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(54) Title: PROCESS FOR FORMING POLYACRYLAMIDE MEMBRANES

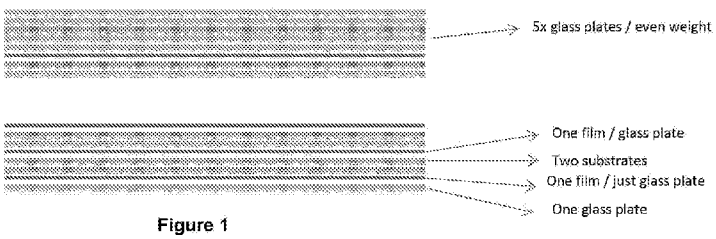

Figure 1

(57) Abstract: A process for producing a plurality of polyacrylamide membranes comprising (a) providing acrylamide monomer solution and crosslinking agent to a membrane casting vessel; (b) providing a casting plate to the bottom of the vessel; (c) providing a membrane substrate on the casting plate; (d) providing a casting plate on the membrane substrate; (e) repeating steps (c) to (d) to form a plurality of layers of casting plates and membrane substrates; (f) providing a weighting material to the plurality of layers; and (g) allowing the acrylamide monomer to polymerize to the membrane substrates to form polyacrylamide membranes; wherein at least one casting plate is a heat-resistant plastic film.

PROCESS FOR FORMING POLYACRYLAMIDE MEMBRANES

Technical Field

[001] The present invention relates to an improved process to form polyacrylamide membranes for biological separations using electrophoresis.

Background

[002] Many membranes are manufactured and used for separation of biological samples. A specific electrophoretic separation technology, PriME (Preparative Isolation by Membrane Electrophoresis) has generally used membranes that are thin polyacrylamide membranes which are produced in between two glass plates to achieve a glossy surface with desired thickness. This production process has inherent safety concerns associated with multiple handling of glass plates and the weight of glass can limit the number of membranes produced per batch. The use of an alternative to glass plates such as plastic films has reduce the risk associated with manual handling of glass and increased the batch production scale.

[003] The solution phase polymerisation process to manufacture membranes, such as thin polyacrylamide hydrogel membrane with defined pour size, involves the free radical co-polymerisation of acrylamide monomer and polyfunctional crosslinking agent N, N'-methylene-bis-acrylamide (Bis). A redox initiator system ammonium persulphate (APS) with *N,N,N',N'* –tetramethylethylenediamine (TEMED) is used to initiate the free radical polymerisation. The membranes may be formed on a substrate of polyethyleneterapthalate (PET) by casting polyacrylamide polymer gel between two glass plates. Manual production is limited to small scale batches when glass plates are used due its heavy weight and thickness, also handling the fragile glass plates is a safety concern.

[004] The present inventors have developed a process resulting in improved polyacrylamide membranes suitable for biological separations using electrophoresis.

Disclosure of Invention

[005] In a first aspect, the present invention provides a process for producing a plurality of polyacrylamide membranes, the process comprising:

(a) providing acrylamide monomer solution and crosslinking agent to a membrane casting vessel;

(b) providing a casting plate to the bottom of the vessel;

(c) providing a membrane substrate on the casting plate;

(d) providing a casting plate on the membrane substrate;

(e) repeating steps (c) to (d) to form a plurality of layers of casting plates and membrane substrates;

(f) providing a weighting material to the plurality of layers; and

(g) allowing the acrylamide monomer to polymerize to the membrane substrates to form polyacrylamide membranes;

wherein at least one casting plate is a heat-resistant plastic film.

[006] The process may further include:

(h) removing the plurality of layers from the vessel and separating the polyacrylamide membranes from the casting plates to obtain a plurality of polyacrylamide membranes.

[007] In a second aspect, the present invention provides a process for producing a plurality of polyacrylamide membranes, the process comprising:

(a) providing acrylamide monomer solution and crosslinking agent to a membrane casting vessel;

(b) providing a casting plate to the bottom of the vessel;

(c) providing a plurality of alternating layers of casting plates and membrane substrates on top of the casting plate in step (b);

(d) providing a weighting material to the plurality of layers; and

(e) allowing the acrylamide monomer to polymerize to the membrane substrates to form polyacrylamide membranes; wherein at least one casting plate is a heat-resistant plastic film.

[008] The process may further include:

(f) removing the plurality of layers from the vessel and separating the polyacrylamide membranes from the casting plates to obtain a plurality of polyacrylamide membranes.

[009] In some embodiments the membrane casting vessel is a tank having a removable front wall adapted to receive the solution, the casting pates, the membrane substrates and the weighting material.

[010] In one embodiment the acrylamide monomer solution is N, N'- methylene-bis-acrylamide (Bis) and the crosslinking agent is ammonium persulphate (APS) and *N,N,N',N'* –tetramethylethylenediamine (TEMED). Further additives may be included with the solution such as Teric B18 (Surfactant) and buffer components such as MES (2-(*N*-morpholino)ethanesulfonic acid) and Bis-Tris (Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane.

