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(54) Title: BLOOD–RELATED DIALYSIS AND TREATMENT

(57) Abstract

A method of treating blood or plasma of a subject to remove metabolic contaminants, the method comprising: (a) placing blood or plasma from the subject in a first solvent stream, the first solvent stream being separated from a second solvent stream by an electrophoretic membrane; (b) applying an electric potential between the two solvent streams causing movement of metabolic contaminants from the blood or plasma through the membrane into the second solvent stream while cellular and biomolecular components of the blood or plasma are substantially retained in the first sample stream, or if entering the membrane, being substantially prevented from entering the second solvent stream; (c) optionally, periodically stopping and reversing the electric potential to cause movement of any cellular and biomolecular components of the blood or plasma having entered the membrane to move back into the first solvent stream, wherein substantially not causing any metabolic contaminants that have entered the second solvent stream to re–enter first solvent stream; (d) maintaining step (b), and optionally step (c) if used, until the desired amount of removal of the metabolic contaminants from the blood or plasma in the first solvent stream is achieved; and (e) returning the treated blood or plasma in the first solvent stream to the subject.

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Blood-Related Dialysis and Treatment

Technical Field

The present invention relates to methods suitable for treating or processing blood or plasma to remove or reduce the concentration of unwanted components, and particularly dialysis methods applicable to renal dialysis.

Background Art

In healthy individuals, the kidney functions to remove excess water, salts and small proteins from the blood circulation. Nitrogenous wastes removed by the kidney include urea, the final metabolic destiny of excess dietary nitrogen, creatinine which is produced during muscle activity, and uric acid, an endpoint product of nucleotide metabolism. Current renal dialysis technology relies on equilibrium/diffusion principles and transmembrane pressure to remove nitrogenous wastes, salts and excess water from the bloodstream of patients experiencing chronic or acute renal failure. This requires two to three hours of dialysis treatment on three or four occasions each week. There are significant deficiencies in existing dialysis technologies, including sub-optimal biocompatibility of the dialysis membranes used, the inadequacy of existing technology in the removal of some solutes such as phosphates, and poor removal of low molecular weight proteins such as beta-2 microglobulin. GradiFlow technology, or a modification thereof, can be used to perform blood dialysis for purposes of renal replacement therapy, such that these deficiencies in conventional dialysis could be addressed. These deficiencies can be addressed by including the application of an electrical potential through a blood dialysis chamber to accelerate the removal of charged solutes such as phosphate ions and proteins, as well as charged nitrogenous wastes and other salt ions such as sodium, potassium, chloride and so on. The demonstrated protein separation capacity of the GradiFlow technology can be applied to the removal of specific proteins from the blood or plasma circulations, with the intention of treating disease symptoms mediated by those proteins. Examples of such disease states include rheumatoid arthritis and a host of other autoantibody mediated autoimmune diseases, which could be treated by the selective removal of autoantibody or other disease related proteins from the patients blood circulation.

The present inventors have developed a device based on GradiFlow technology (AU 601040) which can be used to selectively remove solutes, metabolites and proteins from either blood or plasma. Such a device can be used as either an add-on module to existing dialysis machines, or as a stand-alone device used to filter the blood of dialysis patients as a specific therapeutic measure to remove metabolites and proteins after conventional dialysis therapy has already been applied.

One of the key advantages of the Gradiflow is its capacity to desalt. In the present system, this is achieved by the retention of the desired macromolecule in a chamber sandwiched between two restriction membranes. Essentially the Gradiflow can be re-configured so that dialysis of a mixture of components is possible.

Internationally, 800,000 people suffer from chronic renal failure which implies that their kidneys can never perform the way they should. In medicine, dialysis is a therapy which eliminates the toxic wastes from the body due to kidney failure. There are two types of dialysis a) haemodialysis and b) peritoneal dialysis.

Haemodialysis is usually performed in dialysis centers, where the treatment entails dialysis for 4 hours three times a week. This sharply interferes with the quality of life of patients and also their productivity to the community at large. The present technology entails the re-routing of blood from the body to a filter made of plastic capillaries. The blood is purified when the waste products diffuse from the blood across the membrane of these tiny capillaries. The blood is then return to the body via the arm. The main advantage to this system is that patient training is not required. The main disadvantages are that dialysis graft failure is common and there is lack of freedom on the part of the patient because of the requirement to report to a center for treatment.

In peritoneal dialysis, the body's own membrane is used as a filter, and the fluid drained in and out of the abdomen replaces the kidneys in getting rid of toxins. There are some great advantages to this system which include the fact that this can be done at home. The domestic use of this, however, requires careful technique and has the added disadvantage of peritonitis and membrane failure.

Gradiflow Technology

The Gradiflow is a unique preparative electrophoresis technology for macromolecule separation which utilises tangential flow across a polyacrylamide membrane when a charge is applied across the membrane. The general design of the Gradiflow system facilitates the purification of proteins and other macromolecules under near native conditions. This results in higher yields and excellent recovery.

In essence, the Gradiflow technology is bundled into a cartridge comprising of three membranes housed in a system of specially engineered grids and gaskets which allow separation of macromolecules by charge and/or molecular weight. The system can also concentrate and desalt/dialyse at the same time. The multimodal nature of the system allows this technology to be used in a number of other areas especially in therapy for the dialysis of blood in situations like renal failure. The configuration of the Gradiflow apparatus allows the possibility of producing a simple portable device which will have the dual capacity of being easy to use and concurrently producing high quality dialysis.

Disclosure of Invention

In a first general aspect, the present invention consists in use of Gradiflow in the processing of blood or plasma from a subject in order to remove or reduce the concentration of unwanted solutes and macromolecules from the blood or plasma.

In a preferred embodiment, Gradiflow is used in renal dialysis, either as a replacement of current dialysis methods or as a supplement to current renal dialysis.

In a second aspect, the present invention consists in a method of treating blood or plasma of a subject to remove or reduce the concentration of metabolic contaminants, the method comprising:

(a) placing blood or plasma from the subject in a first solvent stream, the first solvent stream being separated from a second solvent stream by an electrophoretic membrane;

(b) applying an electric potential between the two solvent streams causing movement of metabolic contaminants from the blood or plasma through the membrane into the second solvent stream while cellular and biomolecular components of the blood or plasma are substantially retained in the first

sample stream, or if entering the membrane, being substantially prevented from entering the second solvent stream;

(c) optionally, periodically stopping and reversing the electric potential to cause movement of any cellular and biomolecular components of the blood or plasma having entered the membrane to move back into the first solvent stream, wherein substantially not causing any metabolic contaminants that have entered the second solvent stream to re-enter the first solvent stream;

(d) maintaining step (b), and optionally step (c) if used, until the desired amount of removal of the metabolic contaminants from the blood or plasma in the first solvent stream is achieved; and

(e) returning the treated blood or plasma in the first solvent stream to the subject.

In a preferred embodiment, the subject is a renal dialysis patient.

The blood or plasma is preferably recirculated between the subject and the first solvent stream.

In a further preferred embodiment of the second aspect of the present invention, the electrophoretic membrane has a molecular mass cut-off close to the apparent molecular mass of metabolic contaminants. It will be appreciated, however, that the membrane may have any required molecular mass cut-off depending on the application.

Preferably, the metabolic contaminants are solutes including phosphates, nitrogenous wastes like urea and uric acid, or macromolecules including beta-2 microglobulin and other unwanted proteins including autoantibodies.

Preferably, the electrophoretic membrane has a molecular mass cut-off of between about 3 and 1000kDa. It will be appreciated, however, that other size membranes may be applicable, depending on the treatment process required. A number of different membranes may also be used in a desired or useful configuration.

The electric potential applied during the method should preferably not substantially adversely effect the cells or proteins present in blood or plasma. An electric potential of up to about 100 volts has been found to be suitable. It will be appreciated, however, that other voltages may be used.

In a third aspect, the present invention consists in a method of renal dialysis, the method comprising carrying out haemodialysis on blood or

plasma of a patient followed by subjecting the blood or plasma of the patient to the method according to the second aspect of the present invention.

As conventional haemodialysis often fails to remove certain metabolic contaminants from the blood of renal patients which can result in the build-up of these contaminants, a second treatment process using the method according to the second aspect of the present invention has the potential to selectively remove these contaminants.

