

## Double layer mixed matrix membrane adsorbers improving capacity and safety hemodialysis

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**Abstract.** Double layer mixed matrix membranes adsorbers have been developed for blood toxin removal by embedding activated carbon into cellulose acetate macroporous membranes. The membranes are prepared by phase inversion method via water vapor induced phase separation followed by an immersion precipitation step. Double layer MMM consisting of an active support and a separating layer. The active support layer consists of activated carbon particles embedded in macroporous cellulose acetate; the separating layer consists of particle free cellulose acetate. The double layer membrane possess an open and interconnected macroporous structure with a high loading of activated carbon available for blood toxins removal. The MMM AC has a swelling degree of 6.5 %, porosity of 53 % and clean water flux of 800 Lm<sup>-2</sup>h<sup>-1</sup>bar<sup>-1</sup>. The prepared membranes show a high dynamic Creatinine (Crt) removal during hemodilysis process. The Crt removal by adsorption contributes to amore than 83 % of the total removal. The double layer adsorptive membrane proves hemodialysis membrane can integrated with adsorption, in which blood toxins are removed in one step.

### 1. Introduction

Blood purification is natural process, but absolutely important to maintain the life [1]. The liver, kidney are responsible for eliminating toxins from the body. When this organs stop working, can not performed their job, the condition is called liver and kidney failure or renal failure, the toxic and harmful substances will accumulate in the blood. Then we need extracorporeal blood purification to correct it. Nowadays, extracorporeal blood purification, particularly hemodialysis are very important in biomedicine. A patient is treated with hemodialysis membrane to remove accumulation of harmful substances in blood, to control fluid balance and remove excess of water. Blood is drawn from your body then circulated to organ support system then clean blood return back to the body [2].

The disadvantage of hemodialysis is removing the toxins depending only on the molecular weight (pore size) with a lack of specificity and they cannot remove middle molecule and macromolecular poisonous substance [3]. Futhermore, the problem with dialysis are cannot remove albumin bonded toxins and needed ultrapure dialysate. Therefore, one big issue is also concern that the hemodialysis membrane is unavoidable from back-transport phenomenon, i.e. endotoxin can transfer from



contaminated dialysate fluid to blood stream leading to potential risk, that stimulate mononuclear cell cytokine production and produce a microinflammatory state. The failure of these therapies has stimulated the development of other or improvement treatments for blood purification.

Modification hemodialysis membrane properties are proposed by increasing membrane pore size and combining with adsorption properties. In fact that among extracorporeal detoxification, kidney support, liver support, and multi organs failure can not possibly work without adsorptive purification of biologic fluid. Historically, resins and charcoals were used for their adsorptive capacity and ability to remove toxic substances from blood. It removes substances of higher molecular weight than standard hemodialysis [4].

Therefore, it is essential to develop or modify a novel hemodialysis (hemofiltration) membrane which integrates filtration and adsorption [5]. Most of the extracorporeal blood detoxification treatments including kidney and liver support do not work without adsorptive purification systems [6, 7]. A new approach to obtain adsorptive hemodialysis membranes is integration of small functionalized particles (sorbents) with hemodialysis membrane. The small adsorbent particles can be putted in biocompatibility porous support may be we can integrate diffusion, convection, and in one step and then you can used for extracorporeal blood purification. The membrane properties can integrate hemodialysis with adsorption. Entrapping smaller particles into porous matrix support could improve surface area, and shorten distance diffusion of toxic compound to active sites. This concept offer possibility to protect release of small micro-particles, particle coalition and possibly fragmentation of the sorbent. Clearly, there is a room for innovations and improvements in capacity and efficacy of hemodialysis membrane as well as in the safety of sorbent systems.

In this paper, the adsorptive hemodialysis membrane offers an alternative approach for blood toxins removal by blood purification membranes meanwhile increasing the biocompatibility of hemoperfusion sorbents (activated carbon) based on mixed matrix membrane (MMM) concept [8]. A major advantage of the membrane concept is the ability to create particle-loaded membranes using any type of particles and almost any type of polymeric material. The activated membranes are prepared by incorporating activated carbon into a biocompatible macroporous polymeric matrix without affecting the activity. The adsorptive membrane are prepared by phase inversion methods. To improve the blood-compatibility, and to avoid mixing of small activated carbon particles with the blood stream, the membran is co-cast with a particle-free cellulose acetate solution with a particle free top layer preventing blood getting in contact with adsorbent particles. Creatinine (Crt) is used as model blood toxin to characterize the membrane adsorber performance.

