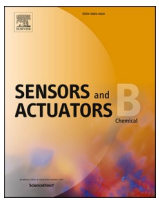
Sensors & Actuators: B. Chemical 426 (2025) 137122

Contents lists available at ScienceDirect

Sensors and Actuators: B. Chemical

journal homepage: www.elsevier.com/locate/snb



Enhanced biosensing by green, switchable photochromic molecularly imprinted polymers

Ana I. Furtado [a,b], Diogo Lobato [a], Sandra Gago [a], Vasco D.B. Bonifácio [b,c,*], Raquel Viveiros [a,**], Teresa Casimiro [a,**]

[a] LAQV–REQUIMTE, Chemistry Department, NOVA School of Science & Technology, NOVA University of Lisbon, Campus de Caparica, Caparica 2829–516, Portugal
[b] iBB–Institute for Bioengineering and Biosciences and i4HB–Institute for Health and Bioeconomy, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1, Lisboa 1049–001, Portugal
[c] Bioengineering Department, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, Lisboa 1049–001, Portugal

ARTICLE INFO

Keywords:
Green chemistry
Supercritical fluid
Photo–switchable materials
Optical sensing
Biorecognition

ABSTRACT

Photochromic Molecularly Imprinted Polymers (PC–MIPs) synthesized using supercritical carbon dioxide ($scCO_2$) represent a key approach to produce green stimuli–responsive polymers. In this work, methacryloyloxyethyl)–3',3'–dimethyl–6–nitrospiro(2H–1benzopyran–2,2'–indoline (SPMA) was incorporated as the functional photochromic monomer (M), L–isoleucine (IsoLEU) as the template (T) molecule, and ethylene glycol dimethacrylate (EGDMA) as the crosslinker (C). Two different PC–MIP systems were explored by changing the crosslinker degree, T:M:C (1:12:50 and 1:12:100). Moreover, enantiomeric separation was evaluated by static binding tests using L–leucine (LEU) and IsoLEU from aqueous solutions. The PC–MIP with high crosslinker degree (PC–MIP1) exhibited the highest affinity and selectivity in template solutions, with a maximum binding capacity of 127 mg IsoLEU/g polymer and an imprinting factor of 1.6. The experiments performed under UV–light (365 nm), both PC–MIPs (PC–MIP1 and PC–MIP2), ranged from white to bluish purple, with reversible color changes upon exposure to visible light. Additionally, both PC–MIPs demonstrated delayed optical responses upon incorporation of the target molecule into the polymeric matrix, enabling real–time detection. This study underscores the potential of PC–MIPs as green and cost–effective optical sensors for bioprocess monitoring, offering high sensitivity and selectivity for IsoLEU detection in aqueous media.

1. Introduction

Stimuli–responsive polymers have been gaining significant attention in the field of materials science because their response to specific stimuli makes them highly versatile and valuable for various applications. These polymers suffer variations in their properties (physical and chemical) by changing their environment (temperature, pH, light stimulus, among others) [1]. Indeed, photo–responsive polymers, also known as photochromic polymers, are stimuli–responsive polymers that undergo color change when exposed to light. The design of these polymers is dependent on the target application since different moieties of photoresponsive molecules can be designed, providing various responses by environmental changes. Many different photoresponsive groups may be incorporated in conventional polymerization reactions, including irreversible photoresponsive monomers (pyrenylmethyl ester, *o*–nitrobenzyl ester, coumarinyl ester, *p*–methoxyphenacyl ester) and reversible monomers (diazonaphthoquinone, azobenzene, spiropyran, dithienylethene, coumarin, truxillic acid, cinnamic ester, and anthracene) [1]. Each system is characterized by its reversible response to light irradiation, promoting photocleavage, photoisomerization, or photocycloaddition [2]. The 1'–(2–methacryloyloxyethyl)–3',3'–dimethyl–6–nitrospiro (2H–1benzopyran–2,2'–indoline) (SPMA) molecule has a spiropyran (SP) scaffold that is photoresponsive. SP has three molecular states and can work as a sensor, specifically by chemical (acid or base) or light (visible or UV–light) stimuli [3]. More specifically, SPMA can switch from a non–polar SP form to a polar merocyanine form (MC) under UV–light irradiation, changing the material's color from colorless to blue/purple (MC λ_{abs} *ca.* 500–700 nm). MC is reversibly transformed to SP by visible light

* Corresponding author at: iBB–Institute for Bioengineering and Biosciences and i4HB–Institute for Health and Bioeconomy, Instituto Superior Técnico, University of Lisbon, Av. Rovisco Pais, 1, Lisboa 1049–001, Portugal.
** Corresponding authors.
E-mail addresses: vasco.bonifacio@tecnico.ulisboa.pt (V.D.B. Bonifácio), raquel.viveiros@fct.unl.pt (R. Viveiros), teresa.casimiro@fct.unl.pt (T. Casimiro).

https://doi.org/10.1016/j.snb.2024.137122
Received 7 October 2024; Received in revised form 10 December 2024; Accepted 11 December 2024
Available online 12 December 2024

irradiation [3,4].

The application of these molecules can be challenging due to low stability, fatigue (repeated cycling between states can degrade the material over time, reducing its effectiveness), and lack of control in the response time. However, the incorporation of these molecules into a polymeric matrix allows their photo–response reversibility, overcoming the low stability [5,6]. Photochromic Molecularly Imprinted Polymers (PC–MIPs) combine the unique properties of photochromic agents and molecularly imprinted polymers (MIPs) to create advanced materials with tailor–made recognition ability and reversible optical responses [7]. Several conventional PC–MIPs have been reported for affinity–based separation devices, demonstrating an effective ON–OFF and selective response, adsorbing the target molecule when exposed to visible light and desorbing it, when exposed to UV–light [4,5]. Typically, the synthesis of the conventional PC–MIPs is based on bulk polymerization using volatile organic solvents (*e.g.*, toluene, chloroform, acetone). Therefore, advances in synthetic approaches are needed, by choosing green, sustainable and scalable methodologies to enable large–scale production of PC–MIPs without compromising their functional properties, seems to be a key future direction. As sustainability becomes a priority, new greener approaches to produce these materials are sought. Supercritical carbon dioxide ($scCO_2$) has been implemented in many processes as alternative solvent. $ScCO_2$ is non–toxic, non–flammable, inert, odorless, can be easily removed without any additional energy input, and can be reused. $ScCO_2$ is easily achieved due to its mild critical point ($p_C = 73.8$ bar; $T_C = 31.1$ °C). Above this point, CO_2 combines the best properties of gas and liquid, such as gas–like diffusivity and viscosity, liquid–like density, high mass transport capacity, high diffusivity, and zero surface tension. Besides, $scCO_2$ is aprotic thus has low interference with the interaction template–monomers at the beginning of the polymerization and turns into gas upon depressurization leading to a ready–to–use solvent free material. These properties turn CO_2 an excellent medium for carrying out polymerizations and are advantageous in MIPs development [8]. The production of photochromic materials using $scCO_2$ is still limited to one study, where spiropyran–based interpenetrating polymer networks (IPNs) incorporation of functional monomers (including SPMA) into silicone elastomers, were developed for drug delivery [9].

