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New mixed matrix membrane for the removal of urea from dialysate solution

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ABSTRACT

Urea removal is one of the biggest challenges in dialysate regeneration in Wearable Artificial Kidney (WAK) devices. In this work, a new Mixed Matrix Membrane (MMM) is developed for urea removal in WAK applications. The MMM consists of polystyrene-based ninhydrin particles within a polyethersulfone/polyvinylpyrrolidone polymer blend matrix. The MMM is prepared via dry-wet spinning technique and characterized in terms of its morphology via electron microscopy and clean water permeance. Urea removal is studied both in static and in dynamic conditions. Thanks to the good dispersion of small size ninhydrin particles (size < 63 μm), the MMM removed under static conditions, at 70 °C, 2.1 ± 0.1 mmol of urea per grams of particles at 24 h, while urea removal by the particles in suspension reached 1.7 ± 0.1 mmol/g under the same conditions. Importantly, in continuous recirculation experiments, performed at 70 °C using a laboratory scale module, the MMM removed 3.4 ± 0.3 mmol of urea per grams of particles, in 4 h, due to the high particle accessibility by urea within the membrane. Based on these results it is estimated that only 215 g of MMM are needed for removing the daily produced urea from spent dialysate (400 mmol) making MMM suitable for application to WAK, where miniaturization and lightweight are required.

1. Introduction

Hemodialysis (HD) treatment is a life-sustaining therapy for the treatment of end stage kidney disease (ESKD) patients waiting for or not being suitable for kidney transplantation. However, due to the intermittent treatment, performed mostly in the clinics 4 h − 3 times per week, accumulation of toxins and excess water occurs in between dialysis sessions [1,2].

Portable (PAK) and wearable artificial kidney (WAK) devices have been proposed to improve blood purification as well as patients' quality of life. These systems would provide continuous or semi-continuous blood purification, mimicking closer the natural kidneys, leading to higher clearance of toxins and higher removal of excess water [3,4]. Moreover, they would allow for dialysis at home, improving patients' mobility and participation in social life and would significantly lower the amount of water needed for the treatment [5–7]. In fact, for a standard HD session of 4 h 280 – 500 L of water is used to generate 120 L of pure dialysate [8,9]. In contrast, for a PAK and/or WAK a small volume of spent dialysate (preferably 0.5 L or lower) would be continuously regenerated and recirculated to guarantee portability and wearability [6,7]. In order to regenerate the spent dialysate, ions such as phosphate and potassium, small organic waste solutes such as creatinine and urea and middle molecules such as β2-microglobulin need to be removed [10]. Ion-exchangers can be used for the removal of phosphate and potassium, while activated carbon can efficiently remove most organic waste solutes [3,5,10,11]. However, the biggest challenge is the removal of urea [10] which is the main waste product of nitrogen metabolism (waste solute with the highest daily molar production of 240 to 470 mmol, depending on protein intake) [12,13]. High urea plasma concentrations (in the range 20–30 mM, as for the ESKD patients) are associated with toxicity, including insulin resistance, disruption of the gastrointestinal barrier, production of radical oxygen species and endothelial changes promoting atherosclerosis [14]. Urea removal from spent dialysate is difficult because of its small molecular weight (MW =

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60 g/mol) and because of the absence of charges at physiologic pH, which does not allow for the formation of ionic bonds [10,15]. Many strategies have been proposed for its removal from spent dialysate. For example, enzymatic decomposition of urea by means of ureases is very efficient and was applied in the REDY (Recirculation of DialYsis) device. However, this system was withdrawn from the market because of production of toxic byproducts among which ammonia, high costs and lower efficiency compared to single-pass HD [16–20]. Electrooxidation of urea, which converts urea into nitrogen, carbon dioxide and hydrogen gases, is quite efficient too. The formed gases can be easily removed by a bubble trap. Nevertheless, toxic side products, as nitrate, nitrite, ammonia, chloramines and active chlorine species are formed [10,21–24]. Several sorbents have also been proposed for the removal of urea from spent dialysate via physisorption (non-covalent binding), coordination or covalent binding (chemisorption) [10]. Activated carbon [25–28], silica [29], zeolites [25,30] and MXenes nanosheets were used for urea removal via physisorption [31]. However, they have rather low affinity for urea in water because they rely on hydrogen bonding, van der Waals and dipole interactions [10]. Urea removal via chitosan complexed with copper ions has also been proposed [32–35]. However, although various studies reported high urea binding capacity, the toxicity derived by potential copper leaching is a concern [10]. Several other studies report the synthesis of urea-molecular imprinted polymers for urea detection and/or removal [36–39]. There, competition by other dialysate components for binding is avoided. However, as for the work of Alizadeh et al. [37], many questions arise from the high urea binding capacity compared to the much lower theoretical maximum urea binding capacity of the imprinted polymer [10]. Carbonyl-type sorbents, although not selective towards urea, possess high urea binding capacity, are not toxic when immobilized in the dialysate circuit and can form irreversible covalent bond between the electrophilic carbonyl groups and weakly nucleophilic urea molecules [40]. Several sorbents containing urea-reactive carbonyl groups have been reported in literature, such as aldehydes, α-ketoaldehyde hydrates, ninhydrins, α-ketoesters and glyoxaldehydes [41–50]. The urea binding kinetic is slow, but it can increase at higher temperatures [10,41,50,51]. Thus far, carbonyl-type sorbents have been investigated for urea removal only in suspension or in bed column systems [41–48,51,52], where high pressure drop, rather suboptimal particle dispersion and aggregation could limit the amount of available binding sites, thus reducing their removal capacity.