## 2. Materials and Methods

Cellulose acetate with an average acetate content of 38.8 mol % was purchased from Aldrich (M.W $\approx$ 30.000) and used as membrane material without further modification. Acetone (Merck) was employed as solvent. Ultra pure water, prepared using a Millipore Milli-Q plus purification unit, was used as additive in the polymer solution and as non-solvent in the coagulation bath. Activated carbon Norit A Supra EUR, kindly supplied by Norit Netherlands B.V. was used as adsorbent particles. Creatinine was used as a model blood uremic-toxin to investigate the adsorption capacity of the MMM. The buffer solutions were freshly prepared in ultrapure water. Tyrode buffer was chosen as representing adequately the ionic solutes, pH and glucose concentrations of the blood plasma. The Tyrode buffer composed of 137 mM NaCl, 5.4 mM KCl, 1.8 mM CaCl<sub>2</sub>, 0.5 mM MgCl<sub>2</sub>, 11,9 mM NaHCO<sub>3</sub>, 5.5 mM glucose (pH=7.4). The dialysate buffer contained of 140 mM NaCl, 2 mM KCl, 1.5 mM CaCl<sub>2</sub>, 0.25 mM MgCl<sub>2</sub>, 35 mM NaHCO<sub>3</sub> and 5.5 mM glucose (pH=7.4).

#### *a. Membrane preparation and characterization*

To obtain membranes with adsorptive properties, activated carbon was dispersed into a homogenous solution containing 12 wt% cellulose acetate (CA) and 20 wt% water in acetone. The water, being a non-solvent additive was added to the casting solution in order to facilitate instantaneous demixing, which improves the membrane morphology. The dispersion was stirred over night at room temperature to break down possible particles clusters. The MMM were prepared by water vapor induced phase separation. The cast polymer solution was exposed to water vapor (temperature 30-33 °C and relative humidity of 98 %) prior to immersion in a water containing coagulation bath. The polymer solutions were co-cast on a glass plate using a double casting knife with adjustable height. The height of the first and the second slit was 475 and 550  $\mu\text{m}$  respectively. After the formation process, the membranes were washed with milli-Q water at room temperature to remove the residual solvent traces. The membranes were overnight dried in air and stored in a vacuum oven at 30 °C.

The membrane structures were characterized by Scanning Electron Microscopy (SEM) using a JEOL TM 220 A. The membrane top surfaces with and without co-casting layer as well as the cross sections and bottom surfaces were examined. Cross-section samples were prepared by cryogenic breaking fresh samples in liquid nitrogen. The samples were allowed to dry overnight under vacuum at room temperature and then gold-coated using a Balzers Union SCD 040 sputter coater.

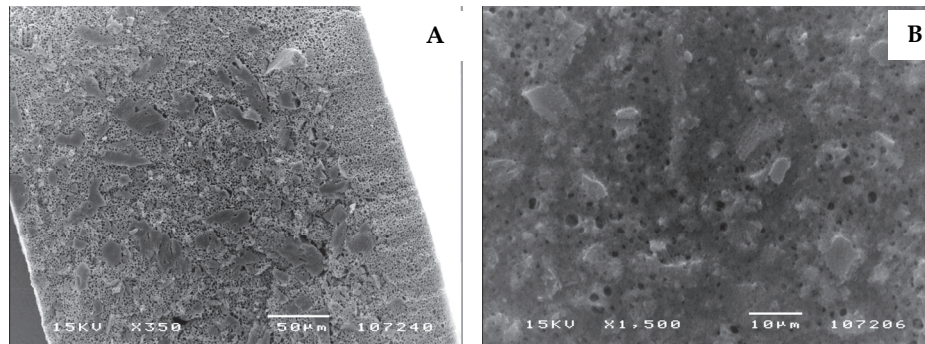
#### *b. Haemodialysis test*

The diffusive transport of Crt was investigated using a two-compartment dialysis cell at room temperature. The first compartment was filled with Crt in the Tyrode buffer and the second compartment was filled with dialysate buffer. The compartments were separated by the MMM AC. The volume of each compartment was 105 ml and the active membrane area was 19.6  $\text{cm}^2$  (0.25 gram). Both solutions were recirculated at flow rate of 3 ml/minute using a peristaltic pump. During the experiment, 1 ml samples were taken as function of time to determine the Crt concentration in both compartments. Immediately after measuring the UV absorbance, at 230 nm the samples were returned to the compartments. The dialysis experiments were evaluated for six hours before the process were stopped.

