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Highly porous polytriazole ion exchange membranes cast from solutions in non-toxic cosolvents

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Abstract

The development of highly functionalized porous materials for protein separation is important for biotech processes. We report the preparation of highly porous polytriazole with sulfonic acid functionalization. The resulting ion exchange membranes are selective for protein adsorption. The starting material was a hydroxyl-functionalized polytriazole, which is an advantageous platform for further modification. The polymer was dissolved in a mixture of 1-ethyl-3-methylimidazolium acetate ([C2mim]OAc) and dimethyl carbonate (DMC), which can be both considered green solvents. The polymer solubilization was only possible due to an interesting effect of cosolvency, which is discussed, based in phase diagrams. Membranes were prepared by solution casting, followed by immersion in a non-solvent bath. We then grafted sulfone groups on the membranes, by reacting the hydroxyl groups with 1,3-propane sultone and 1,4-butane sultone. Lysozyme adsorption was successfully evaluated. Membranes modified with 1,4-butane sultone adsorbed more protein than those with 1,3-propane sultone.

Keywords: polytriazole, high porous membrane, green solvents, ion exchange chromatography

1. Introduction

Biopharmaceutical products have become the therapeutics of choice for treatment of many important diseases such as cancer, autoimmune diseases, diabetes, among others [1]. The growing number of products that are entering the production pipeline requires a reliable process with high yield and high purity and, most important, lower production cost. In addition, the emergence of biosimilar increases the need for a more economical downstream process.

Conventional protein purification is performed in packed bed columns using porous beads as chromatographic supports. Microporous membranes could offer a valid alternative to resins as they can be operated at higher flow rates and lower pressure drops, thus reducing both processing times and pumping requirements [2]. These advantages have the potential to overcome the limitation of membranes in terms of low capacity. More importantly, membranes do not require extensive costs associated with packing protocols and validation of the bed, a major burden in industrial scale chromatography equipment. This is clearly supported by the current implementation of ion exchange membrane adsorbers as single-use or disposable units in the polishing steps [3].

One of the objectives of this work is the preparation of novel membranes with high porosity and high binding capacity to be used as a support for ion exchange chromatographic separation of proteins.

Protein adsorption on the membrane surface is mostly based on ion-exchange mechanism, which involves the electrostatic interaction between the charges of the macromolecule to be separated and the adsorber surface. The most common ion exchange membranes used in industry are mainly cation exchange membranes having sulfonic acid and/or carboxylic acid groups, and anion exchange membranes with quaternary ammonium groups [4]. In this work we developed new highly porous hydroxyl-functionalized polytriazole (PTA-OH) ion exchange membranes for selective protein adsorption. Lysozyme was used as a model protein to evaluate the binding capacity of the modified polytriazole membrane.

Membrane technology is a sustainable alternative with low energy consumption, reduced environmental impact, simple scale-up and operational conditions [5, 6]. One of the main challenges in guaranteeing the sustainability of these separation processes is the membrane preparation itself since most membranes are currently manufactured from solutions in organic

solvents, such as N-methyl-2-pyrrolidone (NMP), N,N-dimethylformamide (DMF), N,N-dimethylacetamide (DMA), tetrahydrofuran (THF) and 1,4-dioxane [7-9], which are considered harmful with major impact on health and environment [10, 11]. Therefore, when developing new membranes it is important to propose processes, which use non-hazardous, recyclable solvents. Good examples of safer less toxic solvents are methyl and ethyl lactate [12, 13], triethylphosphate [14], dimethyl sulfoxide [15] and γ -butyrolactone [6]. Ionic liquids can be considered as green solvents to replace the traditional ones, due to their low volatility, chemical stability, non-flammability and non-combustibility, and relatively low toxicity, even though in some cases their classification as green solvents has been contested when their whole synthesis process is taken into consideration or the toxicity of a few of them in water [16-19]. Ionic liquids are organic salts, which are liquid at room temperature, and are formed from organic cations (imidazolium, pyridinium, quaternary ammonium, or quaternary phosphonium) and inorganic or organic anions (halogenate, borate, phosphate or acetate). They have been more widely used in organic synthesis to replace traditional solvents, as catalysts or in extraction steps [20-22]. For membrane application ionic liquids have been effectively incorporated to promote carbon dioxide separation [23, 24]. As solvent for membrane preparation, the ionic liquid 1-butyl-3-methylimidazolium thiocyanate ([BMIM]SCN) was used for cellulose acetate [25]. 1-Ethyl-3-methylimidazolium acetate ([C2mim]OAc) was used for polybenzimidazole [26, 27] or for polybenzimidazole/polyimide blend membranes for ultrafiltration separation [28]. Our group has been proposing ionic liquids as new solvent systems for the most relevant commercial membrane polymers. We have reported the use of [C2mim]OAc to manufacture cellulose multilayer membranes for nanofiltration and ultrafiltration separation, for the preparation of polyacrylonitrile and for cellulose acetate hollow fibers for forward osmosis application [29, 30]. We have pioneered the use of 1-ethyl-3-methylimidazolium dimethylphosphate as solvent for the preparation of polyethersulfone membranes [31]. We used different ionic liquids to guide self-assembly in the formation of isoporous block copolymer membranes [32].

