoelectric Focusing of water soluble charged molecules and particles comprising a cathode and an anode wherein a portion of the cathode that comes into contact with an electrolyte solution when in use, is coating with a metal protective layer and a polymer protective layer and a portion of the anode that comes into contact with the electrolyte solution when in use, is coating with a metal protective layer and a polymer protective layer.

[0015]. Preferably the the cathode forms one wall of a chamber for Free-Flow Isoelectric Focusing of water soluble charged molecules and particles, and the anode forms another opposite wall of the chamber.

[0016]. Preferably the cathode and the anode comprise a soft, high conductivity, oxidation resistant materials selected from the group consisting of brass, carbon-amorphous, graphite, copper, copper graphite, copper tellurium, copper tungsten, copper zirconium diboride, gold or gold alloy, electrographite, metal graphite, molybdenum, palladium or palladium alloys, platinum or platinum alloys, plated base metal, resin bonded graphite, silver or silver alloys, silver copper, silver cadmium oxide, silver graphite, silver molybdenum, silver nickel, silver tin oxide, silver tungsten, silver tungsten carbide, tungsten, and tungsten carbide.

[0017]. Preferably the metal protective layer comprises a sintered powder of an oxy-compound of any one of titanium, tantalum, zirconium, vanadium, niobium, hafnium, aluminum, silicon, tin, chromium, molybdenum, tungsten, lead, manganese, beryllium, iron, cobalt, nickel, platinum, palladium, osmium, iridium, rhenium, technetium, rhodium, ruthenium, gold, silver, cadmium, copper, zinc, germanium, arsenic, antimony, bismuth, boron, scandium and metals of the lanthanide and actinide series.

[0018]. Preferably the polymer protective layer comprises a conductive polymer.

[0019]. The conductive polymer may include a charged polymer, a polyacetylene, a polypyrrole or a polyaniline. Such as a negative charged polymer resin, a positively charged polymer resin, an electropolymerized pyrrole propylic acid Polypyrrole/polyacrylonitrile, polyaniline/polyacrylonitrile, or poly(3,4-ethylenedioxythiophene)/polyacrylonitrile

[0020]. Another aspect of the invention provides a System for Free-Flow Isoelectric Focusing of water soluble charged molecules and particles comprising the electrode of the invention and a series of homogenous membranes applied in parallel between the cathode and the anode.

[0021]. Preferably the homogenous membrane comprises a polyimide, a PPO (2, 6-dimethyl-1,4-phenylene oxide), a polysulfone, or a polyethersulfone modified with -COOH , -SO_3 , -NH_4 , $\text{-N(CH}_3)_4^+$, -PO_3 or -NR_2 functional groups.

[0022]. Another aspect of the invention provides a buffer solution for Free-Flow Isoelectric Focusing of water soluble charged molecules in a system of the invention comprising a synthetic polyelectrolyte a multi -COOH groups and a multi -NH_2 groups.

[0023]. Preferably the multi -COOH groups and the multi -NH_2 groups are amino acids, short peptides and/or derivatives of amino acids.

[0024]. Other aspects of the invention include those apparent to a person skilled in the art with reference to the description and figures of the preferred embodiments.

[0025]. **Brief Description Of The Drawings**

[0026]. **Figure 1** The schematic diagram of the FFIEF device CEM is a commercial dense cation exchange membrane which resists the protein from the anode. AEM is a commercial dense anion exchange membrane which resists the protein from the cathode.

[0027]. **Figure 2** Multi-chamber and electrode designs for industrial scale FFIEF.

[0028]. **Figure 3** Design of individual chamber and inlets, outlets module

[0029]. **Figure 4** The design of electrodes

[0030]. **Figure 5** Proteins standards and egg white components after pre-treatment.

[0031]. **Figure 6** HPLC results of egg white components separated by the M-IN membrane in the FFIEF system at 30mA for **A.** 10 hours, **B.** 18 hours and **C.** 27 hours

[0032]. **Detailed description**

[0033]. This system is designed, and fabricated for industrial scale FFIEF equipment that produces high flux, high selectivity bio-macromolecule products preferably at low cost.