In earlier studies, we have shown that the application of a Mixed Matrix Membrane (MMM), consisting of activated carbon sorbent particles embedded within a polyethersulphone (PES)/polyvinylpyrrolidone (PVP) polymer matrix, can remove a high amount of uremic toxins from blood plasma. The optimal distribution of small sorbent activated carbon particles in the MMM can achieve optimal particle accessibility leading to high toxins removal [53–55]. Here, we develop of a new MMM hollow fiber (HF) for urea removal from dialysate solution. This MMM consists of a carbonyl-type sorbent that contains reactive ninhydrin groups and is prepared from polystyrene particles (PS-Nin particles) embedded within a PES/PVP porous polymer matrix. The synthesis of the PS-Nin particles and their application for urea removal from dialysis fluid have been reported elsewhere [48,51]. They have quite high theoretical urea binding capacity of approximately 2.7 mmol/g, considering that approximately 55 % of the phenyl groups of polystyrene are transformed in ninhydrin units, as assessed by Smakman et al. [48,51]. Moreover, this material can be sterilized, does not leach any compounds into the dialysate and has been even suggested for oral use [51]. Here, we hypothesize that the optimal dispersion of small size PS-Nin sorbent particles within the MMM would minimize particle aggregation and lead to high particle accessibility and therefore high urea removal kinetics and maximum urea binding. The MMM hollow fiber was produced via dry-wet spinning technique and is characterized for morphology (via scanning electron microscopy) and transport properties (clean water flux). The effects of sorbent particle size, temperature, and incorporation in the polymer matrix on urea removal were first studied in static experiments. PES/PVP HF (without particles) was used as control to verify that the embedded PS-Nin particles were mainly responsible for urea removal. Finally, dynamic urea removal experiments, where a small volume of dialysate model solution spiked with urea was recirculated for 4 h through the MMM HF, were performed to assess the urea removal by the MMM under conditions better mimicking PAK or WAK systems.

2. Materials and methods

2.1. Polystyrene-ninhydrin (PS-Nin) beads

Polystyrene beads containing indanone groups (PS-Ind beads), synthesized as described by Smakman et al. [48,51], were obtained from Innovista (Nigtevecht, the Netherlands). The indanone groups in the particles were oxidized into ninhydrin groups using a method described previously (90 °C, 1.0 eq. iodine and hydrogen iodide, 24 h) [50]. To confirm the oxidation of the indanone groups to ninhydrin groups, the sorbent PS-Nin beads were characterized by means of FTIR spectroscopy and compared to the FTIR spectrum of ninhydrin (see Fig. 2S of Supplementary Information). Also, the ability of the ninhydrin groups on the PS-Nin beads to bind urea was investigated via FTIR spectroscopy by comparing the infrared spectra of the PS-Nin beads after reaction with urea with that of the ninhydrin-urea complex (Fig. 2S of Supplementary Information). These particles have theoretical urea binding capacity of 2.7 mmol/g, considering that the predicted amount of phenyl groups of polystyrene transformed in ninhydrin units is 55 %, as reported by Smakman et al. [48,51]. The PS-Nin beads have an average diameter of 483 ± 282 μm (see Fig. 1S of Appendix A). The surface area (SBET) and the pore volume of the PS-Nin beads, as determined by N_2 sorption isotherm studies, are 23.7 m^2/g and 0.14 mL/g, respectively (see Fig. 3s of Appendix A).

2.2. Membrane fabrication

Prior to membrane fabrication, PS-Nin beads were grinded using a mortar and pestle. Afterwards, the grinded PS-Nin particles were sieved through a 63 μm sieve. Throughout the manuscript, “PS-Nin beads” indicate the large polystyrene-ninhydrin beads before grinding and sieving and “PS-Nin particles” indicate the small polystyrene-ninhydrin particles obtained after grinding and sieving. The HF MMM was prepared by incorporating PS-Nin particles within PES/PVP polymer matrix. The HF MMM was produced via dry-wet spinning technique. The polymer dope solution was prepared by dissolving Ultrason E6020 PES (BASF, Ludwigshafen, Germany) and PVP K90 (MW ≈ 360 kDa, Sigma-Aldrich Chemie GmbH, Munchen, Germany) in ultrapure N-methylpyrrolidone (NMP) (Acros Organics, Geel, Belgium) (see dope composition on Table 1). PS-Nin particles were added to the dope solution to have a final weight of particles equal to 55% of the weight of the membrane. The PES/PVP/PS-Nin particles mixture was stirred for two days at 60 °C to ensure well-dispersion of the particles in the polymer solution. Afterwards the dispersion was transferred into stainless-steel syringes and left to degas for 24 h. The concentrations of PES, PVP and PS-Nin particles and the spinning parameters used in the study are specified in Table 1. After degassing, the syringe was connected to a high-pressure syringe pump and to a designed spinneret for preparing the HF (specifications given in Table 1). Ultrapure water was used as bore forming solution. The airgap between the spinneret and the coagulation bath was adjusted to 5.5 cm. The HF was left to free-fall in the water coagulation bath. The fabricated membrane was washed with demineralized-water and stored in demineralized-water for further use. Besides the MMM, we also fabricated PES/PVP HF without sorbent particles (see fabrication protocol in Appendix A) to be used as control.