In addition to ionic liquids, another solvent, which could replace the traditional ones, is dimethyl carbonate (DMC), due to non-toxicity and biodegradability, being inert and safe under ambient conditions [10, 33]. DMC has been frequently used as eco-friendly alternative as methylation and carbonylation agent in organic synthesis reactions, compared to dimethyl sulfate, methyl halide and phosgene, which are highly toxic [34, 35]. In this work we used a

mixture of DMC and [C2mim]OAc for the membrane preparation. This system is an interesting case of co-solvency, where each single component is a non-solvent for PTA-OH, while with the appropriate solvent ratio a successful casting solution can be obtained. The thermodynamic reasons for that are here discussed.

2. Experimental part

2.1 Materials

1-Ethyl-3-methylimidazolium acetate ([C2mim]OAc), dimethyl carbonate (DMC), lysozyme, trisma base (Tris), 1,3-propane sultone (1,3-PS), 1,4-butane sultone (1,4-BS) and all the other chemicals were purchased from Sigma Aldrich. All the chemicals were used as received. Polytriazole with free OH groups (98% degree of functionalization (PTA-OH)) was synthesized in our lab by polycondensation reaction in two steps: the first step consists in the synthesis of the polyoxadiazole precursor; the second step consists in dissolving this precursor in 1-methyl-2-pyrrolidinone and reacting with 4-aminophenol in the presence of small amount of polyphosphoric acid at 195°C for 15 h [36-38].

2.2 Hydroxyl-polytriazole (PTA-OH) membrane

Porous functionalized polytriazole membranes were obtained by phase inversion. Hydroxyl-functionalized polytriazole (Scheme 1) was dissolved in different mixtures of [C2mim]OAc / DMC. The resulting solution was cast on a glass plate using a casting knife with 200 μm gap and then immersed in a water bath. The obtained hydroxyl-functionalized polytriazole membranes were activated, by reacting with 1,3-propane sultone (1,3-PS) and 1,4-butane sultone (1,4-BS). For that, the polytriazole membranes with free OH groups were immersed in 5 wt% solution of sultone derivatives in water with sodium hydroxide at 65 °C for 6 h. After modification the membranes were washed with water several times.

Scheme 1. The structure of polytriazole with free OH groups (PTA-OH)

2.3 Thermodynamic aspects

[C2mim]OAc and DMC are non-solvents for PTA-OH, while appropriate mixtures exhibit cosolvency. For the optimization of the membrane preparation, a polymer/solvents ternary phase diagram was calculated based on the Gibbs energy. The Gibbs energy was determined by using the Flory – Huggins equation [39]:

$$\frac{\Delta G_m}{RT} = n_1 \ln \Phi_1 + n_2 \ln \Phi_2 + \chi \Phi_1 \Phi_2 (n_1 + m n_2) \quad (1)$$

where ΔG_m is the free energy, n_1 and n_2 are the number of moles of solvent and polymer, respectively, Φ_1 and Φ_2 are the volume fraction of solvent and polymer, m is the ratio of molar volumes of the polymer and solvent, χ is the Flory-Huggins interaction parameter, R is the gas constant ($8.314 \text{ J mol}^{-1} \text{ K}$) and T is the temperature in $^\circ\text{K}$.

The Flory-Huggins interaction parameters between PTA-OH and the solvent mixture were estimated from Hansen solubility values, δ , values:

$$\chi_{PS} = \frac{V A_{PS}}{RT} + 0.34 \quad (2)$$

where V is the solvent molar volume; and A was calculated from the weight average of the different δ contributions:

$$A_{PS} = (\delta_{DS} - \delta_{DP})^2 + (\delta_{PS} - \delta_{PP})^2 + (\delta_{HS} - \delta_{HP})^2 \quad (3)$$

where δ_{iS} and δ_{iP} are Hansen solubility parameters for the solvents and PTA-OH, respectively.

Hansen solubility parameters (δ) for polytriazole and 1-ethyl-3-methylimidazolium acetate ([C2mim]OAc) were determined by testing the solubility of each of them, separately in 46 solvents. In the case of PTA-OH the solubility test was performed at low concentrations (0.1 – 1%) and in the case of [C2mim]OAc was performed by testing the miscibility in a ratio of 60 wt% [C2mim]OAc and 40 wt% of other solvents of known δ values. The δ values for PTA-OH and [C2mim]OAc were then calculated using the HSPiP software (4th Edition 4.1.07) [40].

2.4 Chemical and morphological characterization

The interaction between solvents and between solvents and polytriazole were investigated by Fourier Transform Infrared Spectroscopy (FTIR). The FTIR spectra were collected by recording 16 scans. The spectral resolution was 4 cm⁻¹ and the spectra were recorded at room temperature on a Nicolet 6700 FTIR equipment.

Scanning electron microscopy (SEM) was used to investigate the membranes morphology, using a Quanta 600 microscope. For imaging a voltage gap of 3–5 kV and a working distance of 5 – 10 mm were used. The samples were coated with iridium, using a Quorum Q150TES equipment, before performing the measurements.

The pore size distribution of the membranes was measured on a Porolux^{TM} 1000 (POROMETER, Belgium). Perfluoroether (Porefill, surface tension 16 mN m⁻¹) was used to fill the pores of the membranes and nitrogen gas flow with the pressure up to 34.5 bar was used to displace the liquid.

The porosity ε of the membranes was calculated, according to equation (4):

$$\varepsilon = \frac{(m_1 - m_2)/\rho_w}{(m_1 - m_2)/\rho_w + m_2/\rho_p} \quad (4)$$

where m_1 and m_2 are the weights of the wet and dry membranes, respectively; ρ_w and ρ_p are the densities of water and polymer.

