**Sustainable fabrication and pervaporation application of bio-based membranes:
combining a polyhydroxyalkanoate (PHA) as biopolymer and Cyrene™ as green solvent**

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Abstract

Within the prospect to develop a more sustainable solution for the membrane fabrication sector, this work aims to investigate a new combination of bio-based materials, including a polyhydroxyalkanoate (PHA) as a polymer and Cyrene™ as a solvent, for the phase inversion process. The herein studied poly(hydroxybutyrate-co-hydroxyvalerate) (PHBV) is a microbial biopolymer with excellent biocompatible, biodegradable and solvent resistance properties; while Cyrene™ is a non-toxic, biodegradable and renewable alternative to most of the traditionally used polar aprotic solvents.

Several parameters of the phase inversion process were studied in order to identify the different membrane microstructure possibilities and thus their final performances. The various studied process parameters include the evaporation time before coagulation, the use of different traditional and green additives, the concentration of polymer and additives into the dope solution and the molecular weight of the additives. Polyethylene glycol (PEG),

polyvinylpyrrolidone (PVP) and an epoxidized broccoli vegetable oil (EBO) were successfully used as pore former agents for these membranes. Developed membranes were fully characterized in terms of morphology, topography, surface wettability, pore size, porosity, thermal degradation, mechanical resistance and stability. After all, membranes exhibiting different architectures (from porous to dense) were obtained. In order to prove their applicability, dense membranes were finally successfully applied in pervaporation (PV) for the separation of an organic/organic azeotropic mixture.

Keywords: bio-based membranes; PHBHV; CyreneTM; green solvents; biopolymers; pervaporation

1 Introduction

In the last decades, the transition towards a circular economy, in order to face the current global concerns, spurred to a shift in the production system from the use of petroleum-derived products to the use of new chemicals deriving from renewable, recyclable and biodegradable resources. Sustainable chemistry is at the basis of the new research of chemicals efficiently exploiting the natural resources and minimizing carbon dioxide emissions and wastes production [1]. Pursuing the criteria of the process intensification strategy [2] and green chemistry design [3,4], it is possible to develop and re-design new and more efficient processes which can operate with a lower energy requirement and a reduced footprint tapping into natural raw materials. Membrane operations, thanks to their numerous advantages, such as flexibility, low footprint, modest energy requirement, simplicity of operation, absence of chemicals to achieve the separation [2], well meet the needs of the sustainable development and process intensification strategy. However, the way the membranes are produced still suffers from the use of chemicals (polymer, solvents and additives) derived from fossil based sources or classified as toxic for humans and harmful for the environment [5]. In order to establish a virtuous cycle, the entire membrane process should be fully sustainable: starting from the preparation of the membrane to its final disposal. Lawler et al. [6] studied, through a life cycle assessment, the environmental impact of a reverse osmosis (RO) module used for water desalination, finding out that the membrane production is the main contributor of CO₂ emissions to the atmosphere accounting for the 45% of emissions for each module produced. Moreover, the materials employed for the membrane preparation process (they considered polysulfone and polyamide as polymers together with N,N-dimethylformamide (DMF) as solvent) are the components, during a RO module manufacturing, with the highest negative impact on the environment in terms of ecotoxicity, ozone and fossil depletion, marine and freshwater eutrophication. In addition, the life cycle assessment of the fabrication of cellulose acetate membranes using N-methyl-2-

pyrrolidone (NMP) confirmed that the solvent has a major contribution on the overall environmental impacts [7].

The choice of the proper polymer-solvent system is crucial in the formation of the membrane and in determining the final morphology and properties. Currently, the most employed polymers in porous membrane preparation, at laboratory and industrial scale, are polysulfone (PSf), polyethersulfone (PES), polyvinylidene fluoride (PVDF), polytetrafluoroethylene (PTFE) and polyacrylonitrile (PAN) which are all fossil-based materials. They are soluble in traditional polar aprotic solvents, such as NMP, N, N-dimethyl acetamide (DMA) and DMF. All these solvents, despite their large use in membrane manufacturing, can pose serious risks for the environment and humans health. The USA Environmental Protection Agency (EPA), for instance, recently proposed to ban NMP due to its possible reproductive toxicity, while DMF and DMA are included in list of Substances of Very High Concern (SVHC) [8].

During the last years, a part of the research on membrane preparation started to shed the light on new benign and green solvents which could be used for the preparation of polymeric membranes and on reducing or abolishing the use of toxic solvents [9].

Seminal works started to explore a series of more sustainable or fully green solvents such as triethyl phosphate (TEP) [10], dimethyl isosorbide (DMI) [11], Rhodiasolv® PolarClean [12,13], ethyl lactate [14], *p*-Cymene [15] and Cyrene™ [16] to be used for the preparation of more sustainable membranes using common polymers (e.g. PES and PVDF). Complementary to the use of a green solvent, is the employment of sustainable materials such as biopolymers able to provide a series of undoubtable benefits including biodegradability, biocompatibility, easy processability, low cost and safe waste disposal [17]. The production of membranes based on biopolymers is a growing field of interest and it is particularly attractive in biomedical and pharmaceutical applications. Polylactic acid (PLA) [18], polybutylene succinate [19], polyhydroxyalkanoates (PHA) [20–22], chitosan [23], alginate [24], cellulose acetate [25] and

starch [26] have been widely investigated as biopolymers for the production of membranes applied in different sectors. However, biopolymers selection is not completely tailored for membrane applications and their use is often challenging and problematic.

To fully respond to the requirements related to the reduction of waste generation and in accordance with the basic principles of the Green Chemistry, this work aims to develop innovative fully sustainable membranes exploiting raw bio-based materials (polymer, solvent and additive) for targeted applications.

The biopolymer selected, poly(hydroxybutyrate-co-hydroxyvalerate) (PHBHV), is an industrially available representative of the family of PHA, which are microbial biopolymers with excellent biocompatible and biodegradable properties [27]. PHBHV is a linear copolymer that is receiving growing attention due to its versatile thermomechanical properties tailored by its hydroxyvalerate (HV) content [28,29].

PHBHV, moreover, differently from the most of the above-mentioned biopolymers, has the advantage to be directly extracted from its biomass and displays remarkable organic solvents resistance properties. In particular, PHBHV (especially when valerate content is low) is generally soluble in chlorinated solvents but presents poor solubility in most common organic solvents (e.g. ethanol, acetone, DMA, ethyl acetate or THF) under atmospheric pressure and at moderate temperature [30,31].

After a preliminary evaluation screening carried out on different green solvents (e.g. Tamisolve™, Rhodiasolv® PolarClean, dimethyl carbonate), dihydrolevoglucosenone (Cyrene™) has been selected as the best option to solubilize PHBHV thanks to its ability to completely solubilize the polymer and to form stable and homogenous dope solutions. Cyrene™ is an innovative bio-based solvent derived from ligno-cellulosic biomass feed-stock and produced by the Circa Group [32]. The synthesis of Cyrene™ occurs through the intermediate 1,6-anhydro-3,4-dideoxy-D-glycero-hex-3-enopyranos-2-ulose (or

Levogluconone) that is converted into the final product by dehydrogenation [33]. Cyrene™ is a polar aprotic solvent with a chemo-physical profile comparable to traditional solvents (NMP, DMA and DMF) [34]. Furthermore, Cyrene™ is non-toxic, biodegradable and renewable. The most relevant physical-chemical properties and eco-toxicological information of Cyrene™ respect to traditional solvents (NMP, DMF and DMA) are reported in Table S1. In this work, the bio-based membranes were prepared by changing different phase inversion parameters to evaluate the effect on membrane properties and performance. The influence of polymer concentration, evaporation time and additives type was studied. The membranes have been fully characterized in terms of morphology, topography, thickness, porosity, mechanical properties, surface wettability and pure water permeability. The application of selected membranes in pervaporation (PV) for the separation of a methanol (MeOH)/methyl tert-butyl ether (MTBE) azeotropic mixture, at different operating temperatures, has been investigated. Finally, long-term stability tests of two selected membranes were performed in sea water and in enzymatic solution at room temperature and at 37°C, for up to 105 days, in order to assess the membrane stability or microstructural changes overtime in aqueous medium. To the best of our knowledge this is the first time that fully sustainable membranes, based on polymers of the PHA family, are produced and characterized. This work aims to shed a new light in a more and more sustainable and green preparation of polymeric membranes without scarifying their properties and performance.