### **3. Results and Discussion**

#### *3.1. Membrane preparation and characterization*

Figure 1 shows SEM images of the MMM, which have been prepared with activated carbon (AC) as adsorbent. The MMM AC was prepared from a solution of 12 % CA, 20% water in acetone containing 60 wt% loading activated carbon, under exposure to water vapor environment. Regular membrane structures were obtained by means of these compositions. The prepared MMM AC shows good mechanical properties and no particle loss is observed during membrane formation. The membranes possess open and interconnected macroporous structures. The membranes display open structures both at bottom and top surface. The top layer of the membranes consists of a highly interconnected macroporous structure, which forms a continuous structure with the sub-layer. The average pore size of the membranes as determined by bubble point measurement is 0.234  $\mu\text{m}$ . The MMM AC has a swelling degree of 6.5 %, porosity of 53 % and clean water flux of 800  $\text{Lm}^{-2}\text{h}^{-1}\text{bar}^{-1}$ .

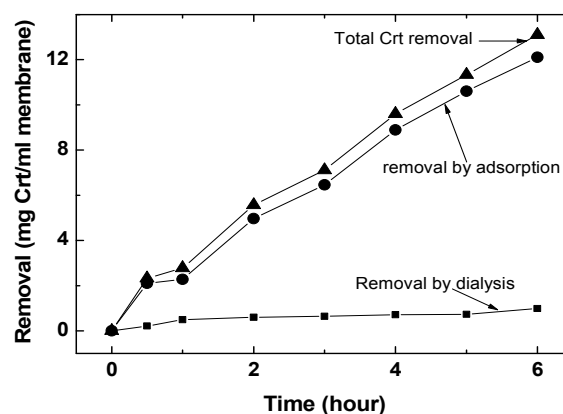


**Figure 1.** SEM micrographs of co-cast MMM prepared by water vapor induced phase separation and immersion precipitation.

Figure 1 indicated a cross section, magnification x350, the size bar indicates 50µm, MMM with embedded activated carbon particles (A), and a bottom surface, magnification x1500, the size bar indicates 10 µm MMM (B).

### 3.2. Dynamic creatinine removal

The MMM AC offers the possibility to integrate adsorption and hemodialysis treatments in one step. We investigated the performance of the MMM AC for this purpose. The adsorptive membrane was placed in a dialysis cell, which separates two compartments: one containing the Crt solution and the other containing the dialysate. The total Crt removal from the sample solution and the Crt concentration in the dialysate side were determined in time. The amount of Crt removed by adsorption onto the MMM AC can be calculated by subtraction the increase in Crt in the dialysate cell from the Crt that is removed from the sample side. Figure 2 presents the Crt removal during six hours of dialysis. Crt is significantly removed during the dialysis process by a combination of adsorption in the MMM AC and diffusion across the membrane.



**Figure 2.** Creatinine removal during adsorptive dialysis. Starting concentration of Crt was approximately 0.1 mg/ml.

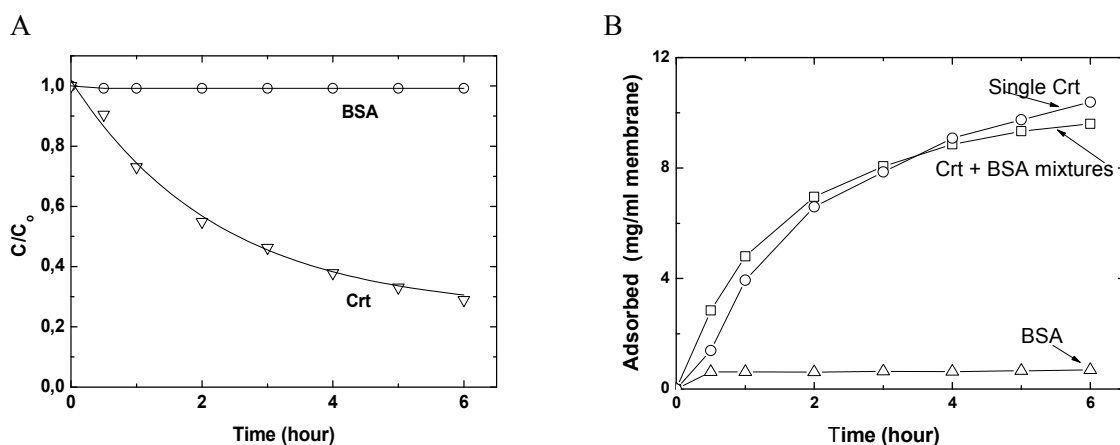
The relative contribution of adsorption and dialysis to the total removal of Crt can clearly be observed (figure 2). This indicates that most of Crt is captured by active carbon when diffusion through the membrane. The Crt removal by adsorption contributes to more than 83 % of the total