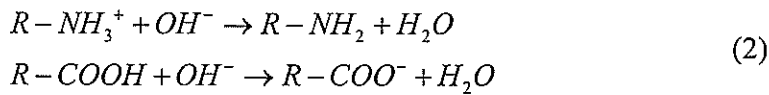
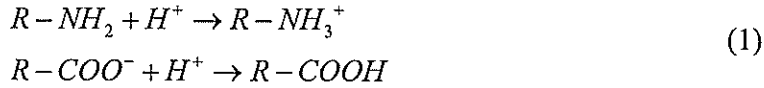
[0034]. The advantages of the system include Low capital investment and low operation cost compared to systems currently available. Particularly these costs are much lower than the conventionally used HPLC technology for drug preparation purposes; a combination of industrial ion exchange membranes and free-flow isoelectric focusing (FFIEF) technology for high-flux and high-selectivity protein separation, in which we use industry scale ion exchange membranes as the separation media. Extensive experiments have been conducted in the laboratory scale FFIEF device, where the feasibility, effectiveness, efficiency, reproducibility has been verified. Here we provide a unique design of industrial equipment for bulk amount biomedical loading for separation of high purity products. The design of half flat electrodes with anti-corrosive layers, avoids a short circuit occurring in chamber circulations, the design for the synergetic effect of pH control system and high capacity buffer solutions have been combined in the FFIEF industrial equipment.

[0035]. All water soluble charged molecules and particles could be purified in this process. Such molecules and particles include: Proteins, DNAs, RNAs, heparin and most charged bio-molecules; Vitamins (Vitamin C, B6, B12, etc.), hormones; Antibiotics e.g.(penicillin, cephalosporin, erythromycin, colistin sulphate, gibberellin, etc.); Bio-pesticides purification and concentration (Ninamycin, Polyoxorim, Kasugamycin, etc.); Recombinant gene expressed drugs; Most organic acids and organic bases; Most organics having clear dipoles; or Enantiomeric mixture.

[0036]. **Working principle of the FFIEF system**

[0037]. The net charge of proteins is the sum of all positive and negative charges provided by amino acid chains. When the pH value of a protein solution is lower than the isoelectric point (pI) of the protein (i.e., $\text{pH} < \text{pI}$), the protein molecules carry positive charges, as shown in Eq. (1). On the contrary, when the pH value of a protein solution is higher than the

pI value of the protein (i.e., $\text{pH} > \text{pI}$), the protein molecules carry negative charges, as shown in Eq. (2).



[0038]. The theoretical protein separation device is demonstrated in Fig.1. The model mixture of BSA (bovine serum albumin) and Mb (myoglobin) is applied here to illustrate the mass transfer through the membrane. As shown in Fig.1, the feed and permeate chambers are partitioned by an Ultra filtration (UF) on an ion exchange membrane (IEM) which only allows the opposite charged ions to pass through due to the electrostatic attraction. Meanwhile, this membrane may prevent the back flow from the permeate side. When bovine serum albumin (BSA) and myoglobin (Mb) proteins are mixed in the feed chamber with a pH value of 7.0, BSA molecules carry negative charge, while Mb molecules is zero charged due to its pI value of 7.0. Therefore, Mb molecules stay at the feed chamber due to zero electric driving force, whilst BSA molecules with positive charges diffuse through the porous ion exchange membrane under the electric driving force and reach the permeate chamber (with a pH value of 4.8). The net charge of BSA molecules reduces to zero in the permeate chamber, thus preventing BSA molecules from further migration. Therefore, in principle, a high-purity BSA may be obtained at the permeate chamber.

[0039]. Main concept for industrial design

[0040]. This is a combination of membrane technology and IEF electrophoresis technology. It employs membranes as mass transfer media instead of gel strip in electrophoresis, enabling electrophoresis concept enlarged to industrial scale. This process provides measures for purifications of bio-macromolecular products in pharmaceutical applications, such as protein drugs, DNAs, RNAs, heparin and other recombinant gene expressed drugs and all those listed above or known to those skilled in the art.