2 Material and method

2.1 Chemicals

PHBHV pellets were supplied by Tianan Biologic Material (China), under the trade name Enmat Y1000P. They were purified by dissolution in chloroform and precipitation in methanol. The white purified PHBHV powder was characterized by gel permeation chromatography (GPC) equipped with three successive columns (2 × ResiPore and 1 × PL gel Mixed C from

Agilent) and a Waters UV detector working at a wavelength of 241 nm. The molecular weight was determined to be 116 000 g.mol⁻¹. Differential scanning calorimetry (DSC) (TA Instruments - Q10 DSC) was used to measure its crystallinity degree, χ_c = 62%, (see Fig. S1). The crystallinity ratio (X_c) of the polymer was calculated based on the melting enthalpy measured at the first cycle (ΔH_m = 89,99 J/g). The following equation (Eq. 1) was used:

$$X_c (\%) = (\Delta H_m / \Delta H_{PHB}^0) \times 100 \quad (1)$$

with ΔH_{PHB}^0 = 146 J/g, the melting enthalpy of the polymer 100% crystalline.

The HV monomer content was determined by ¹H NMR analysis (Bruker 400 MHz) and was equal to 2.3 mol%. This PHBHV has a T_g of 28 °C, a T_m of 172°C and a T_c around 120-130 °C. Cyrene™ was used as the solvent and purchased from Sigma Aldrich. Conventional additives, polyethyleneglycol (PEG) 200 g.mol⁻¹ (PEG200) and 600 g.mol⁻¹ (PEG600) were supplied by Sigma Aldrich while polyvinylpyrrolidone (PVP) 17,000 g.mol⁻¹ (PVPK17) and 30,000 g.mol⁻¹ (PVPK30) were supplied by BASF.

Epoxidized broccoli oil (EBO) was investigated as a bio-based additive (see molecular structure in supplementary information, Fig. S2) and it has previously been demonstrated to act as a plasticizer for PHBHV [35].

It was produced by the epoxidation of a by-product generated during the manufacturing of a food complement extracted from broccoli bean. EBO was gently provided by a research team from the *Institut des Sciences Chimique de Rennes* [35]. It has a molecular weight of 1,017 g.mol⁻¹.

All the chemicals were used as received.

2.2 Membrane preparation

The schematic overview of the membrane fabrication is shown in Figure 1. First, dope solutions were prepared by dissolving the additives and PHBHV into Cyrene™. The solutions were stirred at 130°C for 3 hours. The different solution compositions of the fabricated membranes

are given in Table 1. The dope solutions were then automatically cast with a gap size of 300 μm on a glass plate kept at 140°C and left for various delayed times (evaporation induced phase separation, EIPS step) before being coagulated in water (the environmental measured relative humidity (RH) was $50 \pm 4 \%$). Then, the cast solutions were soaked into distilled water at 20°C to complete the phase inversion process by non-solvent induced phase separation (NIPS). Thus, the membranes were prepared by NIPS (when they were immediately immersed in water after casting) or by a combination of EIPS and NIPS when the evaporation step (from 0.5 to 5 min) was introduced before immersion in the coagulation bath (Table 1).

Afterward, to remove the remaining additives and solvent, the membranes were washed through three consecutive baths of distilled water at 60°C. Finally, the membranes were dried in an oven at 40°C for 24 hours (for measuring the water permeability the membranes were not dried but kept in water after preparation until their use).

Figure 1. A schematic overview of the membrane fabrication process.

The reference names and the fabrication parameters of the different membranes discussed in this study are shown in Table 1. The fabrication parameters that have been investigated include the dope solution composition (polymer and additive composition, additive nature) and the evaporation times.

Table 1. References and compositions of the various fabricated membranes. Concentrations are in weight percentage (wt%) in respect to the total dope solution.

Membrane reference	PHBHV concentration (wt%)	Additive		Evaporation time	Variable investigated
		Nature	Concentration (wt%)		
M-0				0	
M-0.5				0.5 min	Evaporation time
M-1.5	20%	None	/	1.5 min	
M-2.5				2.5 min	
M-5				5 min	
M-1.5-5PVPK17	20%	PVP17000	5%	1.5 min	Additive type at two different evaporation times
M-5-5PVPK17				5 min	
M-1.5-5PEG200	20%	PEG200	5%	1.5 min	
M-5-5PEG200				5 min	
M-1.5-5EBO	20%	EBO	5%	1.5 min	Additive and polymer concentration
M-5-5EBO				5 min	
M-1.5-7PEG200	20%		7%		
M-1.5-17/5PEG200	17%	PEG200	5%	1.5 min	
M-1.5-17/7PEG200	17%		7%		Additives molecular weights
M-1.5-17/7PVPK17		PVP17000			
M-1.5-17/7PVPK30	17%	PVP30000	7%	1.5 min	
M-1.5-17/7PEG600		PEG600			

The effect of EIPS before NIPS was studied through various evaporation times between 0 min and 5 min. The PHBHV concentration into the dope solution was between 17 wt% and 20 wt%. Various additives were investigated, including PEG200, PEG600, PVPK17, PVPK30 and EBO.

The concentrations of the additives into the dope solution that were investigated are 5 wt% or 7 wt%.

The tube tilting method was used to determine the sol-gel transition temperature for the PHBHV/CyreneTM system. Different polymer concentrations (5, 10, 15 and 20 wt%) were, thus, dissolved into the solvent at high temperature (130 °C). The temperature was, then, gradually decreased (5°C every hour) until the gel phase was achieved.

The viscosity of dope solutions (20 wt% of polymer), prepared with and without additives (5 wt%), was measured at 90°C using a rotational rheometer (Brookfield, Synchro-Lectric viscometer model: LV).

2.3 Characterization

2.3.1 Structural properties

The surface and cross section structures of each membrane were observed by SEM using a Zeiss EVO, MA10. For the cross-section analyses, the samples were freeze-fractured in liquid nitrogen. The samples were coated with a thin layer of gold prior analyses (sputter machine Quorum Q 150R S).

Surface roughness of selected membranes was measured by means of atomic force microscopy (AFM) (Bruker Multimode 8 with Nanoscope V controller). Data were acquired in tapping mode, using silicon cantilevers (model TAP150, Bruker). The images were collected in a scan size of 5 µm x 5 µm.

The average pore size of selected membranes was determined using a PMI Capillary Flow Porometer (CFP1500 AEXL, Porous Materials Inc., USA) using the wet up/dry up method. The membranes samples were initially wetted using Fluorinert FC-40 as a wetting liquid (surface tension 26 dyne/cm) for 2 h.

The membrane porosity was calculated by means of the following equation (Eq. 2):

$$Porosity (\%) = \left(\frac{\frac{W_w - W_d}{\rho_k}}{\frac{W_w - W_d}{\rho_k} + \frac{W_d}{\rho_p}} \right) * 100 \quad (2)$$

where W_w is the weight of the wet membrane, W_d is the weight of the dry membrane, ρ_k is the density of kerosene (0.80 g.cm^{-3}) and ρ_p is the density of the polymer (1.25 g.cm^{-3}). Three pieces of each membrane were weighted before and after their immersion in kerosene for 24 h.

2.3.2 Physical-chemical and mechanical tests

The water contact angles of the membranes were evaluated on the top surfaces using ultrapure water with the sessile drop method. The apparatus was a CAM200 instrument (KSV Instrument LTD, Finland). The mean and standard deviation values were calculated with five measurements.

The thermogravimetric experiments were carried out with a TGA Instruments SDT-Q600 apparatus at a scanning rate of $10^\circ\text{C.min}^{-1}$ under air with a temperature ramp from 20°C to 700°C .

The mechanical properties of the membranes were evaluated using a Zwick Roell Z 2.5 apparatus. Each sample was stretched unidirectionally at a steady velocity of 5 mm.min^{-1} . The initial distance between the clamps was of 50 mm. The mean of three samples was performed.

2.3.3 Pure water permeability (PWP)

The PWP was evaluated using a typical laboratory cross-flow cell purchased from DeltaE S.R.L. (Italy). Pure water at 20°C was pumped across the membrane (surface: 8 cm^2) by using a gear pump (Tuthill Pump Co., California). First, the membranes were conditioned with a transmembrane pressure of 2 bar until the water flow stabilized. Then, the PWP ($\text{L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$) was calculated by means of the following equation (Eq. 3):

$$PWP = \frac{Q}{S \times TMP} \quad (3)$$

where Q is the water flux ($\text{L}\cdot\text{h}^{-1}$), S the membrane surface area (m^2) and TMP the transmembrane pressure (bar).