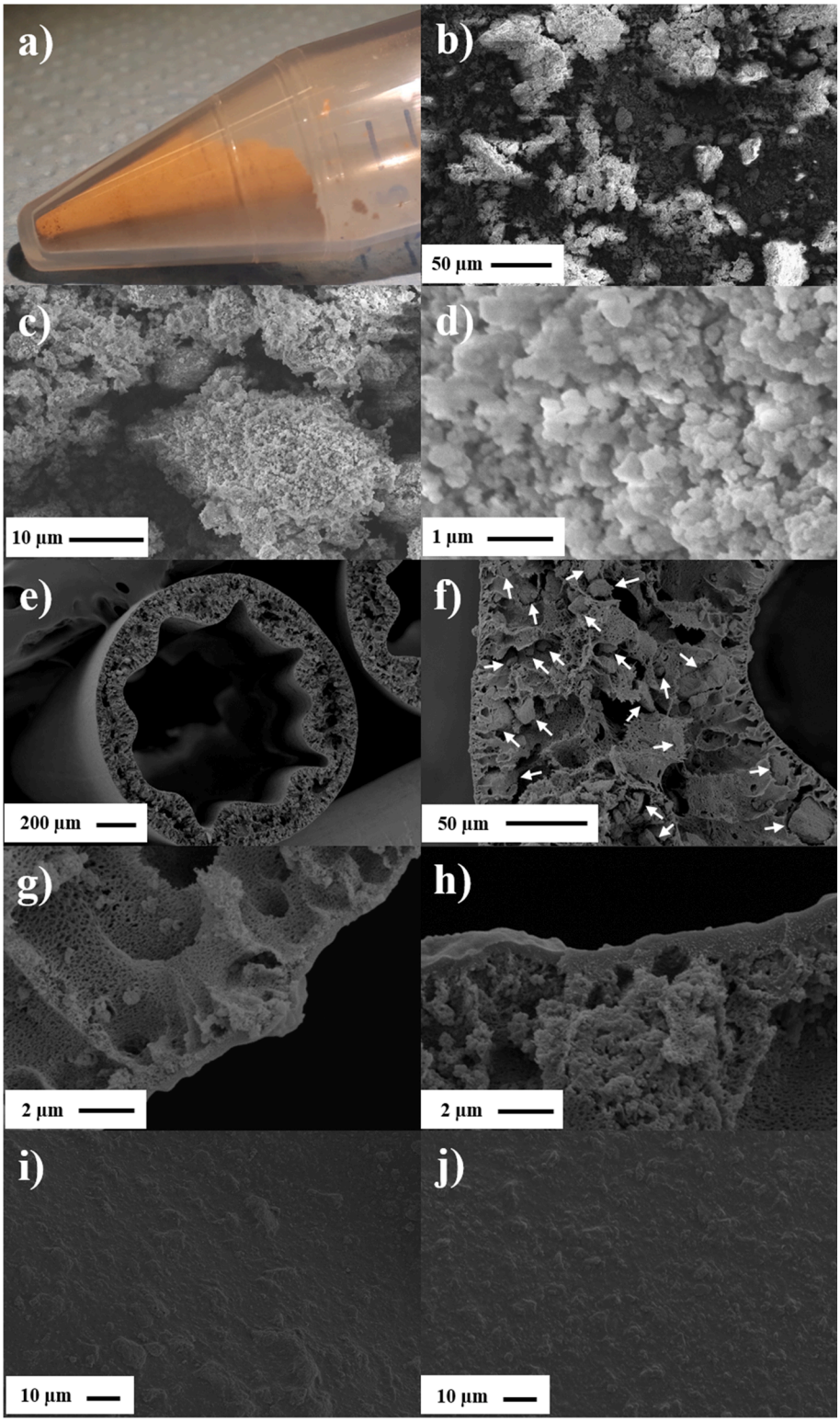

Fig. 1. a) Picture of PS-Nin particles after grinding and sieving. SEM images of PS-Nin particles after grinding and sieving at different magnifications: b) 370X, c) 2300X, d) 20000X. SEM images of the MMM: e) cross-section, f) magnification of the wall, g) magnification of the lumen layer, h) magnification of the outer layer, i) magnification of the lumen surface, j) magnification of the outer surface.