Hence, to advance sensing applications, green PC–MIPs were developed using a $scCO_2$–assisted polymerization having SPMA as the functional monomer and L–isoleucine (IsoLEU) as the template to obtain a highly sensitive optical biosensing device. Enantiomeric separation and biosensing are explored by evaluating the binding performance using aqueous media of IsoLEU *vs.* L–leucine (LEU) for real–time detection with high selectivity and sensitivity. The color obtained by UV–light stimulus was evaluated as a real–time optical indicator of the presence of the target molecule within the polymeric matrix. The PC–MIPs biosensors have potential use in *in situ* monitorization of bioseparation processes.

2. Materials and methods

2.1. Materials

L–Leucine (LEU, > 98 %), L–isoleucine (IsoLEU, ≥ 98 %), ethylene glycol dimethacrylate (EGDMA, 98 %), ninhydrin (NIN, ≥ 95 %), ethanol (≥ 99.8 %), triethylamine (≥ 99.5 %), hydrochloric acid (HCl, 37 %), dichloromethane (CH_2Cl_2 ≥ 99.8 %), sodium carbonate (Na_2CO_3, ≥ 99.5 %), sodium chloride (NaCl, ≥ 99.0 %), sodium hydroxide (NaOH) 1 M solution, 2–hydroxyl–5–nitrobenzaldehyde (98 %), 2,3,3–trimethyl–3H–indole (98 %), potassium hydroxide (KOH, ≥ 85 %), magnesium sulfate anhydrous ($MgSO_4$, 98 %) were purchased from Sigma–Aldrich. The brine solution consists in a saturated aqueous solution of NaCl. The solvent ethyl acetate (EtOAc, ≥ 99.5 %) and diethyl ether (Et_2O, ≥ 99.8 %), were purchased from Honeywell. 2,2′–Azobis(2,4–dimethylvaleronitrile) (V–65, 98 %) and barium sulfate were purchased from Wako Pure Chemical Industries. Methacryloyl chloride (MeCN, ≥ 97 %) was purchased from Fluka Analytical. Acetonitrile (ACN, ≥ 99.9 %) and *n*–hexane (HEX, ≥ 95 %) were purchased from CARLO ERBA. 4–(Dimethylamino) pyridine (DMAP, 99 %) was purchased from Alfa Aesar. 2–Bromoethanol (> 95.0 %) was purchased from Tokyo Chemical Industries. RC Syringe Filter was purchased from Sartorius®. SnakeSkin™ dialysis tubing was purchased from Thermo Scientific. Carbon dioxide was obtained from Air Liquide with purity better than 99.998 %. All commercial reagents were used as received. Water was purified using a Millipore Milli–Q water system.

2.2. $ScCO_2$–assisted production of IsoLEU–photochromic molecularly imprinted polymers (PC–MIPs)

For the PC–MIPs synthesis, IsoLEU was used as template (T) (3.00 mg), the SPMA as functional monomer (M) (115.40 mg) and EGDMA as crosslinker (C) (216 μL or 432 μL). The SPMA used as a monomer in the polymerization reactions was synthesized following a reported protocol [10]. The SPMA synthesis is described in detail, in Section 1 of supplementary information, and the reaction scheme can be found in Fig. S1, and its purity was confirmed by FTIR analysis (see Fig. S2) and ^{1}H–NMR (see Fig. S3). The SPMA monomer displayed the desired photochromism (see Fig. S4). PC–MIPs were synthesized using two different crosslinking degrees, resulting in the following molar ratios of T:M:C, 1:12:100 (PC–MIP1) and 1:12:50 (PC–MIP2). Additionally, 2 wt% of V–65 (11.43 mg) was used as the thermal initiator.

Amino acids are polar molecules, with low solubility in $scCO_2$. Reported studies show that EtOAc is the best cosolvent to enhance amino acids solubilization in $scCO_2$ [8]. Therefore, IsoLEU was previously stirred for 4 h with a small amount of EtOAc (500 μL). All the reactants were placed in a 33 mL high–pressure cell with a magnetic stir bar. The cell was then immersed in a thermostatic water bath set to 45 °C and pressurized up to 200 bar with CO_2, using a Knauer 1900 liquid pump. The polymerization reaction was carried out under stirring for 24 h. The polymer was subsequently washed with fresh high–pressure CO_2 for 1 h to remove any unreacted reagents. The non–imprinted polymer (NIP) was synthesized using the same procedure, but without the addition of IsoLEU.

2.3. $ScCO_2$–assisted IsoLEU desorption

The IsoLEU desorption was performed packing each pre–synthesized PC–MIP on a stainless–steel tubular column coupled to a 33 mL high–pressure cell containing 3 mL of EtOAc at 40 ºC and 200 bar in continuous flow for 3 h. Both high–pressure cells were immersed in a thermostatted water bath at 40 ºC, and CO_2 was bubbled through the cell containing the co–solvent (bottom to top) and the mixture CO_2–EtOAc was passed through the tubular reactor using a Knauer – 1900 liquid pump. To evaluate the template–desorption efficiency, 20 mg of desorbed MIP was crushed and placed in a vial with 3 mL of Milli–Q water and kept under stirring for 24 h. After that, the sample was filtered through a 0.2 μm filter and quantified by high–pressure ionic chromatograph (HPIC), as described in detail in Section 2.8. No residues of IsoLEU were detected by the HPIC analysis (detection limit of 0.5 ppm).