Preferably, the method comprises:

(a) carrying out haemodialysis on blood or plasma of the patient;

(b) placing blood or plasma from the haemodialysed patient in a first solvent stream, the first solvent stream being separated from a second solvent stream by an electrophoretic membrane;

(c) applying an electric potential between the two solvent streams causing movement of metabolic contaminants from the blood or plasma through the membrane into the second solvent stream while cellular and biomolecular components of the blood or plasma are substantially retained in the first sample stream, or if entering the membrane, being substantially prevented from entering the second solvent stream;

(d) optionally, periodically stopping and reversing the electric potential to cause movement of any cellular and biomolecular components of the blood or plasma having entered the membrane to move back into the first solvent stream, wherein substantially not causing any metabolic contaminants that have entered the second solvent stream to re-enter the first solvent stream;

(e) maintaining step (c), and optionally step (d) if used, until the desired amount of removal or reduction of the metabolic contaminants from the blood or plasma in the first solvent stream is achieved; and

(f) returning the treated blood or plasma in the first solvent stream to the patient.

The contaminants can be phosphates or proteins such as beta-2 microglobulin or autoantibodies. It will be appreciated, however, that other unwanted metabolic contaminants can also be removed in this process.

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 element, integer or step, or group of elements, integers or steps, but not the exclusion of any other element, integer or step, or group of elements, integers or steps.

In order that the present invention may be more clearly understood a preferred form will be described with reference to the accompanying drawings.

Brief Description of Drawings

Figure 1. Removal of Urea in PBS from upstream to downstream of GradiFlow.

Figure 2. Removal of endogenous and exogenous Urea from plasma by passive diffusion.

Figure 3. The rate of Urea removal is dependent on the membrane molecular weight cutoff.

Figure 4. Increasing temperature increases the rate of Urea removal in the GradiFlow device.

Figure 5. The rate of Urea removal, expressed in mg Urea removed per minute, is proportional to the Urea concentration on the sample stream.

Figure 6. Creatinine was rapidly removed from the upstream of the GradiFlow instrument at 25V. Creatinine entered the downstream of the GradiFlow, but was not retained by the 3kDa restriction membrane, so the downstream concentration was also rapidly depleted.

Figure 7. Creatinine removal is dependent on pH, with lower pH conditions resulting in more rapid removal of creatinine from aqueous solutions.

Figure 8. The application of increasing voltage in the GradiFlow system accelerated the removal of creatinine from the sample stream.

Figure 9. Increasing the size of the membrane molecular mass cutoff value allowed creatinine removal to proceed at a progressively faster rate.

Figure 10. Creatinine was removed from plasma using 10 and 20V potentials.

Figure 11. Uric acid was rapidly removed from the upstream, passing through the downstream to reach the buffer stream.

Figure 12. Increasing voltages resulted in more rapid removal of Uric acid from Hepes/Imidazole buffer.

Figure 13. Increasing the molecular mass cutoff of the GradiFlow membranes resulted in more rapid removal of Uric acid from the sample stream.

Figure 14. The addition of NaCl caused a dose-dependent decrease in the rate of uric acid removal.

Figure 15. Increasing buffer temperature resulted in more rapid removal of Uric acid.

Figure 16. Uric acid was readily removed from human plasma in a voltage dependent manner.

Figure 17. Phosphate ions were found to migrate from the upstream, through the downstream, into the buffer stream in a voltage dependent manner.

Figure 18. Phosphate was rapidly removed from plasma using a 50V electric potential.

Figure 19. Native PAGE analysis of proteins removed from whole blood using the GradiFlow system. Lanes 1 and 10 are molecular weight markers, with size in kDa shown at the right side. Lane 2 is diluted plasma. Lane 3 is red cell lysate, predominantly haemoglobin. Lane 4 shows albumin and other smaller proteins removed from blood that had been passed through the GradiFlow 10 times with an applied voltage of 50V at 4C. Lanes 5 and 6 show proteins removed from blood using 100V at 4C after 5 and 10 passes respectively. Lanes 7,8 and 9 show proteins removed from whole blood after 10 passes at room temperature, using 0, 50 and 100V respectively.

Figure 20. The accumulation of protein removed from plasma. The triangles indicate the A280 (total protein absorbance) in the downstream. The squares indicate the relative amount of beta-2 microglobulin in the downstream.

Modes for Carrying Out the Invention

APPLICATIONS

Urea Removal by passive diffusion

Demonstration of the removal of Urea from aqueous solutions

Method

One mg/mL Urea was dissolved in phosphate buffered saline (PBS) and placed in the upstream of a GradiFlow device. PBS buffer, chilled to 4^0C with ice, was recirculated in the buffer stream. The up and down streams were pumped through the GradiFlow device at 20mL/min and samples taken from both streams at 10 minute intervals. No voltage or current was applied during this procedure. The timed samples were then assayed for urea content.

Results

The data in Figure 1 show the concentration of Urea in the upstream (solid squares) and in the downstream (hatched squares). The concentration of Urea in the upstream decreased over time, while the concentration of urea in the downstream increased. This result indicates that urea can be removed from aqueous solution by passive diffusion.

Removal of Urea from plasma

Method

Unmodified human plasma, or human plasma to which 1mg/mL Urea had been added, was placed in the upstream of a GradiFlow device. PBS buffer, chilled to 4^0C with ice, was recirculated in the buffer stream. The up and down streams were pumped through the GradiFlow device at 20mL/min and samples taken from both streams at 10 minute intervals. No voltage or current was applied during this procedure. The timed samples were then assayed for urea content.

Results

The data in Figure 2 show the concentration of endogenous Urea in the upstream (solid diamonds) and in the downstream (hatched diamonds) when unmodified plasma was used in this experiment. The concentration of Urea in the upstream and downstream when exogenous Urea was added to the sample is shown in red squares and pink triangles respectively. As shown above for aqueous solution, the concentration of Urea in the upstream decreased over time, while the concentration of urea in the downstream increased. This result indicates that urea may be removed from plasma by passive diffusion.

Factors affecting the removal of Urea from aqueous solutions

Method

Urea was dissolved in an appropriate buffer and placed in the sample stream of a GradiFlow device, with the GradiFlow cartridge constructed in dialysis configuration. The circulating buffer stream was selected to match the solution in which Urea had been dissolved. The starting Urea concentration, buffer pH, salt concentration, temperature of the system and applied voltage/current were varied systematically to determine the effect each variable had on the rate of Urea removal. The Urea solution was pumped through the GradiFlow device at 20mL/min, with samples generally

being taken at 10 minute intervals. The timed samples were then assayed for urea content.

The effect of applied current on Urea removal

One mg/mL Urea was dissolved in Tris Borate buffer, pH 9.0 and processed through the GradiFlow as described above. Electrical currents from 0 to 1.5 Amps were applied to the system, however, no change in Urea removal rate was observed, indicating that the rate of Urea removal was insensitive to the applied current.

Voltage dependence of Urea movement

One mg/mL Urea was dissolved in Tris/Borate buffer at pH 9.0 and circulated in the sample stream of a GradiFlow cartridge constructed in the dialysis configuration. Various electrical potentials from 0 to 100V were applied to the GradiFlow system. Varying the applied voltage resulted in no significant alteration to the rate of Urea removal from the sample stream.

pH dependence of Urea removal

One mg/mL Urea was dissolved in GABA/acetic acid buffer pH 3, Hepes /Imidazole buffer pH 6.0 and Tris/Borate buffer pH 9.0, and processed through the GradiFlow with an applied electrical potential of 50V. No significant difference in the rate of Urea removal was observed as a function of changes in buffer pH.

The effect of NaCl concentration on Urea removal

One mg/mL Urea was dissolved in 20mM phosphate buffer containing 0 to 150mM NaCl and processed through the GradiFlow in dialysis configuration using 50kDa cutoff membranes. No electrical potential was applied in these experiments. The presence of increasing concentrations of NaCl had no effect on the diffusion of Urea in the GradiFlow instrument.

The effect of membrane pore size on the removal of Urea from aqueous solutions

One mg/mL Urea was dissolved in PBS and processed in the GradiFlow using membranes with molecular weigh cutoff values between 3 and 75kDa. Ten minute time samples were taken during these runs and the slope of these curves determined as the rate of Urea removal. Figure 3 shows the relationship between membrane molecular weight cutoff and the rate of Urea removal from aqueous solutions.

The effect of temperature on the removal of Urea

One mg/mL Urea was dissolved in PBS and processed in the GradiFlow as previously. The buffer temperature was maintained at temperatures between 4 and 37^{0}C and the removal of Urea determined. Figure 4 shows that increasing buffer temperature increased the rate of Urea removal, consistent with a passive diffusion phenomenon.