2.3. Scanning electron microscopy (SEM)

The morphology of the HF MMM and of the PS-Nin particles after grinding and sieving was analyzed by SEM (JEOL JSM-IT 100, Tokyo, Japan). Membrane samples were dried in air and fractured in liquid nitrogen for the imaging of the cross-sections. Prior to SEM imaging, the samples were gold sputtered using the Cressington 108 auto sputter (Cressington Scientific Instruments, Watford, UK).

2.4. Water transport experiments

Membrane modules composed of 3 HF with a total surface area of

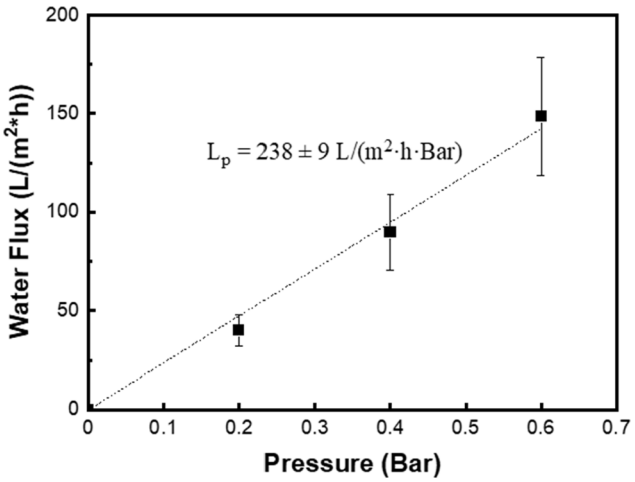

Fig. 2. Water flux across the MMM at various TMP. The slope of the graphs represented the MMM water permeance (n = 6; average ± standard deviation).

13.9 ± 0.1 cm^2 were used. A 2-compontent epoxy glue (Griffon Combi Snel-Rapide, Bison International, Goes, the Netherlands) was used for the preparation of the modules. Before water transport experiments, the HF modules (n = 6) were pre-wetted with ethanol for 30 min at a *trans*-membrane pressure (TMP) of 0.2 Bar and pre-compacted with ultrapure water at a TMP of 0.6 Bar for 30 min. Afterwards, the amount of permeated water was measured over time at TMP of 0.2, 0.4 and 0.6 Bar. The resulting water permeance was calculated as the slope of the linear fit of the flux (L/(m^2·h)) versus the TMP (Bar).

2.5. Static urea removal studies

2.5.1. Effect of particle size on urea removal kinetics

In order to study the effect of the particle size on urea removal, kinetic urea removal experiments were performed at 70 °C on PS-Nin beads and on the PS-Nin particles. Both (15 mg, each) were incubated in urea solution (1.5 mL, 30 mM) in PBS (phosphate buffer saline, pH 7.4). The samples (n = 3 for each time point) were shaken at 70 °C in a water batch and after 1, 2, 4, 8, 16 and 24 h the supernatants were

Table 1
Spinning parameters used for the fabrication of the MMM.

Dope composition (PES/PVP/PS-Nin Particles/NMP)	6.6/3.1/11.9/78.4 wt%
Dope pumping speed	1 mL/min
Spinneret - Thickness dope orifice	0.6 mm
Bore liquid	ultrapure water
Bore liquid pumping speed	1 mL/min
Spinneret – Diameter bore needle	1.35 mm
Air gap	5.5 cm
Coagulation bath composition	ultrapure water
Fiber's collection	free falling