2.4. Scanning electron microscopy (SEM)

All materials were characterized using scanning electron microscopy (SEM) in a Hitachi S–2400 instrument, with an accelerating voltage set to 15 kV. The samples were prepared on aluminum stubs with carbon tape and were gold coated. The magnification used was × 10 000.

2.5. Fourier transform infrared spectroscopy (FTIR–ATR)

The FTIR–ATR spectra were acquired for the synthesized materials, using a PerkinElmer Two spectrometer with 16 scans per second and a

resolution of 1 cm^{-1}, from 4000 to 400 cm^{-1}.

2.6. Average particle size diameter and particle size distribution

Particle size distribution, as well as average particle size diameters of polymers, was determined using a Morphologi G3 equipment from Malvern. A typical analysis was performed by dispersing the sample using the following conditions: a sample volume of 13 mm^3, SDU settings (injection pressure: 4 bar, injection time: 40 ms, and setting time: 120 s), and an optic selection of 50 ×. The analyses were performed in triplicate from three different dispersions with at least 30 000 particles counted.

2.7. Accelerated surface area and porosimetry (ASAP)

The specific surface area and the average pore size diameter of the materials were determined by N_2 adsorption according to the Brunauer–Emmett–Teller (BET) method. An accelerated surface area and porosimetry system (ASAP 2010 Micromeritics) was used under N_2 flow. The analyses were performed at a temperature of −195.8 °C, and the degasification was under vacuum atmosphere until 150 °C for more than 3 h.

2.8. Static binding tests

The binding capacity of the produced materials was evaluated using 0.5 mg/mL of IsoLEU aqueous solutions as template solution to evaluate the affinity of the polymers, and 0.5 mg/mL of LEU aqueous solutions as analogue molecule solution to evaluate the selectivity. The tests were performed in triplicated assays. Briefly, 20 mg of each polymer was weighted and placed into SnakeSkin™ dialysis membranes which were then introduced in 25 mL of amino acid solution, for 24 h under stirring (100 rpm) (IKA KS 4000 I Control shaker with an orbital movement), at room temperature. After this period, 2.5 mL of each solution were analyzed by UV–VIS spectroscopy by the ninhydrin (NIN) colorimetric method (described in Section 2.7). The binding capacity (Q) was determined using Eq. 1.

$$\boldsymbol{Q} = \frac{(\boldsymbol{C_0} - \boldsymbol{C})\boldsymbol{V}}{\boldsymbol{W}} \quad (1)$$

where Q is the binding capacity (mg amino acid/g polymer), C_0 is the amino acid concentration (mg amino acid/mL) in the solutions measured initially, C is the amino acid concentration (mg amino acid/mL) in the solutions measured after sorption, V (mL) is the volume of the solution and W (g) is the sample polymer weight.

The imprinting factor (IF), that reflects the MIP binding capacity compared to NIP, was calculated using Eq. 2. The Q_{MIP} and Q_{NIP} are the binding capacities of the MIP and NIP, respectively.

$$\boldsymbol{IF} = \frac{\boldsymbol{Q_{MIP}}}{\boldsymbol{Q_{NIP}}} \quad (2)$$

Moreover, the static binding tests under UV–light irradiation were conducted to confirm the effectiveness of IsoLEU adsorption in the absence of light irradiation. For these experiments, the polymers PC–MIP1 and PC–NIP1 were used, following the same procedure described below, but with continuous UV–light irradiation (365 nm UV–lamp, 40 W) during the 24 h stirring period inside a dark chamber.

2.9. Ninhydrin assays

The amino acid (LEU or IsoLEU) content in the binding samples was accessed by a colorimetric method. This method was adapted from literature, in which L–lysine was quantified [11]. The reported conditions were optimized for a concentration range of 0.1–0.5 mg/mL, using 1 mL of NIN in 2.5 mL of amino acid solution. The first step of the procedure was to homogenize the amino acid solution. The samples were kept under stirring (100 rpm) for one minute and then NIN was added under stirring for one additional minute. After that, the samples were introduced in a water bath at 50 °C under stirring (100 rpm) for 10 min. The samples were collected from the water bath and placed in a vessel containing ice to stop the derivation reaction. The samples absorbance (λ = 565 nm) was recorded after 5 min using the Perkin Elmer Lambda 25 UV/VIS Spectrometer.

2.10. High pressure ion chromatography (HPIC)

Amino acid solutions with concentrations lower than 0.05 mg/mL, such as the IsoLEU–desorption efficiency sample, were analyzed using HPIC analysis. The analyzes were carried out using a Dionex ICS3000 equipment with an electrochemical detector – Pulsed Amperometry Detection (PAD), and Aminopac PA10 250 × 4 mm column with a pre–column of 50 × 4 mm as the stationary phase, at 30 °C. The mobile phase contained a NaOH gradient solution (60–90 mM) at a constant flow of 0.8 mL/min. The injection volume of samples was 10 µL. The retention time for IsoLEU was between 10–11 min.

2.11. Diffuse reflectance spectroscopy (DRS)

The photochromic behavior of the polymers was assessed by Diffuse Reflectance UV–VIS spectroscopy using a Shimadzu YV–2501PC equipped with an integrating sphere. For sample preparation, the polymers were placed directly in a powder support. DRS measurements were conducted with barium sulfate as a reference over the 250–800 nm wavelength range. Since the polymers are opaque powders, light absorption was studied in diffuse reflectance mode and the obtained spectra were converted using the Kubelka–Munk Eq. 3 [12]:

$$\boldsymbol{f}[\boldsymbol{R}] = \frac{\boldsymbol{K}}{\boldsymbol{S}} \quad (3)$$

The $f[R]$ is a dimensionless unit, the R is the value of reflectance in percentage. The absorption and scattering coefficients of the Kubelka–Munk are K and S [12]. To understand the range of the photochromic ability, the samples were irradiated by an UV–light (UV–lamp, 365 nm, 40 W) at specific intervals: 0 seconds (sec), 5 sec, 15 sec, 30 sec, 1 min, 2 min, and 4 min, until the other photo–stationary state be reached (~ 40 min). In order to study the reversibility of the photochromic response, the polymers were irradiated with UV–light for 2 min (will turn them in blue), followed by irradiation with visible light (common LED white lamp, 6 W) for 30 min (will turn them in white, the original state). The DRS analysis was performed after each photo–stimulus, and this procedure was repeated three times for each polymer.

