Nafion membranes modified by cationic cyclodextrin derivatives for enantioselective separation

Jana Gaálová [a], Marine Michel [a,b], Mahdi Bourassi [a,c], Bradley P. Ladewig [b,f], Petr Kasal [d], Jindřich Jindřich [d], Pavel Izák [a,e,*]

[a] Institute of Chemical Process Fundamentals of the CAS, v.v.i., Rozvojová 135/1, 165 02 Prague, Czech Republic
[b] Barrer Centre, Department of Chemical Engineering, Imperial College London, Exhibition Road, London SW7 2AZ, United Kingdom
[c] Institute for Environmental Studies, Faculty of Science, Charles University, Benátská 2, 128 01 Prague 2, Czech Republic
[d] Department of Organic Chemistry, Faculty of Science, Charles University, Hlavova 8, 128 43 Prague 2, Czech Republic
[e] MemBrain s.r.o., Pod Vinicí 87, 471 27 Stráž pod Ralskem, Czech Republic
[f] Karlsruhe Institute of Technology, Institute for Micro Process Engineering, Hermann-von-Helmholtz-Platz 1, 76344 Eggenstein-Leopoldshafen, Germany

A R T I C L E   I N F O

A B S T R A C T

Nafion117® membranes modified by three cationic cyclodextrin (CD) derivatives have been prepared by strong ionic bonding. All CD derivatives contained bis(methylimidazolium) (MIM2) cationic anchor covalently bound to the CD unit, either using no spacer or using diethylene glycol (DEG) or tetraethylene glycol (TEEG) spacers. The modified membranes were tested in chiral separation of a model racemic mixture (D/L-tryptophan) from water. Different experimental set-ups for characterising membranes in enantioselective separation – pertraction, two kinds of sorption, and pressure-driven membrane separation – have been described and rigorously compared. The membranes CD-MIM2, CD-DEG-MIM2 have reached the highest enantiomeric excess, 14 and 44% respectively, in 280 days. The lowest performance of the CD-TEEG-MIM2 membrane, with the long spacer, has been visibly ameliorated by applying pertraction; enantiomeric excess rose from 2 to 27% in 80 days. Even though sorption played the main role in pertraction, this process substantially enhanced the separation of racemic mixtures. The pressure-driven approach has allowed the operation to be continuous and faster, which has the potential for continuous large-scale production of enantiopure compounds and could pave the way for many more commercial applications, satisfying the considerable demand for large-scale chiral separation techniques.

1. Introduction

Many pharmaceuticals consist of two enantiomers causing dissimilar biological responses. While one enantiomer can constitute a cure for a specific disease, the other one may have no curative effect or may be even harmful [1,2]. It is essential to evaluate their differences in biological activity as well as toxicity. Tryptophan (Trp) is an α-amino acid that is used in the biosynthesis of proteins. D-Trp cannot be processed by or affect organisms. But L-Trp is an essential amino acid [3]. Moreover, chlorination of water containing these compounds generates byproducts such as dichloroacetonitrile, trihalomethanes, and iodinated trihalomethanes [4–6]. These by-product concentrations are controlled by World Health Organization regulations for drinkable water [7]. Water purification remains crucial to limit this kind of pollutants, which remains a challenge.

The optical resolution, or chiral separation, is an essential undertaking throughout discovery given the importance of chirality in the biological response and the difference of biological activity between two enantiomers. The behaviour of enantiomers differs in terms of spatial arrangement, intrinsic activity, capacity, toxicological implications. Moreover, various stereo-selective drug-drug interactions have been identified [8].

Various methods may be applied for the chiral resolution of racemic mixtures, e.g., chromatography, preferential crystallisation or membrane processes [9,10]. The separation of enantiomers needs to be fast, easy to operate, continuous, cost-effective, and should allow obtaining the enantiomers at a high level of optical purity. Chiral membranes have the advantage of low energy demand, easy scaling, process continuity, or low environmental effect and exhibit high selective efficiency. The use of membranes has been pointed out as a promising methodology for

---

scale-up of enantiomeric separation due to the low energy consumption, continuous operability, variety of materials and supports, simplicity, eco-friendliness, and the possibility to be integrated into other separation processes [11,12]. The membrane is a selective barrier, and the interaction between the chiral environment in the membranes and each enantiomer is decisive. The membrane's chiral resolution performance depends on the specific interaction of the membrane's recognition sites and the enantiomers [13]. Removal of Trp from wastewater has previously been evaluated using activated carbon and different membranes [14,15]. Regarding the limited adsorption capacity of the materials in general, selective adsorption of the harmful and active enantiomer increases the treatment effectiveness. Cyclodextrin (CD) based adsorbents and membranes were tested in wastewater recovery [16,17]. Evaluation of CD-based adsorbents revealed specific adsorption. Furthermore, a study reported a chiral selector for tryptophan enantiomers based on β-CD [18]. This makes CD a good choice for the chiral separation of Trp due to its selectivity towards the two enantiomers.

Ingole et al. reported chiral separation of racemic α-amino acids achieved through enantioselective polymer membranes containing chiral metal–Schiff base complex in a pressure driven process. D-enantiomers of α-amino acids lysine and arginine were found to permeate preferentially through composite membrane. Higher enantio-selectivity (94%) was observed for lysine as compared to arginine (84%). The enantio-selectivity of membrane shows time dependency and increases with time [19].

Another approach is a chitosan membrane crosslinked with glutaraldehyde prepared and used for chiral resolution of (R,S)-2-amino-1-butanol. At an operating pressure of 15 psi and a feed concentration of 500 ppm the enantiomeric excess reached as high as 92% and the separation factor as high as 5.56. [20].

The fundamental study of chiral polyamide-based thin film composite (TFC) membranes over a polysulfone support for enantiomeric separation through chemical modification was studied by Ingole et al. [21]. The enantiomeric excess is varied as concentration of chiral monomer in polymerization. The enantiomeric excess achieved as high as ~92% and the separation factor (α) as high as ~21 in the lysine case and in the asparagine case enantiomeric excess achieved as high as ~68% and the separation factor (α) as high as ~5.2 [21].

The thin film composite membranes prepared by interfacial polymerization of *trans*-1,4-diaminocyclohexane and piperazine (in aqueous phase) with trimesoyl chloride (in non-aqueous phase) on the polysulfone membrane (support for thin chiral selective layer) was reported by Ingole et al. [22]. Chiral selective thin film exhibited enantioselectivity over 78% L-enantiomer of lysine monohydrochloride from aqueous solutions of their racemic mixture in pressure driven reverse osmosis process at 689.42 kPa pressure [22].

In our previous work, different membranes were tested for the chiral separation of tryptophan enantiomers [23,24]. Amino acids are crucial biological substances as they take part in vital functions in a living organism [25] and major metabolic processes [26]. Still, some cannot be synthesised by the organism, e.g., L-Trp. Therefore, it has been studied in membrane enantioseparation research [11]. Recently, positively charged β-CD derivatives, differing in the spacer's length between the positively charged bis(imidazolium) (MIM2) anchor and CD moiety, were prepared and bound to Nafion® membrane, thus inducing its chirality [27]. The previous publication describes in detail especially the preparation of membranes CD-MIM2 (with no spacer in the CD modifier), CD-DEG-MIM2 (with short spacer, diethylene glycol-based), and CD-TEEG-MIM2 (with long spacer, tetraethylene glycol-based). A suitable commercial achiral negatively charged membrane was used as solid support. Onto such membrane, the chiral selectors were attached by a strong ionic bond, using a new type of permanently positively charged "anchors" containing several imidazolium groups bound to a neopentane skeleton. The arrangement ensured a short distance between the positive charges causing the strong ionic binding and high chemical stability of the anchors; the last part of the anchor allowed covalent

binding to a chiral selector using a high yielding "click" reaction. The propargyl group was selected and CuAAC reaction was used to bind the anchor to the azido group containing β-CD derivatives with different lengths of the spacer [27].