[0041]. The Fig. 1 is only a very preliminary study for the feasibility and effectiveness of FFIEF process. The design in Fig.1 is acceptable for an academic research, because no long time continuous operation work is conducted. However, this will not be acceptable for industrial scale work. Therefore, we designed an anti-corrosion half electrode to overcome the problem of reduced conductivity in the system.

[0042]. Compared to our previous work, our new design for industrial applications, solves the problem of 1) electrode corrosion through a covering of two layers of conductive materials; 2) short circuit phenomena in case of online circulations through a new design in circulation loops; 3) The stability of linear pH gradient under longer time operation via the newly designed strong buffer capacities.

[0043]. Firstly, we removed the CEM, and AEM to stop any biomolecules clogging the system due to any affinity to the membranes. CEM is a commercial dense cation exchange membrane which resists the protein from going through to the anode. AEM is a commercial dense anion exchange membrane which resists the protein from going through to the cathode. Removing the CEM and AEM membranes resulted in the electrodes being directly exposed to acidic and basic potions of the electrolyte solutions. To overcome this problem we protected the electrodes with a protective layer of metal and a second protective layer of conductive polymer. The Fig. 2 shows the industrial design of the FFIEF cell.

[0044]. Secondly, a circulation design can unload the precipitate in time and keep the buffer capacity. In a preferred embodiment, a special device for grounding of every circulation loop is included in the design. In the mean time, one can collect the precipitate outside the chamber.

[0045]. Thirdly, a more stable pH gradient is desired for IEF application. Automatic pH adjust system can improve the stability of pH gradient to almost 5 hrs. Combining pH adjust system, ion-exchange membranes and some synthetic multi-electrolyte, the linear pH gradient can last to 15 hrs.

[0046]. Membranes

[0047]. The criterion of selection of membrane materials is that the materials should be easily modified to positive charge or negative charge, such as polyimide, PPO (2, 6-

dimethyl-1,4-phenylene oxide), polysulfone, polyethersulfone and other similar modifiable materials known to those in the art. Functional groups, such as $-\text{COOH}$, $-\text{SO}_3$, $-\text{NH}_4$, $-\text{N}(\text{CH}_3)_4^+$, $-\text{NR}_2$, $-\text{PO}_3$ were used to modify the membrane materials. The ion-exchange capacity (IEC) which is related to separation performance is measurable. Fortunately, we found that porous charged membranes appear to have non-observable fouling phenomena for protein purification process under conditions of FFIEF.

[0048]. Electrode

[0049]. The electrode material is selected from a relatively cheaper metal such as Titanium (Ti). Preferably a Ti metal plate is used for both cathode and anode material. Other suitable electrode materials include any soft, high conductivity, oxidation resistant materials such as brass, carbon-amorphous, graphite, copper, copper graphite, copper tellurium, copper tungsten, copper zirconium diboride, gold or gold alloy, electrographite, metal graphite, molybdenum, palladium or palladium alloys, platinum or platinum alloys, plated base metal, resin bonded graphite, silver or silver alloys, silver copper, silver cadmium oxide, silver graphite, silver molybdenum, silver nickel, silver tin oxide, silver tungsten, silver tungsten carbide, tungsten, and tungsten carbide.

[0050]. The electrode material is treated or coated with anti-corrosion treatments or anti-corrosion materials to make it suitable for use in a free flow isoelectric focusing chamber. The anti-corrosion materials form protective layers on the electrode material. The criterions of selection of protective material are 1) electrical conductivity, 2) ability to cohere to the electrode material, and 3) surface reaction under strong electro-chemical environment. Only the portion of the electrode surface that will come into contact with an electrolyte solution need to have an anticorrosive protection. On a portion of the electrode that may come into contact with the electrolyte solution the electrode portion is modified by coating with a metal protective layer and a polymer protective layer.