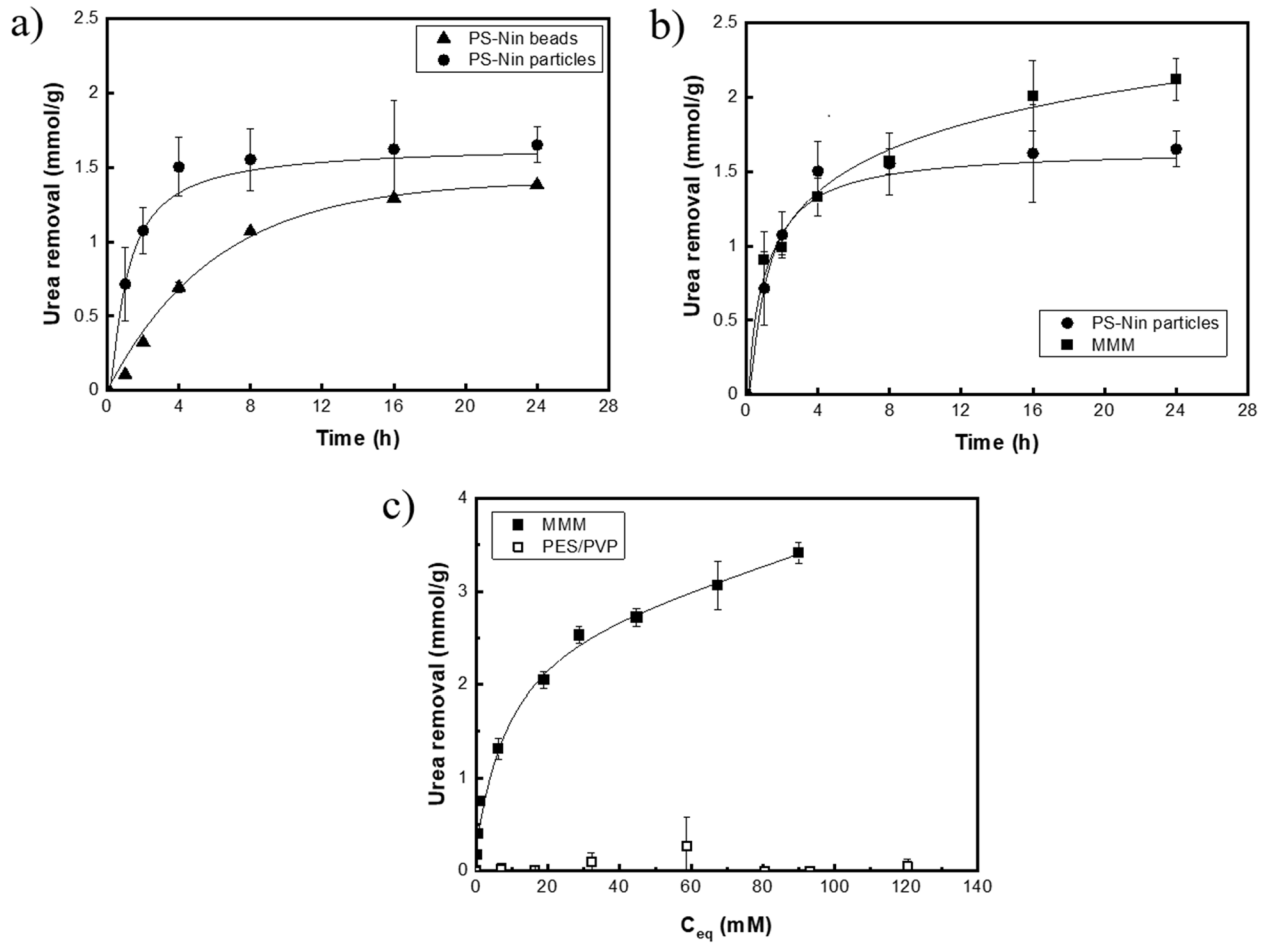

Fig. 3. a) Static urea removal of the PS-Nin beads (483 ± 282 μm) and of the PS-Nin particles (<63 μm). b) Static urea removal of the PS-Nin particles and of the MMM. c) Urea binding isotherm of MMM and PES/PVP control HF. For a), b) and c) the experiments were performed at 70 °C. The data are expressed as average ± standard deviation (n = 3). Urea removal of the MMM is calculated as millimoles of urea removed per grams of particles incorporated in the membrane matrix. Urea removal of the PES/PVP HF is calculated as millimoles of urea removed per grams of membrane.

collected via filtration. Urea concentration in the supernatant was determined as described in section 2.7.

2.5.2. Effect of incorporation of PS-Nin particles in the MMM

A urea removal experiment was performed at 70 °C with the MMM containing PS-Nin particles prepared and characterized as described in section 2.1. Results were compared with the urea removal kinetics of the PS-Nin particles alone (see 2.5.1). The MMM (27 mg containing 15 mg of PS-Nin particles, n = 3) was incubated in dialysate model solution (1.5 mL), consisting of urea (30 mM), 2 mM KCl, 140 mM NaCl, 1.5 mM $CaCl_2$, 0.25 mM $MgCl_2$, 35 mM $NaHCO_3$ (all from Sigma-Aldrich Chemie GmbH, Schnelldorf, Germany) and 5.5 mM glucose (Life Technologies Europe BV, Bleiswijk, the Netherlands) in ultrapure water at pH 7.4 and shaken at 70 °C in a water bath. After 1, 2, 4, 8, 16 and 24 h, the supernatants were collected, and the urea concentration was determined as described in section 2.7.

2.5.3. Urea binding isotherm of the MMM HF and PES/PVP control HF.

The binding of urea by the MMM and a PES/PVP control HF was measured at various concentrations. PES/PVP control HF was prepared via dry-wet spinning technique as described by Geremia et al. [55] (see Appendix A). 27 mg of the MMM (15 mg of particles in the MMM, n = 3) and 27 mg of the PES/PVP control HF (n = 3) were incubated in Eppendorf tubes in 1.5 mL dialysate model solution (pH 7.4) spiked with urea at different concentrations. The samples were placed in a horizontally shaking water bath at 70 °C. After 24 h, the supernatants were collected and the urea concentration in the supernatants was determined as described in Section 2.7.