This paper compares different experimental set-ups, employing the membranes to resolve the model racemic mixture. Sorption, pertraction, and pressure-driven membrane separation are the keystone of the novel contribution.

2. Experimental methods

2.1. Chemicals

Solvents and chemicals used for membrane preparation and membrane testing were obtained from VWR international, Sigma-Aldrich, Fluorochem, Penta. Methanol (MeOH), Analytical Grade, was purchased from Gelest Inc. and used without purification. All the chemicals were used as received without any further purification.

2.2. Membranes preparation

Membrane material was prepared by ionic binding of imidazolium groups, bound covalently to β-CD, to the SO_3^- groups of Nafion117®. Preparation of charged CD modifiers containing spacers (CD-DEG-MIM2, CD-TEEG-MIM2) is shown in Scheme 1, the derivative without a spacer (CD-MIM2), was prepared by the reaction of azido-CD (prepared from Ts-CD) with the MIM2 anchor.

CD derivatives syntheses and the membranes preparation have been reported in our previous publication [27]. The prepared membranes are named accordingly - CD-MIM2, CD-DEG-MIM2, and CD-TEEG-MIM2. Description of Nafion117® membranes modified with CD derivatives can be found in Table 1.
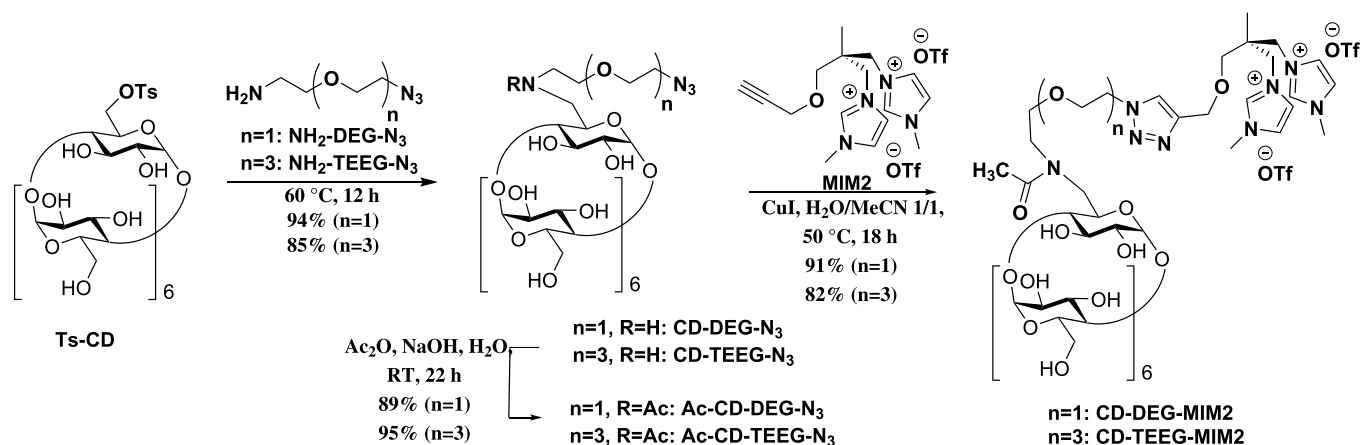
2.3. Membranes characterisation: Chiral separation testing

Nafion membranes (before modification, with CD modifier attached, after sorption experiment, with Trp attached), cyclodextrin (CD) derivatives, and tryptophan (Trp) were characterized by FTIR spectra measured with a Nicolet Avatar 370 FTIR. The method used for CD modifiers and Trp was a diffuse reflectance (DRIFT) in KBr; the method used for measuring Nafion membranes was Attenuated Total Reflectance (ATR) with Ge crystal. IR absorptions are given in wavenumbers as cm^{-1} (see Figs. 1–11 in Supporting Information).

The differences in IR spectra confirm the binding of CD modifiers on the Nafion membrane. Nevertheless, due to the small difference in spectra of Trp and CD modified Nafion membranes, the stability of electrostatic binding of CD modifier during the sorption experiments (i. e., in the presence of an excess of Trp) was confirmed by the additional experiment utilizing a fluorescent tag.

A fluorescent group - *N*-propyl-naphthalimide (PNI) - was attached via the hexamethylenediamine linker [28] to the CD-TEEG-MIM2 using isothiocyanate intermediate [27], and the PNI-tagged CD modifier was attached to the Nafion membrane using the same method used for other charged CD modifiers. The membrane was then treated with 2% Trp water solution (i.e., conditions used for Trp sorption experiments, see for the experimental setup). To speed up the eventual desorption of the charged modifier, the system was heated to 50 °C. Nevertheless, no fluorescence signal of the PNI group was detectable in the Trp solution even after 12 days of heating (the membrane staying fluorescent). Thus, the strong electrostatic binding of multiply charged modifiers to the Nafion membrane was confirmed.

The three, previously described [27], chiral membranes were tested using pertraction, pressure-driven permeation, as well as two different set-ups for preferential sorption (in Czech Republic "Preferential Sorption CZ" and in United Kingdom "Preferential Sorption UK"). These experiments were performed to evaluate the performance in enantio-

Scheme 1. Synthesis of CD modifiers CD-DEG-MIM2 and CD-TEEG-MIM2.

Table 1
Description of modified Nafion117® membranes modified with CD derivatives.

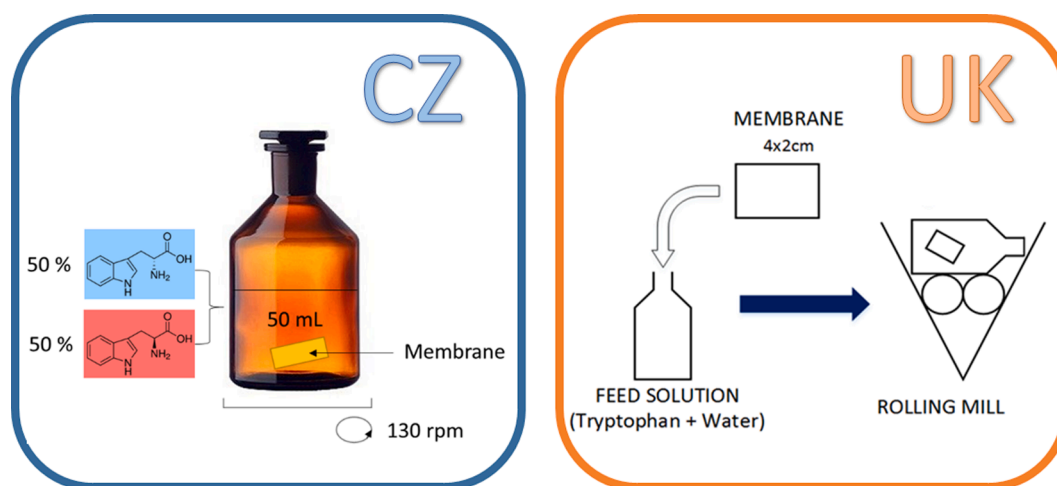| Membrane	CD-MIM2	CD-DEG-MIM2	CD-TEEG-MIM2
CD modifier			
Specific rotation of CD modifier	$[\alpha]^{25}_D + 71.4°$	$[\alpha]^{25}_D + 90.0°$	$[\alpha]^{25}_D + 95.9°$

Fig. 1. Preferential sorption of tryptophan racemic mixture (D-enantiomer in blue + L-enantiomer in red) "CZ" [19] and "UK" [23]. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

separation, compare the different set-ups, and assess the experiments' reproducibility. A racemic mixture of tryptophan (2 mg/mL) in water was used for these experiments as a model compound.