2.5 Protein adsorption measurement

The capacity of the membranes to adsorb proteins, in particular lysozyme, was measured in batch equilibrium experiments performed at 25 °C. Samples of modified membranes with area in the range of 2–3 cm² were equilibrated overnight in 50 mM Tris buffer at pH 8, before the adsorption experiment. The membranes were immersed in a known volume (in the range of 3 to 10 mL) of 0.35 g/L lysozyme solution in 50 mM Tris at pH 8 and left until no change of lysozyme concentration in solution was detected. The value was constant after 24 h. The concentration was measured by UV. The amount of lysozyme adsorbed was calculated by a mass balance, by measuring the difference between the mass of protein in the initial solution and that present at equilibrium. Protein concentration in solution was measured by UV readings, on a Thermo Scientific NanoDrop 2000c Spectrophotometer. The membrane binding capacity was calculated per unit area (q_{ads}), according to equation 5:

$$q_{ads} = m_{asd} / A \quad (5)$$

and per unit volume (q'_{ads}), according to equation 6:

$$q'_{ads} = m_{asd} / V \quad (6)$$

where m_{ads} is the mass of the adsorbed protein (mg); A is the surface area of the membrane (cm²) and V is the membrane volume (cm³).

3. Results and discussions

Primary requirements for membrane chromatography are the availability of highly porous materials with large pore size and ion exchange capacity to promote efficient protein adsorption and elution. High density of ion exchange sites were provided by post-functionalization of the OH groups of the PTA-OH chosen as basic material in this work. PTA-OH with 90 % of the repeating units containing hydroxyl functionalization was prepared by adding phenol aniline as reactant for the polycondensation.

3.1 Cosolvency

Membrane preparation with high porosity and adequate morphology is mostly done by phase inversion, a solution process, which consists of casting followed by immersion in a non-solvent bath. Therefore the first step after the polymer synthesis was the identification of the best suitable solvent system. An important criterion is the toxicity, since there is an increasing

concern with the environmental impact of industrial processes. We could not identify a single ionic liquid to solubilize PTA-OH. It could not be solubilized in DMC either. However we found out that a mixture of [C2mim]OAc and DMC could be a suitable solvent for PTA-OH. By measuring the cloud points for the ternary PTA-OH/[C2mim]OAc/DMC system at room temperature, we determined the phase diagram and identified a narrow cosolvency window.

The experimental ternary (PTA-OH/ [C2mim]OAc / DMC) phase diagram is shown in Fig. 1a. [C2mim]OAc and DMC (without polymer) are not miscible in 65/35 ratios or with lower [C2mim]OAc content. Above 65 wt% [C2mim]OAc the binary solutions are homogeneous. Small amount (around 1 wt%) of PTA-OH could be dissolved in mixtures with [C2mim]OAc content higher than 65 wt%. With higher polymer concentration (3 wt%), a homogeneous solution is obtained even for the solvent ratio of 60/40 [C2mim]OAc/DMC, which is not miscible without polymer. This characterizes a case of cosolvency, with the polymer acting as a compatibilizer for the two single solvents. This behavior can be explained by the strong dipole-dipole interactions between the carbonyl groups, which can promote the dimerization of DMC molecules. The carbonyl group is then screened by the methyl groups and become less available to interact with [C2mim]OAc. As the concentration of [C2mim]OAc increases, it dilutes DMC and self-association is disfavored. For the solvent ratio of 60/40 [C2mim]OAc/DMC, by adding PTA-OH the tendency of forming hydrogen bonding increases and reduces the chances of dimerization. More free carbonyl groups become available for interaction with the PTA-OH. A classic example of cosolvency, with some analogy to this, is polystyrene/acetone/methyl cyclohexane [43].

Fig. 1. (a) Ternary phase diagram expressed in weight % of PTA-OH, [C2mim]OAc and DMC; (b) Gibbs energy as a function of [C2mim]OAc/DMC ratio for different polymer concentrations.

The strong interactions between [C2mim]OAc and DMC can be emphasized by studying the FTIR spectra of pure and mixtures with different ratios of [C2mim]OAc and DMC (Fig. 2a). The absorption peaks at 1261 cm^{-1} and 1749 cm^{-1} (associated with carbonate and carbonyl groups, respectively) are characteristic of DMC [42]. They are shifted and their intensity decreases as the [C2mim]OAc concentration increases. These changes in the spectra can be correlated with the formation of hydrogen bonds, which are more favorable in specific ratios, leading to miscibility. Moreover, the absorption peaks at 1376 cm^{-1} and 1561 cm^{-1} (associated with symmetric and asymmetric acetate group) are characteristic of [C2mim]OAc. They are shifted, also suggesting that the interactions between [C2mim]OAc and DMC become strong [44].

Fig. 2. (a) FTIR spectra for single and mixtures with different ratios of [C2mim]OAc and DMC; (b) FTIR spectra for 65/35 [C2mim]OAc/DMC with different concentrations of dissolved PTA-OH; 3D representation of hydrogen bonding in PTA-OH/[C2mim]OAc/DMC.

The hydroxyl-functionalized polytriazole itself plays an important role in the cosolvency for the ternary system investigated here. This can be explained by taking into consideration the hydrogen bonds between the polytriazole OH groups, which are H-donor, and both solvents, which contain H-acceptors. The formation of hydrogen bonds between the polytriazole and the solvent system can be followed by FTIR. Fig. 2b shows the FTIR spectra for 65/35 [C2mim]OAc/DMC and different concentration of dissolved PTA-OH. There is a decrease of intensity and slight shift of the peaks at 1261 cm^{-1} and 1561 cm^{-1} with increasing polymer concentration. They are characteristic for DMC and [C2mim]OAc, respectively. This indicates that there is a strong interaction between polytriazole OH groups and the carbonate and acetate groups of the solvents.