2.3.4 Swelling experiments

The swelling degree of the dense membranes selected for the PV experiments was evaluated into methanol (MeOH), methyl tert-butyl ether (MTBE) and a mixture of them (50/50 w/w). First, small pieces of the membrane were weighed and introduced into each solvent solution. The samples were then left at 30 °C for 48 h. Afterward, the swollen pieces were removed from the solution, the excess of solvent on their surfaces was eliminated gently by using absorbent paper and they were then directly weighed. Experiments were carried out using a digital balance (Gibertini, Crystal 500, Italy) with the accuracy of 0.0001 g. The swelling degree for each membrane was calculated using the following equation (Eq. 4):

$$\text{Swelling degree (ml/g)} = \frac{1}{\rho_s} \left(\frac{W_w - W_d}{W_d} \right) \quad (4)$$

where ρ_s is the solvent density (MTBE: 0.74 g/ml; MeOH: 0.79 g/ml; MeOH/MTBE 50/50 w/w 0.765 g/ml), W_w is the weight of the wet piece and W_d is the weight of the dry piece. Swelling was determined as described by Geens et al. [36].

2.3.5 Pervaporation (PV) experiments

The PV performance of selected membranes showing a dense morphology was studied for the separation of a MeOH/MTBE organic/organic solution at the azeotropic point (MeOH/MTBE, 14.3/85.7, w/w).

The membrane cell of the laboratory scale set-up (see Fig. S3) was made of two stainless-steel half-cylinders hermetically sealed with nuts and bolts between which the membrane was placed on a porous stainless-steel plate. The effective surface of the membrane was 8.55 cm^2 . The cell was equipped with a mechanical stirrer and was thermally regulated with a double jacketed water circulation system. On the permeate side, the vapor was trapped into a glass condenser cooled by liquid nitrogen. A vacuum of 0.5 ± 0.1 mbar was applied on the permeate side by

means of a rotary vane pump RV5 (Edwards) and it was monitored using an Active Pirani Gauge (APG-M-NW16 AL, Edwards) manometer.

For each PV experiment, 200 mL of the azeotropic mixture were added into the cell and kept at 25°C, 35°C or 45°C. Once the steady state was reached, permeate samples were collected on a duration of at least 2h 30min. The total permeate flux J_t ($\text{Kg}\cdot\text{m}^{-2}\cdot\text{h}^{-1}$) was calculated as follows (Eq. 5):

$$J_t = \frac{\Delta m}{S \times \Delta t} \quad (5)$$

where Δm is the weight of the collected permeate (Kg), S the effective surface area of the membrane (m^2) and Δt the permeate collection time (h).

The composition of the feed and permeate samples were determined by measuring their refractive index with an Abbe 60DR refractometer (Bellingham and Stanley) at 25°C. The membrane selectivity factor (α) was then calculated as follows (Eq. 6):

$$\alpha = \frac{y_{\text{MeOH}}/y_{\text{MTBE}}}{x_{\text{MeOH}}/x_{\text{MTBE}}} \quad (6)$$

y_{MeOH} and y_{MTBE} are the respective concentrations of MeOH and MTBE into the permeate.

x_{MeOH} and x_{MTBE} are the respective concentrations of MeOH and MTBE into the feed.

2.3.6 Membrane degradation

The weight loss of two selected membranes in aqueous medium were assessed up to 105 days by membrane incubation in sea water from the Tyrrhenian sea (south west of Italy) and in enzymatic solution, both at 25°C and 37°C. The enzymatic solution was a fungal lipase from *Candida Rugosa*, 150 $\text{U}\cdot\text{mL}^{-1}$ in phosphate buffered solution pH 7.4, with 0.01% NaN_3 . Before the incubation, four different samples (2x2 cm) from each batch were dried in a vacuum oven and their initial weight (W_i) exactly measured. Then the samples were immersed in 4 mL of sea water or enzymatic solution, which were freshly changed every seven days. At the predetermined time intervals, the samples were washed with copious distilled water, dried in

vacuum oven at 50°C for 4 hours, and their final weight (W_f) precisely measured. The percentage of weight loss (W_{loss}) was determined by the following equation (Eq. 7):

$$W_{loss} (\%) = \frac{W_f}{W_i} * 100 \quad (7)$$

The pH of all the aqueous media incubated with the membranes for the whole time was further monitored.

3. Results and discussion

3.4 Membrane characterization

3.4.1 Effect of the evaporation time

Preliminary tests started with the preparation of membranes resulting from a solution containing 20 wt% of PHBHV in Cyrene™, without any additive. The solutions, heated at 130°C, were cast on a hot glass plate (140°C) and precipitated in water after an evaporation step ranging from 0 (membrane prepared by NIPS) to 5 min (membranes prepared by EIPS+NIPS). The heating of the glass plate was found to be crucial in order to prevent the premature gelification of the solution before NIPS.

A polymer-solvent system is generally studied by means of a phase diagram in relation to cloud point measurements at which a clear polymer solution becomes viscous and opaque [37]. This phenomenon occurs as a consequence of the separation of a polymer-lean phase and a polymer-rich phase (liquid-liquid phase separation). In our PHBHV/Cyrene™ system the solution underwent a phase change (starting to form a viscous gel) as the temperature was decreased. Fig. S4 shows the sol-gel transition temperature of the PHBHV/Cyrene™ system at different polymer concentrations. The results indicate that the sol-gel transition temperature increased (from 43 to about 70°C) as the polymer concentration increased (from 10 to 25 wt%). This can

be explained by the fact that a higher temperature is required to have a stable and homogenous polymer solutions as the polymer concentration is increased.

The membrane prepared only by NIPS (M-0) revealed poor mechanical properties and a great difficulty to be handled without breakage. The introduction of an evaporation step before coagulation, on the contrary, was found to be very beneficial for improving the resistance of the membranes. By looking at the cross-section morphology exhibited by the prepared membranes (Figure 2) it is possible to observe, in fact, that the increase in the evaporation time (from 0 to 5 min) led to a higher compactness especially for the evaporation time of 5 min for which the spherulitic structure is not visible anymore resulting in more mechanically stable membranes [38]. For the M-5 membrane, in fact, the spherulites seems fused together resulting in a less porous membrane.

Figure 2. SEM images of the PHBHV (20 wt%) based membranes prepared with different evaporation times before immersion in the coagulation bath. Top surface on the left and cross sections, with magnification and thickness measurements, on the right.

All the membranes display a smooth surface without visible pores or defects. The cross-sectional views showed dense upper structures, delimited by the white vertical line, associated

to spherulitic sublayers. The M-5 membrane differs slightly from the other membranes due to a sublayer made of more aggregated spherulites.

The structure of the M-0 membrane, prepared only by NIPS, is characterized by a dense top layer and a very porous spherulitic sublayer. The dense top surface is the result of a fast solvent/non-solvent demixing and a rapid polymer precipitation occurring when the cast film is put in contact with water [39]. This phenomenon is usually observed for the membranes prepared by NIPS technique [39–41].

But then, once the top layer is formed, the solvent/non-solvent exchange rate is lowered, so that the demixing in the sublayer is delayed. The spherulitic structure, observed for the sublayer, is typical of membranes prepared by thermally induced phase separation (TIPS) technique with (semi)crystalline polymers [37,41]. It is the result of a solid-liquid (S-L) demixing preceding a sufficiently delayed liquid-liquid (L-L) demixing [42]. It is, moreover, favored in case of high polymer concentrations or low coagulation bath temperatures [43]. In this case, the cast film underwent a quick temperature change, from 140°C to 20°C, when introduced into the coagulation bath, which induced a S-L demixing. This competition encountered between a NIPS and a TIPS process has been previously observed and discussed for PVDF or PHBHV membranes [21,41]. This observation indicates that the phase inversion process that occurred during the fabrication of the membrane in this study is a hybrid TIPS-NIPS process.

When an evaporation step is introduced before the NIPS step, two other phase inversion processes can start and potentially impact the final membrane structure: phase inversion *via* intake of the air humidity (vapor induced phase separation, VIPS) or phase inversion *via* solvent evaporation (EIPS). While VIPS involves, in most of the cases, a dominating non-solvent (water) inflow, EIPS involves a non-solvent outflow. Hence, two major different structures could be obtained.

In VIPS, the thermodynamic system, polymer/solvent/non-solvent, is the same as NIPS but with a slower demixing rate. Thus, the spinodal demixing does not occur and a porous structure without the dense upper layer is usually obtained [44]. On the contrary, in EIPS, the system to be considered is just a binary mixture polymer/solvent and a dense film would form through a S-L phase separation owing to solvent evaporation [45].

So, if the VIPS occurs during the evaporation time, a more porous top layer would be expected and if the EIPS occurs, a denser top layer would be foreseen. Both phenomena, actually, happen during this time interval with prevalence of one over the other depending on the balance between solvent outflow (EIPS) and non-solvent inflow (VIPS) [46]. For example, below a certain humidity level, the water intake rate would be too slow compared to the solvent evaporation rate and dense structures would be obtained [46].