2.4. Preferential sorption "CZ" and "UK"

Both preferential sorption set-ups – performed in the Czech Republic (CZ) and United Kingdom (UK) – were already previously described in detail; see preferential Sorption "CZ" in [23] and preferential Sorption
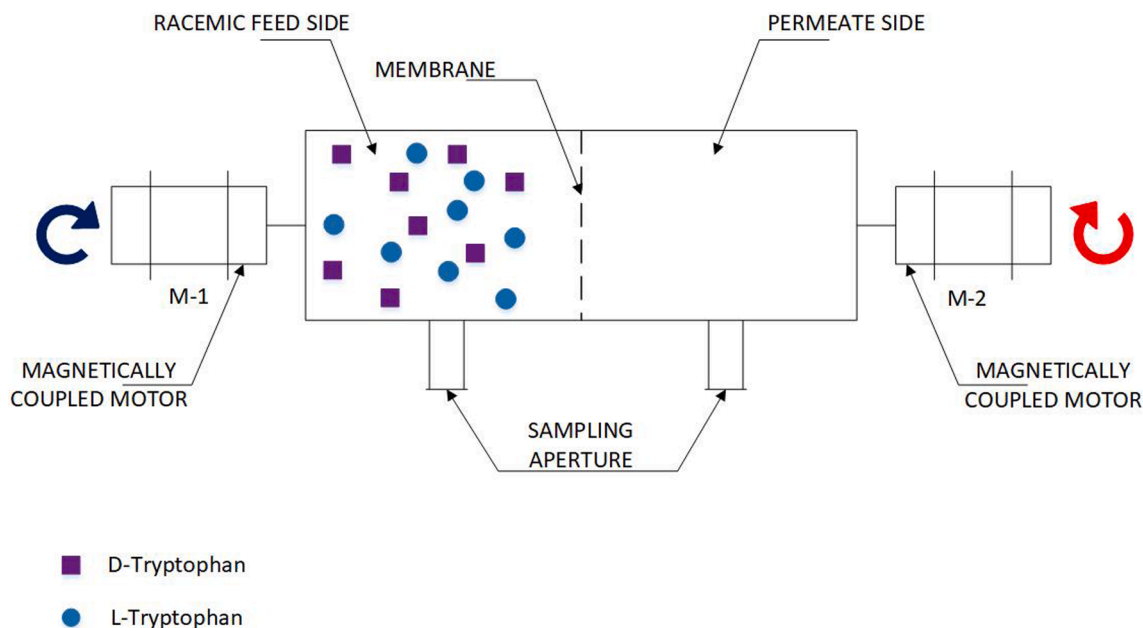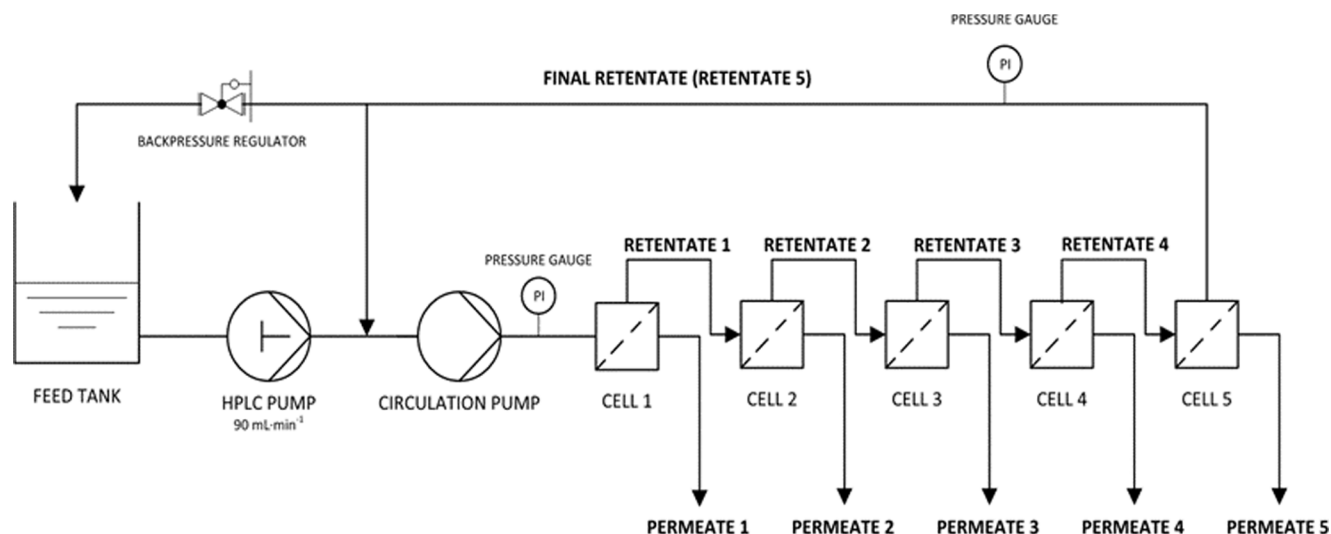
Fig. 2. Pertraction set-up.

Fig. 3. Cross flow apparatus for membrane testing.

"UK" [27]. Fig. 1 gathers two different approaches of measurements to make the differences of set-ups more visible.

All experiments were performed in dark glass bottles. For both types of preferential sorption, the bottle was filled with 0.01 M water solution of DL-Trp (50 mL) to which the chiral membrane cutout (4.0 × 2.0 cm) was added (time = 0) at 25 °C; vertically in CZ, stirred on a GLF 3005 rotator at 130 rpm, and horizontally in UK, in a roller shaker GLF 3005 rotator with a rotational frequency of 118 rot./min. The solution was sampled (1 mL) at regular time intervals and analysed by high-performance liquid chromatography (HPLC). UltiMate3000 spectrometer (Thermo Scientific) was used, equipped with a CHIRALPAK® ZWIX (+) (250 × 3 mm, ID, 3 μm) column (Diacel) in CZ. The mobile phase composed of 98% MeOH and 2% H_2O, containing 50 mM formic acid (HCOOH) and 25 mM diethylamine (Et_2NH) as a buffer. The flow was isocratic at the rate of 0.5 mL/min. The total run time of analysis was 25 min; the peaks of both enantiomers were detected by a UV diode-array detector at 254 nm. An optical resolution column CHIRALPAK® ZWIX (+) (150 × 3 mm ID, 3 μm) commercialised by Daicel Corporation

(Tokyo, Japan) and manufactured at Chiral Technologies Europe (Ill-kirch, France) were used in UK, with the same sampling mode, mobile phase, flow rate, injection volume, detection by UV diode array and run time as in CZ. The signals of both enantiomers were well separated, with elution of D-Trp peak at 8 min and the L-Trp peak at 11 min in both laboratories. The concentrations of each enantiomer were determined using the calibration curve.