This strong solvent-polymer interaction decreases the enthalpy of mixing and favors a lower total free energy of mixing along with the entropic gain, leading to a homogeneous

solution. The ChemDraw scheme depicted in Fig. 2b represents the most probable sites in the polymer for interaction with the solvents, DMC and [C2mim]OAc, to form hydrogen bonds. We can consider PTA-OH as a pseudo-surfactant, which brings the two solvents to form just one phase [45, 46]. 20 wt% PTA-OH is needed to promote miscibility of [C2mim]OAc and DMC into one homogeneous phase if the solvent ratio is 50/50. Continuous addition of PTA-OH cannot further promote miscibility of systems with higher DMC content. When 9 wt% PTA-OH is added to 40/60 [C2mim]OAc/DMC, phase separation takes place: one of the phases is highly diluted and the other is highly concentrated in polymer with high viscosity, practically as a gel.

Fig. 1a shows the maximum concentration of PTA-OH, which could be added to each ratio of [C2mim]OAc/DMC to promote an one-phase solution. The maximum PTA-OH concentration increases from 10 wt% for 85/15 to 20 wt% for 50/50 [C2mim]OAc/DMC.

The experimental results were correlated with values of Gibbs free energy of mixing (ΔG_m), estimated from Flory-Huggins (χ) and solubility parameters (δ_i), using equations 1-3. The δ_i for [C2mim]OAc was determined by testing the miscibility of [C2mim]OAc and a wide variety of solvents in a 60/40 of [C2mim]OAc/solvents ratio. The δ_i value for PTA-OH was estimated by testing solubility in different solvents at 1 wt % concentration. Table 1 shows the values of Hansen solubility parameters and Flory-Huggins interaction parameters, considering different solvent ratios. In general, for a soluble polymer – solvent system, the χ values should be preferentially smaller than 0.5, which corresponds to the theta condition [47, 48].

Table 1. Hansen solubility parameters for PTA-OH and for [C2mim]OAc / DMC mixtures in different ratios; Flory-Huggins interaction parameter between PTA-OH and the solvent mixtures.

	δ_D (MPa) ^{1/2}	δ_P (MPa) ^{1/2}	δ_H (MPa) ^{1/2}	χ_{PS}
PTA-OH	18.1	11.1	10.5	
[C2mim]OAc / DMC				
0/100	15.5	8.6	9.7	0.80
50/50	18.7	12.4	12.6	0.67
60/40	19.4	13.1	13.2	1.03
65/35	19.7	13.5	13.4	1.28
70/30	20.0	13.8	13.7	1.57
75/25	20.3	14.2	14.0	1.92
85/15	20.9	14.9	14.6	2.76
100/0	21.8	16.0	15.4	4.46

δ_D = dispersive contribution; δ_P = polar contribution; δ_H = hydrogen bond contribution

The χ values for PTA-OH in [C2mim]OAc/DMC decrease with decreasing the amount of [C2mim]OAc. This means that the solubility of PTA-OH increases. This finding correlates well with the experimental results. We found that the maximum concentration of PTA-OH, which can be dissolved, increases as the amount of [C2mim]OAc decreases. The highest polymer concentration leading to a soluble system was achieved for the 50/50 solvent ratio, which has the lowest χ values, not far from 0.5. Although systems with other solvent ratios had χ values higher than 0.5 and would be considered a poor solvent, the interaction was still enough to allow solubilization of less than 20 wt% polymer.

The Gibbs free energy was calculated for the binary solvent system and for the ternary system with different PTA-OH concentrations (Fig. 1b). For the binary system, without polymer, the Gibbs energy is strongly positive with a maximum value at 50/50 [C2mim]OAc/DMC. When 1 wt% of PTA-OH is added, the Gibbs energy substantially decreases, becoming negative in broad range of compositions, indicating that the system could be thermodynamically stable as one phase. A homogeneous system is indeed observed from 50/50 to 85/15. PTA-OH reduces the ΔG_m value, by hydrogen bonding, and expands the miscibility window, working as

compatibilizer. On the other hand above 85/15 the Gibbs energy increases and remains positive, even in the presence of polymer.

The application of Hansen and Flory-Huggins theories to ionic liquids is a rough approximation, since they do not include the electrostatic [49] and coulombic interactions, but for the system investigated here there was a good correlation with the experimental observations.

3.2 Membrane morphology

According to phase diagram in Fig. 1a, the window of solubility lays in a polymer concentration below 20wt %, a range useful for membrane preparation. [C2mim]OAc/DMC ratios of 60/40, 65/35 and 70/30 lead to homogenous solutions with a broad variation of polymer concentration. Membranes were prepared by casting 9 wt% PTA-OH solutions in the above mentioned solvent ratios. The surface and cross-section morphologies of the membranes were investigated using SEM (Fig. 3). In all cases highly porous structures were obtained. The porosity was estimated as being around 80%. While the pore size was larger for membranes prepared from solutions in 60/40 [C2mim]OAc/DMC, the pore density and the interconnectivity of the pores increased by increasing the amount of ionic liquid.

Fig. 3. SEM (a) surface and (b) cross-section images of membranes prepared from solutions in optimal [C2mim]OAc/DMC ratios; (c) pore size distribution measured in porometer.