removal. Capacity of the hemodialysis membrane are still available for removal of contaminants after six hours hemodialysis process. An increase in Crt removal by diffusion (dialysis) across the membrane can be expected when all of the active sites in the MMM AC are occupied (breakthrough). Activated carbon based hemoperfusion columns has recently been applied to increase the efficiency of hemodialysis, to remove toxic compounds during kidney or liver failure, autoimmune diseases and encephalopathy[1]. Furthermore, the use of activated carbon hemoperfusion columns are introduced to improve protein-bound and water soluble toxins removal, detoxification by drugs overdoses and poisonous and endotoxin removal [3, 4].

These results proved that the integration of adsorption functions in dialysis membranes significantly improves the performance of a dialysis treatment by preventing the diffusion of toxins between the two fluid streams (compartments). The adsorptive MMM can be adapted to different extracorporeal blood purification treatments, i.e. hemoperfusion, hemofiltration and hemodialysis. The total amount Crt removed during a six hour treatment depends solely on the MMM adsorption capacity. The MMM concept is ensure as safe treatment since only parts of the blood perfuses through the adsorbent and blood cellular component do not interact with the embedded sorbents.

### 3.3. Non-Specific Removal

Protein adsorption at blood-biomaterial interfaces is of prime importance for the biocompatibility of the material. The main factor promoting protein adsorption on surfaces is the hydrophobic interaction force between the sorbent surface and the protein molecule. BSA adsorption on adsorptive membrane is investigated to determine non-specific BSA (a bovine blood plasma protein) adsorption on entrapped active carbon particles. Figure 3 presents the BSA adsorption during six hours process hemodialysis. The BSA adsorption capacity of entrapped activated carbon particles in cellulose acetate membrane is equal to the pure cellulose acetate BSA adsorption capacity. This value is in agreement with data reported by Liu et al.[9], who measured 0.3 mg BSA/g membrane onto pure CA membranes, feed concentration 0.5 mg BSA/ml. The low BSA adsorption of the MMM AC demonstrates that entrapped activated carbon particles in the MMM are not accessible for high molecular weight BSA molecules where low molecular weight Crt molecules have free access. This makes that the MMM AC is very suitable for removal of toxic Crt out BSA containing (plasma) solutions.



**Figure 3.** A. Creatinine removal during adsorptive dialysis. Starting concentration of Crt was approximately 0.1 mg/ml. B. The amount of adsorption single Crt, mixture of Crt and BSA and single BSA on the membrane



The membrane possesses a porous structure and a very good pore interconnectivity so that harmful substances can utilize all active sites. The BSA adsorption of coated particles was approximately 0.8 mg BSA/g sorbent which is close to the non-specific adsorption value of the MMM AC. These results proof that the biocompatibility problems of sorbent hemoperfusion processes can be solved with the MMM approach. Furthermore, the biocompatibility of the blood side of the MMM adsorber can even be further improved by co-casting the MMM with a biocompatible layer.

Marlon et.al [5] 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. 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. A polyethersulfone (PES)/polyvinylpyrrolidone (PVP) polymer blend is used for the preparation of the macro-porous membrane matrix and activated carbon is used as adsorptive particle.

#### 4. Conclusion

The MMM double layers have been prepared by incorporating activated carbon into macroporous cellulose acetate membranes by phase inversion method. To improve the blood-compatibility, and to avoid mixing of small activated carbon particles with the blood stream, the MMM is co-cast with a particle-free cellulose acetate solution with a particle free top layer preventing blood getting in contact with adsorbent particles. The MMM AC has a swelling degree of 6.5 %, porosity of 53 % and clean water flux of  $800 \text{ Lm}^{-2}\text{h}^{-1}\text{bar}^{-1}$ . The prepared membranes show a high dynamic Creatinine (Cr<sub>t</sub>) removal during hemodialysis process. The Cr<sub>t</sub> removal by adsorption contributes to more than 83 % of the total removal. The MMM technology is suitable for integration of dialysis with adsorption in one step.

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