The effect of Urea concentration on the rate of Urea removal

Urea at concentrations between 1 and 50mg/mL was dissolved in PBS and processed through the GradiFlow as above. The rate of Urea removal was determined from time course experiments and calculated in units of urea removed per minute. Figure 5 shows that the rate of Urea removal increases with Urea concentration, again consistent with a passive diffusion phenomenon.

Electrically driven Creatinine removal

Demonstration of the migration of Creatinine

One hundred µg/mL creatinine was dissolved in GABA/acetate buffer, pH 3, and placed in the upstream of a GradiFlow device, using 3kDa restriction membranes and a 50kDa separation membrane. GABA/acetate buffer, chilled to 4^{0}C, was recirculated in the buffer stream. An electrical potential of 25V was applied to the system, using 'reverse polarity'. Samples were collected from the up and down streams at 5 minute intervals and the creatinine concentrations in these samples determined. The results obtained show that creatinine was rapidly removed from the upstream. The transient rise in the downstream creatinine concentration indicates that creatinine moved through the downstream, but was not retained by the 3kDa membrane. Creatinine therefore passed through the restriction membrane into the buffer stream.

Figure 6 shows that Creatinine was rapidly removed from the upstream of the GradiFlow instrument at 25V. Creatinine entered the downstream of the GradiFlow, but was not retained by the 3kDa restriction membrane, so the downstream concentration was also rapidly depleted.

Factors affecting creatinine removal in the GradiFlow

Method

Creatinine was dissolved in an appropriate buffer and placed in the sample stream of a GradiFlow device, with the GradiFlow cartridge constructed in dialysis configuration. The circulating buffer stream was

selected to match the solution in which creatinine had been dissolved. The buffer pH, salt concentration, temperature of the system and applied voltage/current were varied systematically to determine the effect each variable had on the rate of creatinine removal. The creatinine solution was pumped through the GradiFlow device at 20mL/min, with samples generally being taken at 5 minute intervals. The timed samples were then assayed for creatinine content.

The effect of pH on Creatinine removal

One hundred µg/mL Creatinine was dissolved in buffers with pH varying from 3 to 9 and processed through the GradiFlow using an electrical potential of 20V. Creatinine has a pK of 10.4, indicating that creatinine is uncharged at pH 10.4, and positively charged at pH conditions lower than this pK value. Creatinine removal was most rapid at pH 3, and was observed to be progressively slower as the buffer pH was raised to 9.

Figure 7 shows Creatinine removal is dependent on pH, with lower pH conditions resulting in more rapid removal of creatinine from aqueous solutions.

The effect of voltage on Creatinine removal

One hundred µg/mL creatinine was dissolved in GABA/acetate buffer, pH 3, and processed in the GradiFlow as above. Electrical potentials between 0 and 100 V were applied to the system. The increase in applied voltage accelerated the removal of creatinine from the sample stream.

Figure 8 shows the application of increasing voltage in the GradiFlow system accelerated the removal of creatinine from the sample stream.

The effect of NaCl on the removal of Creatinine

One hundred µg/mL creatinine was dissolved in GABA/acetate buffer, pH 3, with the buffer containing NaCl at concentrations between 0 and 150mM. The addition of NaCl caused a slight decrease in the rate of creatinine removal, suggesting that the presence of other charge carrying molecules in the solution reduced the level of electrical force available for driving the removal of creatinine.

The effect of membrane molecular weight cutoff on Creatinine removal

One hundred µg/mL creatinine was dissolved in GABA/acetate buffer, pH 3, and processed in the GradiFlow as previously, using membranes with varying molecular weight cutoff values between 3 and 75kDa. The results generated indicated that the movement of creatinine was influenced by

membrane molecular mass cutoff, with the rate of removal of creatinine becoming progressively faster as the membrane pore size was increased.

Figure 9 shows increasing the size of the membrane molecular mass cutoff value allowed creatinine removal to proceed at a progressively faster rate.

The effect of temperature on the rate of Creatinine removal

One hundred µg/mL creatinine was dissolved in GABA/acetate buffer and processed in the GradiFlow as above. The circulating GABA/acetate buffer was maintained at temperatures between 4 and 37^{0}C to examine the effect of temperature on the rate of creatinine removal. It was observed that the rate of creatinine removal increased with increasing buffer temperature.

Removal of creatinine from plasma

Normal human plasma was made 100µg/mL in creatinine and the plasma processed in the GradiFlow using 10 and 20V potentials. Figure 10 shows that creatinine was successfully removed from human plasma under these conditions.

Electrically driven Uric Acid removal

Demonstration of the removal or Uric acid

Three hundred µg/mL Uric acid was dissolved in Hepes Imidazole buffer, pH 7.26 and placed in the upstream of the GradiFlow instrument. Hepes Imidazole buffer, chilled to 4C, was recirculated in the buffer stream of the GradiFlow device. The membrane cartridge used included 3kDa restriction membranes and a 50kDa separation membrane. When the GradiFlow instrument was run using an electrical potential of 15V, Uric acid was found to be removed from the upstream. The Uric acid was found to accumulate transiently in the downstream, from which it was subsequently removed to the buffer stream.

Figure 11 shows that Uric acid was rapidly removed from the upstream, passing through the downstream to reach the buffer stream.

Factors affecting the removal of Uric Acid

Method

Uric acid was dissolved in an appropriate buffer and placed in the sample stream of a GradiFlow device, with the GradiFlow cartridge constructed in dialysis configuration. The circulating buffer stream was selected to match the solution in which uric acid had been dissolved. The membrane pore size, salt concentration, temperature of the system and

applied voltage/current were varied systematically to determine the effect each variable had on the rate of uric acid removal. The uric acid solution was pumped through the GradiFlow device at 20mL/min, with samples generally being taken at 5 minute intervals. The timed samples were then assayed for uric acid content.

The effect of Voltage on Uric acid removal

Three hundred µg/mL Uric acid in Hepes/Imidazole buffer was processed in the GradiFlow at using electrical potentials from 0 to 100 V. It was observed the Uric acid removal was faster with increasing voltage. Figure 12 shows increasing voltages resulted in more rapid removal of Uric acid from Hepes/Imidazole buffer.

The effect of membrane pore size on Uric acid removal

Three hundred µg/mL Uric acid in Hepes/Imidazole buffer was processed in the GradiFlow as above, using an electrical potential of 10V. The molecular weight cutoff of the membranes used in the GradiFlow cartridge was varied between 3 and 75kDa. It was observed that as the molecular mass cutoff value of the membranes was increased, Uric acid was more rapidly cleared from the GradiFlow sample stream.

Figure 13 shows increasing the molecular mass cutoff of the GradiFlow membranes resulted in more rapid removal of Uric acid from the sample stream.

The effect of NaCl on Uric acid removal

Three hundred µg/mL Uric acid in Hepes/Imidazole was processed in the GradiFlow using 25kDa cutoff membranes and an electrical potential of 10V. NaCl was included in the sample and buffer streams at concentrations from 0 to 150mM. The addition of increasing concentrations of NaCl to the buffer system resulted in a progressive decrease in the rate of uric acid clearance.

Figure 14 shows that the addition of NaCl caused a dose-dependent decrease in the rate of uric acid removal.

The effect of temperature on the rate of Uric acid removal

Three hundred µg/mL Uric acid in Hepes/Imidazole buffer was processed in the GradiFlow as above, using 25kDa membranes and a 10V potential. The recirculating buffer was maintained at temperatures between 4 and 37^{0}C. The rate of Uric acid removal was found to increase with increasing temperature.

Figure 15 shows that increasing buffer temperature resulted in more rapid removal of Uric acid.

The removal of Uric acid from plasma

Normal human plasma was made 300μg/mL in Uric acid. This modified plasma was processed in the GradiFlow as previously, using 25kDa membranes, PBS buffer and using voltages from 10 to 30V. Figure 16 shows that Uric acid was readily removed from human plasma in a voltage dependent manner.

Electrically driven removal of Phosphate ions

Phosphate removal is one of the key deficiencies in existing renal replacement dialysis technology. The capacity of the GradiFlow to rapidly desalt/dialyse aqueous solutions suggested the applicability of the GradiFlow technology in the area of rapid phosphate removal from blood. The GradiFlow system was found to rapidly remove phosphate ions from both aqueous solutions and plasma.