3. Results and discussion

All polymers were obtained as dry, free–flowing, and soft powders, with similar yields (Table 1). The photochromic agents displayed a photo–response toward UV–light (λ = 365 nm), conferring a purple/blue color and a red fluorescence to the polymers upon UV–light irradiation (see Fig. 1 and Fig. S5). The PC–MIPs template desorption was very efficient, since no IsoLEU was detected on the sample by the NIN colorimetric assay, neither by HPIC, ensuring a concentration lower than the detection limit, around 10 ppm.

Table 1 also presents the average particle size diameter, the specific surface area, the pore volume, and pore size of the produced polymers. In general, the average particle size was 1–3 µm, with MIPs showing higher values than the NIPs, which could be related to the presence of LEU in the polymerization step. In terms of material porosimetry, the MIPs exhibited a superior surface area and pore volume compared to their respective NIPs.

The SEM images of PC–MIPs are presented in Fig. 2, and the particle size distribution in Fig. S6 in Supplementary Information. The polymers

Table 1
Molar ratios of IsoLEU (template, T), SPMA (functional monomer, M) and EGDMA (crosslinker, C) used in PC–MIPs synthesis and polymerization yield.

Polymer	T	M	C	Yield (%)	Particle size diameter (μm)	BET surface area ($m^2\ g^{-1}$)	Pore volume ($cm^3\ g^{-1}$)	Pore size (nm)
PC–MIP1	1	12	100	55	2.70 ± 0.12	49.08 ± 1.51	0.20 ± 0.02	16.30 ± 0.35
PC–NIP1	0	12	100	42	1.18 ± 0.09	39.60 ± 1.90	0.17 ± 0.02	17.30 ± 0.67
PC–MIP2	1	12	50	45	2.17 ± 0.14	36.68 ± 1.26	0.16 ± 0.01	17.54 ± 0.50
PC–NIP2	0	12	50	40	1.90 ± 0.13	29.33 ± 1.84	0.14 ± 0.02	18.52 ± 0.95

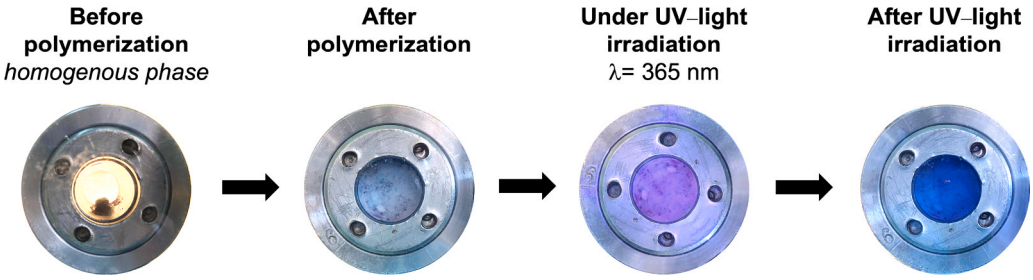


Fig. 1. Synthesis and photochromism of PC–MIPs.

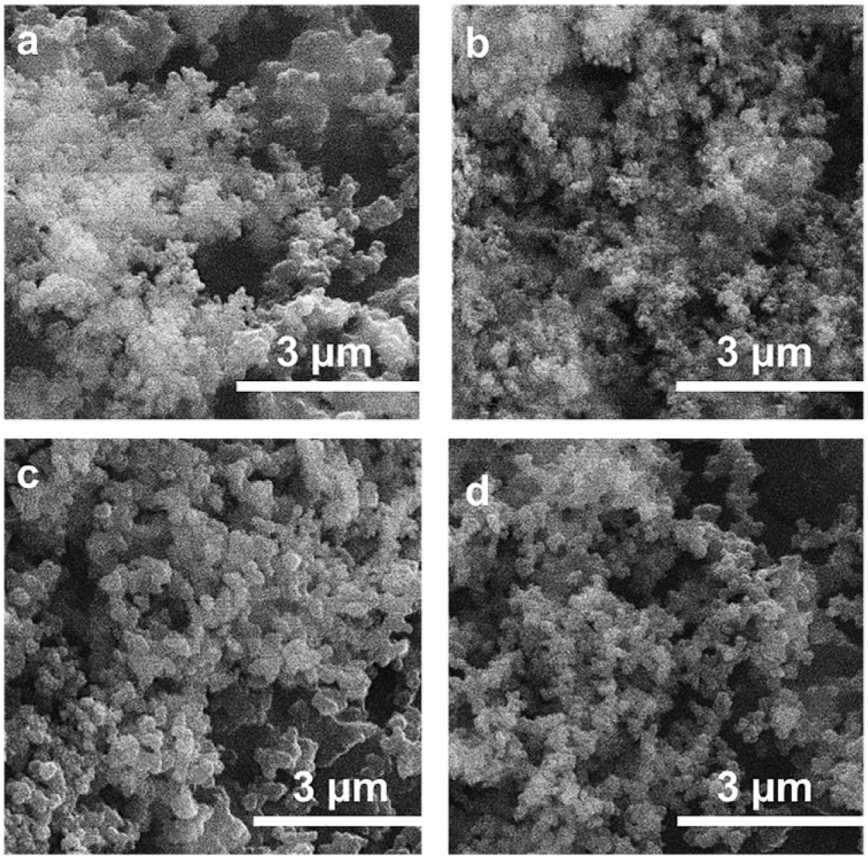


Fig. 2. SEM images of PC–MIPs: PC–MIP1 (a), PC–NIP1 (b), PC–MIP2 (c), and PC–NIP2 (d) (×10 000 magnification).

have a very resembling morphology which presented agglomerates of discrete mesoporous particles, consistent with reported free–radical polymerization reactions in $scCO_2$ [13,14].

The FTIR spectra were similar, clearly showing the characteristic bands of the functional groups of both monomers, SPMA and EGDMA (Fig. S7 in Supplementary Information).