[0051]. The metal protective layer can be formed of metal oxide sintered layer which is inert to electrodic reaction but highly conductive and can be used under acidic anode solution. Alternatively a matrix of sintered powders of an oxy-compound of at least one metal selected from the group consisting of titanium, tantalum, zirconium, vanadium,

niobium, hafnium, aluminum, silicon, tin, chromium, molybdenum, tungsten, lead, manganese, beryllium, iron, cobalt, nickel, platinum, palladium, osmium, iridium, rhenium, technetium, rhodium, ruthenium, gold, silver, cadmium, copper, zinc, germanium, arsenic, antimony, bismuth, boron, scandium and metals of the lanthanide and actinide series can be used.

[0052]. The polymer protective layer should be electrically conductive polymer that can effectively prevent the attachment of proteins or other biomolecules, and may be applied as a secondary protective layer. The main classes of conductive polymers are charged poly-resins, polyacetylene, polypyrrole and polyaniline. Of these conductive polymers, those known to prevent biomolecular attachment include; electropolymerized pyrrole propylic acid Polypyrrole/polyacrylonitrile; polyaniline/polyacrylonitrile; polymers with carboxylate, sulphate, phosphoric, or amine groups attached to the polymer effectively making the polymer become charged either negatively or positively effectively protecting it from protein attachment and poly(3,4-ethylenedioxythiophene)/polyacrylonitrile.

[0053]. In order to obtain the large scale equipment for industrial application, large scale metal plates were applied as both electrodes and structure supports. Effectively the electrodes preferably for a first side of the chamber and a second side of the chamber opposite the first side effectively forming an electric field between the first and second side of the chamber.

[0054]. Buffer

[0055]. The synthetic polyelectrolytes with multi -COOH groups and with multi -NH_2 groups (with $\text{pI}=3.0$ and $\text{pI}=10.0$ individually) are mixed in a certain ratio as a buffer solution. Since their relative small molecular weight, they are easy to be separated from purified proteins in any post treatment. Preferably the multi -COOH groups and the multi -NH_2 groups are amino acids, short peptides and/or derivatives of amino acids.

[0056]. Chamber

[0057]. All of chambers comprise a nylon or other strong plastic material as frames. The electrodes also can form walls of the opposite ends of the chamber. The inlets and outlets are made as standard modules which can be dismounted from chambers. The Fig.3 shows the design of whole electrolyzer and the Fig. 4 shows the design concept of electrodes. As has

been shown in Fig. 3, there is a grounding device inserted in the outlet module, which is connected to a common bus-bar and finally connected to a plug.

[0058]. As shown in Fig. 4, the current from power supply is distributed into few directions along the wings of the distributor. Wires connect between the distributor wings and the T-shape bars, where T bars make the homogenous current distribution on the electrode plate. There is only one side of the electrode surface that will come into contact with the electrolyte solution. Hence the half electrode is strictly sealed to prevent liquid passing. On one half electrode, only the surface facing the electrolyte is modified with metal protective layer and the polymer protective layer.

[0059]. The separation process was controlled by the feedback pH adjusting system. The combination of pH online monitoring and the high capacity polyelectrolyte buffer enables the linear pH gradient to last up to 15 hours. Therefore, a cheaper high capacity buffer allows stable FFIEF processing. We synthesize a group of polyelectrolytes for this purpose.

[0060]. The chamber thickness and buffer capacity could be reduced to improve or enhance the invention. The combination of pH adjusting system and the special buffer we syntheses allow a stable pH gradient in operation period.

[0061]. Ultra thin chamber series with good circulation allows high flux separation finished in short time.

[0062]. The system allows a stable and large size process for bulk amount of protein purification;

[0063]. High efficiency and reproducibility leads to assured high product quality;

[0064]. Example 1

Performance test for egg white protein separation

Materials

[0065]. Brominated poly(2,6-dimethyl-1,4-phenyleneoxide) (BPPO) with 90% (m/m) bromination was obtained from Shandong Tianyi Imp. & Exp. Co., Ltd. China. The egg white powder was procured from Sigma-Aldrich, Singapore. Electrophoresis grade Tris was obtained from Bio-Rad, Singapore. Udel P3500NT polysulfone (PSf) was provided by