[011] The casting plates can be glass plates and heat-resistant plastic films. In one embodiment some or most of the casting plates are heat-resistant plastic films.

[012] The glass pales can have a thickness of about 0.1 to 5 mm or 0.5 to 2 mm. In one embodiment, the glass plates are about 2 mm thick. The heat-resistant plastic films can have a thickness of about 0.1 to 2 mm or 0.1 to 1 mm or 0.1 to 0.5 mm. In one embodiment the heat-resistant plastic films are up to about 1 mm thick. In another embodiment the heat-resistant plastic films are about 0.5 mm thick. It will be appreciated that the glass plates and the plastic films can be thicker or thinner, depending on the supplier specifications.

[013] The heat-resistant plastic films can be made of polyethylene terephthalate (PET), polypropylene or polyethylene terephthalate glycol-modified (PETG), PVC (polyvinyl chloride) film; Polymethylmethacrylate (plexiglass); Teflon PTFE film (polytetrafluoroethylene). In an embodiment the heat-resistant plastic films are PET. An exemplary heat resistant plastic film was the 3M "Black & White Laser transparency film (product no CG3300). The film is a PET coated film suitable for use with laser printers.

[014] In one embodiment the membrane substrate is a PET fibre sheet.

[015] In an embodiment, two PET fibre sheets are placed between the casting plates to form the membrane substrate. In one embodiment, the PET fibre sheets are placed between heat-resistant plastic films to allow smooth glossy membrane surfaces to form.

[016] The weighting material may be one or more glass plates. In an embodiment the weighting material is two or more glass plates. In another embodiment, a plurality of glass plates form the weighting material.

[017] The process can be used to produce 10 or more membranes, 15 or more membranes, 20 or more membranes, 25 or more membranes, 30 or more membranes, 35 or more membranes, 40 or more membranes, 45 or more membranes, 50 or more membranes, 55 or more membranes, 60 or more membranes.

[018] In an embodiment the process is carried out in a controlled atmosphere environment to maintain constant conditions during the casting process. In one embodiment, the membrane casting vessel is placed in a glove box and the process is carried out in the glove box.

[019] In an embodiment the membranes are A4 sheet size or formed in sheets of about 315 mm X 315 mm in size and have a thickness of between about 0.1 to 0.3 mm.

[020] The membranes are adapted for use in Preparative Isolation by Membrane Electrophoresis (PriME) that was originally developed by Gradipore Limited and described in US 6,328,869, US 6,402,913, US 6,919,006, and US 6,800,184. Examples of uses of the membrane separation technology are described in Li G, Stewart R, Conlan B, Gilbert A, Roeth P, Nair H. Purification of human immunoglobulin G: a new approach to plasma fractionation. Vox sanguinis. 2002;83:332-8; Thomas TM, Quindere J, Thomas DE, Gee SC, Bate IM, Rylatt DB. Preparation of monoclonal antibodies using electrophoresis separation instrument, Gradiflow™. Hybridoma and Hybridomics. 2003; 22:47-53; Cheung

GLM, Thomas TM, Rylatt DB. Purification of antibody Fab and $F(ab')_2$ fragments using Gradiflow technology. Protein Expression and Purification. 2003; 32:135-140; Evtushenko M, Wang K, Stokes HW, Nair H. Blood protein purification and simultaneous removal of non-enveloped viruses using tangential-flow preparative electrophoresis. Electrophoresis. 2005;26:28-34; Wang K, Johnson A, Obradovic M, Anderson G, Maclean C, Nair H. TSE clearance during plasma products separation process by Gradiflow™. Biologicals. 2005;33:87-94.

[021] The membranes formed between the heat-resistant plastic films in the casting process were found to have a smoother and more even glossy surface. This resulted in improved batch quality compared with the process only using glass as the casting plates. A further advantage of the use of heat-resistant plastic films in the process is that glass plates are heavy and cumbersome to manipulate in the membrane casting process compared with handling of the heat-resistant plastic films. The heat-resistant plastic films can be discarded after use whereas the glass plates require a rigorous cleaning an process and need to be stored carefully to minimize breakage and scratching.

[022] The use of polyethyleneterapthalate (PET) coated 3M plastic films replacing glass plates has shown promising results with desired performance of the product.

[023] In a third aspect, the present invention provides polyacrylamide membranes produced by the process according to the first aspect of the present invention.

[024] 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.

[025] Any discussion of documents, acts, materials, devices, articles or the like which has been included in the present specification is solely for the purpose of providing a context for the present invention. It is not to be taken as an admission that any or all of these matters form part of the prior art base or were common general knowledge in the field relevant to the present invention as it existed before the priority date of each claim of this specification.