2.5. Pertraction

Pertraction experiments were carried out in the Czech Republic only. The solid membrane separated the two compartments of the pertraction device, and the temperature was maintained at 25 °C (Fig. 2). Membranes (2.5 cm diameter) were cut and loaded in the middle of pertraction test cells. The two chambers of 60 mL each were filled with the racemic solution in water (c_{Trp} = 0.01 M) on the feed side and fresh solvent (ultrapure water) on the stripping phase, respectively. Three pieces of each membrane were tested to assess the repeatability of the
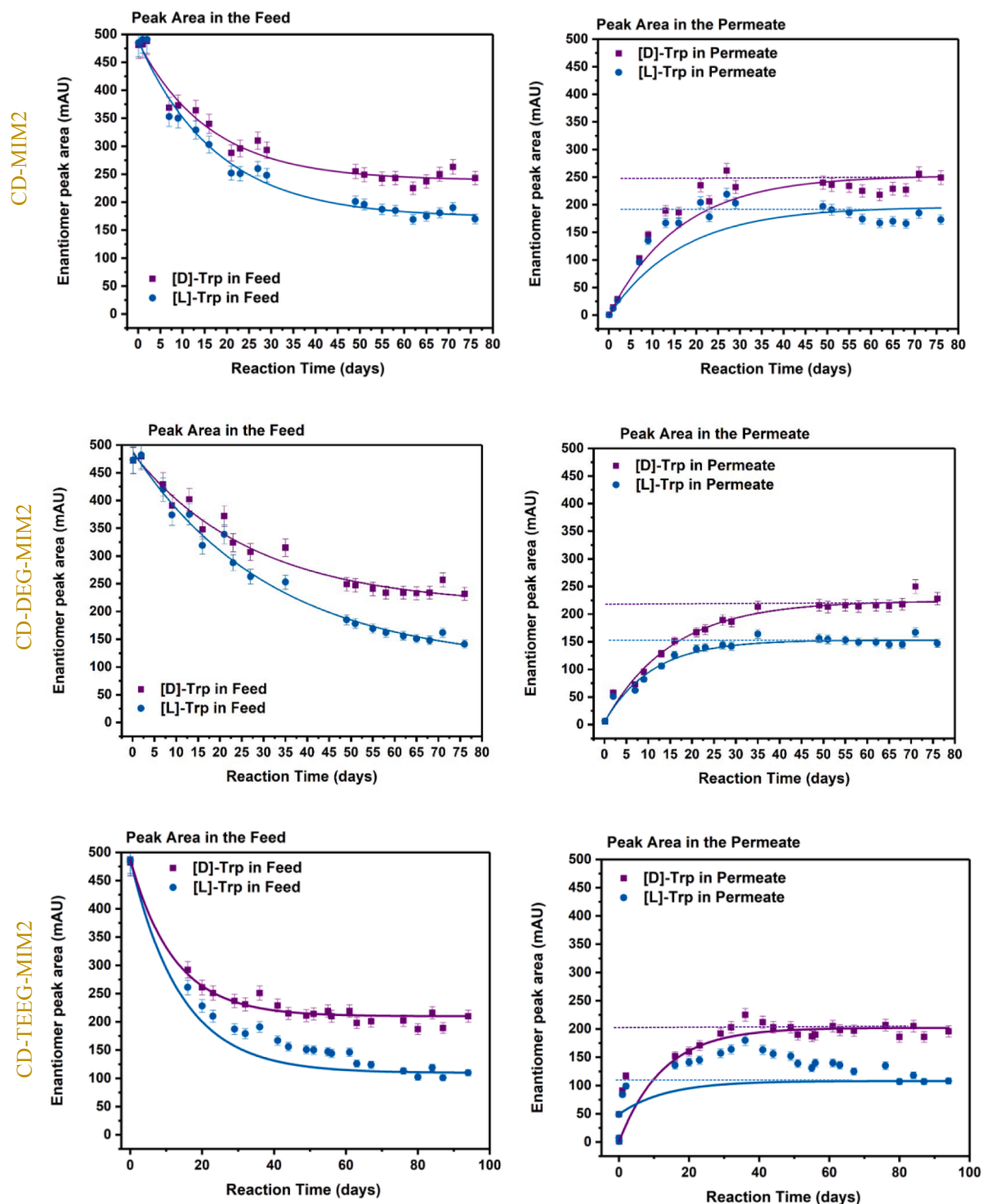
Fig. 4. Evolution of peak area of Trp enantiomers during pertraction experiments. Left graphs – situation in feed, at right – permeate site. The first line for CD-MIM2, the second line for CD-DEG-MIM2 and the third line for CD-TEEG-MIM2 membrane.

results. Each chamber was kept at a stable temperature by a thermostat, and liquids inside the cell were stirred by magnetic bars. The concentration of each of the enantiomer in the permeate was determined by HPLC using the same optical resolution column CHIRALPAK® ZWIX(+) as in the sorption process.

2.6. Pressure driven separation

Nanofiltration experiments were carried out in a cross-flow apparatus (Fig. 3) at room temperature (25 °C) to determine the permeability and the rejection of the membranes. A set of four polyethylene glycols (PEG) of different molecular weight was used to determine preliminary performances of organic solvent nanofiltration (OSN) membranes, such as the steady-state of the membranes. This analysis technique allows rigorous testing of the behaviour of the membranes over extended periods. Discs of the membrane, with an active membrane area of 14 cm^2, were cut, rinsed with the feed solvent, kept in distilled water for 24 h before being placed in the test cells in the
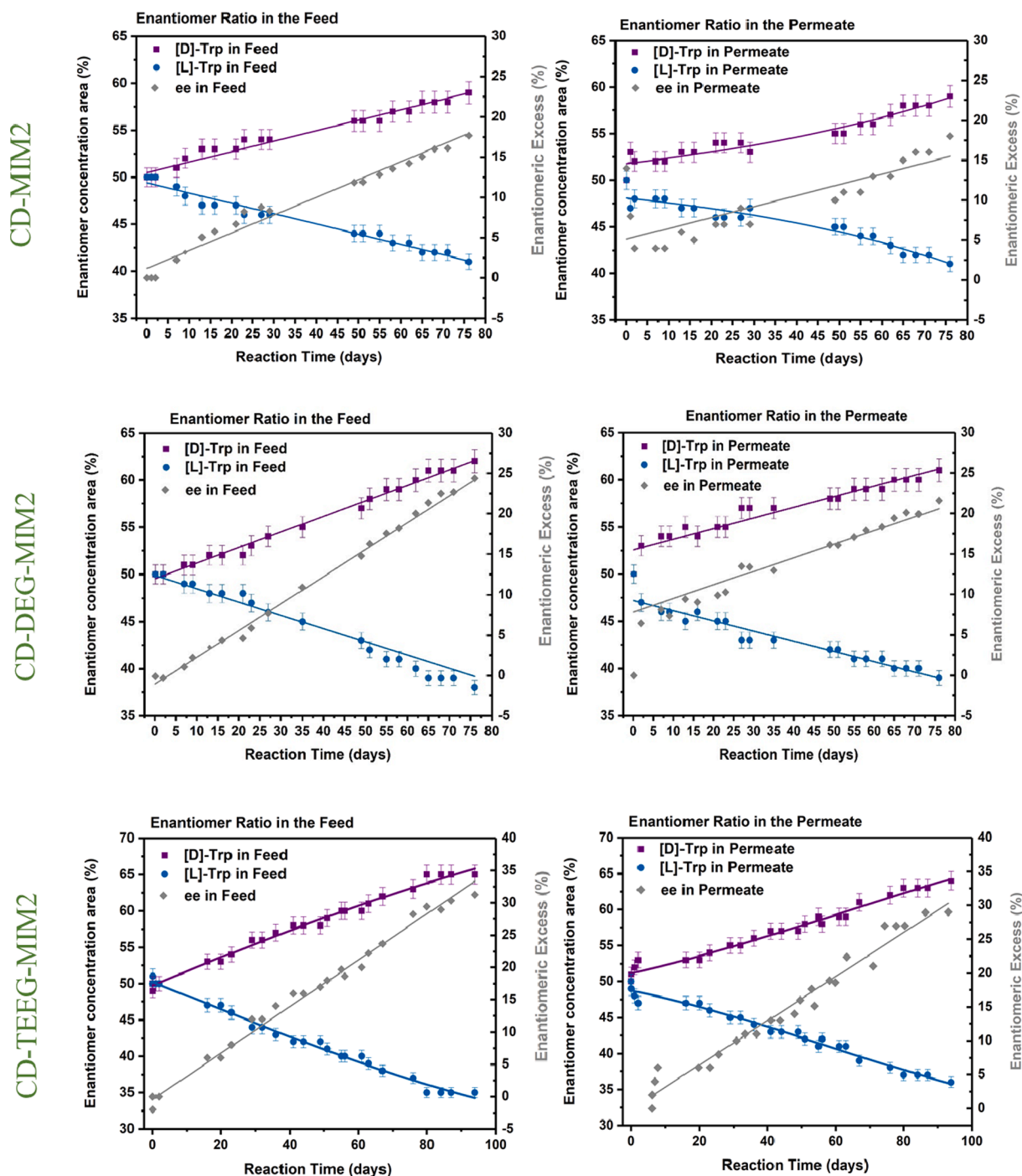
Fig. 5. Evolution of tryptophan enantiomers ratio in the feed and permeate during pertraction separation of a racemic solution of Trp (2 g/L in water). The first line for CD-MIM2, the second line for CD-DEG-MIM2 and the third line for CD-TEEG-MIM2 membrane.