2.6. Dynamic urea removal by the MMM

Membrane modules composed of 3 HF with a total surface area of 23.3 ± 0.4 cm^2 and with a total amount of PS-Nin particles equal to 77 mg were used to study urea removal in dynamic conditions. A 2-compontent epoxy glue (Griffon Combi Snel-Rapide, Bison International, Goes, the Netherlands) was used for the preparation of the modules. Before dynamic urea removal experiments, the HF modules (n = 4) were kept in demineralized water. Urea dynamic experiments were performed in filtration mode (TMP = 0.15 Bar) with 30 mM urea in a dialysate model solution continuously recirculated through the fibers at a flow rate of 20 mL/min using a dedicated set up (Convergence, Enschede, the Netherlands). The removal experiments were performed at 70 °C for 4 h. In order to maintain a temperature of the urea solution of 70 °C inside the recirculation system, the feed solution was heated at 70 °C, the tubing was insulated, and the HF module was immerged in a water bath heated at 90 °C. In that way, the temperature within the module was kept as 70 °C, which was confirmed by measuring the temperature of the urea solution at the exit of the module. Samples of the urea solution were collected every hour for quantification. At the end of the experiment, the HF module (n = 2) was removed from the water bath and was totally emptied. 24.5 mL of ultrapure water at room temperature were recirculated through the module at a flow rate of 20 mL/min for 1 h in order to elute unbound or loosely bound urea from the MMM. Urea concentration was determined as described in section 2.7.

2.7. Quantification of urea concentrations

For the urea removal experiments with the PS-Nin particles, the MMM and the PES/PVP control HF, urea concentrations were determined by the enzymatic assay Urea FS* (Diasys, Holzheim, Germany). For the urea removal experiments with the PS-Nin beads, an AU 5800 routine chemistry analyzer (Beckman Coulter, Brea, CA) for determination of urea concentrations was used. Both methods are based on a coupled enzyme reaction, which results in a colorimetric product proportional to the urea concentration. Via the mass balance, the amount of urea adsorbed was calculated from the depleted amount of urea in the solution.

3. Results and discussion

3.1. Membrane morphology

Fig. 1 presents a photo and SEM images of the PS-Nin particles and of the developed HF MMM. The MMM is composed of blend of PES and PVP, which are polymers already widely used to produce HD membranes. PES has exceptional filtering characteristics, thermal stability, mechanical strength, chemical inertness, and it can withstand all sterilization techniques [56]. In order to develop a membrane with high water transport and low fouling properties, we blended PES with PVP, a non-ionic, highly polar, physiologically inert water-soluble polymer which acts as hydrophilic agent [57].

SEM imaging of the PS-Nin particles (Fig. 1 c-d) reveals that the particles are characterized by a granular morphology, where the biggest particles are composed by stable aggregates of submicron particles. The cross-section image of the MMM (Fig. 1e) shows a corrugated lumen morphology. The grooves are well axially aligned with the flow direction, and they are not expected to disturb mass transfer, flow rate and transmembrane pressure along the fiber. In addition, their presence in the lumen side of the fiber increases the active membrane surface area and therefore the membrane flux. Also, the rather thick membrane wall allows high particles loading, thus enhancing binding properties of the MMM. From the magnification of the wall of the MMM (Fig. 1f), a finger-like macrovoids structure, typical of PES-based membranes [58,59], is visible at the lumen and outer sides of the membrane. These macrovoids vanish along the center of the wall cross-section, where more PS-Nin particles are hosted in the polymer matrix. The sorbent particles are quite well dispersed in the polymer matrix (see white arrows in Fig. 1f). Both the lumen and the outer layers of the MMM (Fig. 1g and 1 h, respectively) present very thin dense layers with no visible macropores at the lumen surface (Fig. 1i) or at the outer surface (Fig. 1j). However, the outer dense layer (Fig. 1h) is slightly thicker (0.5 μm) compared to the inner dense layer (0.2 μm) (Fig. 1g). Finally, it is important to note that we did not observe elution of the particles from the membrane matrix neither during storage in demineralized water nor during the transport, static and dynamic adsorption experiments (results presented later).

3.2. Water transport experiments

Fig. 2 presents the graph of the membrane water flux versus TMP. The membrane water permeance, estimated from the slope, is quite high (238 ± 9 L/(m^2·h·Bar)), typical of a high flux ultrafiltration membrane [60]. This ensures permeability of the membrane for urea, which can therefore reach the PS-Nin particles in the polymer matrix, and fast transit of urea solution through the fibers, which is desired in WAK applications where the spent dialysate must be rapidly recirculated and regenerated. The water transport experiment was performed on 6 different replicates and the standard deviation among the different membrane modules is very low (only 4% of water permeance), thus indicating that the manufacturing process allows to obtain reproducible membranes. The water flux through the membrane increases linearly with pressure, without any indication of membrane compaction and breakage during the experiment. The membrane could, therefore, stands to pressure values up to 0.6 Bar, which is a quite high value for WAK and usually not applied for such applications. Importantly, while performing the water transport experiment, we did not observe any leakage of particles from the membrane. Overall, these results suggest that the MMM has proper morphology characteristics, filtration properties and good mechanical stability to be used for the filtration of urea.

