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A novel approach for blood purification: Mixed-matrix membranes combining diffusion and adsorption in one step

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ABSTRACT

Hemodialysis is a commonly used blood purification technique in patients requiring kidney replacement therapy. Sorbents could increase uremic retention solute removal efficiency but, because of poor biocompatibility, their use is often limited to the treatment of patients with acute poisoning. This paper proposes a novel membrane concept for combining diffusion and adsorption of uremic retention solutes in one step: the so-called mixed-matrix membrane (MMM). In this concept, adsorptive particles are incorporated in a macro-porous membrane layer whereas an extra particle-free membrane layer is introduced on the blood-contacting side of the membrane to improve hemocompatibility and prevent particle release. These dual-layer mixed-matrix membranes have high clean-water permeance and high creatinine adsorption from creatinine model solutions. In human plasma, the removal of creatinine and of the protein-bound solute para-aminohippuric acid (PAH) by single and dual-layer membranes is in agreement with the removal achieved by the activated carbon particles alone, showing that under these experimental conditions the accessibility of the particles in the MMM is excellent. This study proves that the combination of diffusion and adsorption in a single step is possible and paves the way for the development of more efficient blood purification devices, excellently combining the advantages of both techniques.

1. Introduction

The prevalence of end-stage renal disease (ESRD) was ~535,000 in the USA in 2008. Of these patients, ~355,000 were treated with hemodialysis. Despite the high health care costs of dialysis treatment (over €50,000 per patient per year), hemodialysis is only partially successful in the treatment of patients with ESRD. Mortality (15–20% per year) and morbidity of these patients remain excessively high, whereas their quality of life is generally low [1]. This is reflected in the expected remaining life years, which are 25.0 years for the general US population, 15.7 for ESRD patients with a kidney transplant and 5.6 years for ESRD patients receiving dialysis treatment [2].

In the last three decades, sorbent technology has been applied in the treatment of severe intoxication and to increase the efficiency of hemodialysis, or replace it, and as a treatment for fulminant hepatic failure. In hemoperfusion (or plasma perfusion), blood (or plasma) is purified by extracorporeal passage through a column containing the adsorbent which can remove or neutralize the substance of interest. Hemoperfusion cannot fully substitute hemodialysis because it does not remove urea and excess fluid. Sorbents used in hemoperfusion help to remove uremic toxins; however, direct blood contact with the adsorbent often causes hemocompatibility issues, especially on the long term [3]. Activated carbon (AC) has a long record as a sorbent in blood purification in the case of intoxications, acute and chronic renal failure as well as liver failure [3–5]. Uncoated activated carbon is a strong adsorbent for uremic toxins [6] whereas polymeric coatings of activated carbon might

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help to improve hemocompatibility. However, coated activated carbon could still release carbon fragments, even after careful washing, and a double coating process is needed to overcome this problem [7]. In conventional hemoperfusion columns, optimal distribution of blood flow within the packed sorbent bed is very important for adequate use of the adsorption capacity, especially with rather viscous and complex solutions like blood or plasma. Channelling within the column leads to suboptimal adsorption and can induce blood coagulation. Furthermore, micro-particles that can be released into the circulation and can cause emboli are always a concern related to hemoperfusion.

It is obvious that a combination of the strengths of dialysis membranes with the adsorption power of high surface area sorbents can be very beneficial for the blood purification efficacy [8]. In fact, in the late 1970s so-called sorbent membranes were developed. These membranes were even on the market for a certain period, produced by Enka [9–15]. However, due to their quick saturation, manufacturing difficulties, reduced patient convenience and lack of adsorbents with high purity [16–19], they were quickly abandoned. More recently, membrane filtration and adsorption columns are often combined as two separate steps in wearable artificial kidneys [20,21].

In this paper, we propose a novel membrane concept for combining diffusion and adsorption of uremic retention solutes in one step: the so-called mixed-matrix membrane (MMM). In this concept, adsorptive particles are incorporated in a macro-porous membrane matrix. A particle-free membrane layer is introduced on the blood contacting side of the membrane, aiming to improve membrane hemocompatibility and prevent particle release into the circulation and hence emboli formation (see Fig. 1).

Mixed-matrix membranes have been proposed as an alternative for traditional chromatographic columns [22,23]. Compared to conventional columns, they have low flow resistance, which allows the use of smaller particles, resulting in an improved adsorption capacity and adsorption kinetics [24,25]. Furthermore, the particles can be homogenously distributed by embedding them in the matrix, leading to optimal adsorption efficiencies and preventing quick saturation.

Here, for the proof of concept, we prepare and investigate flat sheet MMMs using materials with an excellent record in blood purification. A polyethersulfone (PES)/polyvinylpyrrolidone (PVP) polymer blend is used for the preparation of the macro-porous membrane matrix (PES as a membrane-forming polymer blended with PVP to improve hydrophilicity) and activated carbon is used as adsorptive particle. Creatinine, a small-molecular-weight uremic retention solute, often used as a marker of kidney function, is used as model water soluble solute. The para-aminohippuric acid (PAH) which belongs to the family of hippurates, and is often used as a marker for organic anion transport because of tubular secretion, is used in this study as a model protein-bound solute [26–29].

The study investigates the combination of diffusion and adsorption in a single step, which probably leads to more efficient blood purification devices and will prevent issues related to the use of conventional hemoperfusion columns.