[026] In order that the present invention may be more clearly understood, preferred embodiments will be described with reference to the following drawings and examples.

Brief Description of the Drawings

[027] Figure 1 shows an embodiment of material assembly for casting membranes.

[028] Figure 2 shows a schematic of macromolecule size-based separation using preparative electrophoresis membrane based technology.

[029] Figure 3 shows a schematic macromolecule charge-based separation using preparative electrophoresis membrane based technology.

Mode(s) for Carrying Out the Invention

MATERIALS AND METHODS

Chemicals

[030] The chemicals acrylamide, N, N'- methylene-bis-acrylamide (Bis), ammonium persulphate (APS) and *N,N,N',N'*–tetramethylethylenediamine (TEMED) were obtained from Sigma-Aldrich Australia and used without further purification.

Casting plates

[031] The casting plates can be glass plates and heat-resistant plastic films. The glass plates (2 mm) were used.

[032] Different types of heat resistant plastic films tested are listed in Table 1.

Table 1. Plastic films

Substrate	Supplier	Type	Thickness (mm)
PET_3M	3M, USA	Transparent laser film	0.1
PET_China	Lichi Mechinary, China	Transparent inkjet film	0.1
Polypropylene	Conservation Resources, Australia	Transparent pliable films	0.1
PETG		Clear films	0.5

[033] The better performing heat resistant plastic film was the 3M "Black & White Laser transparency film (product no CG3300). The film is a PET coated film but the percentage of coating is 3M proprietary information. The coating percentage may determine the transparency level and may be partly thermal properties of the film. Other films that have similar properties could be used for membrane manufacturing process. However, the surface of the membranes could be slightly less or more glossy and intact depending on the type of films used without affecting intrinsic properties of membrane.

[034] The films were developed for use in other areas and include useful features such as being waterproof, transparent, rigid, excellent mechanical strength, environment friendly

and high temperature durable. Application areas include laser printing, offset printing, plate-making, overhead projecting and advertising printing.

Membrane substrate

[035] Polyethyleneterapthalate (PET) fibre sheets are used as a substrate for casting the membranes. These fibers are utilized to produce wicking media with open-cell pore structures that control liquid volume capacity and fluid transfer rates. The fibers offer a wide assortment of extruded profile geometries and can be engineered to meet various density, permeability, and wicking performance requirements. PET fibre sheets are FDA compliant for use in various biological assays and separation processes.

[036] The substrates are engineered for excellent fluid transfer properties, have high thermal bonding properties for use in higher temperature applications. Good thermal bonding properties maintain part integrity during normal transportation, storage, and use. The substrates are highly inert and resistant to chemicals and may be utilized with many acidic, basic, and organic solvents. Mechanical properties provide excellent part rigidity. PET porous media may also be engineered for part softness or rigidity as required for the end-use application. It offers excellent tensile strength, which is beneficial for product that is supplied on reels and intended for high volume assembly automation.

[037] The substrates can have one or more of the following characteristics and structure:
engineered for excellent fluid transfer properties; high thermal bonding properties for use in higher temperature applications; good thermal bonding properties and maintain part integrity during normal transportation, storage, and use; chemical resistance: highly inert and resistant to chemicals and may be utilized with many acidic, basic, and organic solvents. Mechanical properties: exhibiting part rigidity. PET porous substrates may also be engineered for part softness or rigidity as required for the end-use application. PET porous substrates offer excellent tensile strength, which is beneficial for product that is supplied on reels and intended for high volume assembly automation.

[038] The substrates come in sheets or rolls and were purchased from Shangshai Bolting cloth manufacturing, China.

Chemistry

[039] The properties of the polyacrylamide gels are depended on the composition of reacting monomer, cross-linker, initiator as well as polymerisation environment such as oxygen level and temperature. The experiments were carried out at different initiator concentrations to produce membranes with desired properties using different substrates. The experiments were also carried out under controlled and uncontrolled environment with

optimising the initiator concentration. The acrylamide (monomer) and the cross-liker (N, N'-methylene-bis-acrylamide (Bis) concentrations were kept constant to retain the desired pore size of particular membranes. Table 2 shows the composition of initiator for 250 kDa membrane formulation using different plastic films. The initiator concentration for the glass plate formed membranes was used as reference standard. Table 3 shows the composition of initiator for 75 kDa membrane formulation using different plastic films in uncontrolled environment.

Table 2. The composition of initiator using different plastic films for 250 kDa membrane.