3.3. Static urea removal studies

The covalent binding of urea is a thermally activated process and the reaction rate increases with temperature. Here, we performed urea binding studies by PS-Nin beads at various temperatures (37, 50 and 70 °C) which are below the Tg of PS [49] and that of PES [61]. From the rate constants at these temperatures, it was determined that the activation energy (E_A) and pre-exponential factor (A) for the reaction of urea with the ninhydrin groups in PS-Nin were 10.0 ± 2.7 kcal/mol and 17.7 ± 4.4·10^6 $M^{-1}h^{-1}$, respectively (see Figure S5 and Table S1 of Appendix A). These values are consistent with activation energy and pre-exponential factor earlier reported for the reaction of urea with ninhydrin groups in polyvinylindanone (10.8 ± 0.5 kcal/mol and 48.4 ± 2.1·10^6 $M^{-1}h^{-1}$, respectively) [50]. The urea removal kinetics is the highest at 70 °C (see Fig. 4S of Appendix A), therefore all static and dynamic urea removal experiments were performed at 70 °C.

Fig. 3a presents the static urea removal at 70 °C by the PS-Nin beads (483 ± 282 μm) and PS-Nin particles (<63 μm). Urea removal is significantly faster for the small particles; for example, at 4 h urea removal by the PS-Nin particles is more than double that by the big beads. The faster binding kinetics and thus higher removal rate of urea for the PS-Nin particles is most likely due to the increased specific surface area of the grinded particles. Nevertheless, the maximum theoretical binding capacity (2.7 mmol/g, considering that the amount of phenyl groups of polystyrene transformed into ninhydrin units is 55 %, as reported by Smakman et al. [48,51]) was not reached, perhaps due to particle aggregation and/or poor dispersion of the particles in the suspension, leading to limited accessibility of the ninhydrin moieties to urea. Despite that, our results clearly indicate the advantage of using small particles to achieve faster and higher urea removal.

Fig. 3b compares the static urea removal of the PS-Nin particles and of the MMM containing these PS-Nin particles at 70 °C over a period of 24 h. At 24 h, urea removal by the particles in suspension is 1.7 ± 0.1 mmol/g, while the MMM showed removal up to 2.1 ± 0.1 mmol/g. The latter could be due to the better particle dispersion without aggregation within the MMM (SEM image, Fig. 1). Moreover, the polymer matrix does not limit the accessibility of the particles for urea. As a matter of fact, the polymer matrix surrounding the particles is highly porous (Fig. 1) as also indicated by the very high water permeance of the membrane (Fig. 2).

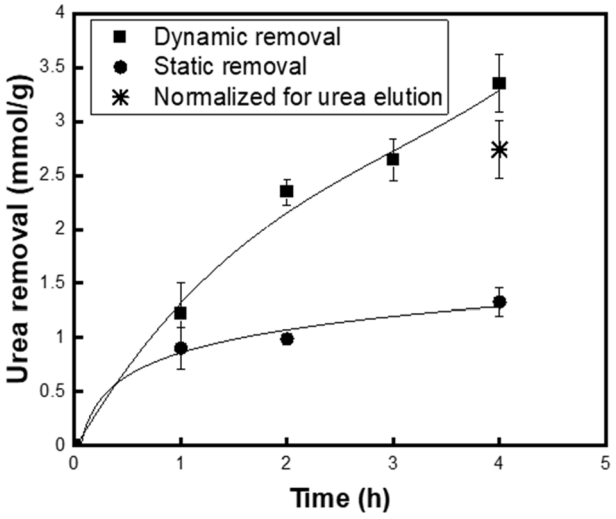

Fig. 4. Dynamic removal of urea by the MMM at 70 °C (n = 4, average ± standard deviation) and comparison with urea removal by the MMM in static conditions at 70 °C. Urea removal by the MMM is calculated as millimoles of urea per grams of particles incorporated in the membrane matrix. * at 4 h represents the calculated remaining urea in the MMM after eluting loosely bound urea (n = 4, average ± standard deviation).

Fig. 3c presents the sorption isotherm of urea on the MMM and on the PES/PVP control HF at 70 °C versus equilibrium urea concentration. Urea binding is expressed as mmol of urea per grams of sorbent particles for the MMM and per grams of membranes for the PES/PVP control HF. The PES/PVP control HF does not remove urea, proving that the particles in the MMM are solely responsible for urea binding. Interestingly, at the equilibrium concentration of 90.1 mM, urea removal by the MMM corresponds to 3.4 ± 0.1 mmol/g, which is much higher than the maximum theoretical urea binding (2.7 mmol/g [48,51]). This exciting result suggests that there is another mechanism playing a role in urea removal by the MMM besides chemisorption of urea to the ninhydrin moieties. This may be based on physisorption, possibly via hydrogen bonding between multi-layered urea molecules, similar to what was described by Cheah et al. for mesoporous silica [29]. Possibly, the local urea concentration within the MMM polymer network and around the particles is high leading to enhanced hydrogen bonding between urea molecules and high urea physisorption between urea and the formed urea/ninhydrin complexes.