2. Materials and methods

2.1. Materials

Polyethersulfone (PES) (ULTRASON, E6020P, BASF, the Netherlands) was used as membrane material. Polyvinylpyrrolidone (PVP) (K90), (MW ≈ 360,000, Fluka, Sigma–Aldrich, Germany) and extra pure N-Methylpyrrolidone (NMP) (Acros organics, Belgium) were used as additive and solvent, respectively. Ultrapure water, prepared with a Millipore purification unit, was used as non-solvent in the coagulation bath. Activated carbon (Norit A Supra EUR, Norit Netherlands B.V., the Netherlands) was sieved with a 45 μm sieve (Fritsch GmbH, Germany) and used as adsorbent particles (median size 27 μm). The following chemicals were purchased from Fluka, Sigma–Aldrich. Creatinine was dissolved in Tyrode's buffer (pH 7.4) composed of 137 mM NaCl, 5.4 mM KCl, 1.8 mM $CaCl_2$, 0.5 mM $MgCl_2$, 11.9 mM $NaHCO_3$ and 5.5 mM glucose in ultrapure water.

2.2. Membrane preparation

The particle-free membrane layer was prepared using a 15 wt.% PES and a 7 wt.% PVP in NMP solution which was stirred at a roller bank overnight at room temperature. For the MMM, first a mixture of 14 wt.% PES and 1.4 wt.% PVP in NMP solution was prepared and stirred at a roller bank overnight at room temperature, then different amounts of dry activated carbon particles were added. Loadings of 50, 60 and 70 wt.% activated carbon in relation to the amount of PES in the mixed-matrix membrane layer were applied, calculated as:

$$\text{Loading}(\%) = \frac{W_{AC}}{W_{AC} + W_{PES}} \cdot 100 \quad (1)$$

where W_{AC} is the dry weight of activated carbon particles (g) and W_{PES} is the dry weight of PES (g). The mixtures were stirred at least overnight and ultrasound was applied for at least 15 min to break down possible particle clusters. After degassing overnight, all the membranes were prepared by immersion precipitation.

Solutions were cast on a glass plate using a casting knife. A slit of 300 μm and 150 μm for single-layer MMMs and single particle-free membranes were used respectively. An adjustable co-casting knife was used for dual-layer MMMs, see Fig. 2. The heights of the slits of the first and second knife were 300 and 450 μm respectively. Casting was immediately followed by immersion into the coagulation bath, containing 60 wt.% NMP in ultrapure

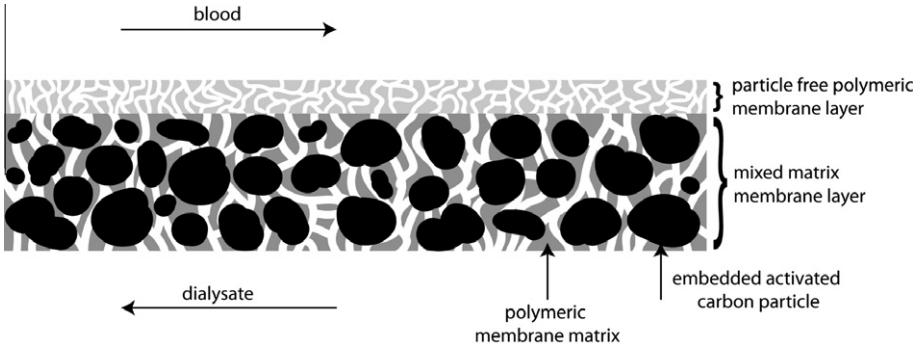

Fig. 1. Concept of dual-layer mixed-matrix membranes for blood purification.

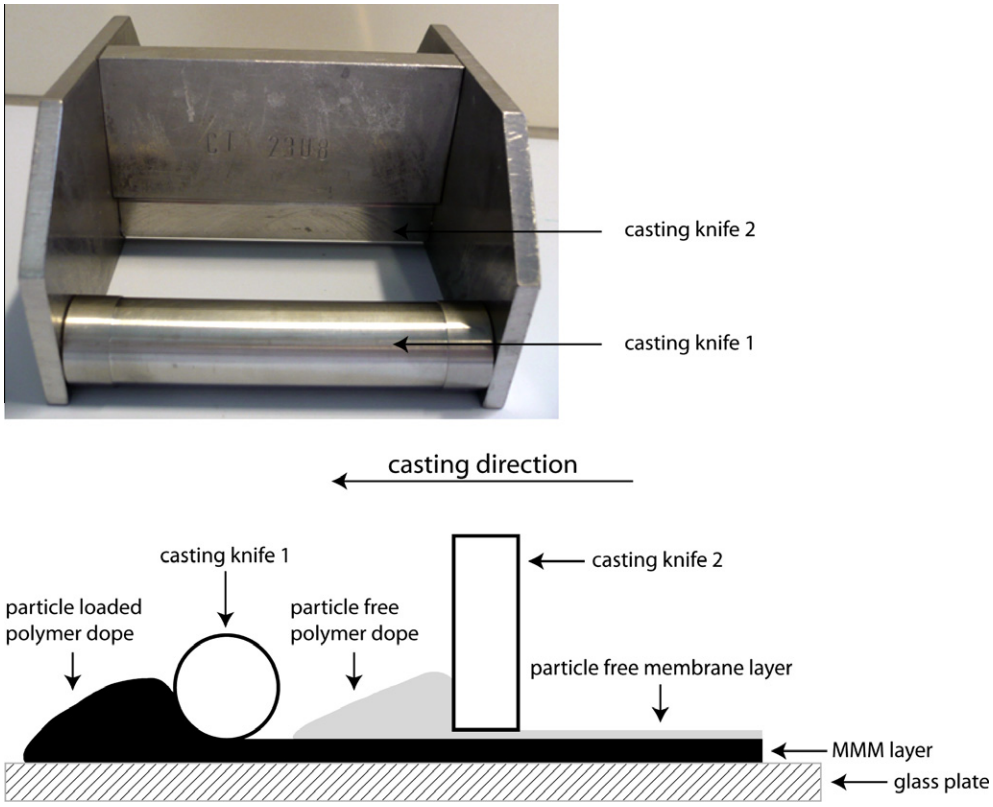

Fig. 2. Picture and schematic drawing of the co-casting knife. It consists of two attached casting knifes with 300 and 450 μm slits. The particle-free polymer dope is cast by casting knife 2 on top of the particle-loaded polymer dope casted by casting knife 1 to form dual-layer membranes.

water at room temperature. After the membrane formation process, the membranes were rinsed with ultrapure water to remove residual solvent traces, and stored in ultrapure water upon further use.