Demonstration of phosphate removal from aqueous solution

One hundred μg/mL sodium phosphate was dissolved in Hepes/Imidazole buffer and placed in the upstream of the GradiFlow device. Hepes/Imidazole buffer, pH 7.2 was placed in the downstream and buffer stream of the GradiFlow instrument. The membrane cartridge used included 3kDa restriction membranes and a 10kDa separation membrane. Electrical potentials from 0 to 50V were applied and the changes in phosphate concentration monitored as a function of time. When a voltage was applied, phosphate ions were found to leave the upstream and enter the downstream. The quantity of phosphate in the downstream was also rapidly depleted, indicating that the phosphate ions continued to migrate towards the positive electrode, leaving the downstream and entering the recirculating buffer stream. The rate of phosphate removal was also observed to be dependent on the applied voltage.

Figure 17 shows phosphate ions were found to migrate from the upstream, through the downstream, into the buffer stream in a voltage dependent manner.

Removal of phosphate from plasma

Unmodified human plasma, or plasma containing an additional 0.1mg/mL phosphate, was processed in the GradiFlow. The membrane cartridge was constructed in dialysis configuration using 10kDa restriction

and separation membranes. Hepes/Imidazole buffer, pH 7.2, and an electrical potential of 50V. Samples of the plasma were taken every 5 minutes and assayed for phosphate content. The results shown in Figure 18 demonstrate the rapid removal of phosphate from human plasma.

Removal of proteins from plasma and whole blood

General protein removal (using human serum albumin (HSA) as an example)

The ability to eliminate disease related proteins from the circulation of patients relies on the capacity of the GradiFlow system to remove proteins from whole blood. Albumin was chosen as a target blood protein to demonstrate the process according to the present invention. In practice, however, proteins like autoantibodies (typically IgG or IgM classes) will be targeted for removal from blood or plasma. To demonstrate this phenomenon, whole blood was circulated in the upstream of a GradiFlow device, with PBS buffer placed in the downstream and in the recirculating buffer tank, which was maintained at either 4^0C or room temperature. Either 50 or 100V potential was applied in the GradiFlow system. Samples of the downstream were collected and analysed by native PAGE on a 4-20% polyacrylamide gel. Figure 19 shows that albumin (the most abundant protein in blood) is readily removed after passing a volume of blood through the GradiFlow, and that the quantity of protein removed appears to be dependent on the temperature and voltage applied in the GradiFlow system.

Removal of beta-2 microglobulin

Beta-2 microglobulin is a normal component of MHC Class I molecules, which are found on the surface of all nucleated cells. This protein is frequently released in to the blood circulation during episodes of immunological activity, such as infections. Normal plasma contains very low concentrations of beta-2 microglobulin, in the order of 3μg/mL. This concentration is raised in renal dialysis patients, firstly due to the increased frequency of infections experienced when on dialysis, and secondly due to the poor capacity of conventional renal dialysis technology to remove this protein. As a result of the inability of conventional renal replacement therapy to remove beta-2 microglobulin, the concentration of this protein increases in the blood circulation of renal dialysis patients. The primary consequence of this accumulation of beta-2 microglobulin is the development of beta-2 microglobulin amyloid fibrils in the bones and other

tissues of renal dialysis patients, which affects bone structure and bone marrow function.

The present inventors have tested the ability of the GradiFlow to remove beta-2 microglobulin from normal human plasma. Forty mL of plasma was diluted 1:1 in Tris/borate buffer pH 9 and processed in the GradiFlow using 3kDa restriction membranes and 25kDa separation membranes. A maximum potential of 250V was applied to the system, with the circulation buffer maintained at 4C. The absorbance at 280nm of the downstream was measured at 30 minute intervals, and the beta-2 microglobulin content of the downstream was determined by and ELISA method in samples taken every hour. The ELISA method employed a rabbit polyclonal antiserum specific to detect beta-2 microglobulin specifically. Figure 20 shows that low molecular weight proteins were rapidly removed from plasma, and that beta-2 microglobulin was detectable in the downstream. The gradual reduction in total protein in the downstream (A280 points) may relate to the gradual electrophoresis of very small proteins and peptides through the 3kDa restriction membranes or the adhesion of proteins to the bottom restriction membranes.

SUMMARY

Urea removal has been shown to be independent of voltage, current, pH and salt concentration. Urea removal has been shown to be dependent on temperature, membrane molecular weight cutoff and the starting concentration of urea. Urea removal from plasma has been demonstrated. Urea, being an uncharged molecule, does not move in response to electrical field variations, rather its movement is due entirely to passive diffusion phenomena. The ability of the GradiFlow system to remove Urea is of significance to the GradiFlow renal dialysis application, as urea is the major nitrogenous waste that must be removed. This is also the first demonstration of passive diffusion phenomena in the GradiFlow system, indicating the GradiFlow may be used for the removal and/or purification of uncharged solutes while simultaneously removing charged molecules by electrophoretic means.

Creatinine is a charged nitrogenous waste material which has been shown to be removed from plasma, and whose rate of removal has been shown to be dependent on voltage, pH, salt concentration, temperature and membrane pore size. The capacity of the GradiFlow system to rapidly

remove charged nitrogenous wastes is significant to the GradiFlow capacity in renal dialysis.

Uric acid was removed from aqueous solutions and from plasma. Removal of Uric acid was shown to be dependent on voltage, membrane pore size, temperature and salt concentration. Uric acid removal is another example of electrically driven dialysis which allows rapid removal of nitrogenous wastes from plasma.

The removal of phosphate ions from blood and plasma is a critical application of GradiFlow technology to the field of renal dialysis. The inability of current dialysis technologies to remove phosphate ions is an area that could be readily addressed by a variation of the GradiFlow technology using electrically driven dialysis to remove charged solutes. The general principle of removing charged ions which is demonstrated here can also be considered to apply to other salt ions such as sodium, potassium, chloride and so on. The removal of excess concentrations of these ions would also be made more rapid using electrically driven dialysis systems.

The demonstration of the ability of GradiFlow technology to remove proteins, specifically albumin and beta-2 microglobulin, from whole blood and plasma implies that, using the correct conditions of membrane molecular weight cutoff, voltage and buffer solution, individual disease related proteins may be removed from blood or plasma for therapeutic purposes. This potential should not be restricted to the two proteins for which the principle has been demonstrated. In theory, any protein for which a specific combination of electrical field and membrane selectivity can be specified, could be removed from blood or plasma for therapeutic purposes.

CONCLUSIONS

It is apparent from the data presented that the GradiFlow system is useful for the removal of nitrogenous wastes, phosphate ions, and proteins such as albumin and beta-2 microglobulin, from aqueous solutions, plasma and blood. The ability to remove waste or unwanted materials from blood or plasma by the simultaneous use of diffusive and electrophoretic principles in a single cartridge system is an advantage. For example, urea can be removed on the basis of latent diffusion while other waste materials can be removed on the basis of charge during the same process. The capacity of the basic GradiFlow system to perform these functions indicates the potential applications of the GradiFlow system in the field of renal dialysis and other

blood purification applications which require the selective removal of proteins and other charged or uncharged species from circulating blood or plasma. Modified versions of the GradiFlow device can be constructed which could be used either as a complete renal dialysis device, addressing all renal replacement therapy needs including removal of salts, phosphate, nitrogenous wastes, excess water balancing blood pH and removing beta-2 microglobulin. Alternatively, a simpler device may be constructed to function as an addition to existing renal dialysis systems, whose function is to address the deficiencies of the existing systems, ie the removal of phosphate and beta-2 microglobulin from either blood or plasma. The present inventors have demonstrated that the GradiFlow system is capable of removing all these solutes and proteins. The correct combination of membrane chemistry, dialysis solution, voltage and current conditions, cartridge and tubing materials, pump design etc are all integral to the functioning of the system.

Furthermore, given that individual proteins may be removed from blood and/or plasma, it will be feasible to construct a version of the GradiFlow which is designed to selectively remove proteins such as autoantibodies, which may be related to autoimmune diseases such as rheumatoid arthritis, lupus and so on, as well as other proteins which may be causative factors in other diseases. Examples of other proteins or blood contaminants may include the removal of bacterial endotoxins or specific lipoproteins from blood or plasma as a therapeutic measures for treating septic shock or lipid metabolism disorders respectively.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:

1. A method of treating blood or plasma of a subject to remove or reduce the concentration of metabolic contaminants, the method comprising:

(a) placing blood or plasma from the subject in a first solvent stream, the first solvent stream being separated from a second solvent stream by an electrophoretic membrane;

(b) applying an electric potential between the two solvent streams causing movement of metabolic contaminants from the blood or plasma through the membrane into the second solvent stream while cellular and biomolecular components of the blood or plasma are substantially retained in the first sample stream, or if entering the membrane, being substantially prevented from entering the second solvent stream;

(c) optionally, periodically stopping and reversing the electric potential to cause movement of any cellular and biomolecular components of the blood or plasma having entered the membrane to move back into the first solvent stream, wherein substantially not causing any metabolic contaminants that have entered the second solvent stream to re-enter the first solvent stream;

(d) maintaining step (b), and optionally step (c) if used, until the desired amount of removal of the metabolic contaminants from the blood or plasma in the first solvent stream is achieved; and

(e) returning the treated blood or plasma in the first solvent stream to the subject.