Formulation	Substrate	Temperature (˚C)	Oxygen level (%)	APS concentration (mM)	TEMED concentration (mM)
Run 1					
250 kDa	Glass plate	~ 22	0.5-0.7	0.183	0.183
	PET_3M			0.183	0.183
	PET_China			0.183	0.183
	Polypropylene			0.183	0.183
Run 2					
	Glass plate	~ 22	0.5-0.7	0.366	0.366
	PET_3M			0.366	0.366
	PET_China			0.366	0.366
	Polypropylene			0.366	0.366
Run 3					
	Glass plate	~ 22	0.5-0.7	0.549	0.549
	PET_3M			0.549	0.549
	PET_China			0.549	0.549
	Polypropylene			0.549	0.549
Validation runs					
	PET_China			0.366	0.366
	Polypropylene			0.366	0.366

Table 3. The composition of initiator using different plastic films for 75 kDa membrane in uncontrolled environment.

Formulation	Substrate	Temperature (°C)	Oxygen level (%)	APS concentration (mM)	TEMED concentration (mM)
Run 1					
75 kDa	Glass plate	Not controlled	Not controlled	0.183	0.183
	PET_3M			0.183	0.183
	PETG			0.183	0.183
Run 2					
	Glass plate			1.647	1.647
	PET_3M			1.647	1.647
	PETG			1.647	1.647
Validation runs					
	PET_3M			1.647	1.647
	PETG			1.647	1.647

Membrane casting vessel

[040] The membrane casting vessel was a tank having a removable front wall adapted to receive the solution, the casting pates, the membrane substrates and the weighting material. A small scale casting vessel that makes A4 size membrane sheets and a large scale casting vessel that makes 315 mm x315 mm membrane were used. Both vessels were made of acrylic plate having a bottom and three fixed side walls. The fourth side wall was removable to assist with removing the cast membranes.

Membrane casting

[041] Membranes were prepared in a membrane casting vessel in the form of a tank by assembling the glass plates or plastic films as appropriate in the acrylamide reaction mixture solution. Figure 1 shows an example of the assembly of materials for casting membranes. This procedure was carried out either in a controlled/ uncontrolled environment in a membrane casting tank containing the reaction mixture. The polymerisation starts within 25-30 minutes after adding the initiator. Therefore, the

assembly should be completed within about 20 minutes of adding initiator to the monomer solution. The reaction is completed within about 2 hours. The membranes can be left curing overnight for up to about 18 hours. The cast membrane and casting plate block is removed from the vessel and the membranes were separated after curing and stored in appropriate storage buffer.

Membrane cassette

[042] Membranes were cut to size and assembled into a membrane cassette for use in the electrophoresis apparatus. The cassette has an outer housing unit component containing gasket. A restriction membrane was placed within the housing unit followed by a support grid having channels. A separation membrane was then placed on the grid. A collar of the grid faced the restriction membrane and the grid channels face upwards towards the separation membrane. An another grid was placed on the separation membrane so that the channels face towards the separation membrane. Another restriction was placed on top of the grid followed by inner housing component (containing a gasket) on top of the cartridge stack. Clips (6 in total) on the inner and outer housing are aligned and gently pressed until they are fully clipped. Buffer stream inlet tubes were then provided to the assembled cassette.

Electrophoresis apparatus

[043] The PrIME technology is a preparative electrophoresis membrane based technology based on two major techniques. One is polyacrylamide membrane and the another is protein electrophoreses. By choosing a selected pore size of the separation membrane and a suitable buffer (or buffers), it separates protein molecules based on their molecule weights and isoelectric point within a particular electrical field and buffer environment. The separation process is simple and efficient and the working principle is demonstrated by Figure 2 showing macromolecule size-based separation and Figure 3 showing macromolecule charge-based separation.

[044] The BF400 (NuSep Ltd) is a laboratory scale biological separations apparatus incorporating the PrIME technology. With a processing volume of between 5 and 50 ml, it is a versatile system capable of processing samples from a diverse range of biological complexes. The control panel has time setting key, voltage key, buffer pump start key, stream pump start key, cover indicator, electrical reverse key and start and stop key. The apparatus has a fixed stream pump and buffer pump, stream pump gives a fixed stream flow rate to both stream 1 and 2 at 20 ml/min, the buffer pump circle the buffer at 2 l/min.

Electrophoresis conditions

[045] The following example shows albumin separation from human plasma using BF400 electrophoresis apparatus and membranes produce according to the present invention.

[046] Frozen pooled plasma (Serologicals, Atlanta, GA) from healthy donors was thawed at 37°C. One millilitre of plasma was diluted in 1:10 with Tris–Borate buffer pH 8·9 [32 mM (3·88 g/l) Trizma®Base, 96 mM(5·93 g/l) and placed into stream 1 (S1) of the BF400. A membrane cartridge, comprising a 150 kDa pore size separation membrane sandwiched between two 5-k Da pore size restriction membranes, was used in the separation unit. Tris-Borate buffer (1·8 l) was circulated in the buffer tank and kept cold with ice. An electric potential of 250 V with the positive electrode configured at stream 2 (S2), was placed across the membrane sandwich to perform the electrophoresis. The product in S2 was harvested every 60 min for a total of 360 min. After each harvest, 10·ml of fresh buffer was used to replenish S2.