PC–MIPs were designed as a new and high–specific tool for the detection of target biomolecules, such as IsoLEU. Therefore, with this aim, the affinity binding performance was evaluated using IsoLEU aqueous solutions, and the selectivity was evaluated using the isomer LEU. The binding capacities and their respective *IFs* from the static binding tests (SBTs) are presented in Fig. 3. Despite the materials have the same composition and similar surface morphology, the polymeric conformation may differ since the produced materials showed differences in their photo–response (Fig. S8–S10 in Supplementary Information) as well as in their binding capacity (Fig. 3).

Both MIPs (PC–MIP1 and PC–MIP2) showed higher binding capacity (Q) in template solution (IsoLEU) over the corresponding control materials (NIPs), indicating that a successful imprinting process occurred within the crosslinked materials produced. The PC–MIP with a higher

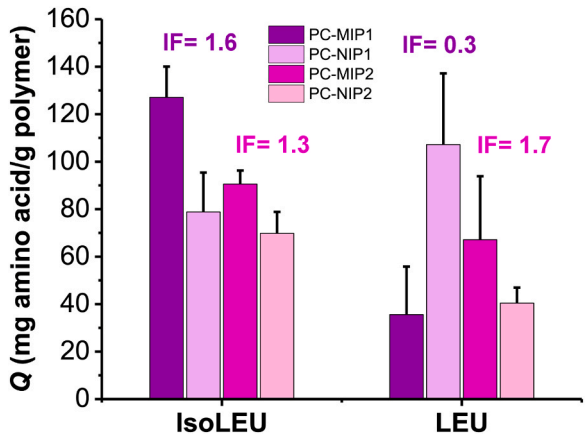


Fig. 3. Static binding performance of PC–MIPs: affinity and selectively for IsoLEU and LEU aqueous solutions.

crosslinking degree (PC–MIP1) has high selectivity since it has 3–fold lower binding performance with the analogue molecule (LEU) when compared with the template molecule, while its counterpart control (NIP) had a similar performance in both solutions. The system with a lower crosslinking degree (PC–MIP2 and control PC–NIP2), has a better binding performance in the template solution compared to the analogue solution. However, PC–MIP2 is less selective than PC–MIP1, as the difference in binding performance between IsoLEU and LEU solutions is not as pronounced with PC–MIP2 as it is with PC–MIP1.

As can be seen, the crosslinking degree in MIPs is an important factor that affects their binding performance. The positive trend between binding performance and the degree of crosslinking observed here follows other EGDMA–based MIPs obtained by precipitation polymerization [15,16]. The decrease in crosslinking density affects the hydrogen bonding interactions between monomers and the template in the pre–polymerization mixture. This results in changes inside the polymer network that may lead to a decrease in the rebinding capacity of the template [15]. The selectivity is also associated with a high degree of crosslinking, since during the imprinting process, the strongest bonds are formed when the functional monomers and the template are aligned in the most favorable conformation, and to maintain this alignment for later rebinding, it is crucial to keep the polymeric conformation ensured by the crosslinker [17]. Therefore, the polymer chains are immobilized with a high crosslinking degree, creating highly confined molecularly imprinted cavities with less structural flexibility for rebinding processes. However, it is worth highlighting other factors such as the choice of the functional monomer, the amount of initiator, and the nature of the template itself that can influence the binding performance [17].

Previous studies evaluated the ability of photosensitive molecules incorporated in materials, like SPMA, to bind target molecules for separation and sensing applications [4,5]. According to these studies, PC–MIPs can bind target molecules without UV–light exposure. However, when irradiated with UV–light, they lose their binding ability due to the conformational change in SPMA to its MC form, which is not favorable for binding [7]. To test if the produced polymers in this study follow the same behavior, binding tests were performed under UV–light in the same conditions. These tests were conducted in a black box with the samples continuously irradiated with UV–light at 365 nm for 24 h. As expected, the polymers lost their ability to adsorb molecules. Both polymers (PC–MIP1 and PC–NIP1) showed the same concentration of LEU and IsoLEU in aqueous solutions before and after the UV–assisted static binding test, indicating a loss of binding capability as reported in conventional SPMA–based MIPs. The SP inside the binding cavities was transformed into MC, resulting in unfavorable translocation carboxyl group for amino acid binding [4,18].

The goal of this work was to achieve real–time detection sensors for target amino acids with high selectivity and sensitivity. The color change induced by UV–light was evaluated as an optical indicator of the presence of the target molecule within the polymeric matrix, using DRS measurements for solids. Initially the effects of the response of the produced polymers by the UV–light irradiation were studied over time. The Figs. S8 and S9 in Supplementary Information, present the variations of the Kubelka–Munk converted UV–VIS diffuse reflectance spectra of the polymeric powders, under UV–light irradiation ($\lambda = 365$ nm). All polymers were irradiated until their photostationary state is reached, with a maximum absorption around 590 nm, typical of the MC form [3]. The DRS measurements following the previous procedure of the polymers from the static binding tests, i.e., the polymers containing the target molecule (IsoLEU) at concentrations between 0.02–0.11 mg/mL, were also performed. By following the coloration kinetics at 590 nm of the irradiated samples, as shown in Fig. 4, the difference between the photo–response of PC–MIP and PC–NIP was more evident in the system with lower crosslinking degree (PC–MIP2 and PC–NIP2). PC–NIP2 has a faster conversion of SP to MC than PC–MIP2. It is also evident a slower conversion to reach the photostationary state for PC–MIP2 +IsoLEU compared to the other polymers studied, respectively, which may be explained by additional mobility constraints for photoconversion caused by the inclusion of the target molecule inside of the polymeric imprinted cavities.

To study the fatigue resistance performance of PC–MIPs, three cycles with alternating UV and visible light irradiations were performed (Fig. 5), and the results are presented in Fig. 6. The PC–MIPs, with and without IsoLEU, as well as the control polymers (PC–NIPs), were exposed to UV light for 2 min followed by 30 min of visible light. As it was already reported for conventional SPMA–based MIPs, when the PC–MIPs are irradiated with UV–light the SP form is converted to the MC form, further reverted upon visible light irradiation, but difficult to achieve in the solid state [19,20].