below cross-flow apparatus. At least three discs of each membrane were tested in order to assess the repeatability of the results. To avoid concentration polarisation on the membrane surface, the feed solution was continuously stirring. To minimise the variation of concentration in the feed solution during the permeation test (sampling), an excess amount of feed (about 500 mL) was used.

This cross-flow apparatus contains up to five test cells in series (Fig. 3). The HPLC pump 305 (Gilson) was set at 30 mL/min. The circulation pump is a Micropump purchased from Michael Smith Engineers Ltd. The operating pressure, as well as the pressure drop across the membranes, were controlled using pressure gauges located before and after the cells. Pressure gauges were purchased from Wika, connector

and back pressure regulator (24.1–51.7 bar) from Swagelok. The back pressure was set up at 10, 20, 30 or 40 bar according to the experiment. The circulation pump (10–30 mL/min) allows to have a good cross-flow on membrane surfaces, decreases concentration polarisation and improves fluid dynamics. The pressure drop between before and after the membrane is reduced. The HPLC pump (which could operate at up to 90 mL/min) was used to minimise the amount of liquid which is alternatively pressurised and depressurised.

2.7. Evaluation of separation processes

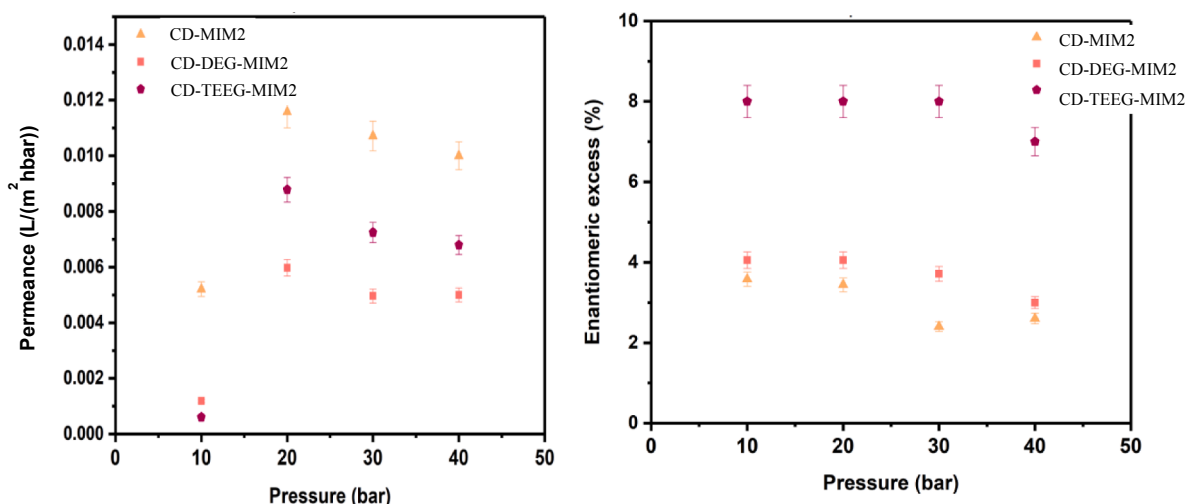The performance of a membrane is usually described by permeability

Fig. 6. Evolution of the permeance of tryptophan solution accross CD modified membranes and the enantiomeric excess of D-Trp over L-Trp in a pressure driven separation set-up.

FLUX

PERTRACTION   PRESSURE DRIVEN PERMEATION
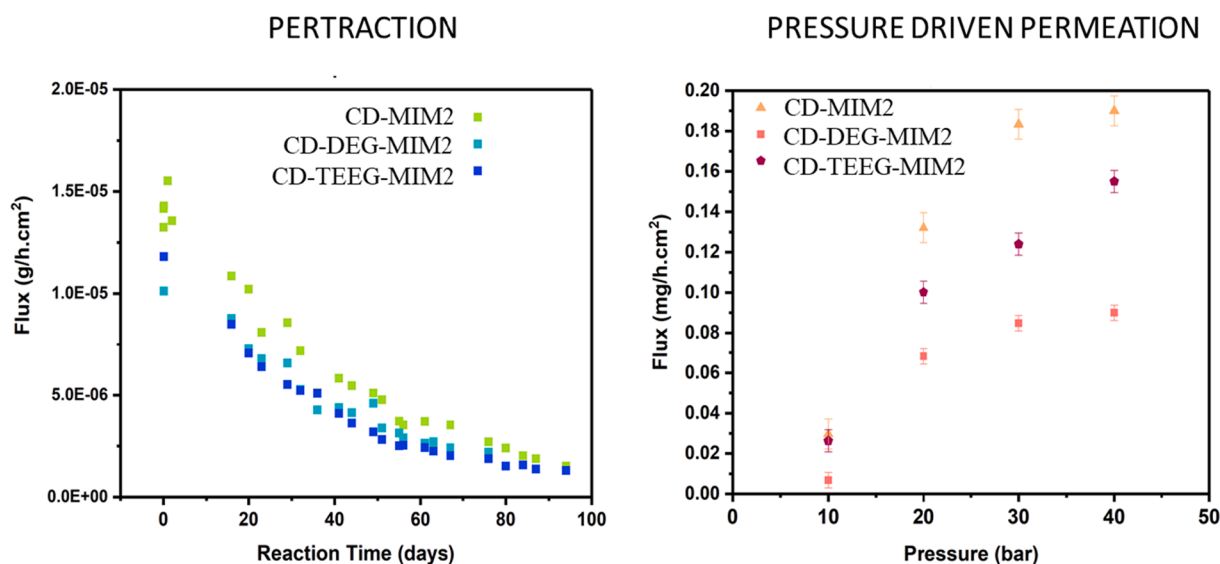

Fig. 7. Evolution of the flux of Trp solution across CD modified membrane in pertraction (left) and a pressure driven separation set-up (right).

and selectivity. The permeability evaluates the productivity of the process, and the selectivity measures the efficiency of the process.