Solvay, Germany. Tetrahydrofuran (THF) and triethylamine (TEA) were from Fisher Scientific, UK; N-methyl-2-pyrrolidone (NMP), was from Panreac E.U.; isopropanol (IPA) was from Chedelco Pte. Ltd. Singapore; Methanol, phosphoric acid (H₃PO₄), sodium hydroxide (NaOH) trifluoroacetic acid (TFA), dichloromethane (DCM), chlorosulfonic acid and potassium chloride (KCl) were all in analytical grade (AR) and purchased from Merck Singapore. For high performance liquid chromatography (HPLC) analyses, GR (guaranteed reagent) grade ACN and water were used, while for other occasions, deionized water (electrical resistance $R > 18.2\text{M}_\Omega$) was used. The ultrapure water used for HPLC was produced by a “Milli-Q plus 185” pure water system in our lab.

[0066]. Ovalbumin occupies 54% (w/w) of the dry mass of the composition of egg white. Since this large component may interfere with the separation of other components, the protein solution was pretreated to remove ovalbumin and reduce the complexity using the following procedure. The egg white aqueous solution was adjusted to pH= 4.5 and stored in a refrigerator at 5 °C overnight so that most ovalbumin could be precipitated and hence easily removed by filtration. Figure 5 demonstrates the HPLC results of the protein solution after pre-treatment and compares with the standards of reagent grade ovalbumin, ovotransferrin, lysozyme and ovomucoid peaks. Comparing those spectra between the feed and standards in Figure 5, four protein peaks in the egg white mixture can be identified. They are ovomucoid at 6.8 min, lysozyme at 10.5 min, ovotransferrin at 11.0–12 min, and ovalbumin at 14.5–17.0 min. The analyses of commercial protein products also imply that it is very difficult to separate ovomucoid from ovalbumin.

[0067]. In order to investigate the evolution of fractionation as a function of operation time, samples from the FFIEF process partitioned by the membrane M-IN were collected at 10 h, 18 h and 27 h. The corresponded HPLC results were summarized in Figure 6. The protein separation performance after 10-h operation of FFIEF under a constant direct current of 30 mA is shown in Figure 6A. The egg white protein can be partially separated by MIN and three relatively purer components can be obtained after a 10-h operation; they are ovalbumin, ovomucoid and at least one unknown component. As the FFIEF process proceeds to 18 h, Figure 6B four purer components are obtained and they are ovotransferrin, lysozyme, ovomucoid and at least one unknown component. A continuation of the FFIEF process to 27

h elucidates much higher protein purities within each individual chamber as shown in Figure 6C they are ovotransferrin, ovomucoid and one unknown component. In summary, at least five pure proteins can be obtained from the M-IN partitioned FFIEF.

[0068]. Example 2

[0069]. Performance test for plasma separation

[0070]. A similar experiment was conducted on plasma to extract the different protein components in plasma.

[0071]. Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. The invention includes all such variation and modifications. The invention also includes all of the steps, features, formulations and compounds referred to or indicated in the specification, individually or collectively and any and all combinations or any two or more of the steps or features.

[0072]. Each document, reference, patent application or patent cited in this text is expressly incorporated herein in their entirety by reference, which means that it should be read and considered by the reader as part of this text. That the document, reference, patent application or patent cited in this text is not repeated in this text is merely for reasons of conciseness.

[0073]. Any manufacturer's instructions, descriptions, product specifications, and product sheets for any products mentioned herein or in any document incorporated by reference herein, are hereby incorporated herein by reference, and may be employed in the practice of the invention.

[0074]. The present invention is not to be limited in scope by any of the specific embodiments described herein. These embodiments are intended for the purpose of exemplification only. Functionally equivalent products, formulations and methods are clearly within the scope of the invention as described herein.

[0075]. The invention described herein may include one or more range of values (eg size, concentration etc). A range of values will be understood to include all values within the

range, including the values defining the range, and values adjacent to the range which lead to the same or substantially the same outcome as the values immediately adjacent to that value which defines the boundary to the range.