[047] The purity of the samples was determined using sodium dodecyl sulphate–polyacrylamide gel electrophoresis (SDS–PAGE). SDS–PAGE was performed using Tris–glycine NG 4–20% NuSep iGel (NuSep Ltd), which were stained with NuSep NuBlue Coomassie Blue stain (NuSep Ltd).

Membrane characterisation

[048] The membranes were visually inspected for bubbles, white patches, thickness and appearance. The membranes are then characterised with respect to its pore size using specific proteins following PRIME instrument and technology. The pore size is determined from the transfer rate of the specific protein through the membranes.

EXAMPLE 1

[049] Production of polyacrylamide membranes includes the following steps:

Preparation of casting vessel

i. Ensure that the surfaces to contact the monomer solution are clean and ready for use.

ii. Check that each casting plate has no residual gel, grease marks or other contaminants before use. Spray with ethanol and wipe dry with lint-free paper (Kim wipes) to remove any remaining smudges if needed.

iii. When not in use, the casting plates should be separated with clean A4 paper and stored in a suitable container.

iv. Assemble casting vessel by screwing the front panel to the open wall space.

Loading

i. Gently lay a glass plate onto the bottom of the casting vessel

ii. Add the monomer solution to the membrane tank

iii. Place the desired membrane substrate sheets over the reaction mixture. Pin the left side of the substrates with a casting plate. With a second plate, gently run along the surface from one side to the other to remove any air bubbles. Repeat if necessary until you are satisfied that there are no trapped bubbles.

iv. Lay a casting plate/film on top of the substrates and gently press.

v. Repeat Steps iii and iv until all sheets of substrate are loaded. Place another glass plate on top of the last casting plate/film.

vi. Leave the reaction mixture to polymerise in the tank.

Unloading

i. Remove the tank from the glove box and disassemble the tank by removing the front panel. Carefully scrape and discard any excess gel.

ii. Using an appropriate tool, carefully lift each casting plate and remove the polyacrylamide membranes. Any excess gel from the edges of the membrane can be cleaned away.

EXAMPLE 2

[050] A batch of membranes prepared as described in Example 1 using 15 glass plates had a total weight in excess of 20 kg. The components and weights used for manufacturing a batch of membrane is shown in Table 4.

Table 4. Total weight of components used for manufacturing one batch of membranes using glass plates

Component	**Weight per unit (kg)**	**Quantity**	**Sub-total (kg)**
Reaction chamber	5.6	1	5.6
Large glass plate	0.86	15	12.9
Monomer solutions	1.5	1	1.5
substrate	0.005	26	0.13
Total weight (kg)			20.13

EXAMPLE 3

[051] A batch of membranes prepared as described in Example 1 using 12 PET film casting plates, 3M (Black & White Laser Transparency Film), and 3 glass plates had a total weight less than 10 kg. The resultant membranes had glossy and shiny surface comparable to glass plate based membranes. The protein transfer test results are also comparable, i.e. the desired pore size of the membranes have not been affected. The protein transfer results are provided in Table 5.

[052] Three batches of membranes were produced using both glass plates and the plastic films. The product performance was confirmed by physical inspection and protein transfer QC test and the results were comparable. The smooth surface of coated plastic films have produced membranes with glossy surface comparable with glass plates.

Table 5. Protein transfer comparative results

Glass plate			**Plastic film**		
Batch	**Sample**	**Protein transfer (%) Specification 15-20%**	**Batch**	**Sample**	**Protein transfer (%) Specification 15-20%**
75K280512	1	15.87	75K140312	1	17.21
	2	19.38		2	16.39
75K090512	1	20.28	75K210312	1	15.58
	2	16.68		2	16.17
75K140612	1	15.53	75K220312	1	16
	2	14.21		2	19.03

[053] The total weight of the reaction chamber was significantly reduced as well as significantly reduced concern regarding the presence of broken glass during manufacturing and cleaning process. The thickness of each plastic film (0.1 mm) is 20 times thinner than the glass plate, i.e. batch size can be increased significantly, 40 membranes per batch have been produced.

[054] Considering the weight and thickness more than 40 membranes could have been produced.

Results

[055] The initiator concentration was successfully optimised to produce membrane using suitable plastic films. The validation batches reproduced the membranes with desired properties. Table 6 show the results for 250 kDa in controlled environment. The standard initiator concentration is used for polymerisation for batch HN250K061113. The polymerisation time was longer and the gel consistency was more elastic. This could be due the dissolved oxygen in the plastic film which inhibits polymerisation. The gel was sticking to 3M films (could not be separated) and also on the glass plates. Polypropylene shows best results: produced membrane with no dry patches and easy to separate. However the batch was failed due to the consistency of the gels. The initiator concentration was optimised to 2 times (Batch: HN250K141113_1) and 3 times (Batch: HN250K141113_2) higher than the standard. The batch HN250K141113_1 produced membranes with desired properties. Batch: HN250K141113_2 failed due to early polymerisation. The validation batches produced reproducible results.