To understand the formation mechanism of highly porous membranes and optimize the preparation, we investigated the effect of solvent evaporation and composition of the non-solvent bath. The [C2mim]OAc/DMC ratio was fixed as 65/35 for the membrane preparation. Fig. 4a shows the SEM images for membranes prepared by immersion in water, after different evaporation times. The membranes morphologies are similar. At longer evaporation times turbidity starts to be observed in the solution before the immersion in water, indicating that phase separation is initiated before immersion in water, as DMC, the volatile co-solvent, evaporates. At the same time the polymer concentration increases and the [C2mim]OAc/DMC ratio is shifted to a condition of higher Gibbs energy, out of the solubility window in Fig. 1a. In addition to that we could not completely exclude the possibility that humidity from the environment could be introduced to the system, also contributing to the phase separation.

Fig. 4. SEM images of the surface of PTA-OH membranes, prepared in 65/35 [C2mim]OAc/DMC: (a) using different evaporation times before immersion in water; (b) immediately immersed in water/ethanol coagulation baths with different volume % ratios.

For most commercial membranes the pore formation results from phase separation induced by immersion in water. The phase separation follows the mechanism of spinodal decomposition if the water-solvent exchange is fast and a composition corresponding to the two-phases region of the phase diagram is rapidly achieved [50]. The polymer diluted phase gives rise to the pores. In the specific system investigated here, while [C2mim]OAc easily moves into the water bath, DMC has very low solubility in water (13.9 g / 100 ml water). Therefore if DMC evaporation is not completed before immersion in water, a large amount of DMC might remain trapped inside the casting solution layer. The fact that DMC remains in the solution instead of

diffusing into water could be an advantageous aspect for obtaining larger pores and high surface area, characteristics, which are beneficial for membrane chromatographic separations.

Fig. 4b shows the morphology of the membranes obtained by using a mixture of water – ethanol, as non-solvent bath, to facilitate the extraction of both DMC and [C2mim]OAc after immersion, as the phase separation proceeds, forming the membrane. The membranes obtained by using water as non-solvent or a mixture of 75/25 or 50/50 (volume ratios) water/ethanol are compared. Analogous membranes prepared with 50/50 water/ methanol coagulation bath (not shown here) had similar morphology. Highly porous structures are obtained in all cases, however the pore sizes decrease as the ethanol concentration increases, probably facilitating the transport of DMC into the bath. An extreme case is seen when the ethanol concentration increases to 75 %, leading to a practically dense membrane.

3.3 Membrane activation and protein adsorption capacity

Sulfonic groups are considered strong cation-exchanger. After optimizing the morphology, the membranes were turned into ion-exchangers by anchoring sulfonic groups to the pores surfaces. An important advantage of using PTA-OH is the availability of free OH groups, which can be easily modified. Therefore, PTA-OH can be considered a straightforward material with respect to currently commercial polymers (polysulfone, poly(ether sulfone), poly(vinylidene difluoride), polyethylene, and polypropylene), which does not have analogous functional groups and need to be functionalized by using γ -ray radiation, UV or plasma treatment, with the risk of scission of chemical bonds and ultimately degradation of the membranes [51, 52]. The chosen reaction was based on the ring opening mechanism between free OH groups of the PTA-OH and 1,3-propane sultone (1,3-PS) or 1,4-butane sultone (1,4-BS) (Fig. 5).

Figure 5. Functionalization of porous polytriazole membranes with sulfonic groups.

The success of the functionalization was confirmed by FTIR (Fig. 5). The FTIR spectra of polytriazole membranes show a new intense band at 1040 cm⁻¹ after the functionalization, which is associated with asymmetric stretching vibrations of O=S=O [53], and other new bands at 2885 and 2945 cm⁻¹, which are associated with aliphatic C–H stretching vibration from sulfones structure [54], indicating that the sulfonic groups are attached to the membrane. The broad band characteristic of OH groups, present in the pristine PTA-OH in the range of 3500–3000 cm⁻¹, became sharper, more intense and shifted to 3430 cm⁻¹ in the case of sulfonic-modified polytriazole. The shifting of this band is associated with the hydrogen bond formation between unreacted OH groups and sulfonic groups attached to the polymer chains [53, 55]. Comparing the FTIR spectra of polymer membranes modified with 1,3-PS and with 1,4-BE it can be observed that the intensity of the band associated with SO₃⁻ and the band associated with aliphatic C–H are slightly higher, suggesting that, when 1,4-BE is used, the density of attached sulfone groups is larger than when 1,3-PS is used. The ratio of the absorbance at 1040 cm⁻¹ ($A_{1040 \text{ cm}^{-1}}$ associated to O=S=O vibration) to that at 1172 cm⁻¹ ($A_{1172 \text{ cm}^{-1}}$ associated to C–F vibration), which remain unaltered after 1,3-PS or 1,4-BE grafting, for 1,3-PS grafting is 0.4 and for 1,4-BE grafting is 0.5. All spectra show an intense band at 1514 cm⁻¹, which is characteristic of triazole ring [36].

Fig. 6. FTIR spectra of pristine PTA-OH obtained from casting solutions in 65/35 [C2mim]OAc/DMC; membrane spectra after modification with 1,3-propane sultone (1,3-PS) and 1,4-butane sultone (1,4-BS). .

The morphologies of membranes activated with 1,3-PS and 1,4-BS are presented in Fig. 7. High porosity with interconnected and uniform pores can be seen even after modification with sulfonic groups, a clear indication that the functionalization effect on pore size was minimum.

Fig. 7. Morphology of (b) pristine and (a, c) sulfonic-modified polytriazole membranes prepared from casting solutions in 70/30 [C2mim]OAc/DMC; (a) membrane activated with 1,3-propane sultone (1,3-PS), and (c) with 1,4-butane sultone (1,4-BS).