In our case, increasing the evaporation time, a dense upper layer was always present, suggesting the absence of VIPS and the membrane formation by EIPS (Figure 2).

More details about the characteristic thicknesses of the membranes and their porosities are displayed in Table 2.

Table 2. Characteristics of the PHBHV based membranes made without additive and with different evaporation times.

	M-0	M-0.5	M-1.5	M-2.5	M-5
Evaporation time (min)	0	0.5	1.5	2.5	5
Thickness overall structure (μm)	239 ± 3	210 ± 4	94 ± 2	118 ± 3	72 ± 2
Dense upper structure (μm)	15 ± 2	15 ± 2	15 ± 2	16 ± 3	11 ± 1
Porosity (%)	<i>Not measurable Too fragile</i>	<i>Not measurable Too fragile</i>	70 ± 1	67 ± 3	43 ± 2
Water contact angle (°)	75 ± 2	74 ± 4	61 ± 1	69 ± 2	72 ± 4
Elongation at break (%)	1.3 ± 0.4	1.9 ± 0.1	2.0 ± 0.3	1.8 ± 0.3	3.3 ± 0.6

The overall membrane thickness varied from 72 μm, for the M-5 membrane, to 239 μm, for the M-0 membrane. The dense upper layer thickness varied from 11 μm to 16 μm among the membranes. The membranes prepared at the lowest evaporation times, M-0 and M-0.5, were

too fragile to make the porosity measurements possible. The other three membranes, M-1.5, M-2.5 and M-5, displayed respective overall porosities of 70 %, 67% and 43%. This decrease in porosity, as the evaporation time is increased, is clearly visible from the cross-section of the membranes that shows more compact structure at higher evaporation times (Figure 2). In the cross-section of the M-5 membrane, showing the lowest degree of porosity (43%), the spherulites underneath the top layer are fused together making the overall membrane more compact and less porous (as also evidenced by the lower membrane thickness (72 μm)). These membranes displayed relatively hydrophilic surfaces with contact angle values ranging from 61° to 75° . Since all the membranes have the same chemical composition, the variations in contact angle values can be related to variations in their surface roughness which may depend on their formation conditions [47].

AFM results (Fig. 3), carried out on M0, M1.5 and M5 membranes, in fact, confirm different surface roughness for the investigated membranes. The topography of the membranes was expressed in terms of root mean square (Sq), average roughness (Sa) and peak to peak value (Sz). M0 displayed the higher values of roughness on its surface which can be responsible of the highest value of contact angle exhibited by this membrane. The roughness is, in fact, related to contact angle by the following equation (Eq. 8):

$$\cos \theta_m = r \cos \theta_y \quad (8)$$

where r is the ratio of projected surface area to real surface area ($r = 1$ for a smooth surface and $r > 1$ for a rough surface), and θ_y is the Young contact angle, which is equal to θ_m if $r = 1$ [48]. According to this equation the hydrophobicity of a membrane is reinforced by surface roughness [49].

Therefore, the lower contact angle values shown by M1.5 and M5 membranes can be related to their lower roughness degree respect to M0.

Figure 3. AFM images and roughness parameters of M-0, M-1.5 and M-5 membranes

It is also important to highlight that the wettability degree of PHBHV membranes reported in literature is not unanimous varying from hydrophobic values of 96° [50] to hydrophilic values of 76° and 72.5° [51,52]. These discrepancies could be related to the different content of HV in the polymer and to the topography of each membrane.

The mechanical properties of the investigated membranes are reported in Fig. 4.

Figure 4. Influence of the evaporation time on the mechanical properties of the PHBHV based membranes.

As expected, the Young's modulus and tensile strength of the membranes increased with the evaporation time, offering better mechanical properties. The Young's modulus varied from 94

MPa to 490 MPa while the tensile strength ranged from 0.87 MPa to 13 MPa. These results are similar when compared to PVDF membranes which usually display a Young's modulus from 100 MPa to 600 MPa and a tensile strength from 0.2 MPa to 15 MPa [41,53].

The measured values of elongation at break (Table 2) are significantly lower than the ones reported for PVDF or PSf membranes, usually between 10 % and 500 % [41,54,55]. The low elongation at break is related to the properties of the used PHBHV, which has a crystallinity of 62% with rigid amorphous parts and a glass transition temperature of 28°C, responsible of the fragility of the polymer [56,57].

The improvement of the mechanical properties, Young's modulus, tensile strength and elongation at break is linked to the morphological changes induced by the introduction of the evaporation step during membrane preparation. By increasing the evaporation time, more interconnected spherulites are obtained (Figure 2), leading to lower porosities (Table 2) and finally increasing the mechanical parameters. Voids and pores, in fact, represent weak points in the polymer matrix making the membranes more fragile in comparison to denser structures [58]. The membranes exhibiting satisfying mechanical properties (M-1.5, M-2.5 and M-5) were tested for PWP but none of them was permeable to water as consequence of their dense top layer.

3.4.2 Effect of the dope solution composition

3.4.2.1 Nature of the additive

Within the prospect of tailoring the membrane microstructure and potentially membrane performance, different additives, PEG200, PVPK17 and EBO, were included into the dope solution. PEG and PVP have been both previously used for membrane fabrication with Cyrene™ as a solvent [59]. They act as pore former and hydrophilic agents, enhancing the water membrane permeability. PEG is a biodegradable, non-toxic compound with good

433 chemical and thermal stability. PVP is a fossil-based and non-toxic additive. On the other hand,
434 the EBO is a valorized co-product obtained from the synthesis of a food active ingredient,
435 already explored as a plasticizer for PHA based materials [35].
436 The PHBHV concentration was kept at 20 wt% and the additives concentration was fixed at 5
437 wt%. On the basis of the previous tests, two different evaporation times have been evaluated:
438 1.5 and 5 min. Below 1.5 min, in fact, the membranes demonstrated poor mechanical properties,
439 while 1.5 and 2.5 min produced membranes with similar structure and properties. The SEM
440 images of the membranes prepared with the three additives are shown in Fig. 5.

441

442

443

444 **Figure 5.** SEM images of the PHBV based membranes prepared with different additives and different evaporation times before immersion in
 445 the coagulation bath (dope solutions with 20 wt% PHBV and 5 wt% additive (PVPK17, PEG200 or EBO)). Top surface, with magnification,
 446 on the left and cross section, with magnification and thickness measurements, on the right.

For the membranes prepared with the three additives and an evaporation time of 1.5 min, M-1.5-5PVPK17, M-1.5-5PEG200 and M-1.5-5EBO, the SEM images show porous surfaces with a spherulitic sublayer visible along the cross-section as the results of the hybrid TIPS-NIPS mechanism. Hence, the effect of the additives is clearly visible on the surfaces of the membranes where pores with various sizes are present. The membrane with PEG200 as an additive, M-1.5-5PEG200, is the only one with finger like macrovoids above the spherulitic part. This result can be related to the lower MW of PEG200 (200 g.mol^{-1}) compared to EBO ($1,017 \text{ g.mol}^{-1}$) and PVPK17 ($17,000 \text{ g.mol}^{-1}$), which affects the viscosity of the dope solution. Since the dope solution viscosity increases with the additive molecular weight, the lower viscosity of the dope solution with PEG200 could result in a faster L-L demixing promoting the formation of macrovoids [60]. These results are, in fact, confirmed by viscosity measurements carried out on the solutions prepared with and without additives. The lower viscosity value was found for the dope solution formed by only polymer (20 wt%) and solvent which was 2461 cP. The viscosity was increased as PEG200 (5 wt%) was added in the dope solution (5532 cP). A further viscosity increase (6,539 cP) was, then, observed when PVPK17 (5 wt%) was used, as a consequence of its higher molecular weight. Finally, the addition of EBO led to a drastic increase in solution viscosity (88,511 cP) due to its high density.

It is also worth noting that EBO leads to the formation of more and larger surface pores compared to PEG200 and PVPK17 (Fig. 5). However, the EBO membrane appears to have the lowest porosity (Table 3). These observations are not contradictory as the SEM picture only allows to observe surface pores while porosity measurements account for the whole membrane volume including the upper and sublayers.

As previously observed, regardless of the nature of the additive, the overall membrane microstructure tends to be less porous when the evaporation time is increased from 1.5 min to 5 min. For the M-5-5PEG200 and M-5-5EBO membranes, the spherulitic sublayer is not visible

any more and it is replaced by a denser cross-sectional structure. The spherulitic substructure is still visible only for the M-5-5PVPK17 membrane, even if the spherulites seem to be fused together respect to the membranes prepared at 1.5 min. Some pores on the surface can be observed for the M-5-5PVPK17 and M-5-5PEG200 membranes.