2. The method according to claim 1 wherein the subject is a renal dialysis patient.

3. The method according to claim 1 or 2 wherein the blood or plasma is recirculated between the subject and the first solvent stream.

4. The method according to any one of claims 1 to 3 wherein the metabolic contaminants are removed or reduced by electrophoretic or diffusive means.

5. The method according to any one of claims 1 to 4 wherein the metabolic contaminants are selected from the group consisting of urea, creatinine, uric acid, and phosphate ions.

6. The method according to any one of claims 1 to 4 wherein the metabolic contaminants are proteins.

7. The method according to claim 6 wherein the proteins are selected from the group consisting of beta-2 microglobulin and autoantibodies.

8. The method according to any one of claims 1 to 7 wherein the electrophoretic membrane has a molecular mass cut-off close to the apparent molecular mass of metabolic contaminants.

9. The method according to claim 8 wherein the electrophoretic membrane has a molecular mass cut-off of between 3 and 1000kDa.

10. The method according to any one of claims 1 to 9 wherein the electric potential applied does not substantially adversely affect the cells or proteins present in the blood or plasma.

11. The method according to claim 10 wherein the electric potential applied is up to 100 volts.

12. A method of renal dialysis to remove or reduce the concentration of unwanted metabolic contaminants from blood or plasma of a renal patient, the method comprising:

(a) carrying out haemodialysis on blood or plasma of the patient;

(b) placing blood or plasma from the haemodialysed patient in a first solvent stream, the first solvent stream being separated from a second solvent stream by an electrophoretic membrane;

(c) applying an electric potential between the two solvent streams causing movement of metabolic contaminants from the blood or plasma through the membrane into the second solvent stream while cellular and biomolecular components of the blood or plasma are substantially retained in the first sample stream, or if entering the membrane, being substantially prevented from entering the second solvent stream;

(d) optionally, periodically stopping and reversing the electric potential to cause movement of any cellular and biomolecular components of the blood or plasma having entered the membrane to move back into the first solvent stream, wherein substantially not causing any metabolic contaminants that have entered the second solvent stream to re-enter the first solvent stream;

(e) maintaining step (c), and optionally step (d) if used, until the desired amount of removal or reduction of the metabolic contaminants from the blood or plasma in the first solvent stream is achieved; and

(f) returning the treated blood or plasma in the first solvent stream to the patient.

13. The method according to claim 12 wherein the blood or plasma is recirculated between the renal patient and the first solvent stream.

14. The method according to claim 12 or 13 wherein the metabolic contaminants are removed or reduced by electrophoretic or diffusive means.

15. The method according to any one of claims 12 to 14 wherein the metabolic contaminants are selected from the group consisting of urea, creatinine, uric acid, and phosphate ions.

16. The method according to any one of claims 12 to 14 wherein the metabolic contaminants are proteins.

17. The method according to claim 16 wherein the proteins are selected from the group consisting of beta-2 microglobulin and autoantibodies.

18. The method according to any one of claims 12 to 17 wherein the electrophoretic membrane has a molecular mass cut-off close to the apparent molecular mass of metabolic contaminants.

19. The method according to claim 18 wherein the electrophoretic membrane has a molecular mass cut-off of between 3 and 1000kDa.

20. The method according to any one of claims 12 to 19 wherein the electric potential applied does not substantially adversely affect cells or proteins present in the blood or plasma.