To evaluate the binding capacity of the new cation exchange membrane, we used lysozyme, due to the positive charge at pH below its isoelectric point ($pH=11.2$). The protein adsorption tests were performed in batch. Fig. 8 shows the adsorption of lysozyme in batch with the modified membranes, which were prepared by casting 9 wt% PTA-OH solutions in 60/40, 65/35 and 70/30 [C2mim]OAc/DMC. After modification with 1,3-PS and 1,4-BE, they were immersed in 10 mL of 0.3 g/L of lysozyme solution in 50 mM Tris buffer at pH 8. The binding capacity was measured after the equilibrium was reached. In all cases higher protein adsorption capacity was obtained for membranes modified with 1,4-BE than with 1,3-PS, which could be correlated with the presence of more sulfone groups, as observed in FTIR spectra ($A_{1040\text{ cm}^{-1}}/A_{1172\text{ cm}^{-1}} = 0.5$ for 1,4-BE grafting comparing to $A_{1040\text{ cm}^{-1}}/A_{1172\text{ cm}^{-1}} = 0.4$ for 1,3-PS grafting). The protein adsorption capacity of the modified membranes obtained from different ratios of [C2mim]OAc/DMC increases, with increasing the amount of [C2mim]OAc (Fig. 8). This behavior could be correlated with the high porosity and interconnectivity of the membranes, as observed in Fig. 3, which corresponds to a higher internal surface area and therefore to a higher density of binding sites available for protein adsorption.

Fig. 8. Lysozyme adsorption on membranes prepared from 9 wt% polymer casting solutions in 60/40, 65/35 and 70/30 [C2mim]OAc/DMC.

Conclusions

We developed highly porous polytriazole-based ion exchange membranes. For the membrane preparation the polymer was dissolved in non-toxic solvents such as 1-ethyl-3-methylimidazolium acetate ([C2mim]OAc) and dimethyl carbonate (DMC), followed by phase inversion in water. Cosolvency was observed. The PTA-OH is not soluble in the pure solvents, but is soluble in a mixture of them. The optimal ratios of [C2mim]OAc/DMC were 60/40, 65/35 and 70/30, allowing to work with a broader range of polymer concentrations. We investigated the morphology of membranes prepared with different evaporation times and different precipitation baths. The membranes were activated by incorporation of sulfone groups for lysozyme adsorption, by using the following grafted agents: 1,3 propane sultone and 1,4-butane sultone. The best ion exchange performance was obtained for the membranes prepared from 70/30 [C2mim]OAc/DMC and modified with 1,4-butane sultone. The advantages of having high density of free OH groups, large and homogeneous pores, as well as the fact that the solvents are non-toxic, make the new polytriazole membranes a convenient platform for further functionalization with different ligands and groups other than sulfonic, for protein separation.

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References

- [1] The Global Use of Medicine: Outlook through 2018. Report by the IMS Institute for Healthcare Informatics. <<http://www.imshealth.com/en/thought-leadership/quintilesims-institute/reports/global-outlook-for-medicines-through-2018#ims-form>>, 2016 (accessed 30.11.2016.).
- [2] V. Orr, L. Zhong, M. Moo-Young, C.P. Chou, Recent advances in bioprocessing application of membrane chromatography, *Biotechnology Advances* 31(4) (2013) 450-465.
- [3] B. Cristiana, D. Simone, Advances in Membrane Chromatography for the Capture Step of Monoclonal Antibodies, *Current Organic Chemistry* 20 (2016) 1-7.

- [4] T. Sata, Ion exchange membranes: preparation, characterization, modification and application, Royal Society of Chemistry 2004.
- [5] E. Drioli, A. Brunetti, G. Di Profio, G. Barbieri, Process intensification strategies and membrane engineering, *Green Chemistry* 14(6) (2012) 1561-1572.
- [6] A. Figoli, T. Marino, S. Simone, E. Di Nicolo, X.M. Li, T. He, S. Tornaghi, E. Drioli, Towards non-toxic solvents for membrane preparation: a review, *Green Chemistry* 16(9) (2014) 4034-4059.
- [7] C. Jimenez-Gonzalez, D.J.C. Constable, C.S. Ponder, Evaluating the "Greenness" of chemical processes and products in the pharmaceutical industry-a green metrics primer, *Chemical Society Reviews* 41(4) (2012) 1485-1498.
- [8] K. Vanherck, G. Koeckelberghs, I.F.J. Vankelecom, Crosslinking polyimides for membrane applications: A review, *Progress in Polymer Science* 38(6) (2013) 874-896.
- [9] P. Marchetti, M.F. Jimenez Solomon, G. Szekely, A.G. Livingston, Molecular Separation with Organic Solvent Nanofiltration: A Critical Review, *Chemical Reviews* 114(21) (2014) 10735-10806.
- [10] R.K. Henderson, C. Jimenez-Gonzalez, D.J.C. Constable, S.R. Alston, G.G.A. Inglis, G. Fisher, J. Sherwood, S.P. Binks, A.D. Curzons, Expanding GSK's solvent selection guide - embedding sustainability into solvent selection starting at medicinal chemistry, *Green Chemistry* 13(4) (2011) 854-862.
- [11] A. Amelio, G. Genduso, S. Vreysen, P. Luis, B. Van der Bruggen, Guidelines based on life cycle assessment for solvent selection during the process design and evaluation of treatment alternatives, *Green Chemistry* 16(6) (2014) 3045-3063.
- [12] C.R. Cannon, Cellulose acetate membranes, U.S. Patent 3,460,683 1969.
- [13] Y. Medina-Gonzalez, P. Aimar, J.F. Lahitte, J.C. Remigy, Towards green membranes: preparation of cellulose acetate ultrafiltration membranes using methyl lactate as a biosolvent, *International Journal of Sustainable Engineering* 4(1) (2011) 75-83.
- [14] M.-m. Tao, F. Liu, B.-r. Ma, L.-x. Xue, Effect of solvent power on PVDF membrane polymorphism during phase inversion, *Desalination* 316 (2013) 137-145.
- [15] I. Soroko, Y. Bhole, A.G. Livingston, Environmentally friendly route for the preparation of solvent resistant polyimide nanofiltration membranes, *Green Chemistry* 13(1) (2011) 162-168.