Table 6. The pour results for 250 kDa membranes using different plastic films.

Batch No	**Substrate**	**Temp (°C)**	**Oxygen level (%)**	**APS/ TEMED conc (mM)**	**Visual inspection**	**Protein transfer (%),Specification: 14± 4%**
Run 1						
HN250K061113	Glass plate	~ 22	0.5-0.7	0.183	Fail	NA
	PET_3M			0.183	Fail	NA
	PET_China			0.183	Fail	NA
	Polypropylene			0.183	Fail	NA
Run 2						
HN250K141113_1	Glass plate	~ 22	0.5-0.7	0.366	Pass	14.41
	PET_3M			0.366	Pass	13.36
	PET_China			0.366	Pass	16.11
	Polypropylene			0.366	Pass	10.85
Run 3						
HN250K141113	Glass plate	~ 22	0.5-0.7	0.549	Fail	NA

Batch No	Substrate	Temp (˚C)	Oxygen level (%)	APS/ TEMED conc (mM)	Visual inspection	Protein transfer (%),Specification: 14± 4%
_2						
	PET_3M			0.549	Fail	NA
	PET_China			0.549	Fail	NA
	Polypropylene			0.549	Fail	NA
Validation runs						
HN250K041213_1	PET_China	~ 22	0.5-0.7	0.366	Pass	13.84
	Polypropylene			0.366	Pass	WIP*
HN250K041213_2	PET_China			0.366	Pass	15.67
	Polypropylene			0.366	Pass	WIP
HN250K041213_3	PET_China			0.366	Pass	17.75
	Polypropylene			0.366	Pass	WIP

*work in progress

[056] Table 7 show the results for 75 kDa membrane production in uncontrolled environment using different plastic films kDa. The initiator concentration was optimised to 9 times than the standard to produce membrane with desired properties.

Table 7. The pour results for 75 kDa membranes using different plastic films in uncontrolled environment.

Batch No	Substrate	Temp (°C)	Oxygen level (%)	APS /TEMED concentration (mM)	Visual inspection	Protein transfer (%),Specification: 15-20 %
Run 1						
75K160512	Glass plate	Not controlled	Not controlled	0.183	Fail	NA
	PET_3M			0.183	Fail	NA
	PETG			0.183	Fail	NA
Run 2						
75K280512	Glass plate			1.647	Pass	17.63
75K140312	PET_3M			1.647	Pass	16.8
HN75K191212_PETG	PETG			1.647	Pass	17.59
Validation runs						
75K100312	PET_3M			1.647	Pass	15.8
75K220312	PET_3M			1.647	Pass	17.51

Conclusion

[057] Membranes can be manufactured successfully using plastic films replacing glass plates. The product performance was confirmed by physical inspection and protein transfer QC test and the results were comparable. The smooth surface of plastic films has produced membranes with glossy surface comparable with glass plates.

[058] Production capacity can also be increased. With reference to Table 4, a batch of membranes prepared using 15 glass plates had a total weight in excess of 20 kg. When produced a batch of membranes using 12 PET film casting plates/ 3M (Black & White Laser

Transparency Film)/ polypropylene/ PETG and 5 glass plates had a total weight less than 10 kg. The resultant membranes had glossy and shiny surface comparable to glass plate based membranes. The protein transfer test results are also comparable as the desired pore size of the membranes were not affected. The total weight of the membrane reaction chamber was significantly reduced. The thickness of each plastic film (PET) (0.1 mm) is 20 times thinner than the glass plate so the batch size can be increased significantly. Considering the weight and thickness of the plastic film more than 40 membranes can been produced in one batch.

[059] The plastic films are disposable, therefore reducing the processing time significantly. The cleaning of glass plates involves hand washing, washing in the dishwasher 3 times and drying, also further cleaning with ethanol.

[060] The total weight of the reaction chamber was significantly reduced when glass plates were replaced with plastic films which reduces safety concern regarding handling heavy weights for the manufacturer as well as significantly reduced concern regarding the presence of broken glass during manufacturing and cleaning process.

[061] The membranes formed between the heat-resistant plastic films in the casting process were found to have a smoother and more even glossy surface. This resulted in improved batch quality compared with the process only using glass as the casting plates. A further advantage of the use of heat-resistant plastic films in the process is that glass plates are heavy and cumbersome to manipulate in the membrane casting process compared with handling of the heat-resistant plastic films. The heat-resistant plastic films can be discarded after use whereas the glass plates require a rigorous cleaning an process and need to be stored carefully to minimize breakage and scratching.