The solvent flux J (mol/(cm^2.h)) (permeance) was obtained by measuring the amount of substance permeating through the membrane and using the following formula [29,30]:

$$J = \frac{Q}{\Delta tA} = \frac{\Delta CV}{\Delta tA} \qquad (4)$$

where Q is the quantity of the solute permeated for a given time, ΔC is the change in concentration, Δt is the permeation time, V is the downstream volume, and A is the effective membrane area.

The transmembrane fluxes (J) of each enantiomer across the membrane were calculated by measuring the concentration changes of each of them in the feed and the permeate as a function of the time interval Δt and can be written as follow:

$$J_{D-Trp} = \frac{V\Delta C_{D-Trp}}{\Delta tA} \qquad (5)$$

$$J_{L-Trp} = \frac{V\Delta C_{L-Trp}}{\Delta tA} \qquad (6)$$

A is the effective membrane area, and V is the volume of the cell compartment considered. C_{D-Trp} and C_{L-Trp} are the D-Trp and L-Trp concentrations in the permeate phase and J_{D-Trp} and J_{L-Trp} are fluxes of the two enantiomers.

The permeance values given in this chapter will be in L/(m^2.h.bar), using the formula:

$$\frac{J}{\Delta C\Delta p} = \frac{V}{A\Delta t\Delta p} \qquad (7)$$

The permeability coefficient P (cm^2/s) is given by:

$$P = \frac{Jd}{C_f - C_p} \qquad (8)$$

where d is the membrane thickness and $C_f - C_p$ is the concentration difference between feed and permeate compartments.

In the case of a membrane-based enantioseparation process, the enantioselectivity replaces the selectivity and is measured using the "enantiomeric excess" (*ee*) of one enantiomer in the permeate [30]. The enantiomeric excess of permeates was determined from the areas of their two enantiomers D-isomer (A_D) and L-isomer (A_L):

$$ee(\%) = 100 * \frac{A_D - A_L}{A_D + A_L} \tag{9}$$

The degree of enantiomeric separation was also evaluated using the resolutions (R_s) and separation factors (α):

$$R_s = 2 * \frac{(t_{R2} - t_{R1})}{(w_1 + w_2)}, \tag{10}$$

$$\alpha = \frac{C_D}{C_L} = \frac{1 - ee}{1 + ee}; \quad \alpha = \frac{k_1}{k_2} \tag{11}$$

where t_{R1}, t_{R2} and w_1, w_2 are, respectively, the retention times and peak widths of the first and second eluted peaks, and k_1 and k_2 indicate capacity factors of the first and second eluted peaks.

2.8. The mechanism of enantio-separation by chiral membranes

The different binding affinities of two enantiomers may be the result of different hydrogen bonding, hydrophobic, Coulomb, van der Waals interactions and steric effects with the chiral sites. Two mechanisms have been perceived in enantiomeric separations: facilitated and retarded transport [31]. Based on our experimental results the retarded transport is preferred.

The membrane follows the retarded transport mechanism where the driving force is a pressure gradient. In contrast to the facilitated transport mechanism, retarded transport retains the adsorbed enantiomer in the membrane phase, while permitting the other enantiomer to pass through the membrane more easily due to its lower affinity for the chiral recognition site. Membranes that function based on the retarded transport mechanism are called adsorption-enantioselective membranes and they usually incorporate chiral selectors [32]. In an adsorption-enantioselective membrane, the binding affinity between chiral recognition sites and enantiomers is stronger than that of a diffusion-enantioselective membrane, and this interaction force always exists between one enantiomer and one chiral site. Separation efficiency of these membranes is mainly determined by the binding capacity. The adsorption-enantioselective membranes are expected to simultaneously possess relatively high flux and high enantioselectivity, and thus have more potential than diffusion-enantioselective membranes to carry out industrial-scale productions of optically pure compounds [32].

3. Results and discussion

3.1. Preferential sorption experiment

The feed solution of racemic tryptophan was analysed before the preferential sorption experiments in both countries via HPLC to confirm the ratio of 50:50 D-Trp and L-Trp. One part of the results (from CZ laboratory [23]) was previously published: enantiomeric excess of 14, 44, and 8% for CD-MIM2, CD-DEG-MIM2, and CD-TEEG-MIM2 membranes, respectively, in 280 days. Newly, the identical sampling mode of preferential sorption in time in slightly different set-up (in UK) revealed the same results within the experimental error of ±1%. The membrane modified by CD derivative with no spacer, CD-MIM2, separated racemic mixture with a medium enantiomeric excess (14%), possibly due to the CD moiety being positioned too close to the Nafion membrane surface. That might prevent access of Trp enantiomers to the CD ring and thus inhibit the enantio-separation. CD-DEG-MIM2, CD modifier with the short spacer, exhibited the best separation performance (44%). For CD-TEEG-MIM2, CD modifier with the long spacer, the weakest separation

effect was confirmed, just 8% *ee*. The CD moiety is potentially hanging too far from the membrane, which causes problems for Trp enantiomers to reach the CD ring and remain adsorbed there. The short spacer CD derivative was, therefore, yet again optimal.

Three new preferential sorption experiments proved practically equal results in two independent laboratories, comprising the different analytical tools. Enantiomeric excess reached in 80 days 4, 13, and 2% for CD-MIM2, CD-DEG-MIM2, and CD-TEEG-MIM2, respectively. Another new result was the validation that the pristine membrane without CD modification did not perform any enantiomeric separation. Therefore, enantiomeric excess is exclusively attributed to CD modification.

All these results were necessary to compare other membrane processes – pertraction (in CZ) and pressure-driven separation (UK) – without the intervention of possible mismatched results due to experimental procedure or analytical methods.

3.2. Pertraction experiment

The membranes CD-MIM2, CD-DEG-MIM2 and CD-TEEG-MIM2 were repeatedly tested in CZ as separation material in pertraction of Trp racemic mixture, following the protocol detailed in Experimental Methods. The feed solution of racemic Trp in ultrapure water (c_{Trp} = 0.01 M, equivalent to 2 g/L) was analysed before the experiments via HPLC to confirm the equal ratio of D-Trp and L-Trp, 50:50. The evolution of peak area (perfectly corresponding to the concentration) of Trp enantiomers during pertraction is traced in Fig. 4 for all membranes. Graphs for the feeds are on the left, for permeates on the right. The first line shows the results of CD-MIM2, the membrane with no spacer in CD modifier, the second one is attributed to CD-DEG-MIM2 with the short spacer, and the third belongs to CD-TEEG-MIM2 with the long spacer.

For the three types of membranes, the equilibrium of Trp in two chambers has been reached within the experimental time (80 days). In accordance with the preferential sorption tests, all membranes preferentially sorb L-Trp. However, a certain trend was observed according to the membrane type. To complete the following discussion, Table 2 shows the weight of the membranes fresh and used, with the weighted or calculated amount of CD derivates and Trp enantiomers, influencing the weight of separation materials.