- [16] R.D. Rogers, K.R. Seddon, Ionic Liquids--Solvents of the Future?, *Science* 302(5646) (2003) 792-793.
- [17] J. Lu, F. Yan, J. Texter, Advanced applications of ionic liquids in polymer science, *Progress in Polymer Science* 34(5) (2009) 431-448.
- [18] E. Garcia-Verdugo, B. Altava, M.I. Burguete, P. Lozano, S.V. Luis, Ionic liquids and continuous flow processes: a good marriage to design sustainable processes, *Green Chemistry* 17(5) (2015) 2693-2713.
- [19] P.C. Marr, A.C. Marr, Ionic liquid gel materials: applications in green and sustainable chemistry, *Green Chemistry* 18(1) (2016) 105-128.
- [20] P. Kubisa, Ionic liquids as solvents for polymerization processes—Progress and challenges, *Progress in Polymer Science* 34(12) (2009) 1333-1347.
- [21] T. Erdmenger, C. Guerrero-Sanchez, J. Vitz, R. Hoogenboom, U.S. Schubert, Recent developments in the utilization of green solvents in polymer chemistry, *Chemical Society Reviews* 39(8) (2010) 3317-3333.
- [22] H. Olivier-Bourbigou, L. Magna, D. Morvan, Ionic liquids and catalysis: Recent progress from knowledge to applications, *Applied Catalysis A: General* 373(1–2) (2010) 1-56.
- [23] J. Zhou, M.M. Mok, M.G. Cowan, W.M. McDanel, T.K. Carlisle, D.L. Gin, R.D. Noble, High-Permeance Room-Temperature Ionic-Liquid-Based Membranes for CO₂/N₂ Separation, *Industrial & Engineering Chemistry Research* 53(51) (2014) 20064-20067.
- [24] W.M. McDanel, M.G. Cowan, J.A. Barton, D.L. Gin, R.D. Noble, Effect of Monomer Structure on Curing Behavior, CO₂ Solubility, and Gas Permeability of Ionic Liquid-Based Epoxy–Amine Resins and Ion-Gels, *Industrial & Engineering Chemistry Research* 54(16) (2015) 4396-4406.
- [25] D.Y. Xing, N. Peng, T.-S. Chung, Formation of Cellulose Acetate Membranes via Phase Inversion Using Ionic Liquid, [BMIM]SCN, As the Solvent, *Industrial & Engineering Chemistry Research* 49(18) (2010) 8761-8769.
- [26] D.Y. Xing, S.Y. Chan, T.-S. Chung, Molecular interactions between polybenzimidazole and [EMIM]OAc, and derived ultrafiltration membranes for protein separation, *Green Chemistry* 14(5) (2012) 1405-1412.

- [27] D.Y. Xing, S.Y. Chan, T.-S. Chung, The ionic liquid [EMIM]OAc as a solvent to fabricate stable polybenzimidazole membranes for organic solvent nanofiltration, *Green Chemistry* 16(3) (2014) 1383-1392.
- [28] D.Y. Xing, S.Y. Chan, T.-S. Chung, Fabrication of porous and interconnected PBI/P84 ultrafiltration membranes using [EMIM]OAc as the green solvent, *Chemical Engineering Science* 87 (2013) 194-203.
- [29] S. Livazovic, Z. Li, A.R. Behzad, K.V. Peinemann, S.P. Nunes, Cellulose multilayer membranes manufacture with ionic liquid, *Journal of Membrane Science* 490 (2015) 282-293.
- [30] D. Kim, N. Moreno, S.P. Nunes, Fabrication of polyacrylonitrile hollow fiber membranes from ionic liquid solutions, *Polymer Chemistry* 7(1) (2016) 113-124.
- [31] D. Kim, O.R. Salazar, S.P. Nunes, Membrane manufacture for peptide separation, *Green Chemistry* 18(19) (2016) 5151-5159.
- [32] P. Madhavan, R. Sougrat, A.R. Behzad, K.-V. Peinemann, S.P. Nunes, Ionic liquids as self-assembly guide for the formation of nanostructured block copolymer membranes, *Journal of Membrane Science* 492 (2015) 568-577.
- [33] P. Tundo, M. Selva, The Chemistry of Dimethyl Carbonate, *Accounts of Chemical Research* 35(9) (2002) 706-716.
- [34] M. Fabris, V. Lucchini, M. Noè, A. Perosa, M. Selva, Ionic Liquids Made with Dimethyl Carbonate: Solvents as well as Boosted Basic Catalysts for the Michael Reaction, *Chemistry – A European Journal* 15(45) (2009) 12273-12282.
- [35] T.N. Glasnov, J.D. Holbrey, C.O. Kappe, K.R. Seddon, T. Yan, Methylation using dimethylcarbonate catalysed by ionic liquids under continuous flow conditions, *Green Chemistry* 14(11) (2012) 3071-3076.
- [36] S. Chisca, P.H.H. Duong, A.H. Emwas, R. Sougrat, S.P. Nunes, Crosslinked copolyazoles with a zwitterionic structure for organic solvent resistant membranes, *Polymer Chemistry* 6(4) (2015) 543-554.
- [37] L.F. Villalobos, S. Chisca, H. Cheng, P.-Y. Hong, S. Nunes, K.-V. Peinemann, In situ growth of biocidal AgCl crystals in the top layer of asymmetric polytriazole membranes, *RSC Advances* 6(52) (2016) 46696-46701.
- [38] S. Chisca, G. Falca, V.E. Musteata, C. Boi, S.P. Nunes, Crosslinked polytriazole membranes for organophilic filtration, *Journal of Membrane Science* 528 (2017) 264-272.