[062] 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 process for producing a plurality of polyacrylamide membranes, the process comprising:

(a) providing acrylamide monomer solution and crosslinking agent to a membrane casting vessel;

(b) providing a casting plate to the bottom of the vessel;

(c) providing a membrane substrate on the casting plate;

(d) providing a casting plate on the membrane substrate;

(e) repeating steps (c) to (d) to form a plurality of layers of casting plates and membrane substrates;

(f) providing a weighting material to the plurality of layers; and

(g) allowing the acrylamide monomer to polymerize to the membrane substrates to form polyacrylamide membranes;

wherein at least one casting plate is a heat-resistant plastic film.

2. The process according to claim 1 further including:

(h) removing the plurality of layers from the vessel and separating the polyacrylamide membranes from the casting plates to obtain a plurality of polyacrylamide membranes.

3. The process according to claim 1 or 2 wherein the membrane casting vessel is a tank having a removable front wall adapted to receive the solution, the casting pates, the membrane substrates and the weighting material.

4. The process according to any one of claims 1 to 3 wherein the acrylamide monomer solution is N, N'- methylene-bis-acrylamide (Bis) and the crosslinking agent is ammonium persulphate (APS) and *N,N,N',N'* –tetramethylethylenediamine (TEMED).

5. The process according to claim 4 further including additives selected from Teric B18 as surfactant, buffer components including MES (2-(*N*-morpholino)ethanesulfonic acid) and Bis-Tris (Bis(2-hydroxyethyl)amino-tris(hydroxymethyl)methane.

6. The process according to any one of claims 1 to 5 wherein the casting plates are glass plates and heat-resistant plastic films.

7. The process according to claim 6 wherein most of the casting plates are heat-resistant plastic films.

8. The process according to any one of claims 1 to 7 wherein the heat-resistant plastic films are made of polyethylene terephthalate (PET), polypropylene or polyethylene terephthalate glycol-modified (PETG), polyvinyl chloride (PVC), polymethylmethacrylate (plexiglass) or Teflon polytetrafluoroethylene (PTFE).

9. The process according to claim 8 wherein the heat-resistant plastic films are PET.

10. The process according to any one of claims 1 to 9 wherein the membrane substrate is a PET fibre sheet.

11. The process according to claim 10 wherein two PET fibre sheets are placed between the casting plates to form the membrane substrate.

12. The process according to any one of claims 1 to 11 wherein the weighting material is one or more glass plates.

13. The process according to claim 12 wherein the weighting material is a plurality of glass plates.

14. The process according to any one of claims 1 to 13 carried out in a controlled atmosphere environment to maintain constant conditions during the casting process.

15. The process according to claim 14 wherein the membrane casting vessel is placed in a glove box and the process is carried out in the glove box.

16. A process for producing a plurality of polyacrylamide membranes, the process comprising:

(a) providing acrylamide monomer solution and crosslinking agent to a membrane casting vessel;

(b) providing a casting plate to the bottom of the vessel;

(c) providing a plurality of alternating layers of casting plates and membrane substrates on top of the casting plate in step (b);

(d) providing a weighting material to the plurality of layers;

(e) allowing the acrylamide monomer to polymerize to the membrane substrates to form polyacrylamide membranes; wherein at least one casting plate is a heat-resistant is plastic film.

17. The process according to claim 16 further including:

(f) removing the plurality of layers from the vessel and separating the polyacrylamide membranes from the casting plates to obtain a plurality of polyacrylamide membranes.

18. The process according to claim 16 or 17 wherein the casting plates are glass plates and heat-resistant plastic films.