Both the presence of additives and the evaporation time played a role in the EIPS process (Fig. 5). From a thermodynamic point of view, additives can be compared to a non-solvent. In that case, the binary system polymer/solvent turns into the ternary system polymer/solvent/additive. Hence, during the solvent evaporation, instead of a direct S-L demixing, a L-L demixing, between a polymeric rich phase and an additive rich phase, can occur [45]. The additive rich phase would grow and coalesce to finally give the final pores of the membrane, herein observed on the surface, while the polymeric rich phase would yield to the membrane matrix.

More detailed characteristics of the membranes made with additives are displayed in Table 3.

Table 3. Characteristics of the PHBHV based membranes made with additives and different evaporation times.

	M-1.5- 5PVPK17	M-1.5- 5PEG200	M-1.5- 5EBO	M-5- 5PVPK17	M-5- 5PEG200	M-5- 5EBO
Evaporation time	1.5 min	1.5 min	1.5 min	5 min	5 min	5 min
Additive	PVPK17	PEG200	EBO	PVPK17	PEG200	EBO
Thickness overall structure (μm)	160 ± 14	201 ± 5	131 ± 3	156 ± 7	62 ± 1	97 ± 2
Upper structure (μm)	24 ± 2	17 ± 4	20 ± 4	/	/	/
Water contact angle ($^\circ$)	70 ± 2	63 ± 3	84 ± 1	74 ± 7	70 ± 1	75 ± 1
Young's modulus (MPa)	120 ± 48	187 ± 45	71 ± 4	457 ± 91	300 ± 35	419 ± 51
Tensile strength (MPa)	1.4 ± 0.5	1.7 ± 0.4	1.8 ± 0.3	21 ± 5	11 ± 3	8.6 ± 0.7
Elongation at break (%)	2.0 ± 0.5	1.7 ± 0.4	1.3 ± 0.1	3.1 ± 0.1	3.0 ± 1.0	4.1 ± 2.3
Porosity (%)	70 ± 2	67 ± 1	61 ± 1	48 ± 2	52 ± 4	44 ± 4

The overall membrane thickness ranged from 62 μm , for the M-5-5PEG200 membrane, to 201 μm , for the M-1.5-5PEG200 membrane. The upper structure thickness was determined for the membranes showing well distinguished limits between the upper structure and the spherulitic sublayer. The upper structure varied from 17 μm to 24 μm among the membranes made with 1.5 min evaporation time.

Despite the thickness differences, whether for the overall thickness or the upper structure thickness, no clear trend is depicted with the additive nature or membrane porosity. One potential explanation could be a variation of the closed porosity microstructure among these membranes for the different additives tested. Indeed, since the closed cells can not be measured with the porosity measurement technique employed here, it is possible that the closed porosities among these membranes are different, thus what would impact the membrane thickness.

The surfaces of these membranes display a different hydrophilic character as demonstrated by the water contact angles ranging from 63° to 84°. The membranes prepared with PEG200 revealed to be the ones with a more hydrophilic moiety respect to the membranes prepared with EBO as an additive which presented the highest values of contact angle. PEG and PVP are hydrophilic additives widely employed to improve the porosity and pore size of the membranes. The measured contact angle values of the membranes prepared with these two additives are slightly higher respect to pristine membranes (Table 2). This can be due to the different surface topography of the membranes prepared with and without additives.

As evidenced from Fig. 6, in fact, the membranes prepared with PVP and PEG presented higher values of Sq, Sa and Sz respect to the analogue membrane prepared without additives (M1.5). The addition of PVP and PEG, in fact, promoted the formation of porous surfaces that, according to Cassie-Baxter model, entrap more air pockets inside the pores inducing higher contact angle values [61].

The higher contact angle value (84°) for the M-1.5-EBO membrane can be also related to its higher values of roughness (Sq and Sa) with respect to the other membranes as a consequence of the presence of large and numerous pores on its surface as evidenced in SEM images.

Figure 6. AFM images and roughness parameters of M-1.5-5PVPK17, M-1.5-5PEG200 and M-1.5-5EBO membranes

The mechanical parameters, the Young's modulus, tensile strength and elongation at break were enhanced when the evaporation was increased from 1.5 min to 5 min. Indeed, the Young's modulus varied from 71 MPa to 187 MPa for the membranes obtained with 1.5 min of evaporation time and from 300 MPa to 457 MPa for the membranes obtained with 5 min of evaporation time. Coherently, both tensile strength and elongation at break risen significantly with the evaporation time extension (Table 3). This is related to the decrease of porosity observed at evaporation time of 5 min independently on the presence of additives (Fig. 7).

Figure 7. Porosities of the PHBV based membranes as a function of the evaporation time (dope solutions with 20 wt% PHBV without additive (no additive) or with 5 wt% additive (PVPK17, PEG200 or EBO)).

PWP tests highlighted that only the M-1.5-5PEG200 membrane was permeable to water with a value of $200 \pm 80 \text{ L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$. This membrane combining large pores on its surface (Fig. 5) and high porosity (67 %) (Table 3) displayed a PWP value in the ultrafiltration (UF) range although highly variable. It should be also noted that the other membranes are not permeable to water suggesting the absence of open and interconnected pores.

3.4.2.2 Influence of polymer concentration, additives concentration and additives molecular weight

The influence of the PHA concentration as well as additive concentration and molecular weights have been also investigated. To this purpose, the evaporation time was fixed at 1.5 min and PEG200 has been selected as a pore forming additive. The polymer concentration was lowered to 17 wt% meanwhile the additive concentration was increased to 7 wt%. Fig. 8

displays the surface SEM images of the PHBHV based membranes prepared by using different polymer and PEG200 concentrations.

Figure 8. Top surface SEM images of the PHBHV based membranes prepared with different polymer and additive (PEG200) concentrations (evaporation of 1.5 min).

All the membranes display a porous surface. When PEG200 concentration increases from 5% to 7%, the size and number of surface pores increase. Similarly, when the PHBHV concentration decreases from 20% to 17%, an increase of the size and number of pores is observed. In other words, by decreasing the PHBHV concentration and increasing the additive concentration, more and bigger pores over the surface can be observed without any variation in the morphology of the cross-section that maintained a spherulitic substructure (see Fig. S5). During the partial evaporation of the solvent at the surface, an increase of the additive concentration enriches the additive phase volume, thus yielding more porous structures and pores with bigger size. Moreover, from a kinetic point of view, the viscosity tends to decrease with the reduction of the polymer/additive concentrations [62], which also favors the formation

and growing of the additive rich phase, thus encouraging the formation of pores. The Table 4 gives more details about the properties and PWP of these membranes.

Table 4. Characteristics of the PHBHV based membranes prepared with different concentrations of PHBHV and PEG200 (evaporation time of 1.5 min).

	M-1.5-5PEG200	M-1.5-7PEG200	M-1.5-17/5PEG200	M-1.5-17/7PEG200
PHBHV Concentration	20 wt%	20 wt%	17 wt%	17 wt%
PEG200 Concentration	5 wt%	7 wt%	5 wt%	7 wt%
Thickness overall structure (μm)	201 ± 5	183 ± 5	323 ± 9	204 ± 21
Upper structure (μm)	17 ± 4	14 ± 5	17 ± 3	15 ± 3
Porosity (%)	67 ± 1	63 ± 2	74 ± 4	72 ± 1
Water contact angle ($^\circ$)	63 ± 3	67 ± 5	78 ± 5	63 ± 2
PWP ($\text{L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$)	200 ± 80	12 ± 7	76 ± 21	21 ± 5

The overall membrane thickness ranged from 183 μm , for the M-1.5-7PEG200 membrane, to 323 μm , for the M-1.5-17/5PEG200 membrane.

With the 5 wt% PEG200 concentration, the membrane thickness increased from 201 μm to 323 μm when the PHBHV concentration decreased from 20 wt% to 17 wt%. This observation is in good agreement with the higher porosity of the M-1.5-17/5PEG200 membrane (74%) compared to the M-1.5-5PEG200 membrane (67%). Same trend can be observed for the membranes prepared with the higher concentration of PEG200 (7 wt%).

Indeed, when the porosity increases, the number of voids inside the membrane microstructure increases too, what finally favours a thicker membrane. Such increase of the membrane porosity when the polymer concentration in the dope solutions decreases has previously been reported [13,63].

Among these membranes, the upper structure varied from 14 μm to 17 μm , with very similar values.