These materials revealed a high potential to be used as optical sensors with a quite fast response for the detection of the target molecule. While PC–MIPs could be reused three cycles without losing performance with good reversibility, PC–NIPs revealed slight fatigue after the second cycle. This can be attributed to the faster photoresponse of the PC–NIP, thus reaching an earlier photostationary state. When a material in solid state consistently operates near this state, it becomes more susceptible to performance degradation due to repetitive cycling. Repeated or prolonged exposure to UV light could further reduce the material's ability to respond effectively to light, as observed in other studies [9]. Nonetheless, in comparison to previous reports, our results indicate that the reversible isomerization of the SP−MC scaffold in all polymers demonstrates a considerable resistance to fatigue [5,9].

Reversible photo–switchable SP–based materials, to regulate the assembly and release of amino acid derivatives by light, have already been reported [7,21]. However, SPMA–based MIPs that explore these features using amino acids are quite nonexistent. SPMA–based MIPs, designed for separation applications in liquid solutions and using other templates, have proven to be effective for adsorption in visible light and desorption in UV–light as photo–switchable MIPs at least for three UV–VIS cycles [6,10]. Herein, we present a MIP system displaying an optical response in the presence of entrapped IsoLEU with a photo–switchable behavior above three UV–VIS cycles.

In addition, we provide a greener and non–toxic approach, compared to conventional solutions that use large amounts of organic solvents, such as chloroform, keeping the same binding properties [4,5]. Also, the imprinting factors of our PC–MIPs compare to reported values (around 1–2).

4. Conclusion

In this study, photochromic molecularly imprinted polymers (PC–MIPs) were successfully synthesized using L–isoleucine as the template, SPMA as the functional monomer and EGDMA as a crosslinker.

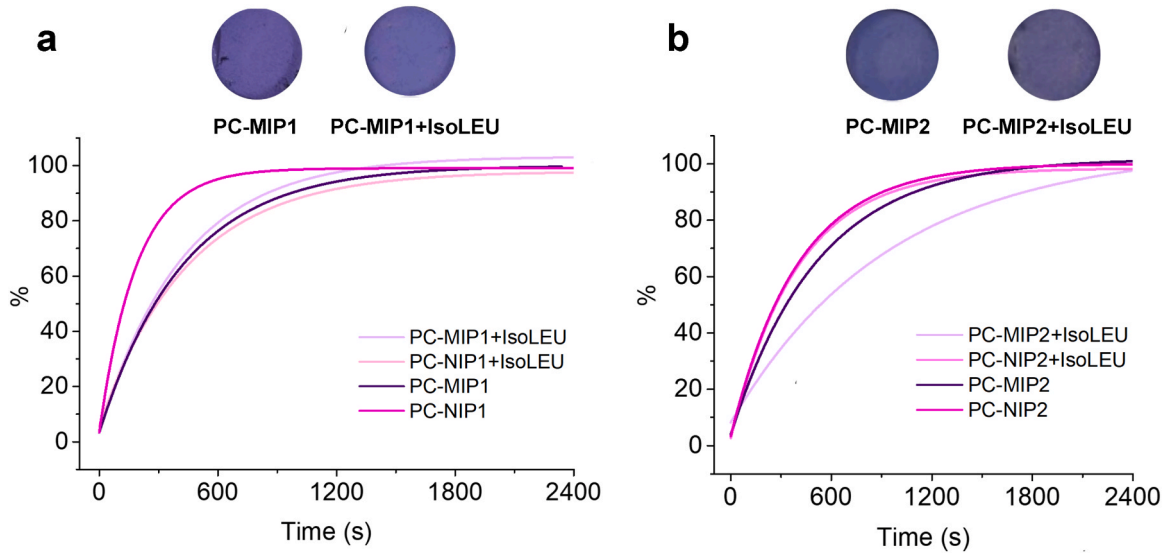


Fig. 4. Time dependence of f[R] at 590 nm with UV–light irradiation of PC–MIPs: higher crosslinking degree (a) and lower crosslinking degree (b). The f[R] data were normalized relative to the maximum f[R] obtained at 590 nm for each polymer.