2.3. Membrane characterization

2.3.1. Scanning electron microscopy

For scanning electron microscopy (SEM), membranes were dried in air at room temperature and cryogenically broken in liquid nitrogen. The obtained cross-sections were dried overnight under vacuum at 30 °C and gold coated using a BalzersUnion SCD 040 sputter coater (OerlikonBalzers, Belgium). Coated membrane samples were examined using a JEOL JSM-5600LV scanning electron microscope (JEOL, Japan).

2.3.2. Membrane transport properties

Clean-water fluxes of the membranes were tested at room temperature using a nitrogen pressurized dead-end "Amicon type" ultrafiltration cell and ultrapure water. Flat membranes with an active surface area of 8.04 cm^2 were used. First, membranes were pre-pressurized for at least 0.5 h at the highest applicable pressure, which was 1.00 bar. Subsequently, pressures of 0.25, 0.5, 0.75 and 1.00 bar were applied for at least 20 min and the clean-water flux at each pressure was determined. The membrane permeance was calculated from the slope of the linear part of the flux vs. transmembrane pressure relation.

The BSA sieving coefficient of the single and dual-layer membranes was measured at room temperature using a nitrogen pressurized dead-end Amicon ultrafiltration cell. BSA was dissolved in ultrapure water with an initial concentration of 1 mg $ml^{-1}$and was pressurized through the membranes (with active surface area of 12.57 cm^2) at 0.5 bar for 30 min. The BSA sieving coefficient (SC) was calculated using the equation:

$$SC = \frac{C_p}{C_f} \tag{2}$$

where C_p and C_f are the BSA concentrations in the permeate and feed solution, respectively.

The BSA concentrations were determined by spectrophotometric analysis (Varian, Cary 300 Scan UV–visible spectrophotometer) at 278 nm. The student *t*-test was used for statistical testing ($p < 0.05$).

2.3.3. Creatinine adsorption capacity

The creatinine adsorption capacity of the prepared membranes was determined by batch adsorption experiments with model creatinine solutions. Known amounts of dry membranes were equilibrated in solutions with different creatinine concentrations in a shaking water bath at 37 °C for 24 h. The equilibrium creatinine concentration (C) was determined by spectrophotometric analysis (Varian, Cary 300 Scan UV–visible spectrophotometer) at 230 nm with 2 mm quartz cuvettes at 25 °C. Via the mass balance the amount of adsorbed creatinine was calculated from the depleted amount of creatinine in the solution. The adsorption capacity (q) was expressed as mg adsorbed creatinine per g of adsorptive particle. For this the proportion of activated carbon particles in the membrane was estimated (see Supplementary for details). Dry membrane weight was multiplied by this proportion and the obtained amount of activated carbon particles in the membrane was used to relate with the amount of adsorbed creatinine. Origin 7.5 was used for non-linear curve fitting of the isotherm in order to obtain a Langmuir fit according to the following equation:

$$q = \frac{q_m \cdot C}{K_d + C} \quad (3)$$

In which q is the adsorption capacity (mg g^{-1} AC), C is the equilibrium concentration of creatinine (mg ml^{-1}) in the solution, q_m is the maximum adsorption capacity (mg g^{-1} AC) and K_d is the dissociation constant (mg ml^{-1}).

2.3.4. Adsorption from human blood plasma

Human plasma was obtained from six patients who underwent plasma exchange because of acute renal disease. 25 mg activated carbon, MMM and dual-layer MMM, which contained ~25 mg activated carbon based on the proportion of activated carbon particles in them and a particle-free membrane of similar size as the dual-layer MMM, were incubated in 4 ml of six different plasmas. In the case of small deviations from the 25 mg sorbent weight, the amount of plasma was adjusted so that the sorbent-volume proportion would be similar to 25 mg in 4 ml. These test samples and plasmas without sorbents were incubated on a roller bank for 4 h. After incubation, samples were centrifuged at 4 °C for 10 min. The supernatant, ~3 ml per sample, was collected and stored in micro-cups at 4 °C for analysis later. Osmolarity, pH, total protein and creatinine concentrations were measured according to the protocol of manufacturer of the kit and/or device (see Table 1) whereas the PAH concentration was measured following the protocol described elsewhere [30].

Since the initial concentrations of creatinine and PAH in plasma were different for every patient, and to avoid large variation by taking averages of the absolute concentrations, we used relative concentrations. The absolute initial creatinine concentrations in the six different plasmas were: 495.4, 1299.2, 332.6, 44.6, 276.4 and 60.9 μmol l^{-1}. For the PAH, only two plasmas had reasonable baseline concentrations (49.8 and 72.4 μmol l^{-1}); therefore only two plasmas were used for these experiments. The relative change in concentration of the various solutes was calculated as follows:

$$\text{Relative concentration} = \frac{C_s}{C_b} \quad (4)$$

where C_s is the concentration in the plasma incubated with a sample (AC or membranes) and C_b is the concentration in the blank solution (plasma without sorbents or membranes). Likewise, the relative osmolarity and relative pH were calculated. Statistical differences were determined using a one-way ANOVA and Dunnett's test for the creatinine, pH, osmolarity and total protein concentrations. For PAH, due to the low number of plasma samples no statistical analysis was performed.