According to what has been observed in the SEM images (Fig. 8), the membrane porosity increased when the polymer concentration decreased (from 20 to 17 wt%) for both concentrations of additives employed (Table 4).

This behavior is in accordance with the results obtained with PVDF or cellulose acetate membranes [13,63].

It is interesting to note that the increase of additive concentration from 5 wt% to 7 wt% did not significantly influence the membrane porosity (Fig. 8). The membranes with 20 wt% of PHBHV, M-1.5-5PEG200 and M-1.5-7PEG200, showed respective overall porosity values of 67% and 63% while the membranes with 17 wt% of PHBHV, M-1.5-17/5PEG200 and M-1.5-17/7PEG200, gained a porosity of 74% and 72%, respectively. Usually, the increase in concentration of a such additive would tend to increase the membrane porosity [41,54]. However, the additive might have a counter effect on the porosity since it increases the dope solution viscosity [64,65]. Hence, it might be possible that the variation in PEG200 concentration from 5 wt% to 7 wt% would have hindered the internal porous network due to a potential higher viscosity. These four membranes made from different PHBHV and PEG200 concentrations were all permeable to water.

However, no clear correlation can be depicted between the membrane permeability and the membrane microstructure, which includes the membrane thickness, porosity and surface pores. This could be the consequence of the formation of non-through pores (pore opened on one side but closed on the other side). Thus, such pores would be visible on SEM pictures and considered in the porosity determination by the gravimetric method (the pores are accessible through diffusion when soaked into kerosene) but can not contribute to the permeate production as they are closed on one side (i.e. water can not cross the membranes through these pores). In addition,

605 differences of pore tortuosity might exist for the different membranes. A more detailed analysis
606 of the membranes micro/nano structure using advanced methods such as micro/nano X-ray
607 tomography could help to elucidate the structure-performance relationships for such
608 membranes but was out of the scope of this study.

609 Finally, the impact of the PEG and PVP molecular weights on membrane properties has been
610 also investigated (Fig. 9). Previous studies, in fact, showed that the molecular weight of the
611 additive can greatly influence the thermodynamic and kinetic of the demixing process thus
612 affecting the membrane microstructure [54,66,67]. The increase of the molecular weight might,
613 for instance, enhances the membrane porosity [54,67], changes the macrovoids shape [67] or
614 hinder the formation of macrovoids [66]. By keeping PHBHV and additives concentrations
615 fixed at 17 wt% and 7 wt%, respectively, two different PVP and PEG molecular weights have
616 been considered: a 30,000 g.mol⁻¹ PVP (PVPK30) was compared to the 17,000 g.mol⁻¹ PVP
617 (PVPK17), while a 600 g.mol⁻¹ PEG (PEG600) was compared to the 200 g.mol⁻¹ PEG
618 (PEG200).

Figure 9. SEM images of the PHBV based membranes prepared with PVP or PEG of different molecular weights (dope solutions with 17 wt% PHBV concentration and 7 wt% additive concentration with an evaporation time of 1.5 min). Top surface, with magnification, on the left and cross section, with magnification, on the right.

SEM images of the top surfaces show a porous structure in all cases even if different pore sizes and densities are depicted. As seen before with other concentrations (Fig. 5), the PVP led to smaller pores on the surface compared to PEG. This is also confirmed by the mean pore diameter measurements performed on M-1.5-17/7PVPK17 and M-1.5-17/7PEG200 membranes. Indeed, the membrane with PVP displayed a mean pore diameter of 0.04 ± 0.01 μm while the membrane with PEG200 had a bigger mean pore diameter of 0.12 ± 0.01 μm . These results placed these membranes in the UF/MF range. For both PVP and PEG, when the additive molecular weight increased, the size of the surface pores seems to decrease. Indeed, the M-1.5-17/7PVPK30 and M-1.5-17/7PEG600 membranes have smaller pores than the respective M-1.5-17/7PVPK17 and M-1.5-17/7PEG200 membranes.

The cross-section views mainly reveal spherulitic structures for all the investigated membranes. A structure made of more aggregated spherulites is found in the membranes made with PVPK17 or PVPK30 while M-1.5-17/7PVPK30 membrane exhibits closed cells within the upper layer and the spherulites. On the contrary, the membranes made with PEG200 or PEG600 show cellular structures on their upper parts. Table 5 gives more details about the structures and PWP results of these membranes.

Table 5. Characteristics of the PHBHV based membranes prepared with PVP or PEG of different molecular weights (Dope solutions with 17 wt% PHBHV concentration and 7 wt% additive concentration, with an evaporation time of 1.5 min).

	M-1.5-17/7PVPK17	M-1.5-17/7PVPK30	M-1.5-17/7PEG200	M-1.5-17/7PEG600
Additive nature	PVP	PVP	PEG	PEG
Additive molecular weight (g.mol^{-1})	17000	30000	200	600
Thickness overall structure (μm)	112 ± 8	126 ± 14	204 ± 21	211 ± 10
Upper structure (μm)	14 ± 2	32 ± 5	15 ± 3	14 ± 1
Porosity (%)	77 ± 1	56 ± 3	72 ± 1	73 ± 1
Water contact angle ($^\circ$)	75 ± 3	70 ± 2	63 ± 2	84 ± 2
PWP ($\text{L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$)	37 ± 12	350 ± 93	21 ± 5	Not measurable. Too fragile

The overall membrane thickness varied from 112 μm , for the M-1.5-17/7PVPK17 membrane, to 211 μm , for the M-1.5-17/7PEG600 membrane with an upper structure ranging from 14 μm to 32 μm . Membranes show a similar porosity (72 % - 77 %) besides M-1.5-17/7PVPK30 that exhibits a lower porosity (56%).

For the dope solutions using PVP, the overall membrane porosity decreased with the increase of the additive molecular weight, as confirmed also by SEM images (Fig. 9). This behavior could be due to a slower demixing that limits pores formation when the additive molecular weight increases [62]. Indeed, the additives with lower molecular weights are prone to be more easily leached out from the membrane during the coagulation in the water bath, what may favor the increase of the overall membrane porosity [62].

The water contact angles of these four membranes, between 63° and 84°, still depicted the hydrophilic behavior of the material.

The membrane PWP increased from 37 $\text{L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$ to 350 $\text{L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$ when the PVP molecular weight increased from 17,000 g.mol^{-1} to 30,000 g.mol^{-1} accompanied also by an improvement of membrane hydrophilicity. This increase in PWP can be attributed to the increase in membrane average pore size (from $0.05 \pm 0.01 \mu\text{m}$ for M-1.5-17/7PVPK17 to $0.11 \pm 0.01 \mu\text{m}$ for M-1.5-17/7PVPK30). The average pore size has been measured by using a capillary flow porometer and thus directly relies on the extrusion of the wetting liquid from the membrane pores. In other words, the pore size obtained by this technique is based on a fluid permeability through the material and thus the measurement does not account for non-through pores.

Noteworthy, is that the pore size of both membranes, as shown in Fig. S6, has a very narrow distribution for the M-1.5-17/7PVPK17 membrane (98% of the pores at 0.05 μm) and a good distribution for the M-1.5-17/7PVPK30 membrane (65% of the pores at 0.11 μm).

The M-1.5-17/7PEG200 membrane showed a PWP of $21 \text{ L.m}^{-2}.\text{h}^{-1}.\text{bar}^{-1}$ whereas the M-1.5-17/7PEG600 was too fragile to be tested. Indeed, it has been observed that increasing the concentration and molecular weight of the additive, the homogeneity of the membranes was lost. A decrease of the additive solubility into the solvent (Cyrene™) could be the cause of these observations.

3.5 Pervaporation results

The potentiality of the newly developed bio-based membranes has been demonstrated in PV applications for the separation of organic/organic solutions. PHA based membranes, in fact, have already shown their resistance towards a series of organic solvents and have been already used in such applications [68]. The pervaporative performance of a selection of three of the produced dense membranes were studied for the separation of a MeOH/MTBE mixture at the azeotropic point (14.3/85.7 w/w). The influence of the additive nature and feed temperature on the membranes' performances were investigated. The interest in separating such a mixtures lies in the fact that an excess of MeOH is employed during the synthesis of MTBE, an octane booster used in motor gasoline [69]. The advantage of PV, over conventional technologies such as column distillation, consists in the possibility to break the forming azeotrope allowing the complete separation of the organic/organic mixture.