- [39] R.F. Blanks, J.M. Prausnitz, Thermodynamics of Polymer Solubility in Polar and Nonpolar Systems, *Industrial & Engineering Chemistry Fundamentals* 3(1) (1964) 1-8.
- [40] Hansen-solubility website <<https://www.hansen-solubility.com/buy-HSPiP-software.php>>, (accessed 26.03.2017.).
- [41] L.B. Favero, B.M. Giuliano, S. Melandri, A. Maris, P. Ottaviani, B. Velino, W. Caminati, CH $\cdots$ O and CH $\cdots$ F Links Form the Cage Structure of Dioxane–Trifluoromethane, *The Journal of Physical Chemistry A* 109(33) (2005) 7402-7404.
- [42] S.K. Reddy, S. Balasubramanian, Liquid Dimethyl Carbonate: A Quantum Chemical and Molecular Dynamics Study, *The Journal of Physical Chemistry B* 116(51) (2012) 14892-14902.
- [43] J.M.G. Cowie, J.T. McCrindle, Polymer cosolvent systems—I. Selective adsorption in methyl cyclohexane (1), acetone (2), polystyrene (3), *European Polymer Journal* 8(10) (1972) 1185-1191.
- [44] X. Tan, X. Li, L. Chen, F. Xie, Solubility of starch and microcrystalline cellulose in 1-ethyl-3-methylimidazolium acetate ionic liquid and solution rheological properties, *Physical Chemistry Chemical Physics* 18(39) (2016) 27584-27593.
- [45] P.A. Hunt, C.R. Ashworth, R.P. Matthews, Hydrogen bonding in ionic liquids, *Chemical Society Reviews* 44(5) (2015) 1257-1288.
- [46] Y.-k. Ahn, B. Kim, J. Ko, D.-J. You, Z. Yin, H. Kim, D. Shin, S. Cho, J. Yoo, Y.S. Kim, All solid state flexible supercapacitors operating at 4 V with a cross-linked polymer-ionic liquid electrolyte, *Journal of Materials Chemistry A* 4(12) (2016) 4386-4391.
- [47] C.M. Hansen, The three dimensional solubility parameter, *Journal of paint technology* 39(505) (1967) 104-117.
- [48] C.M. Hansen, *Hansen Solubility Parameters: A User's Handbook*, Second Edition, CRC Press 2007.
- [49] A.A.R. Mota, C.C. Gatto, G. Machado, H.C.B. de Oliveira, M. Fasciotti, O. Bianchi, M.N. Eberlin, B.A.D. Neto, Structural Organization and Supramolecular Interactions of the Task-Specific Ionic Liquid 1-Methyl-3-carboxymethylimidazolium Chloride: Solid, Solution, and Gas Phase Structures, *The Journal of Physical Chemistry C* 118(31) (2014) 17878-17889.
- [50] S.P. Nunes, T. Inoue, Evidence for spinodal decomposition and nucleation and growth mechanisms during membrane formation, *Journal of Membrane Science* 111(1) (1996) 93-103.

- [51] X. Zeng, E. Ruckenstein, Membrane Chromatography: Preparation and Applications to Protein Separation, *Biotechnology Progress* 15(6) (1999) 1003-1019.
- [52] B. Bhut, Design of advanced ion-exchange membranes and their performance assessment for downstream chromatographic bioseparations, Clemson University 2010.
- [53] G. Li, C. Zhao, X. Li, D. Qi, C. Liu, F. Bu, H. Na, Novel side-chain-type sulfonated diphenyl-based poly(arylene ether sulfone)s with a hydrogen-bonded network as proton exchange membranes, *Polymer Chemistry* 6(32) (2015) 5911-5920.
- [54] S. Chisca, I. Sava, M. Bruma, Porous polyimide films obtained by using lithium chloride as pore-forming agent, *Polymer International* 62(11) (2013) 1634-1643.
- [55] C.-K. Lin, J.-C. Tsai, The effect of the side-chain ratio on main-chain-type and side-chain-type sulfonated poly(ether ether ketone) for direct methanol fuel cell applications, *Journal of Materials Chemistry* 22(18) (2012) 9244-9252.

Highly porous polytriazole ion exchange membranes cast from solutions in non-toxic cosolvents

Research highlights

- Synthesis of highly porous polytriazole ion exchange membranes.
- Membranes prepared by non-toxic solvent mixture based on ionic liquid.
- Cosolvency of hydroxyl-polytriazole/dimethyl carbonate/ionic liquid.
- Membranes activated with sulfonic groups and tested for protein absorption.