[0076]. Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers. It is also noted that in this disclosure and particularly in the claims and/or paragraphs, terms such as "comprises", "comprised", "comprising" and the like can have the meaning attributed to it in U.S. Patent law; e.g., they can mean "includes", "included", "including", and the like; and that terms such as "consisting essentially of" and "consists essentially of" have the meaning ascribed to them in U.S. Patent law, e.g., they allow for elements not explicitly recited, but exclude elements that are found in the prior art or that affect a basic or novel characteristic of the invention.

[0077]. Other definitions for selected terms used herein may be found within the detailed description of the invention and apply throughout. Unless otherwise defined, all other scientific and technical terms used herein have the same meaning as commonly understood to one of ordinary skill in the art to which the invention belongs.

[0078]. While the invention has been described with reference to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

[0079]. Reference:

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I Claim:

1. An electrode for Free-Flow Isoelectric Focusing of water soluble charged molecules and particles comprising a cathode and an anode wherein a portion of the cathode that comes into contact with an electrolyte solution when in use, is coating with a metal protective layer and a polymer protective layer and a portion of the anode that comes into contact with the electrolyte solution when in use, is coating with a metal protective layer and a polymer protective layer.
2. The electrode of claim 1 wherein the cathode forms one wall of a chamber for Free-Flow Isoelectric Focusing of water soluble charged molecules and particles, and the anode forms another opposite wall of the chamber.
3. The electrode of claim 1 or 2 wherein the cathode and the anode comprise a soft, high conductivity, oxidation resistant materials selected from the group consisting of brass, carbon-amorphous, graphite, copper, copper graphite, copper tellurium, copper tungsten, copper zirconium diboride, gold or gold alloy, electrographite, metal graphite, molybdenum, palladium or palladium alloys, platinum or platinum alloys, plated base metal, resin bonded graphite, silver or silver alloys, silver copper, silver cadmium oxide, silver graphite, silver molybdenum, silver nickel, silver tin oxide, silver tungsten, silver tungsten carbide, tungsten, and tungsten carbide.
4. The electrode of claim 3 wherein the cathode and the anode comprises titanium.
5. The electrode of any one of claims 1 to 4 wherein the metal protective layer comprises a sintered powder of an oxy-compound of any one of titanium, tantalum, zirconium, vanadium, niobium, hafnium, aluminum, silicon, tin, chromium, molybdenum, tungsten, lead, manganese, beryllium, iron, cobalt, nickel, platinum, palladium, osmium, iridium, rhenium, technetium, rhodium, ruthenium, gold, silver, cadmium, copper, zinc, germanium, arsenic, antimony, bismuth, boron, scandium and metals of the lanthanide and actinide series.
6. The electrode of any one of claims 1 to 5 wherein the polymer protective layer comprises a conductive polymer.

7. The electrode of claim 6 wherein the conductive polymer comprises a charged polymer, a polyacetylene, a polypyrrole or a polyaniline.
8. The electrode of claim 6 or 7 wherein the conductive polymer comprises a negative charged polymer resin, a positively charged polymer resin, an electropolymerized pyrrole propylic acid Polypyrrole/polyacrylonitrile, polyaniline/polyacrylonitrile, or poly(3,4-ethylenedioxythiophene)/polyacrylonitrile
9. A System for Free-Flow Isoelectric Focusing of water soluble charged molecules and particles comprising the electrode of any one of claims 1 to 8, and a series of homogenous membranes applied in parallel between the cathode and the anode.
10. The system of claim 9 wherein the homogenous membrane comprises a polyimide, a PPO (2, 6-dimethyl-1,4-phenylene oxide), a polysulfone, or a polyethersulfone modified with—COOH, —SO₃, —NH₄, —N(CH₃)₄⁺, —PO₃ or —NR₂ functional groups.
11. A buffer solution for Free-Flow Isoelectric Focusing of water soluble charged molecules is a system of claim 9 comprising a synthetic polyelectrolyte a multi —COOH groups and a multi —NH₂ groups.
12. The buffer solution of claim 11 wherein the multi —COOH groups and the multi —NH₂ groups are amino acids, short peptides and/or derivatives of amino acids.