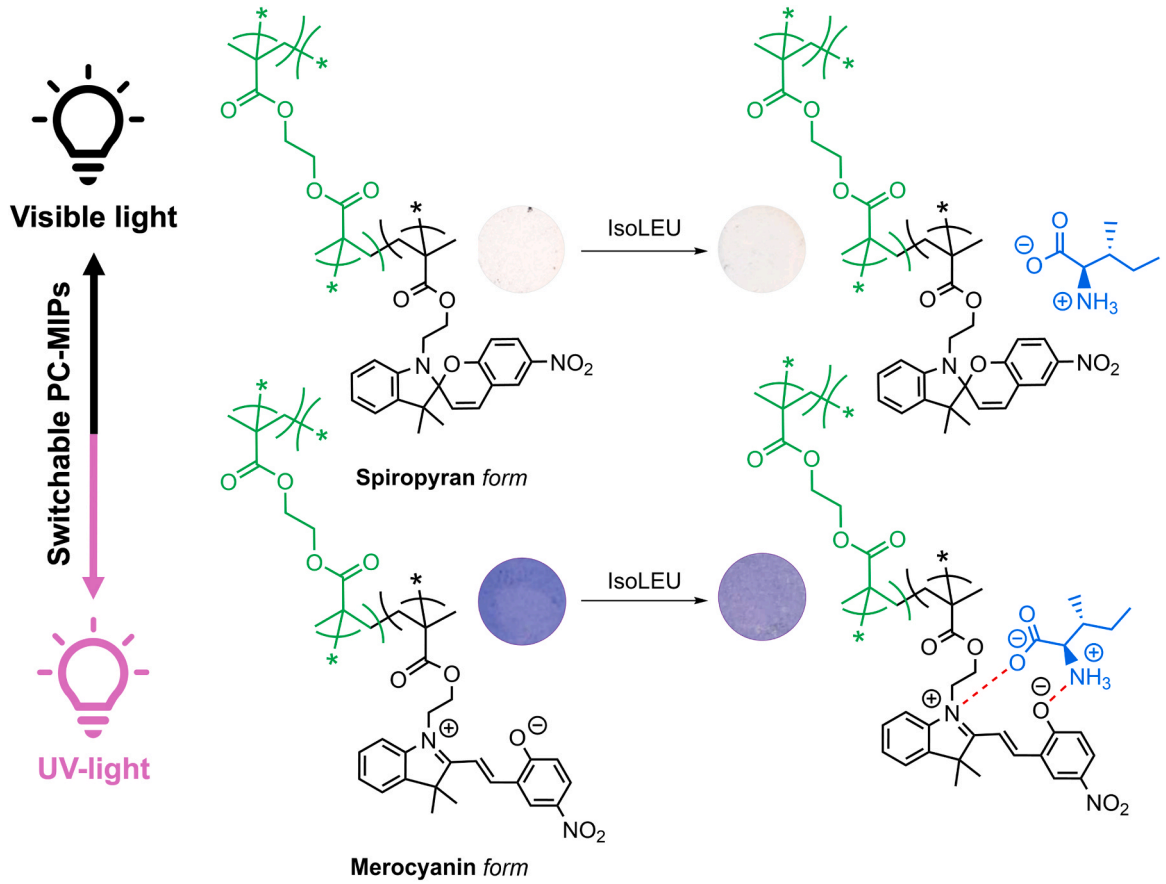


Fig. 5. Chemical structures of PC–MIPs: before (left) and after binding with the IsoLEU (right) when irradiated with visible (top) and UV–light (bottom) and respective polymer appearance after irradiation.

$ScCO_2$ technology was used for both synthesis and template desorption. Two crosslinking degrees were studied, using a monomer:crosslinker molar ratio of 1:2 (PC–MIP1) and 1:1 (PC–MIP2). The produced polymers exhibited a clear photo–response to UV–light, turning purple/blue upon irradiation. Binding tests confirmed that PC–MIPs have affinity to IsoLEU, losing this capability upon UV–irradiation, consistent with previous SPMA–based MIP studies. PC–MIP1, with a higher crosslinking degree, showed greater binding and selectivity performance compared to PC–MIP2, highlighting the importance of the crosslinking density. A maximum imprinting factor of 1.6 was achieved, with a binding capacity of 127 mg IsoLEU/g PC–MIP1. These polymers demonstrated effective sensing (selectivity and sensitivity) for IsoLEU detection, with

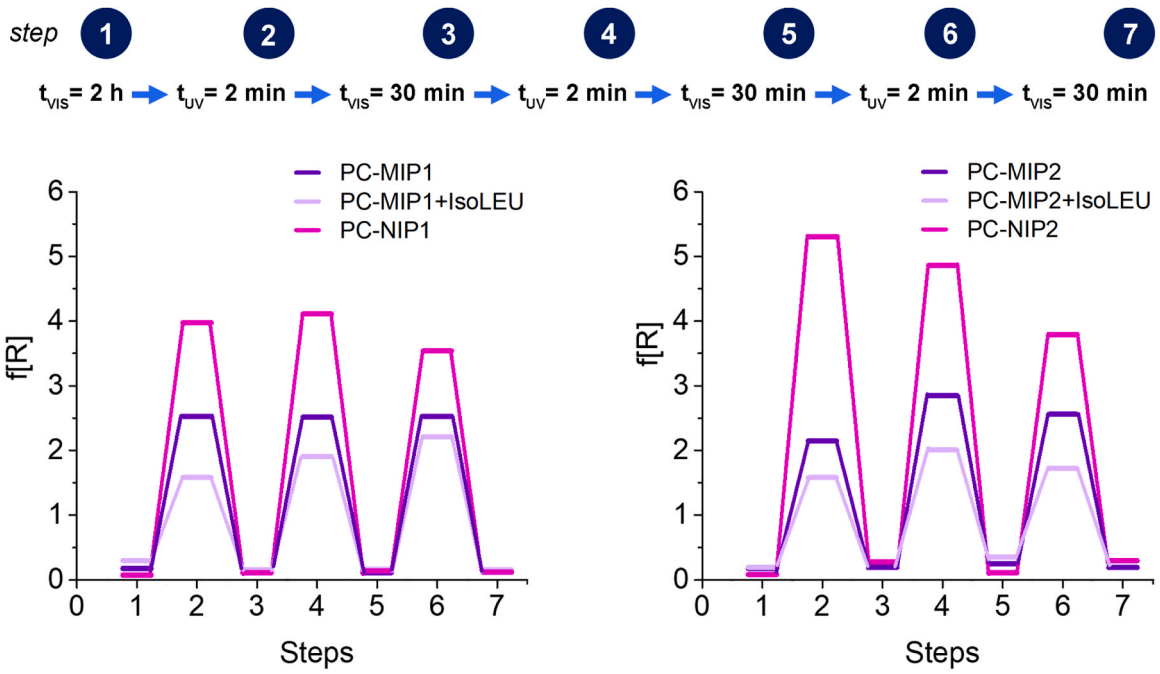


Fig. 6. Photo–response of the produced polymers before and after binding tests by several UV–VIS cycles: polymers with high crosslinking degree (a) and polymers with low crosslinking degree (b).

UV–induced color change acting as a real–time optical indicator. The reversibility of the photochromic response was proven through UV–VIS cycles, indicating potential as reusable optical biosensors. The use of $scCO_2$–assisted synthesis offers a sustainable and greener approach, producing dry, free–flowing, ready–to–use powders, thus avoiding conventional isolation and purification steps. These findings suggest that PC–MIPs are suitable for *in situ* monitoring of bioseparation processes, offering a cost–effective alternative, paving the way for future advancements in molecular recognition and biosensor technologies.

CRediT authorship contribution statement

Teresa Casimiro: Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Ana I. Furtado:** Writing – original draft, Visualization, Validation, Methodology, Investigation. **Diogo Lobato:** Writing – review & editing, Investigation. **Sandra Gago:** Writing – review & editing, Resources, Methodology, Investigation. **Vasco D. B. Bonifácio:** Writing – review & editing, Visualization, Supervision, Resources, Methodology, Funding acquisition. **Raquel Viveiros:** Writing – review & editing, Supervision, Methodology, Conceptualization.