21. The method according to claim 20 wherein the electric potential applied is up to 100 volts.

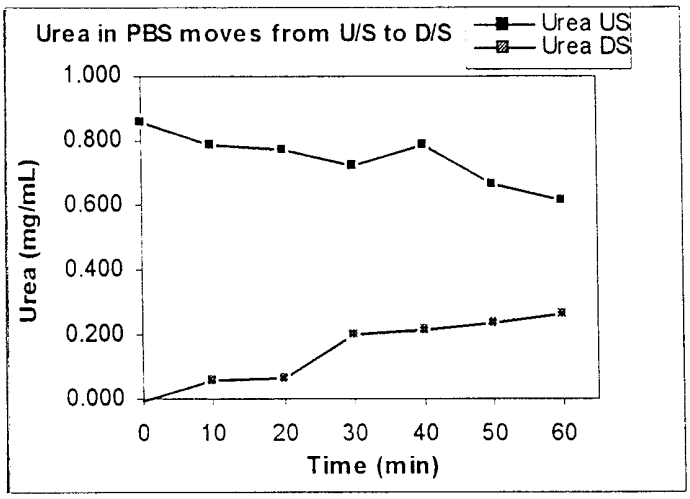


Figure 1

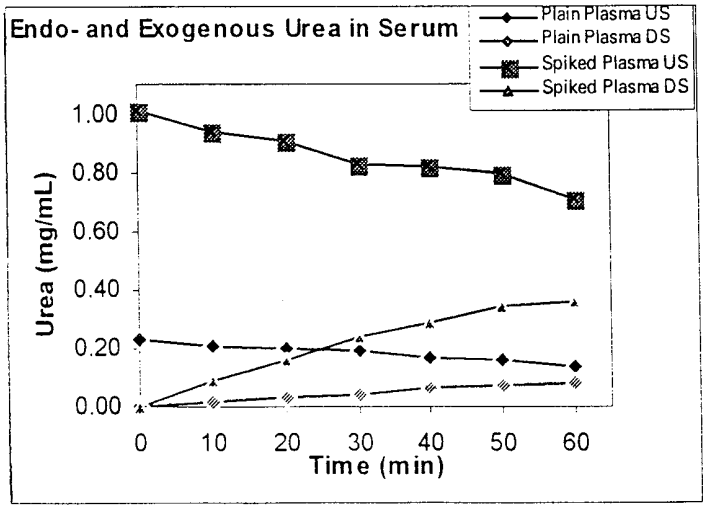


Figure 2

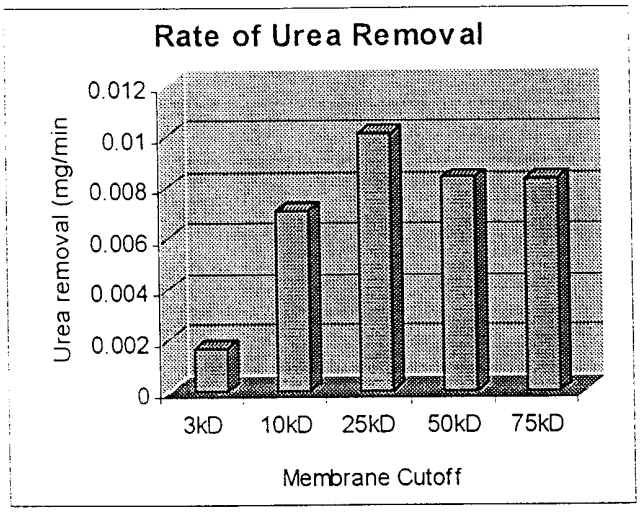


Figure 3

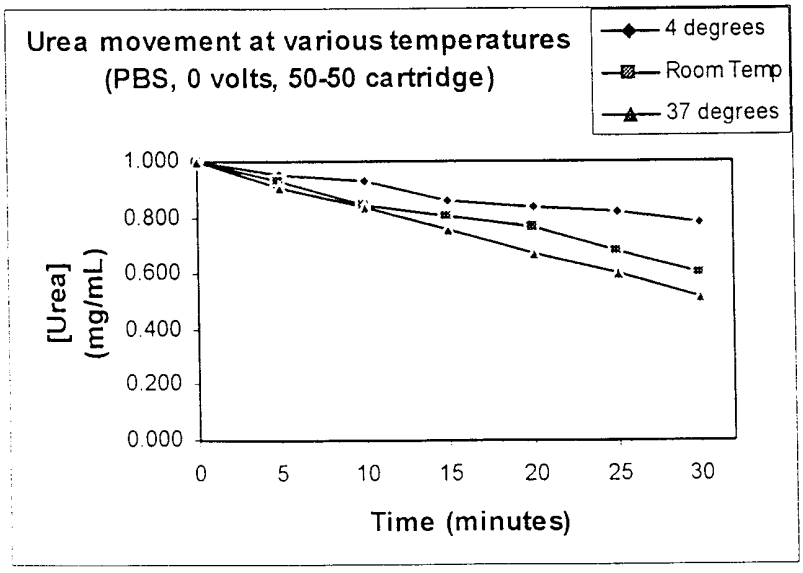


Figure 4

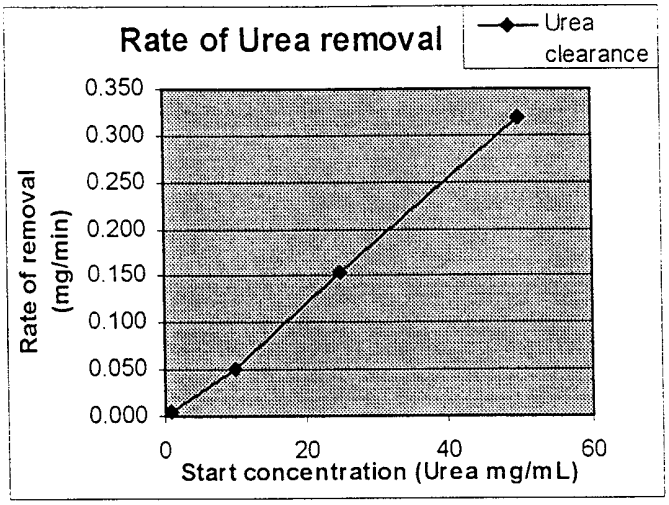
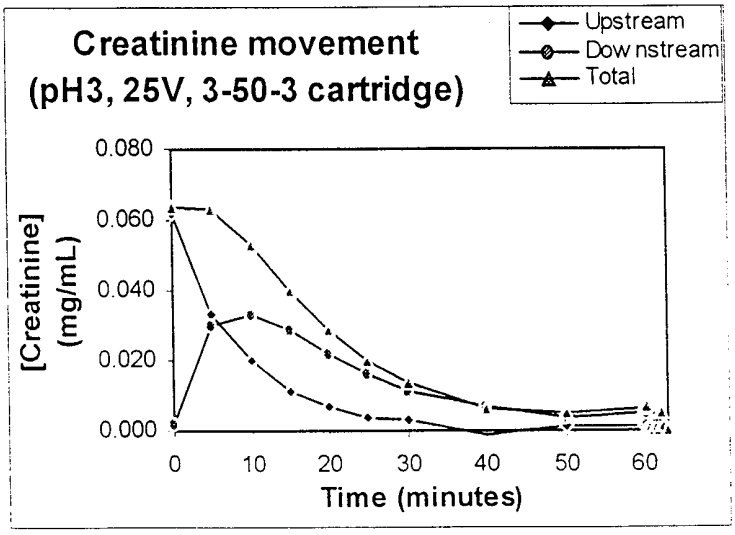


Figure 5



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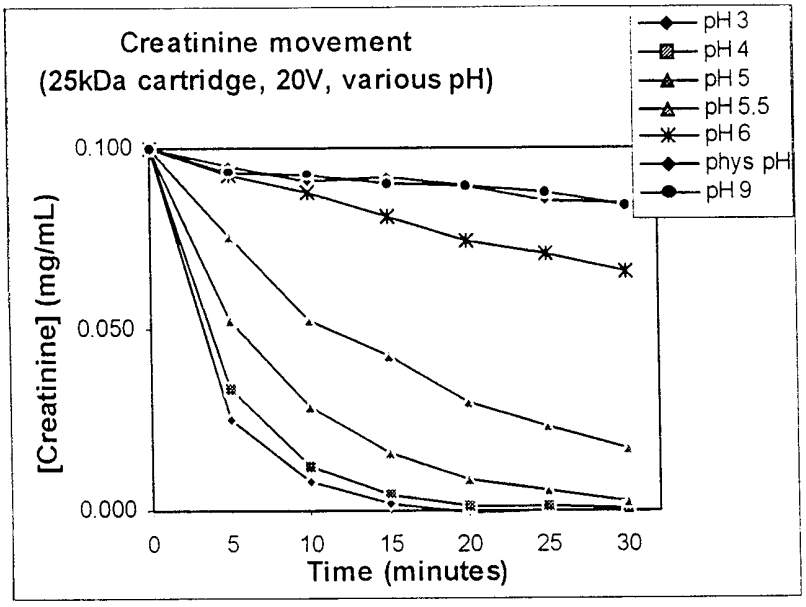