2.3.5. Two-compartment diffusion test

A two-compartment diffusion device [31] was used to measure diffusion and adsorption of creatinine onto dual-layer MMMs at room temperature. Creatinine is a uremic retention solute and is used as an indicator for solute removal by MMMs.The donor compartment was filled with 0.1 mg ml^{-1} creatinine in Tyrode's buffer, whereas the acceptor compartment was filled with pure Tyrode's buffer. The compartments were separated by a dual-layer MMM, with the particle-free layer facing the creatinine containing donor solution. The volume of each compartment was 65 ml and the active membrane area was 3.14 cm^2. Both solutions were stirred at room temperature. During the experiment, 600 μl samples were taken in time to determine creatinine concentrations in both compartments. The creatinine decrease in the donor compartment was considered as total removal. The amount of creatinine that appeared in the acceptor compartment was considered as creatinine which was diffused from the donor compartment. The creatinine deficiency in the mass balance was considered to be adsorbed onto the membrane. This amount was related to the dry membrane weight, which was measured after the experiment.

Table 1
Test methods for analysis of blood plasma.

Parameter	Kit/Device
Osmolarity	Advanced instruments osmometer model 3320
pH	Radiometer Copenhagen PHM lab pH meter
Creatinine	Bio-Rad Microplate reader Benchmark 16-channel photometer DiaSysCreatinine PAP FS (1 1759 99 10 026)
Total protein	Bio-Rad Laboratories GmbH Protein Assay (cat# 500-0006) Bio-Rad Microplate reader Benchmark 16-channel photometer

3. Results and discussion

Here we describe the characterization of the prepared mixed-matrix membranes in terms of morphology and transport properties. First we show the influence of the particle loading on membrane morphology followed by the clean-water permeance measurements and creatinine adsorption isotherms for both the optimized single and dual-layer mixed-matrix membranes. Furthermore, we show adsorption from human blood plasma. Finally, we show creatinine transport results of dual-layer mixed-matrix membranes.

3.1. Membrane particle loading optimization

Different amounts of activated carbon particles were embedded in mixed-matrix membranes. Particle loading is an important parameter: besides influencing membrane morphology, the amount of adsorptive sites in the MMM increases as the particle loading increases. High particle loading can result in too high viscosities for proper casting or result in membranes with low mechanical strength.

Fig. 3 shows cross-sections of MMMs containing 50, 60 and 70 wt.% of activated carbon in relation to the amount of PES. All membranes have a porous structure and no significant loss of particles was observed during membrane fabrication. Membranes loaded with 50 wt.% and 60 wt.% particles (Fig. 3A, B, D and E) have some macro-voids which may reduce mechanical strength of the membrane and may create transport channels. Membranes loaded with 70% particles contain more adsorptive sites per gram of membrane than at lower loadings. Furthermore, membranes with 70 wt.% activated carbon particles possess an open interconnected porous sponge-like structure, without macro-voids across the entire cross-section. In fact, as the particle loading increases, the viscosity of the dope increases as well. Higher dope viscosity restricts growth of the polymer lean phase in the phase separation process, because of a higher mass flow resistance. Likewise non-solvent inflow into the polymer solution is restricted, leading in turn to a slower phase separation process. The high viscosity prevents the formation of macro-voids and leads to the formation of smaller pores [32]. Furthermore, the particles probably act as a nucleus in the phase inversion process, leading to sponge-like structures [33]. Fig. 3G, H and I presents bottom surfaces of the different membranes. An increase in particle loading can clearly be observed by the amount of particles there. Activated carbon particles are tightly held together in the porous polymer matrix and no particle clusters are observed. All the obtained membranes possess sufficient mechanical strength for handling and characterization.

In conclusion, membranes loaded with 70 wt.% activated carbon particles contain relatively the highest amount of adsorptive sites, show a porous interconnected structure and have sufficient mechanical strength. Therefore these membranes are selected for

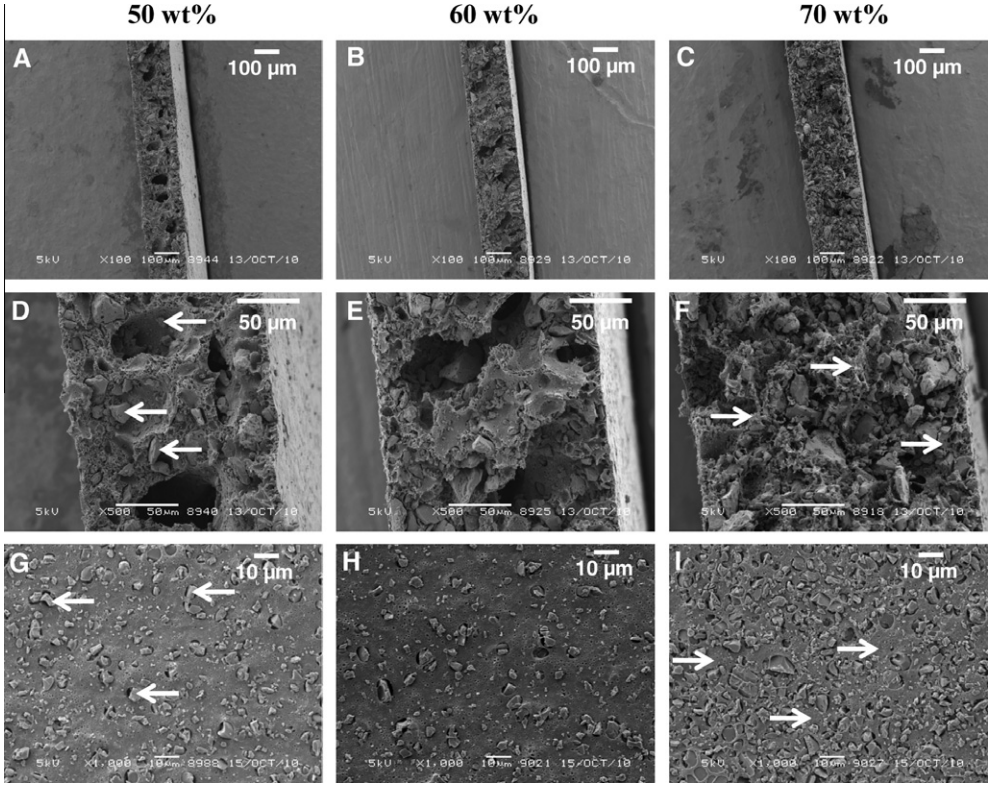

Fig. 3. Scanning electron microscopy pictures of cross-sections (A, B, C, D, E and F) and bottom surface sections (G, H and I) of single-layer mixed-matrix membranes with loadings of 50 wt.% (A, D, G), 60 wt.% (B, E, H) and 70 wt.% (C, F, I). The arrows in D and G indicate the activated carbon particles whereas arrows in F and I indicate the porous polymeric membrane matrix.

further characterization and development of dual-layer mixed-matrix membranes.