19. The process according to claim 18 wherein most of the casting plates are heat-resistant plastic films.

20. A polyacrylamide membrane produced by the process according to any one of claims 1 to 19.

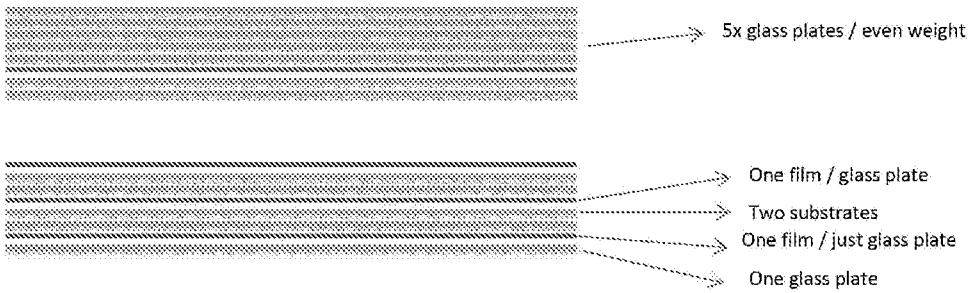

Figure 1

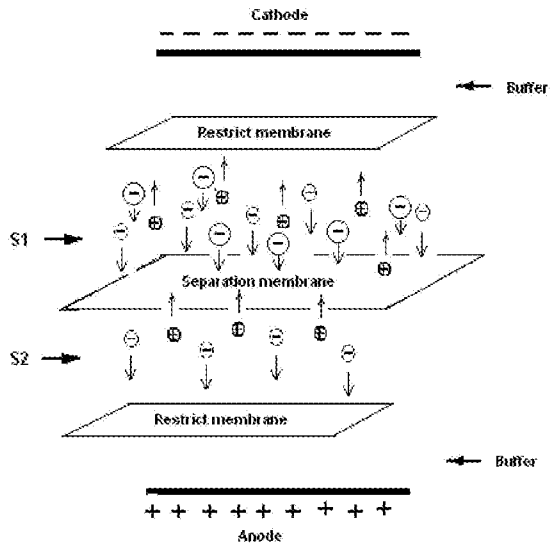

Figure 2

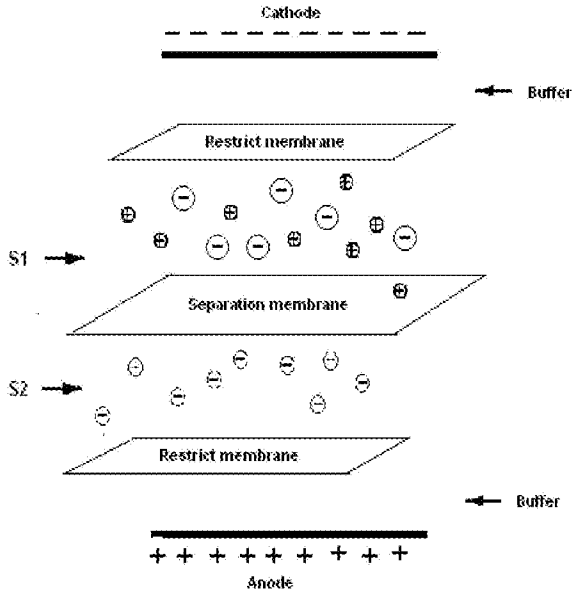
Cathode
Buffer
Restrict membrane
S1
Separation membrane
S2
Restrict membrane
Buffer
Anode

Figure 3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/AU2014/000376

A. CLASSIFICATION OF SUBJECT MATTER

B01D 67/00 (2006.01)

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)

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)

Databases: TXTAU1, TXTCA1, TXTEP1, TXTGB1, TXTSG1, TXTUS0, TXTUS1, TXTUS2, TXTUS3, TXTUS4, TXTUS5, TXTWO1, WPI, EPODOC: IPC's /IC/CC (B01D61, B01D63, B01D65, B01D67, B01D69, B01D71, B01D2323/30, B01D2323/40, B01D2323/42, G01N27/26/LOW) & KEYWORDS (MEMBRANE, POLYACRYLAMIDE, ALTERNATE, PLASTIC, FILM, POLYMERISE, COMPRESS, CROSS LINK AND SIMILAR TERMS).

GOOGLE PATENTS, ESPACENET, THE LENS: KEYWORDS (MEMBRANE POLYACRYLAMIDE CROSS LINK PLASTIC PLATE AND SIMILAR TERMS).
APPLICANT AND INVENTOR SEARCH ON AUSPAT, ESPACENET.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	

[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	Date of mailing of the international search report
20 May 2014	20 May 2014
Name and mailing address of the ISA/AU	**Authorised officer**
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INTERNATIONAL SEARCH REPORT C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		International application No. **PCT/AU2014/000376**
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 4718998 A (OGAWA et al.) 12 January 1988 abstract; column 1, line 55 - column 2, line 68; column 3, line 19 - column 4, line 63; column 8, lines 3-20	1-20
Y	US 4818360 A (HURD et al.) 04 April 1989 column 3, lines 56-61; claims 1-4	1-20
Y	US 6607645 B1 (SARKAR) 19 August 2003 abstract	1-20

INTERNATIONAL SEARCH REPORT Information on patent family members	International application No. **PCT/AU2014/000376**

This Annex lists known 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/s Cited in Search Report		**Patent Family Member/s**	
Publication Number	**Publication Date**	**Publication Number**	**Publication Date**
US 4718998 A	12 Jan 1988	EP 0246873 A2	25 Nov 1987
		EP 0246873 B1	06 May 1992
		JP S62272147 A	26 Nov 1987
		JP S62272148 A	26 Nov 1987
US 4818360 A	04 Apr 1989	None	
US 6607645 B1	19 Aug 2003	AU 5997001 A	20 Nov 2001
		WO 0186030 A1	15 Nov 2001
		CA 2308092 A1	10 Nov 2001
		CA 2614620 A1	10 Nov 2001
		EP 1194615 A1	10 Apr 2002
		JP 2003532609 A	05 Nov 2003
		JP 5030359 B2	19 Sep 2012
		US 2003178307 A1	25 Sep 2003
		US 7182848 B2	27 Feb 2007

End of Annex

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.