During the pertraction experiment, CD-MIM2 let the whole amount of D-Tpr pass through the membrane and 1 g/L is equally distributed between both chambers at the end of the experiment (Fig. 4, the 1st line). Resulting from the HPLC data, a calculated amount of L-Trp adsorbed to the membrane was 14.1 mg (Table 2). Such amount matches the weight gain of the membrane during the experiment because the membrane cannot retained larger amount while Trp was permeating. This is for separation material with no spacer in the CD modifier. The longer is the spacer, the more pronounced is the sorption process (in agreement with preferential sorption); CD-DEG-MIM2 with the short spacer gained 24.9 mg, practically only due to more pronounced L-Trp sorption (Fig. 4, (CD-DEG-MIM2) left graph (the feed side) traces the blue line linked to L-Trp lower than the graph above). The positive effect of pertraction, comparing to simple sorption, is clearly visible in Fig. 4 (CD-TEEG-MIM2). The sorption part of separation process using CD-TEEG-MIM2 membrane was not blocked nor inhibited, and the effect was the most pronounced from all three membranes (with weight increase up to 32.2 mg). Thus, it can be concluded that with the growing length of the spacer, the sorption capacity during the pertraction process visibly increases. The almost ideal coherences between measured and calculated data eliminate the possibility of disappearance of Trp due to the presence of bacteria or similar influence. Fig. 5 shows the kinetics of pertraction of racemic Trp through the CD-MIM2, CD-DEG-MIM2 and CD-TEEG-MIM2 membranes. A detailed evolution of the enantiomer peak area in the feed and in permeate is given along with the enantiomer concentration and the enantiomeric excess for CD-MIM2 (the 1st line), CD-DEG-MIM2 (the 2nd line) and CD-TEEG-MIM2 membrane (the 3rd

Table 2
The weight of the membranes fresh and used, with the weighted or calculated amount of CD derivates and Trp enantiomers.

Membrane	Fresh membrane			Used membrane		
	measured m $_{Nafion}$ (mg)	calculated m$_{CD}$ (mg)	calculated m$_{Nafion + CD}$ (mg)	measured m$_{Nafion + CD + Trp}$ (mg)	measured m$_{Trp\ sorbed}$ (mg)	calculated m$_{Trp\ sorbed}$ (mg)
CD-MIM2	153.6	4.7	158.3	172.2	13.9	14.1
CD-DEG-MIM2	153.6	14.0	167.6	192.7	25.1	24.9
CD-TEEG-MIM2	153.6	17.0	170.6	202.3	31.7	32.2

line). As the enantiomers pass through the membrane, their concentration (peak area) decreased in the feed and increased in permeate. The concentration of both enantiomers decreased, significantly more for the L-Trp, indicating preferential sorption of the L-enantiomer in the membrane, described above. The completely new behaviour of the membranes was observed – the unchanged ratio of enantiomers was transported from the feed to permeate using these membranes. The ratio in the permeate changed from 51:49 to 59:41 (D-Trp: L-Trp) using CD-MIM2, from 51:49 to 61:39 applying CD-DEG-MIM2 and from initial 51:49 up to 63:37 with CD-TEEG-MIM2 membrane. Enantiomeric excess was calculated as 18, 22 and 27% in favour of the D-enantiomer for CD-MIM2, CD-DEG-MIM2 and CD-TEEG-MIM2 respectively. However, the enantio-separation process takes place exclusively during the sorption part of the pertraction process, followed by simple diffusion transport of Trp mixture.

Last, but not least important fact about pertraction can be revealed in comparison with simple sorption experiments. The enantiomeric excess of 44% was reached in the sorption process, using CD modifier with the short spacer. However, the experiment took 280 days. Pertraction tests lasted around 80 days to reach steady-state, which matches to *ee* close to 13% only. Corresponding performance of CD-DEG-MIM2 membrane was higher, *ee* equal 22% and confirm, that pertraction process is more suitable for selective elimination of D-enantiomer of Trp from water.

3.3. Pressure driven permeation experiment

The pressure driven permeation results through the CD membranes are described below in Fig. 6. The evolution of the enantiomeric excess in permeate is given along with permeance of the Trp through the membranes. For the three types of membrane, the data reveals a faster kinetic than for the preferential sorption and pertraction experiments. After 24 h of permeation under pressure, the steady state was reached, and the samples were taken.

A continually significant change in peak area and peak ratio was observed. The same trend for the three membrane types was observed in different extent according to the membrane type. Similarly, as for the pertraction experiments, both enantiomers decreased to a significantly higher degree for the L-tryptophan, indicating preferential sorption of the L-enantiomer in the membrane and a preferential permeation of D-Trp through the membrane. The L-Trp adsorption was confirmed with the calculation of the enantiomeric excess in favour of the D-enantiomer: about 3% for CD-MIM2, 4% for CD-DEG-MIM2 and 7% for CD-TEEG-MIM2. The CD modifier with the long spacer gave the best performances confirming the pertraction results. No spacer, in the case of CD-MIM2, kept the CD ring too close the membrane to be reached by the enantiomers. In the case of CD-DEG-MIM2 and CD-TEEG-MIM2, the steric hindrance is decreased, and the better specific rotation of the CD modified with the longest chain made it more efficient for the chiral separation.

Regarding the impact of the pressure on the chiral separation of Trp enantiomers, the enantiomeric excess seemed to decrease at 40 bar for all three membranes due to coupling effect. When the pressure increased, the quantity of tryptophan going through the membrane increased. Especially, the amount of L-Trp penetrating increased more, which decreased the total enantioselectivity of the membrane. This phenomenon can be explained by the solution-diffusion model [33]. As mentioned earlier, the transport model used to describe the under pressure process included a sorption, diffusion and desorption steps. The selectivity of a dense membrane is then calculated using both sorption and diffusion selectivity. It has been observed that when the pressure increases, the diffusion selectivity usually decreases, eventually decreasing the total selectivity – typical trade of behaviour.

In term of flux (Fig. 7), the same trend was observed for the three types of membranes with a continuous increase of the flux with the operating pressure and permeance increasing until reaching a plateau, contrary to pertraction experiments with the opposite trend.

The flux through CD-MIM2 seems slightly higher, which might be due to a smaller interaction of both enantiomers with the CD ring due to the absence of a spacer. The flux through CD-DEG-MIM2 and CD-TEEG-MIM2 are more similar, which corresponds to their very close specific rotation value.

4. Conclusions and future perspectives

The presence of the modified cyclodextrin in Nafion117® membrane enabled the selective separation of L/D-Tryptophan enantiomers from water. The separation performance is linked to the process used for membrane application. Different experimental set-ups – pertraction, two kind of sorption, and pressure driven membrane separation – revealed the highest performance of membranes in pertraction. Enantiomeric excess reached 18, 22 and 27% in 80 days of pertraction for CD-MIM2, CD-DEG-MIM2 and CD-TEEG-MIM2 membrane respectively. The enantiomeric excess as high as 44% was reached in sorption process, using cyclodextrin molecule with a medium chain. However, the experiment took 280 days, and *ee* close to only 13% corresponds to 80 days of testing time. Corresponding performance of CD-DEG-MIM2 membrane was higher, *ee* equal 22% and confirm, that pertraction process is more suitable for selective elimination of D-enantiomer Trp from water. While the short spacer in CD modifier is optimal for the preferential sorption, the long spacer was the best for performance for the pertraction and the under-pressure permeation processes.

The pressure-driven permeation process has allowed the operation to be continuous and faster, which has the potential for continuous large-scale production of enantiopure compounds and could pave the way for many more commercial applications, satisfying the considerable demand for large-scale chiral separation techniques. However, low enantiomeric excess was observed in 80 days – 3, 4 and 7%. Reducing the flux of enantiomers through the modified membrane might give more time to the enantiomers to differentiate, decrease the coupling effect and thus increases the enantiomeric excess.