3.2. Dual-layer mixed-matrix membranes

To obtain dual-layer MMMs, we co-cast 70 wt.% particle-loaded polymer solution with a particle-free polymer solution. This particle-free layer will be the blood-contacting membrane side to avoid direct blood contact with the embedded sorbents during blood purification. Furthermore, it will prevent particle release into the circulation and consequent emboli formation.

Fig. 4A and B presents a single-layer MMM and dual-layer MMM respectively. The dual-layer MMM has a rather open interconnected porous structure. The particle-loaded layer of the dual-layer MMM possesses a sponge-like structure. Some small round-shaped voids are present in this layer but macro-voids through the entire cross-section are absent. The particle-free layer has a dense top layer and some macro-voids are present below this layer.

Fig. 4C presents the single particle-free membrane, which was cast directly on a glass plate. This single particle-free membrane has a homogenous sponge-like structure, whereas the particle-free layer in the dual-layer MMM has a dense sponge-like top layer but with a more open sub-layer with macro-voids. Besides, the thickness of the two layers of the dual-layer MMM is not in agreement with the casting thicknesses. Probably, the co-casting process, different viscosities of the dopes and different shrinkage of the two membrane layers during phase separation influence the final membrane structure of the dual-layer MMM.

Figs. 5A and B show photos of a single-layer mixed-matrix membrane and dual-layer mixed-matrix membrane respectively. In the dual-layer MMM, the membrane layer with the particles is black and the particle-free layer is white and completely covers the layer with the particles, see Fig. 5B. Fig. 5C shows that the layer with the particles is rather rough whereas the particle-free layer has a more smooth surface and smaller pores.

In dual-layer MMM, the two different layers can clearly be distinguished and are well attached to each other. In fact, no delamination of the two membrane layers was observed. These 70 wt.% loaded single-layer membranes and dual-layer membranes are further characterized.

3.3. Membrane transport properties

Fig. 6 shows the clean-water flux at various transmembrane pressures. For single-layer MMMs, the clean-water permeance is 1839 ± 55 lm^{-2} h^{-1} bar^{-1} based on the slope up to 0.5 bar. Although these membranes were pre-pressurized before the measurement, the flux–transmembrane pressure relationship at higher pressures is not linear. This might be due to membrane compaction during the measurement or possibly relocation of the particles in the matrix which might close the bigger channels. For dual-layer MMMs the permeance is significantly lower, 350.7 ± 69 lm^{-2} h^{-1} bar^{-1} ($p < 0.05$) and the flux–transmembrane pressure relationship is linear in the used pressure range. The decrease in clean-water permeance for the dual-layer MMMs is probably due to the additional particle-free layer, which has a dense sponge-like skin structure.

The single-layer MMMs have a BSA sieving coefficient of 0.8 ± 0.1. The dual-layer MMMs have a significantly lower sieving coefficient of 0.4 ± 0.2 ($p < 0.05$). It seems that the additional particle-free layer tailors the transport through the membrane. For future applications the molecular weight cut-off of the membrane is important and can probably be tailored by optimization of the particle-free layer.

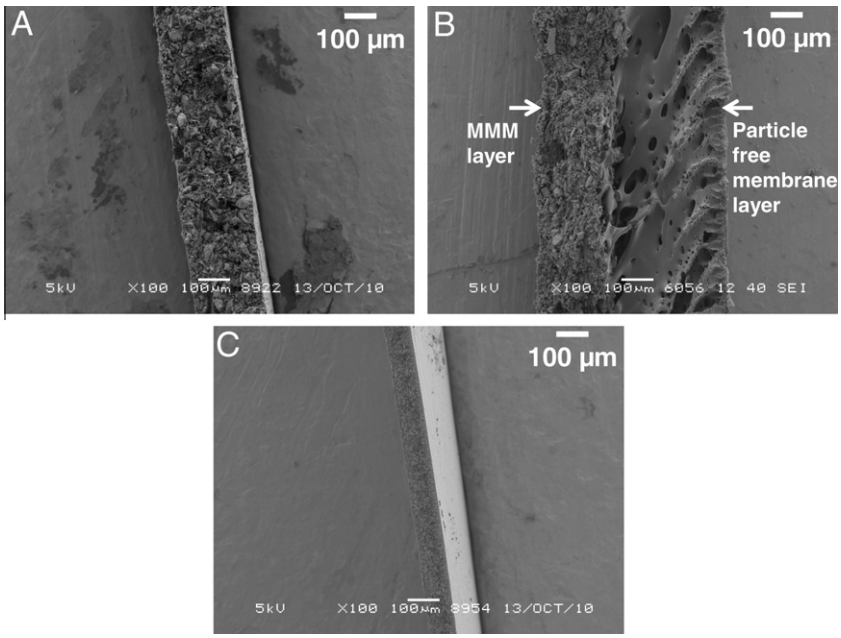

Fig. 4. Scanning electron microscopy pictures of cross-sections of a single-layer MMM (A), dual-layer MMM (B) and particle-free membrane (C).