Figure 7

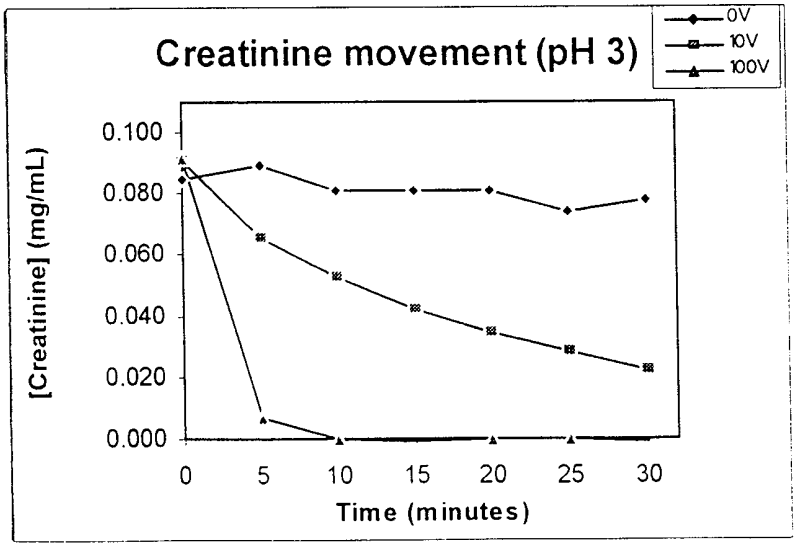

Figure 8

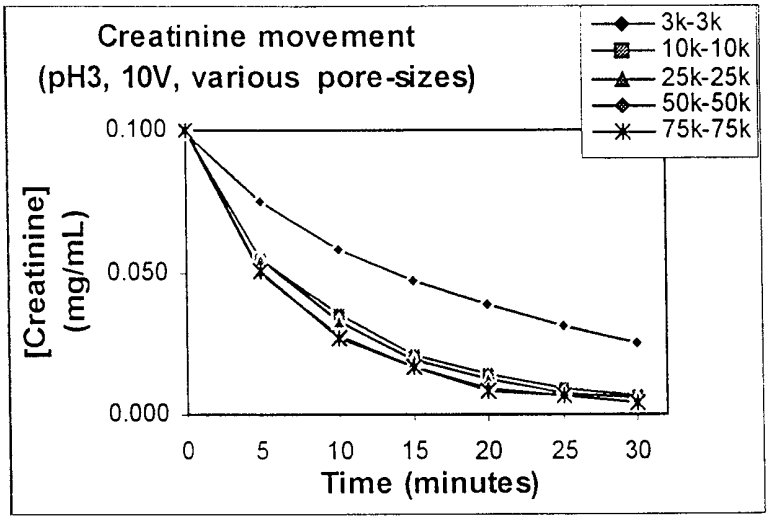

Figure 9

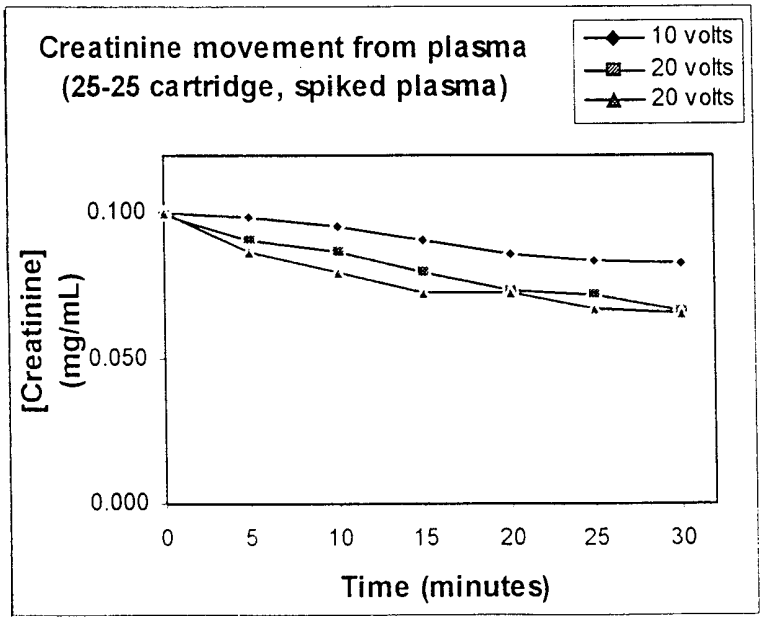

Figure 10

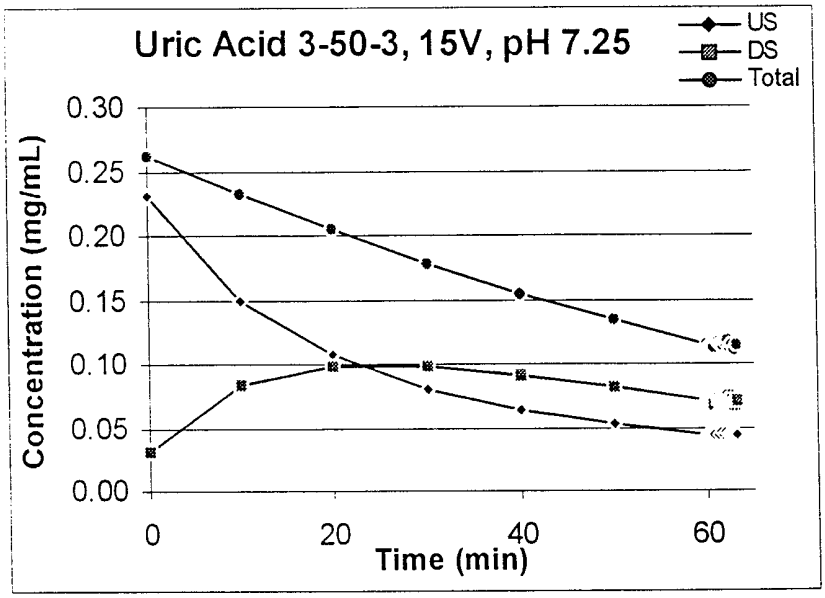

Figure 11

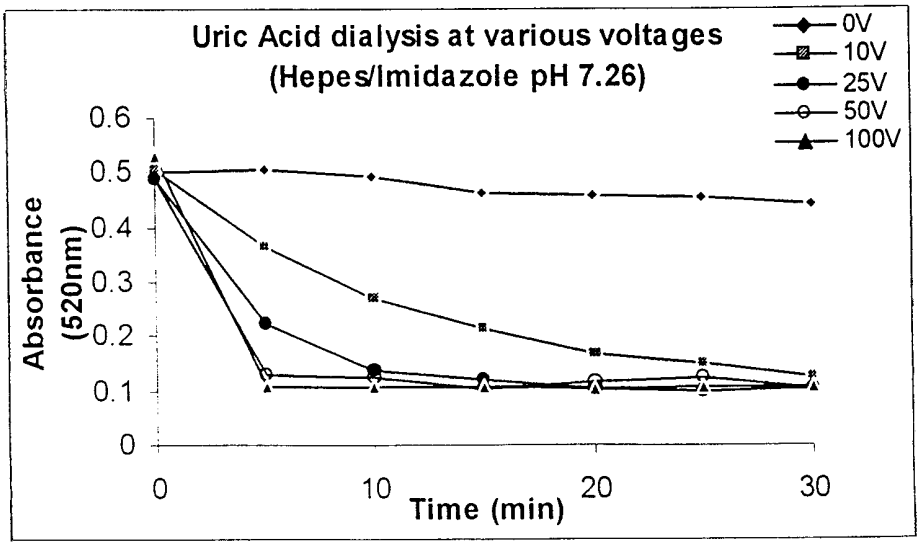

Figure 12

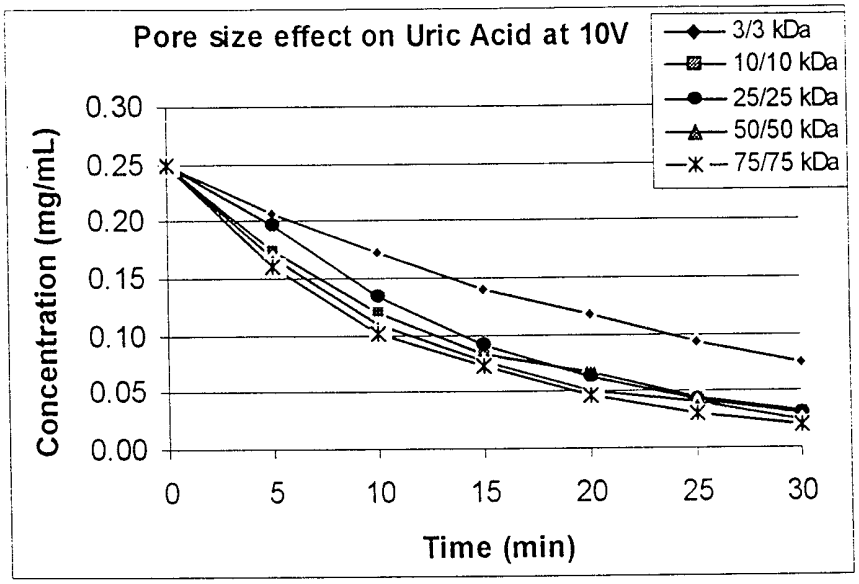

Figure 13

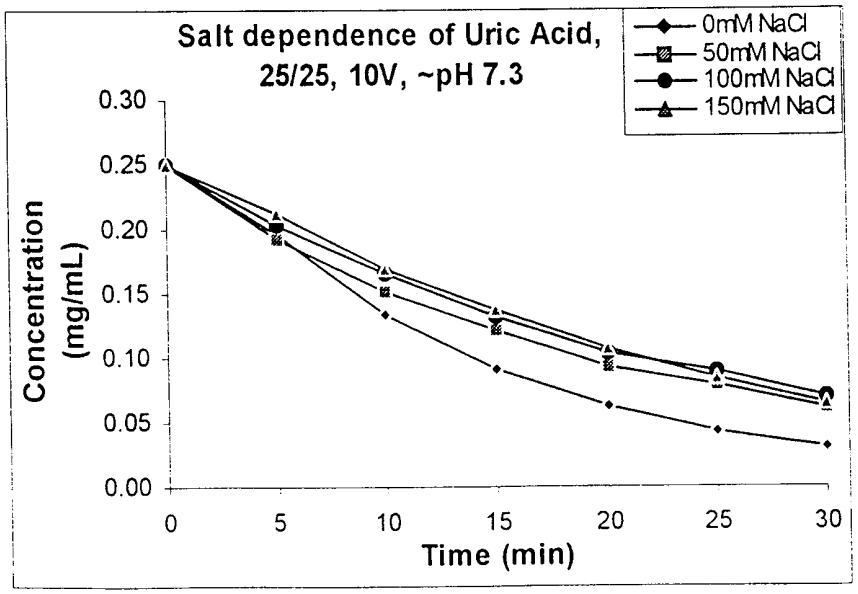

Figure 14

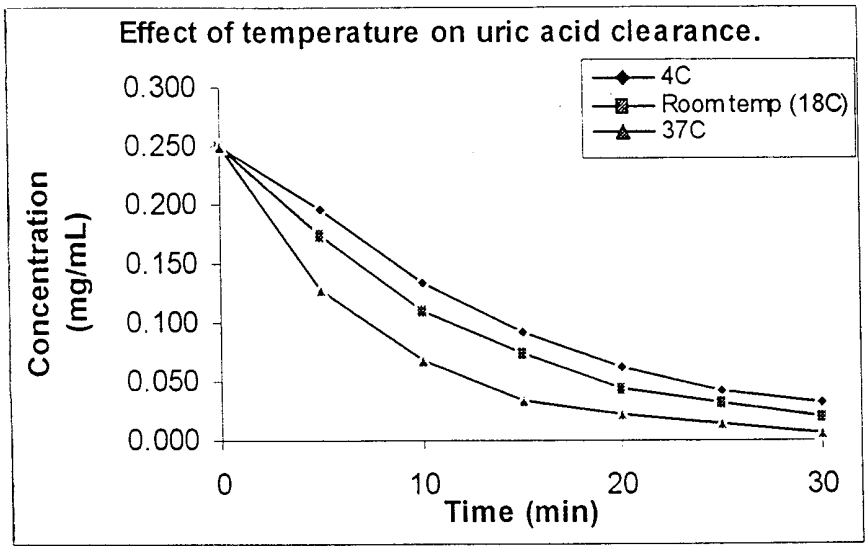

Figure 15

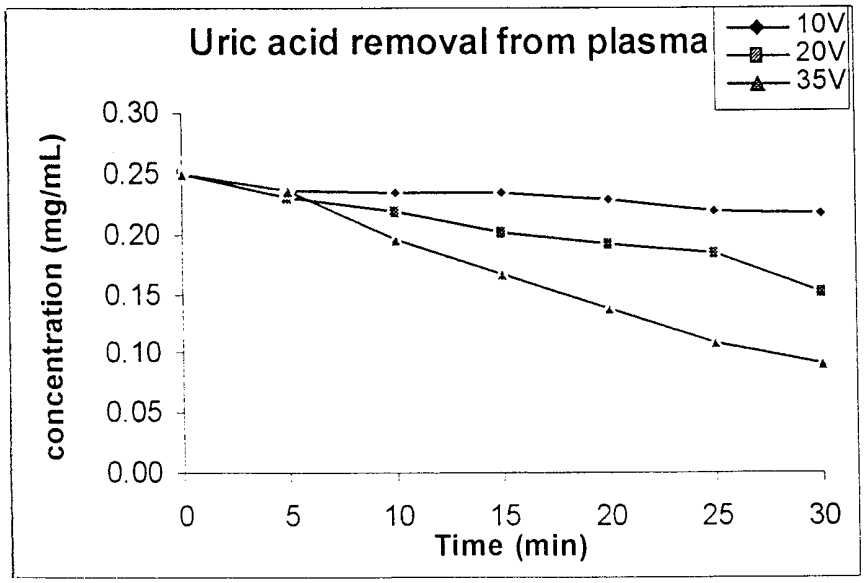

Figure 16

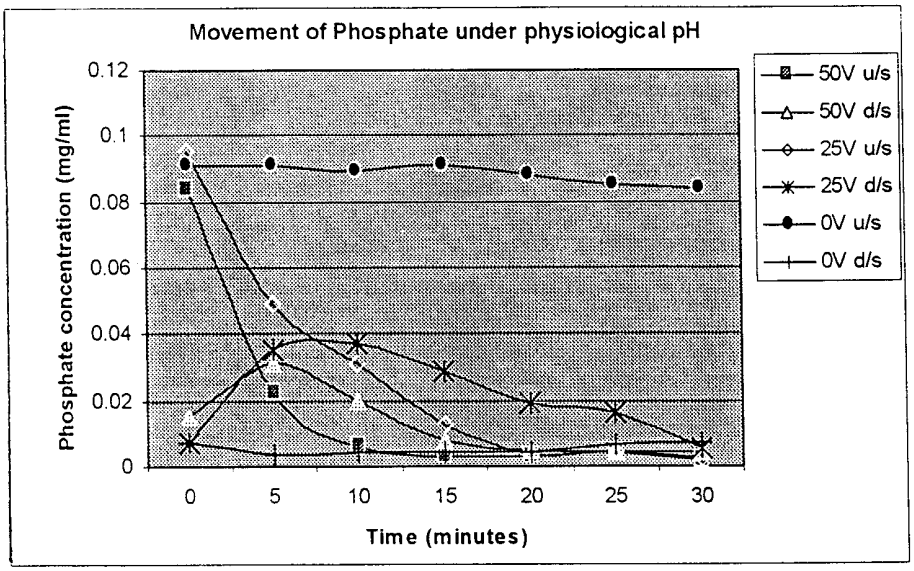

Figure 17

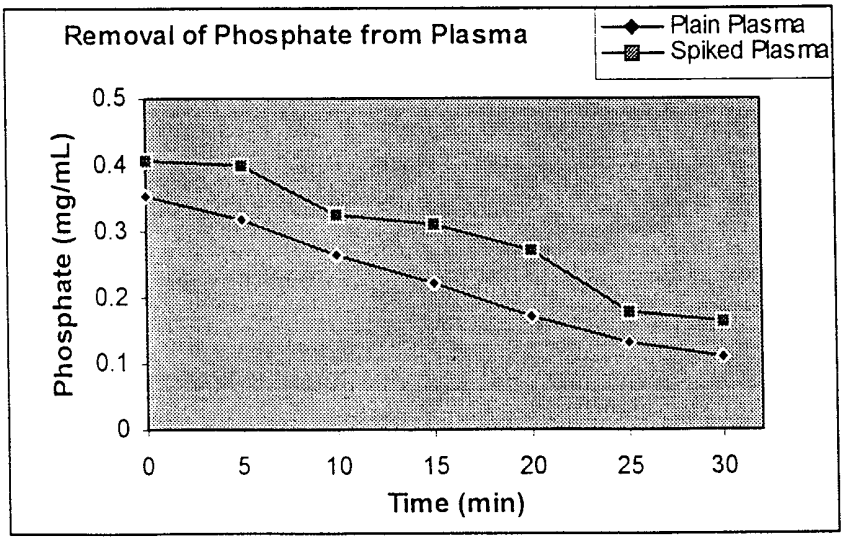

Figure 18

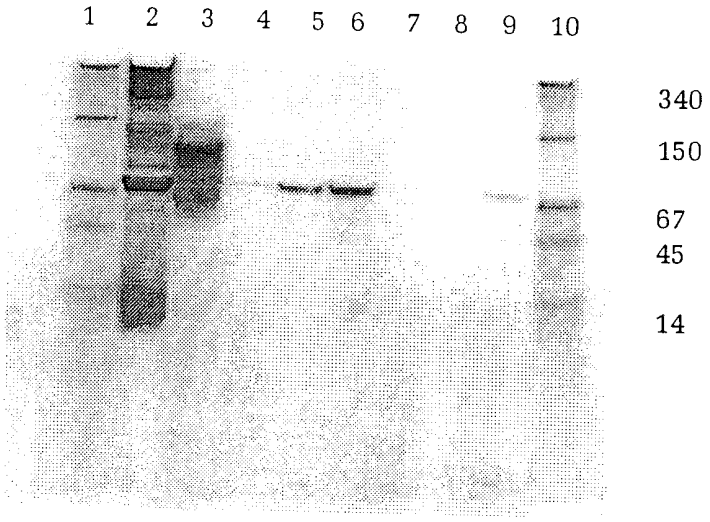

Figure 19

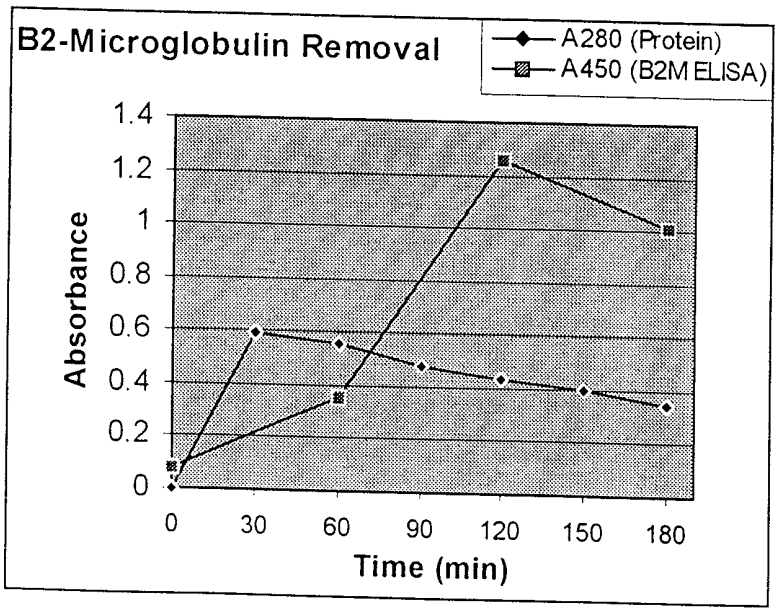

Figure 20

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/01172

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl. [7]: A61M 1/34

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61M 1/-, B01D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT
JAPIO

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88/07406 A (GRADIENT PTY. LTD.) 6 October 1988 Page 3 line 11 to page 4 line 4	1 to 21
Y	US 4461693 A (JAIN) 24 July 1984 Column 1 lines 16 to 29	1 to 21
X,Y	US 5437774 A (LAUSTSEN) 1 August 1995 Column 8 lines 6 to 38	1 to 21

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
24 February 2000

Date of mailing of the international search report
- 7 MAR 2000

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU99/01172

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	US 4043895 A (GRITZNER) 23 August 1977 Column 3 lines 36 to 54	1 to 21

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/AU99/01172

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member		
WO 88/07406	AU 16280/88	EP 352286	US 5039386
US 4461693	DE 3324047	FR 2530027	GB 2123037
US 5437774	AU 13105/95	WO 95/17950	
US 4043895	US 3989613		

END OF ANNEX