After this study we noticed perspectives for future research. The performed separation processes needed long time to achieve required separation factor. When the process is enhanced with pressure to gain in treatment time, we lose in selectivity. Moreover, separation efficiency order of tested membranes switched from sorption to other separation processes. This can be explained only by changing in separation mechanism and involving of diffusion step in separation mechanism. We can also conclude that the chiral modifiers containing multiply positively charged anchor can be safely used for modification of negatively charged membranes, such as Nafion. The ionic binding should be strong and relatively unaffected by pressure, but could be affected by changes in the concentration (especially salt concentration) or pH of the filtering

solution. However during our experiments we did not observed any leaching or instability of the membranes.

Credit authorship contribution statement

J. Gaalova: Conceptualization, Data curation, Formal analysis, Investigation, Funding acquisition, Methodology, Writing - original draft. **M. Michel:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing - original draft. **M. Bourassi:** Writing - review & editing. **B.P. Ladewig:** Writing - review & editing. **P. Kasal:** Formal analysis, Investigation, Methodology, Writing - original draft. **J. Jindrich:** Writing - Funding acquisition, review & editing. **P. Izák:** Project administration, Resources, Supervision, Funding acquisition, Writing - review & editing.

Declaration of Competing Interest

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

Acknowledgments

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2021.118538.

References

[1] N. Vargesson, *Thalidomide-induced teratogenesis: history and mechanisms*, Birth Defects Res. Part C, Embryo Today: Rev. 105 (2) (2015) 140–156.

[2] J.H. Kim, A.R. Scialli, Thalidomide: The Tragedy of Birth Defects and the Effective Treatment of Disease, Toxicol. Sci. 122 (1) (2011) 1–6.

[3] A.T. van der Goot, E.A.A. Nollen, Tryptophan metabolism: entering the field of aging and age-related pathologies, Trends Mol. Med. 19 (6) (2013) 336–344.

[4] H.C. Hong, M.H. Wong, Y. Liang, Amino Acids as Precursors of Trihalomethane and Haloacetic Acid Formation During Chlorination, Arch. Environ. Contam. Toxicol. 56 (4) (2009) 638–645.

[5] X. Yang, et al., Precursors and nitrogen origins of trichloronitromethane and dichloroacetonitrile during chlorination/chloramination, Chemosphere 88 (1) (2012) 25–32.

[6] C. Li, et al., Formation of iodinated trihalomethanes during chlorination of amino acid in waters, Chemosphere 217 (2019) 355–363.

[7] G. WHO, Guidelines for drinking-water quality, World Health Organization 216 (2011) 303-304.

[8] S.W. Smith, Chiral Toxicology: It's the Same Thing…Only Different, Toxicol. Sci. 110 (1) (2009) 4–30.

[9] T.J. Ward, T.M. Oswald, Chiral Separations by High-Performance Liquid Chromatography, in: Encyclopedia of Analytical Chemistry, R.A.M.a.J.G. Dorsey, Editor, John Wiley & Sons, 2006.

[10] W.R. Brode, Optical Rotation of Polarized Light by Chemical Compounds*, J. Opt. Soc. Am. 41 (12) (1951) 987–996.

[11] R. Xie, L.Y. Chu, J.G. Deng, Membranes and membrane processes for chiral resolution, Chem. Soc. Rev. 37 (6) (2008) 1243–1263.

[12] C. Fernandes, M. Tiritan, M. Pinto, Chiral Separation in Preparative Scale: A Brief Overview of Membranes as Tools for Enantiomeric Separation, Symmetry 9 (10) (2017) 206.

[13] A. Higuchi, et al., Polymeric Membranes for Chiral Separation of Pharmaceuticals and Chemicals, Polym. Rev. 50 (2) (2010) 113–143.

[14] B. Belhamdi, et al., The removal and adsorption mechanisms of free amino acid 1-tryptophan from aqueous solution by biomass-based activated carbon by H3PO4 activation: Regeneration study, Phys. Chem. Earth. 114 (2019), 102791.

[15] L.F. Liu, et al., Recovery of L-tryptophan from crystallisation wastewater by combined membrane process, Sep. Purif. Technol. 66 (3) (2009) 443–449.

[16] Q.M. Liu, et al., Novel cyclodextrin-based adsorbents for removing pollutants from wastewater: A critical review, Chemosphere 241 (2020), 125043.

[17] Z. Hammoud, et al., Cyclodextrin-membrane interaction in drug delivery and membrane structure maintenance, Int. J. Pharm. 564 (2019) 59–76.

[18] J. Zou, J.G. Yu, Nafion-stabilized black phosphorus nanosheets-maltosyl-beta-cyclodextrin as a chiral sensor for tryptophan enantiomers, Mater. Sci. Eng. C-Mater. Biol. Appl. 112 (2020), 110910.

[19] P.G. Ingole, et al., Enantioselective permeation of α-amino acid isomers through polymer membrane containing chiral metal–Schiff base complexes, Desalination 281 (2011) 413–421.

[20] P.G. Ingole, et al., Preparation, characterization and performance evaluation of separation of alcohol using crosslinked membrane materials, New J. Chem. 37 (2013) 4018–4024.

[21] P.G. Ingole, et al., Membrane separation processes: Optical resolution of lysine and asparagine amino acids, Desalination 343 (2014) 75–81.

[22] P.G. Ingole, et al., Optical resolution of racemic lysine monohydrochloride by novel enantioselective thin film composite membrane, Desalination 305 (2012) 54–63.

[23] J. Gaálová, et al., Separation of racemic compound by nanofibrous composite membranes with chiral selector, J. Membr. Sci. 596 (2020), 117728.

[24] M. Otmar, et al., Preparation of PSEBS membranes bearing (S)-(−)-methylbenzylamine as chiral selector, Eur. Polym. J. 122 (2020), 109381.

[25] T. Fukushima et al., Enantiomeric Separation of Monosubstituted Tryptophan Derivatives and Metabolites by HPLC with a Cinchona Alkaloid-Based Zwitterionic Chiral Stationary Phase and Its Application to the Evaluation of the Optical Purity of Synthesized 6-Chloro-L-Tryptophan. Int. J. Tryptophan Res. 8 (2015) IJTR. S20381.

[26] M. Barboiu, et al., A new alternative to amino acids transport: facilitated transport of l-phenylalanine by hybrid siloxane membranes with a fixed site macrocyclic complexant, J. Membr. Sci. 161 (1) (1999) 193–206.

[27] P. Kasal, et al., Chiral Nafion membranes prepared by strong electrostatic binding of multiply positively charged β-cyclodextrin derivatives separate tryptophan racemic mixtures, Mater. Today Commun. (2021), https://doi.org/10.1016/j.mtcomm.2021.102234 (in press).

[28] J. Dian, et al., Functionalized materials with fluorescent dyes for chemosensor applications, Monatsh Chem. 148 (2017) 1929–1935.

[29] M. Meldal, et al., Cu-Catalyzed Azide−Alkyne Cycloaddition, Chem. Rev. 108 (8) (2008) 2952–3015.

[30] J.H. Kim, et al., Optical resolution of α-amino acids through enantioselective polymeric membranes based on polysaccharides, J. Membr. Sci. 213 (1–2) (2003) 273–283.

[31] P.G. Ingole, et al., Methods for separation of organic and pharmaceutical compounds by different polymer materials, Korean J. Chem. Eng. 31 (12) (2014) 2109–2123.

[32] E.M. Van der Ent, et al., Design criteria for dense permeation-selective membranes for enantiomer separations, J. Membr. Sci. 185 (2) (2001) 207–221.

[33] J.G. Wijmans, R.W. Baker, The solution-diffusion model: a review, J. Membr. Sci. 107 (1) (1995) 1–21.