The three selected membranes M-5, M-5-5PEG200 and M-5-5EBO are obviously not permeable to water and exhibit a dense top layer or a dense cross-sectional structure (Figure 2 and Fig. 5). This makes them ideal to be employed in PV where dense membranes are required. M-5 is the reference membrane that does not contain any additive. M-5-5PEG200 and M-5-5EBO contain additives of different nature. The membranes are made from a 20 wt% PHBHV concentration and 5 wt% concentration of additive (PEG200 and EBO) into the dope solution. The three membranes display a moderate wettability as highlighted by water contact angles that

are 72°, 70° and 75° for the M-5, M-5-5PEG200 and M-5-5EBO membranes, respectively. The hydrophilic character of these membranes would thus tend to favor the diffusion of MeOH which is more polar than MTBE.

The Hansen Solubility Parameters (HSPs) of the chemicals involved during the separation (membrane materials and solvents to be separated) and kinetic diameters of the solvents are displayed in Table S2. The kinetic diameter of MeOH (0.40 nm) is lower than the one of MTBE (0.62 nm), thus the MeOH is prone to diffuse faster through the membranes than the ether. However, the solubility parameter δ_t of the polymer (20.6 MPa^{1/2}) is closer to the one of MTBE (16.2 MPa^{1/2}) compared to MeOH (29.6 MPa^{1/2}), what means that the polymer has a better predicted affinity with MTBE than MeOH and could hence favor the MTBE sorption and diffusion.

When additives are involved, PEG200 ($\delta_t = 24.3$ MPa^{1/2}) may tend to increase the δ_t of the overall material and thus its affinity with MeOH, while EBO ($\delta_t = 18.6$ MPa^{1/2}) would have the opposite effect.

Swelling degree of the membranes was evaluated into three different solvent solutions: MeOH, MTBE and the mixture MeOH/MTBE (50/50 w/w). The results are displayed in Fig. S7.

For each solvent solution, the M-5 membrane showed the lowest swelling degree (from 0.15 to 0.20 ml/g), followed by the M-5-5EBO membrane (from 0.14 to 0.28 ml/g) and, finally, by the M-5-5PEG200 (from 0.16 to 0.37 ml/g). The swelling degree is in line with porosity values. The higher degree of swelling of the M-5-5PEG200 membrane can be, in fact, related to its higher porosity (52%). Thus, higher porosity leads to higher contact area between the membrane and the solvent which promoted the adsorption of more solvent in the free volumes of the membrane. While M-5 membrane, showing the lowest porosity value (43%), resulted in the lowest swelling degree.

All the membranes swelled more in pure MTBE respect to pure MeOH in agreement with the solubility parameter values. Interestingly, when MeOH and MTBE were used in mixture, the swelling of the membranes was higher respect to the pure solvents, as also observed by S. Zereshki et al. [70] for the same mixture (at 75 wt% of MeOH) with modified poly(ether ether ketone) (PEEKWC) membranes.

In order to investigate the thermal stability of the membranes, a thermogravimetric analysis (TGA) was carried out up to 700°C and the obtained thermograms are plotted in Fig. S8.

The thermal degradation of M-5 membrane, without additive, occurs in one step with an onset temperature of 270 °C. However, the M-5-5EBO membrane displays a two steps degradation curve. The first temperature drop is associated to the PHBHV matrix whereas the second drop is linked to the presence of EBO [35] into the membrane. For the M-5-5PEG200 membrane, the degradation onset temperature is shifted down to 260 °C compared to the M-5 membrane. This may be due to the remaining PEG200 that could decrease the degradation temperature of the material. Finally, none of these membranes display a weight loss within the temperature range used for the PV experiments (25 °C - 45 °C).

The same membrane sample, when tested in PV, at different feed temperatures (25°C, 35°C and 45°C), did not show any visible alteration of its structure (cracks, wrinkles), for more than one week (time necessary to the test the same sample at all investigated temperatures), as a proof of its resistance and stability towards the investigated organic/organic solution.

Fig. 10 displays the membranes performances (total flux and selectivity) as a function of the feed temperatures.

Figure 10. Effect of the feed temperature on: (a) the total permeate flux; and (b) the selectivity for the three investigated membranes.

For all the membranes, as the feed temperature was increased, the flux increased (Fig. 10a) while the selectivity decreased (Fig. 10b). The fluxes varied from $0.017 \text{ Kg.m}^{-2}.\text{h}^{-1}$ to $0.110 \text{ Kg.m}^{-2}.\text{h}^{-1}$ and the selectivity from 3.5 to 31. All the membranes were found to be selective for MeOH showing a selectivity coefficient higher than 1. This could be explained considering two aspects: 1) the smaller kinetic molecular diameter of MeOH (in comparison to MTBE) which can allow its easier penetration through the membrane's free volumes; 2) the hydrophilic nature of the PHBHV based membranes (as shown by contact angle measurements) able to favor the permeation of more polar compounds such as MeOH.

The total flux and selectivity trend, observed in Fig. 10, is typical for PV. The flux increase is, in fact, related to an increase in the thermal motion of membrane's polymer chains, which promotes the formation of free volumes, favoring the permeation of more species [14]. Moreover, the increase in temperature, leads to a gain in the saturated vapor pressures of the species in the feed side, which also foster their permeation through the membrane. However, the larger free volumes, generated by the temperature increase, also facilitate the diffusion of bigger molecules such as MTBE causing a decrease in membrane selectivity [71].

Analyzing the single behavior of each membrane, the M-5 membrane showed the lowest flux and highest selectivity at all investigated temperatures. The presence of additives (PEG200 and EBO) increased the flux of the membranes with a consequent decrease of selectivity. This effect

could be explained considering the plasticizing effect brought by the additives, what can favor the transfer of species, both MeOH and MTBE [72]. The EBO, in fact, has previously shown to be able to increase the free volume in PHA film materials [35]. This additive has a stronger impact on membrane performances in comparison to PEG200. In Fig. 11 the partial MeOH (J_{MeOH}) and MTBE (J_{MTBE}) fluxes are reported as a function of the feed temperature and the total permeate flux.

Figure 11. MeOH (J_{MeOH}) and MTBE (J_{MTBE}) partial fluxes through the investigated membranes as a function of (a) the feed temperature (b) the total permeate flux.

For the M-5 membrane, the MeOH flux was always higher than the MTBE flux. The higher is J_{MeOH} compared to J_{MTBE} , the better is the selectivity. That is why M-5 shows the best selectivity (Fig. 10b). For the M-5-5PEG200 membrane, J_{MeOH} and J_{MTBE} intersect with each other at 35 °C.

Hence, at 45 °C, J_{MTBE} becomes higher than J_{MeOH} . With this membrane, in fact, while the MeOH flux remained almost constant (from about 0.02 to about 0.03 Kg/m² h) with the temperature increase, the MTBE flux was greatly increased (from about 0.01 to about 0.045 Kg/m² h) leading to a drop in membrane selectivity.

Then, in case of M-5-5EBO membrane, the MTBE flux was always higher than MeOH flux, what explains the lowest selectivity observed for this membrane (Fig. 10b).

The Fig. 11b highlights the uneven linear variations of partial fluxes with the total permeate flux. Thereby, for a total flux of 0.044 Kg.m⁻².h⁻¹, the partial fluxes intersect with each other

and, at that point, J_{MTBE} starts to be higher than J_{MeOH} . It means that the higher is the total flux, the higher is the amount of MTBE inside the permeate and the lower is the selectivity. The fact that J_{MTBE} increases faster with the temperature than J_{MeOH} implies a more significative dependency of MTBE on the temperature. This can be verified by calculating the fluxes activation energies. The flux in PV usually follows an Arrhenius type relationship with the temperature that can be expressed by the following equation (Eq. 9):

$$J_i = J_{0,i} \exp\left(-\frac{E_i}{RT}\right) \quad (9)$$

where J_i is the partial flux of the component i , $J_{0,i}$ is the pre-exponential factor (J_i flux at infinite temperature) and E_i is the apparent activation energy for the component i . R is the ideal gas constant and T the absolute temperature.

The sign of E_i shows the way the flux varies with the temperature whereas the absolute value of E_i shows how much it depends on the temperature. In order to determine the activation energies, the natural logarithms of fluxes ($\ln(J_i)$) are plotted according to the reciprocal temperature in Fig. 12.

Figure 12. Arrhenius plot of (a) the MeOH flux (J_{MeOH}), (b) the MTBE flux (J_{MTBE}) and (c) the total flux (J_t).

The obtained linear relationships indicate an Arrhenius dependence of fluxes with the temperature. The curves are decreasing in every case but the slopes are more pronounced for J_{MTBE} than for J_{MeOH} . In order to more clearly compare the dependencies, the activation energies are calculated according to these slopes and using the previous equation (Eq. 9). The obtained values are displayed in Table S3.