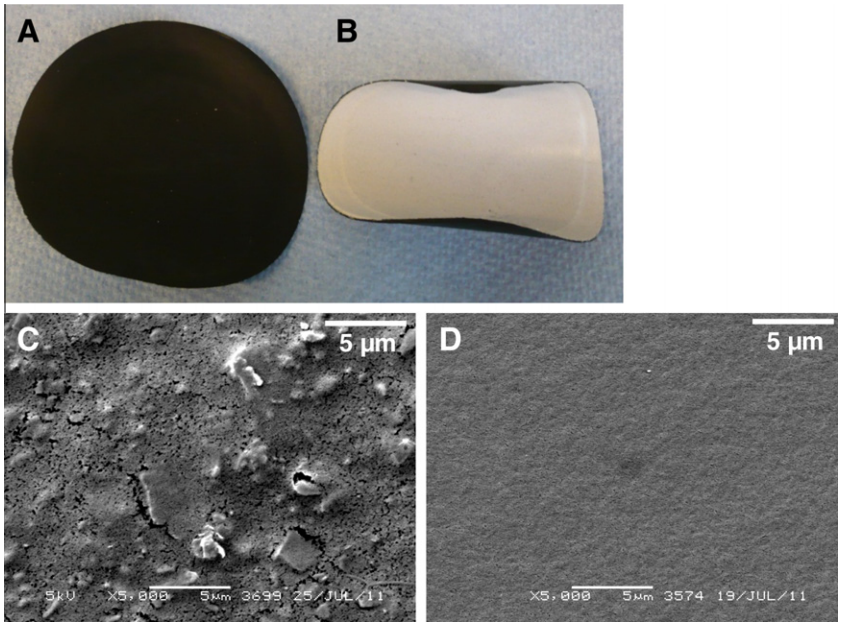

Fig. 5. Surface area pictures and SEM pictures of a single-layer MMM (A, C) and dual-layer MMM (B, D) respectively.

3.4. Creatinine adsorption isotherms

For single and dual-layer MMMs, the creatinine adsorption at various concentrations was measured. The adsorption capacity is expressed in mg adsorbed creatinine per g of activated carbon. Hence the exact particle proportion in the MMM is necessary and is calculated (via the equations in the supplement) to be 0.68 and 0.53 for single- and dual-layer MMMs respectively.

Fig. 7 presents the results expressed in adsorption capacity (q) vs. the equilibrium creatinine concentration (C). The isotherms of single and dual-layer MMMs are almost identical and appear to be of Langmuir type. For the tested concentration range the best Langmuir isotherm curve fit has $q_m = 234\ \text{mg g}^{-1}$ AC and $K_d = 0.351\ \text{mg ml}^{-1}$. The range of creatinine concentrations used here (0–0.15 mg/mL) is relevant because the concentrations are close to the creatinine levels in the normal (0.012 mg/mL) and uremic situation (0.136 mg/mL).

Compared to other studies, the adsorption capacity of the mixed-matrix membranes is high. For example at an equilibrium concentration of 0.05 mg ml^{-1} our MMMs adsorb 29 mg creatinine per g of activated carbon. At the same equilibrium creatinine concentration, Deng et al. reported adsorption of 13 mg creatinine per g of activated carbon [34] for PES and activated-carbon-based hybrid beads, and Ye et al. reported adsorption of 15–20 mg

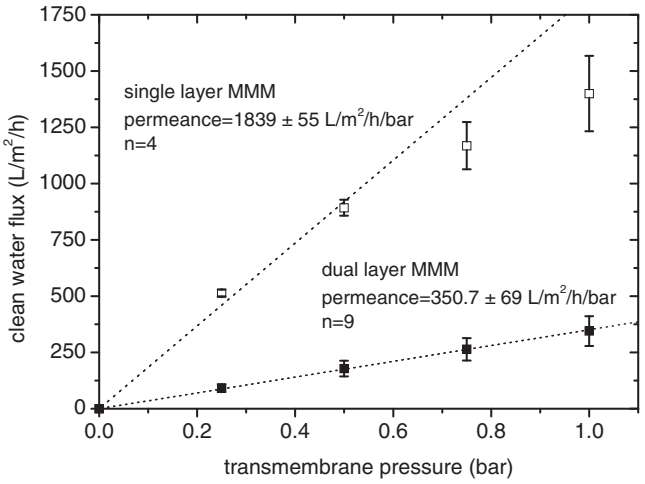

Fig. 6. Average clean-water flux plotted against transmembrane pressure, for single-layer MMMs (□) and dual-layer MMMs (■). The error bars indicate standard deviations.

creatinine per g of activated carbon or carbon nanotube [35]. In another approach using polymeric micro-spheres, the creatinine adsorption was less than 10 mg per gram of micro-sphere [36].

3.5. Human plasma adsorption

Fig. 8A presents average relative creatinine concentrations after incubation in plasma for 4 h, which is an average duration of a hemodialysis treatment. The particle-free membrane does not significantly lower the creatinine concentration. However, more than 80% of the creatinine is removed by the single- and dual-layer membranes, which is in excellent agreement with the removal achieved by the activated particles alone under these experimental conditions. This shows that the accessibility of the particles in the MMM is excellent. These creatinine adsorption results also fit very well to the isotherm obtained with model creatinine solutions (as presented in Fig. 7), suggesting that even for the more complex human plasma the accessibility of our membranes is optimal.