3.4. Dynamic urea removal

Fig. 4 presents the removal of urea by the MMM during filtration and recirculation of a dialysate model solution spiked with urea through the MMM HF at 70 °C and comparison with the removal of urea by the MMM in static conditions (70 °C). The results show that, over a period of 4 h, the membrane removes 3.4 ± 0.3 mmol/g of urea without reaching saturation (mmol of urea removed per gram of particles incorporated in the MMM). Moreover, the removal kinetics is much faster compared to the static conditions (Fig. 4). As a matter of fact, over a period of 2 h the MMM is able to remove more than the double amount of urea removed by the same MMM in static conditions (Fig. 4). It is important to note that some membranes were studied immediately after manufacturing, where others were kept in demineralized water at room temperature for a period up to approximately 3 months before testing. The results were very reproducible among the membrane modules tested.

Besides higher urea binding kinetics, the total amount of urea removed by the MMM under dynamic conditions is higher compared to the static conditions (Fig. 4). Again, the amount of urea removed per gram of particles in the MMM is higher than the maximum removal of urea expected based on the ninhydrin moieties in the particles (2.7 mmol/g [48,51]). As discussed earlier for the binding isotherm, this additional urea removal is probably due to hydrogen bonding of urea molecules with urea molecules covalently bound to the ninhydrin moieties of the particles, thus having multi-layered adsorption [29]. When the MMM (n = 2) were rinsed with ultrapure water at the end of the experiment for 1 h, 0.05 mmol of urea were eluted (see Fig. 4) indicating that there the urea removal by the MMM is a combination of chemisorption and physisorption.

Based on the removal in dynamic conditions at 4 h, considering a daily production of urea equal to 400 mmol/day for a typical western diet [12,13] and assuming comparable urea removal when applied in WAK or portable systems, it is calculated that 215 g of MMM (corresponding to 3.7 m^2 of outer surface area of the fibers) is needed to remove the daily produced urea from spent dialysate at 70 °C. However, in spent dialysate there are also other organic compounds present (such as creatinine and amino acids) which could compete with urea for adsorption to the particles. The PS-Nin particles, as all other carbonyl-based sorbents, are not selective towards urea [10] and can also react with other nucleophilic compounds present in the spent dialysate. To avoid this, an activated carbon (AC) sorbent column upstream of the urea sorbent MMM module [10].

In comparison to literature, the urea removal by our MMM (3.4 ± 0.3 mmol/g, 4 h, 70 °C) is one of the highest values reported [10] (see Appendix Table S2). For example, the reported removal by means of physisorption with AC, graphene oxide, zeolites and MXenes is for most of the systems lower than 1 mmol/g [25,31]. For some sorbent systems

composed of chitosan complexed with metal ions, as copper and zinc, which have higher affinity for urea compared to activated carbon, graphene oxide, zeolites and MXenes nanosheets, urea removal higher than 4 mmol/g was reported [33,35,62,63]. Nevertheless, the toxicity derived by potential copper leaching is a concern which could limit their application [10]. Finally, other studies using oxystarch [41,43], oxycellulose [42] and cyclodextrin [64] as urea sorbents reported urea removal comparable to that of our MMM. However, in contrast to these systems, the carbonyl-type sorbents optimally distributed within the MMM can overcome typical issues of sorbents bed column systems, such as particle aggregation and high pressure drop across the column.

4. Conclusions and outlook

In this work, a new MMM for urea removal from dialysate was developed based on PS-Nin sorbent particles, which can bind urea, embedded in a PES/PVP polymer matrix. Our study should be considered as a proof of concept showing that the incorporation of small sorbent particles in the MMM significantly increases urea removal in comparison to the particles in suspension, thanks to the good dispersion of the sorbents in the MMM without aggregation therewith improving accessibility of urea to the binding moieties. In dynamic removal experiments, the MMM removes approximately 3.4 mmol/g of urea after 4 h of recirculation, which is much higher than the theoretical urea removal expected based on the covalent binding to the ninhydrin moieties on the particles. Elution experiments with water suggest that part of this removal is due to urea physisorption, possibly via urea hydrogen bonding to urea molecules already covalently bound to the sorbent particles (chemisorption). Although our experiments were done using dialysate model solution with only urea, and not any other compounds which could adsorb to the particles, the amount of MMM estimated for the removal of the daily produced urea (215 g) is relatively small demonstrating that this MMM is a promising candidate for application in WAK and PAK systems. Since the results of our study were obtained at 70 °C, application of MMM in a WAK or PAK would require heating up of the MMM module. To achieve urea removal at lower temperature we plan to investigate other urea binding moieties as well as increase the particle inner porosity and adapt the experimental conditions (i. e. higher dialysate flow rate and/or increased *trans*-membrane. Moreover, follow-up characterization could better investigate the effect of the incorporation of the sorbent on polymer packing and crystallinity and how these effects could further optimize urea removal [65].

CRediT authorship contribution statement

Ilaria Geremia: Investigation, Methodology, Formal analysis, Writing – original draft. **Jacobus A.W. Jong:** Investigation, Methodology, Formal analysis, Writing – original draft. **Cornelus F. Nostrum:** Methodology, Formal analysis, Writing – review & editing. **Wim E. Hennink:** Methodology, Formal analysis, Writing – review & editing. **Karin G.F. Gerritsen:** Methodology, Formal analysis, Writing – review & editing. **Dimitrios Stamatialis:** Methodology, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2021.119408.

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