The positive values of the activation energies imply that fluxes increase with the increase of the feed temperature. For M-5 and M-5-5PEG200 membranes, the values for MTBE (respectively

70.6 KJ.mol⁻¹ and 60.8 KJ.mol⁻¹) are much higher than the ones of MeOH (respectively 23.0 KJ.mol⁻¹ and 15.8 KJ.mol⁻¹), what means that MTBE flux increases more significantly than MeOH flux with the temperature. This phenomenon can be explained by an enlargement of the polymer free volume with temperature, which facilitates the passage of larger molecules such as MTBE [73]. On the other hand, for the M-5-5EBO membrane, the values are very similar between MTBE (21.4 KJ.mol⁻¹) and MeOH (22.6 KJ.mol⁻¹), explaining the low selectivity variation with the temperature increase (Fig. 10b).

Fig. 13 compares the performances of the M-5 membrane (the more selective one) with others extracted from the literature [72–77]. As the membranes from the different studies do not all have the same thickness, the selectivity has been plotted against the normalized flux to allow the direct comparison of the different membranes.

Figure 13. Representation of the performances, selectivity and normalized flux, of the herein fabricated PV membranes in comparison to membranes from the literature [72–77]. The MeOH concentration in feed varies from 14.3 to 20 wt%. The feed temperature varies from 25°C to 50°C.

Compared to other biopolymer based membranes, the performances of these membranes are lower than polyvinyl alcohol (PVA) or cellulose acetate (CA) membranes, especially in regard

to their selectivity [72,74]. It may be explained by the more hydrophilic nature of these polymers with regard to the PHBHV, what favors the transfer of polar molecules such as MeOH. This is the reason why the blending of CA with PVA or PVP is a good way to improve the membranes performances [72,74]. However, the M-5 membrane herein reported shows a better selectivity in comparison to PLA and other polyhydroxybutyrate (PHB) based membranes [73,76] and has similar values to the commercial membrane PERVAP® 2256 [75].

3.6 Biodegradability tests

The stability degradation into water-based environments of the M-5 and the M-1.5-5PEG200 membranes with different morphologies and properties was further assessed up to 105 days (Fig. 14) by using sea-water at room temperature and an enzymatic solution of lipase from *Candida Rugosa* (150 U.mL⁻¹) at 25°C and 37°C. Lipases, that are strongly studied for industrial applications, catalyze the hydrolysis reaction of polyesters, such as polylactic acid, polybutylene succinate and some PHA [78,79]. The active center of the lipase catalytic domain have some amino acid sequences in common with PHA depolymerases [80]. The sea water includes different compounds like dissolved salts, organic matters and microorganisms, and represents the type of aqueous environment that the membrane would face for water filtration applications.

Figure 14. Weight loss (%) of M-5 and M-1.5-5PEG 200 membranes in aqueous medium by incubation in sea water and enzymatic solution of Lipase from *Candida Rugosa* (150 U mL⁻¹) over 105 days, at 25°C. Data statistically significant according to ANOVA followed by Bonferroni t'test ($p < 0.05$): (*) M-1.5-5PEG200 sea water vs M-5 lipase and M-1.5-5PEG200 lipase; (§) all vs M-5 lipase; (†) M-1.5-5PEG 200 lipase vs M-5 lipase and M-5 sea water, M-1.5-5PEG 200 sea water and M-5 sea water vs M-5 lipase.

As displayed in Fig. 14, the M-5 membrane exhibits a weight loss of only $0.4 \pm 0.3\%$ and $1.9 \pm 0.7\%$, respectively, after 105 days in both lipase solution and sea water. The M-1.5-5PEG200 membranes show a weight decrease of $0.97 \pm 0.5\%$ and $2.3 \pm 0.7\%$ after 21 and 105 days, respectively, in sea water. Same weight loss percentages were found for membranes incubated at the physiological temperature (data not shown). Coherently, the pH measures of all the aqueous media highlighted small fluctuations with time (Fig. S9), confirming the degradation results ~~good stability~~ of the tested membranes in aqueous solution.

The measured weight loss for the membranes into the lipase solution seems to be very low compared to what has been measured for similar PHBHV films in contact ~~more~~ PHA depolymerases [80]. A fast depolymerizing activity of different commercial lipases from fungal, bacterial and animal origin, has been observed for similar films P(3HB-co4HB) realized

with low polymer concentration (0.1%) [78,81]. In our work we used a polymer concentration (20% PHBHV) of two order of magnitude higher than that reported in these studies. Indeed, our results are in accordance with other reports that show a fairly slow degradation (5%) after 240 days of exposure in lipase solution for 2% PHBHV films [82,83], confirming a direct correlation of the biodegradation rates with the polymer concentration.

For the sea water environments, the weight loss is also relatively low compared with other tests into the real environment of sea water where the weight loss can be 7-20 % after 50-60 days [84,85]—under variable conditions (e.g. temperature variations, potential mechanical stress, constant microorganisms renewal or UV expositions). ~~are omitted.~~

Differently, our findings are in agreement with those reported for the biodegradation of a 10% PHB film, that in static seawater collected from the Pacific Ocean, controlled at 25°C and replaced every 2 days, lost 9% of the initial weight after 10 weeks [86].

The only case differing from the others is the M-5 Lipase, where the membrane weight seems much more stable consistently with the dense structure of the membrane.

Finally, the membranes were extracted from their degradation environments after 105 days and were analyzed by SEM (Fig. S10).

The surface of the M-1.5-5PEG200 and M-5 membranes was not greatly altered after 105 days of ageing into the lipase solution. However, both membranes, after ageing into the sea water-based solution tended to get more pores and rougher surfaces. Consequently, the sea water-based solution seems to have an effect on the polymeric structure.

The sea water-based solution, however, contains a wide variety of bacteria, including the PHA degrading ones, what would favor the biodegradation rate compared to the lipase solution. These surface changes are actually due to the erosion via enzyme catalyzed hydrolysis [78]. During the biodegradation mechanism, first the microorganisms attach to the material surface and then start the depolymerization through the enzyme catalyzed hydrolysis [87]. These two

first steps induce the measured weight loss. Afterward, the bacteria bio-assimilate and mineralize the produced small molecules. Both surface or bulk erosion can occur, however, the surface erosion may be predominant for semi-crystalline polymers, what can explain what is obtained in this case. In the case of the lipase solution environment, because the material morphology does not seem to be impacted after 105 days of ageing, catalyzed hydrolysis with less specific enzymes, like Lipases, appear very low. Thus, the non-catalyzed hydrolysis of this material would be even slower.

At the end, if the weight losses of the membranes within the different investigated environments remain quite low although the sea water-based solution seems to impact the surface morphology. This could influence the long-term performance of the membrane in the perspective of water filtration application in an environment containing lipase enzyme opens a new perspective in the application of these membranes in the filtration of aqueous solutions containing known components.

4 Conclusions

This work aimed to develop a more sustainable and innovative way for the fabrication of polymeric membranes by phase-inversion technique by exploiting a new combination of bio-based materials, PHBHV as the polymer matrix and CyreneTM as a green solvent. Asymmetric membranes with suitable mechanical properties were successfully obtained by introducing a pre-evaporation (EIPS) step before the coagulation bath. Besides the improvement of the mechanical properties, this EIPS step also strongly influenced the membrane morphology, including its cross-sectional microstructure and overall porosity.

A systematic study was, then, carried out by analyzing the impact of different variables, such as: polymer concentration, additive concentration, additive nature and additive molecular weight. In that prospect, PEG, PVP and a vegetable-based additive, EBO, were added as hydrophilic and pore former agents into the dope solution. Thanks to them, more diversified membrane microstructures were observed, including membranes with porous surfaces.

The different membranes architectures obtained resulted in membranes permeable to water (up to 350 L.m⁻².h⁻¹.bar⁻¹), with a pore size in the range of UF/MF, and in asymmetric membranes displaying a dense top layer.

These latter ones, thanks to the solvent resistance properties of PHBHV, were applied in PV for the separation of a MeOH/MTBE organic mixture at its azeotropic point.

All the three membranes resulted selective for MeOH as a consequence of their hydrophilic nature and of the small dimension of the alcohol molecule. When PEG200 and EBO were used, the selectivity decreased (in comparison to the membrane prepared without any additive) due to their plasticizing effect in the membrane matrix which also resulted in a permeate flux enhancement. The performance of most the performing membrane here produced was found to be comparable (or even better) respect to other bio-based membranes made of PLA or PHB.

Porous and dense membranes showed also interesting stability performance when tested under specific degradation environments for long periods of time, suggesting possible applications for aqueous solutions filtration.

5 Conflicts of interest

There are no conflicts to declare.

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