The results of PAH removal by the membrane (see Fig. 8B) are consistent with the results of creatinine. The particle-free membrane does not seem to lower the PAH concentration, whereas the single and dual-layer MMMs seem to remove more than 80% of PAH similar to the removal obtained by the activated carbon particles alone. These results indicate that the developed MMMs can probably remove protein-bound compounds, and the accessibility of the particles there seems to be similar to the accessibility of the particles alone. Protein-bound solutes are thought to contribute to uremic toxicity and are hardly removed by hemodialysis [37,38]. Adsorption has been proposed as a way to improve removal of these toxins [39,40] and it has been shown that the addition of activated carbon to the dialysate compartment of a hemodialyzer can improve protein-bound toxin removal [41,42]. Our first results suggest that the MMM could be suitable for the improvement of protein-bound toxin removal.

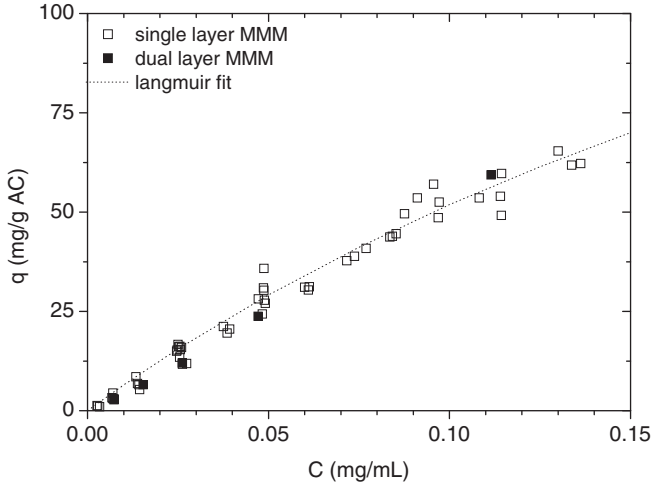

Fig. 7. Creatinine isotherms for single (□) and dual-layer (■) MMMs. The equilibrium adsorption capacity (q) expressed in mg adsorbed creatinine per gram of activated carbon is plotted against the equilibrium creatinine concentration (C). The dotted line represents the calculated Langmuir isotherm fit.

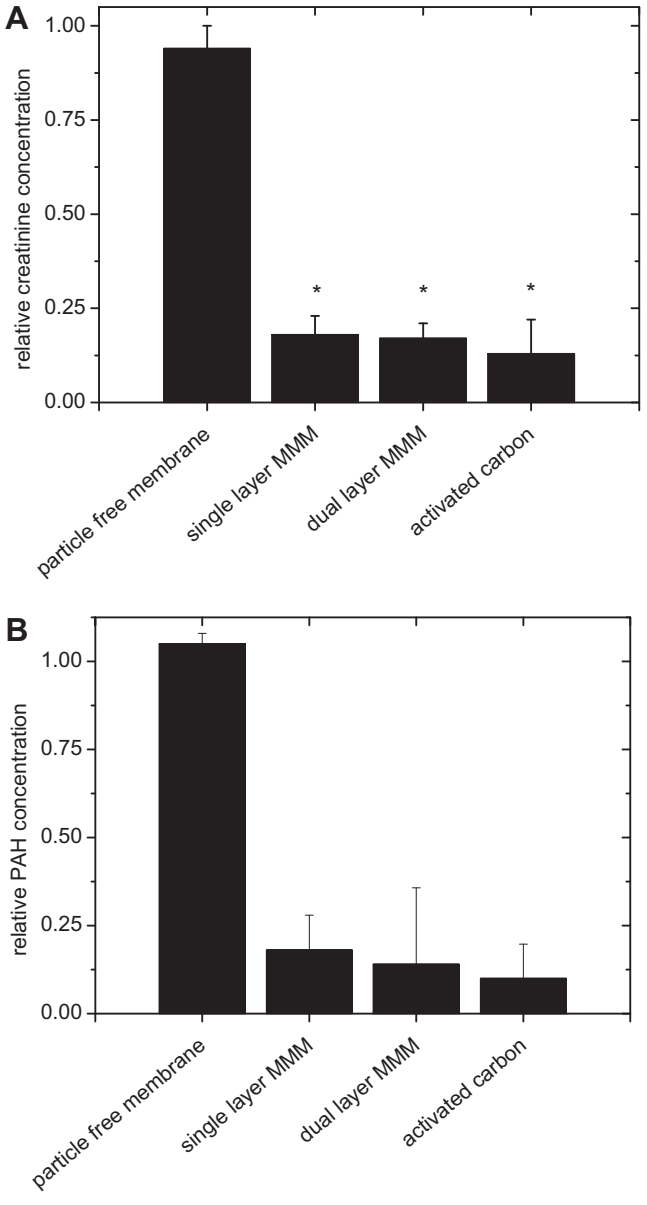

Fig. 8. (A) Average relative creatinine concentration after incubation of a particle-free membrane, single-layer MMMs, dual-layer MMMs or pure activated carbon in plasma ($n = 6$) for 4 h. * indicates $p < 0.05$ in comparison with a particle-free membrane. (B) Average relative PAH concentration after incubation of a particle-free membrane, single-layer MMM, dual-layer MMM or pure activated carbon particles in plasma ($n = 2$) for 4 h.

Table 2
Relative osmolarity, pH and total protein concentration after incubation in human blood plasma ($n = 6$) for 4 h.

Sample	Relative osmolarity	Relative pH	Relative total protein concentration
Activated carbon	0.99 ± 0.01	1.02 ± 0.04	0.93 ± 0.02
Single-layer MMM	1.00 ± 0.01	1.02 ± 0.02	0.94 ± 0.05
Dual-layer MMM	0.98 ± 0.01	1.00 ± 0.01	1.01 ± 0.07
Particle-free membrane	1.01 ± 0.01	1.03 ± 0.02	1.03 ± 0.06

It is also important to note that while under these experimental conditions our single- and dual-layer MMMs seem to remove more than 80% of the creatinine and PAH from the human plasma, they do not cause any significant changes to the plasma pH, osmolarity and the total protein concentration (see Table 2). The latter indicates that undesired general protein binding is limited.

Direct comparison of the performance of our membranes in human plasma with other literature studies is rather difficult since not all experimental conditions are the same.

In this study, 25 mg of adsorbent was incubated in 4 ml plasma. This proportion can be related to the proportion used in hemoperfusion, where ~3000 ml plasma is in contact with a hemoperfusion column containing ~300 gram of activated carbon (Adsorba 300, Gambro). This gives a sorbent–volume proportion of 100 mg per ml of plasma. In our experiments the sorbent–volume proportion is lower, 6 mg per ml of plasma. This indicates that when we would apply a similar sorbent–volume proportion as in hemoperfusion, the obtained relative removals by our MMMs could even be higher.

Perhaps the best way of comparing adsorption results is by means of isotherms at similar equilibrium concentrations of model solutions but which are unfortunately not often discussed in the literature. Nonetheless, there are a few studies with which some comparison can be done. For example, Malchesky et al. developed a blood purification device containing charcoal encapsulated in semi-permeable hollow tubing. A 35 l test solution with an initial concentration of 0.095 mg ml^{-1} creatinine was pumped through a device containing 37 mg activated carbon (sorbent–volume proportion of 0.001 mg ml^{-1}) and 30.1% of the creatinine was removed after 4 h [43]. The Vicenza Wearable Artificial Kidney for peritoneal dialysis contains less than 200 g adsorptive particles, 40% polystyrenic resins, 60% activated carbon with the latter mainly removing creatinine. A 12 l dialysate solution was pumped through the device and 94.2% of the creatinine was removed after 4 h [21].

3.6. Creatinine diffusion through MMMs

For estimating the transport of uremic retention solutes through our membranes, we tested diffusion through and adsorption onto dual-layer MMMs using a two-compartment diffusion device with a 0.1 mg ml^{-1} creatinine feed solution. This concentration is clinically relevant as it is very close to the mean/median uremic creatinine concentration measured in uremia of 0.136 mg ml^{-1} [27]. Fig. 9 shows that after some time the diffused creatinine starts increasing and continues in time. Adsorption of creatinine starts almost immediately, and after more than 24 h seems to reach a plateau. The contribution of creatinine removal after 7 h by adsorption is over 80% of the total creatinine removal. No quick saturation occurs for the developed MMMs under these experimental conditions. The MMM combines uremic retention solute removal via both diffusion as well as adsorption in one single step. This is a novel and promising approach in the extracorporeal blood purification technology. In this paper we showed the first proof of concept; in the future, we foresee several opportunities for MMMs in the use of blood purification. MMMs could be used in a wearable artificial kidney (WAK). The MMM combines two methods of blood purification in one step (adsorption and membrane-based removal). This could enhance the miniaturization of the device. Besides, MMMs might be useful for dialysis with less dialysate (in a WAK), making sure that sufficient amounts of toxins can be removed. Or MMMs could be placed in a separate circuit for regeneration of the dialysate, which could be useful for a WAK system. Furthermore, the MMM may function as an adsorptive barrier for endotoxins, thereby preventing endotoxins from the dialysate entering the patients circulation.

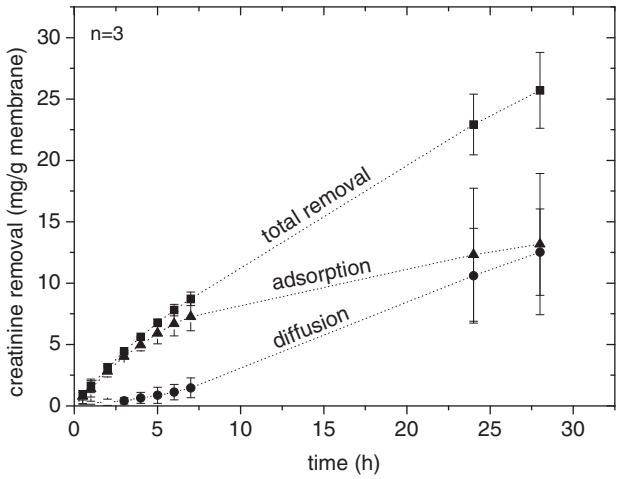

Fig. 9. Average creatinine total removal, diffusion and adsorption plotted vs. time, $n = 3$. Total removal (■) is the amount of creatinine removed from the donor compartment. This removal is mediated by diffusion (●) and adsorption (▲) of creatinine. Error bars indicate standard deviations. The dotted lines are plotted to guide the eye.

4. Conclusions and outlook

This work presents a novel approach for uremic retention solute removal combining diffusion and adsorption. Dual-layer MMMs were formed consisting of a particle-free membrane layer and a mixed-matrix membrane layer containing activated carbon. The dual-layer MMMs have a clean-water permeance of ~350 lm^{-1} h^{-1} bar^{-1}, and show rather high creatinine adsorption of ~29 mg creatinine per g of activated carbon at equilibrium concentration of 0.05 mg ml^{-1}. Moreover, both single- and dual-layer MMMs significantly reduce the creatinine concentration in human blood plasma, without general effects on osmolarity, pH and total protein concentration.

Our future plans will focus on removal of a broad range of uremic toxins including other protein-bound toxins and middle molecules since these are difficult to remove with current hemodialysis. Adsorption-based removal with MMMs might improve the clearances of these toxins as well.

We will also fabricate and optimize dual-layer mixed-matrix hollow fiber membranes with a thin particle-free inner layer. Recent literature studies have shown that it is possible to prepare dual-layer membranes and dual-layer MMMs with different characteristics (material, layer thickness, particle type, etc.) [44–49]. Our focus will be on tailoring the membrane structure by parameters like spinneret design, pumping speeds and polymer and bore liquid compositions to achieve optimal toxin removal.

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Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figs. 2 and 5, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at http://dx.doi.org/10.1016/j.actbio.2012.03.008.

Appendix B. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2012.03.008.

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