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

The authors would like to thank financial support from Fundação para a Ciência e a Tecnologia, Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES), Portugal, through projects PTDC/EQU-EQU/32473/2017, PTDC/MEC-ONC/29327/2017. A.I.F. acknowledges a PhD grant (SFRH/BD/150696/2020) in the aim of the International Year of the Periodic Table – a Protocol established between the Portuguese Chemical Society (SPQ) and the FCT/MCTES. R.V. acknowledges an Individual Support from Scientific Employment Stimulus (CEEC-IND, 2020.00377.CEECIND) from FCT/MCTES. The Associate Laboratory Research Unit for Green Chemistry – Clean Technologies and Processes – LAQV–REQUIMTE is financed by national funds from FCT/MCTES (LA/P/0008/2020 DOI 10.54499/LA/P/0008/2020, UIDP/50006/2020 DOI 10.54499/UIDP/50006/2020 and UIDB/50006/2020 DOI 10.54499/UIDB/50006/2020).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.snb.2024.137122.

Data Availability

Data will be made available on request.

References

[1] O. Bertrand, J.F. Gohy, Photo–responsive polymers: synthesis and applications, Polym. Chem. 8 (2016) 52–73, https://doi.org/10.1039/c6py01082b.
[2] N. Fomina, J. Sankaranarayanan, A. Almutairi, Photochemical mechanisms of light–triggered release from nanocarriers, Adv. Drug Deliv. Rev. 64 (2012) 1005–1020, https://doi.org/10.1016/j.addr.2012.02.006.
[3] F.M. Raymo, S. Giordani, A.J.P. White, D.J. Williams, Digital processing with a three–state molecular switch, J. Org. Chem. 68 (2003) 4158–4169, https://doi.org/10.1021/jo0340455.
[4] N. Murase, T. Mukawa, H. Sunayama, T. Takeuchi, Molecularly imprinted polymers bearing spiropyran–based photoresponsive binding sites capable of photo–triggered switching for molecular recognition activity, J. Polym. Sci. B Polym. Phys. 54 (2016) 1637–1644, https://doi.org/10.1002/polb.24066.
[5] T. Renkecz, G. Mistlberger, M. Pawlak, V. Horváth, E. Bakker, Molecularly imprinted polymer microspheres containing photoswitchable spiropyran–based binding sites, ACS Appl. Mater. Interfaces 5 (2013) 8537–8545, https://doi.org/10.1021/am401958e.
[6] L. Angiolini, T. Benelli, L. Giorgini, F.M. Raymo, Chiroptical switching based on photoinduced proton transfer between homopolymers bearing side–chain spiropyran and azopyridine moieties, Macromol. Chem. Phys. 209 (2008) 2049–2060, https://doi.org/10.1002/macp.200800192.
[7] T. Sajini, B. Mathew, A brief overview of molecularly imprinted polymers: highlighting computational design, nano and photo–responsive imprinting, Talanta Open 4 (2021) 100072, https://doi.org/10.1016/j.talo.2021.100072.
[8] A.I. Furtado, V.D.B. Bonifácio, R. Viveiros, T. Casimiro, Design of molecularly imprinted polymers using supercritical carbon dioxide technology 29 (2024) 926, https://doi.org/10.3390/molecules29050926.

[9] M. Ghani, A. Heiskanen, J. Kajtez, B. Rezaei, N.B. Larsen, P. Thomsen, A. Kristensen, A. Žukauskas, M. Alm, J. Emnéus, On–demand reversible UV–triggered interpenetrating polymer network–based drug delivery system using the spiropyran–merocyanine hydrophobicity switch, ACS Appl. Mater. Interfaces 13 (2021) 3591–3604, https://doi.org/10.1021/acsami.0c19081.
[10] S. Friedle, S.W. Thomas, Controlling contact electrification with photochromic polymers, Angew. Chem. Int. Ed. 49 (2010) 7968–7971, https://doi.org/10.1002/anie.201003985.
[11] H. Bouzid, C.M.C. Filho, J.R.C. Marques, A.J.M. Valente, L.M. Gando–Ferreira, A new approach on the amino acid lysine quantification by UV–visible spectrophotometry, Rev. Chim. 71 (2020) 159–175, https://doi.org/10.37358/rc.20.8.8290.
[12] S. Landi, I.R. Segundo, E. Freitas, M. Vasilevskiy, J. Carneiro, C.J. Tavares, Use and misuse of the Kubelka–Munk function to obtain the band gap energy from diffuse reflectance measurements, Solid State Commun. 341 (2022) 114573, https://doi.org/10.1016/j.ssc.2021.114573.
[13] G. Marcelo, I.C. Ferreira, R. Viveiros, T. Casimiro, Development of itaconic acid–based molecular imprinted polymers using supercritical fluid technology for pH–triggered drug delivery, Int. J. Pharm. 542 (2018) 125–131, https://doi.org/10.1016/j.ijpharm.2018.03.010.
[14] A.I. Furtado, R. Viveiros, V.D.B. Bonifácio, A. Melo, T. Casimiro, Biomolecular fishing: design, green synthesis, and performance of l–leucine–molecularly imprinted polymers, ACS Omega 8 (2023) 9179–9186, https://doi.org/10.1021/acsomega.2c05714.
[15] K. Golker, I.A. Nicholls, The effect of crosslinking density on molecularly imprinted polymer morphology and recognition, Eur. Polym. J. 75 (2016) 423–430, https://doi.org/10.1016/j.europolymj.2016.01.008.
[16] K.Fremielle Lim, C.I. Holdsworth, Effect of formulation on the binding efficiency and selectivity of precipitation molecularly imprinted polymers, Molecules 23 (2018) 2996, https://doi.org/10.3390/molecules23112996.
[17] A. Mueller, A note about crosslinking density in imprinting polymerization, Molecules 26 (2021) 5139, https://doi.org/10.3390/molecules26175139.
[18] Y. Liu, M. Fan, S. Zhang, X. Sheng, J. Yao, Basic amino acid induced isomerization of a spiropyran: towards visual recognition of basic amino acids in water, N. J. Chem. 31 (2007) 1878–1881, https://doi.org/10.1039/b713247f.
[19] Y. Yang, J. He, Z. He, G. Jiang, Enhancement of solid–state reversible photochromism by incorporation of rigid steric hindrance groups, Adv. Opt. Mater. 9 (2021) 2001584, https://doi.org/10.1002/adom.202001584.
[20] L. Kortekaas, W.R. Browne, The evolution of spiropyran: fundamentals and progress of an extraordinarily versatile photochrome, Chem. Soc. Rev. 48 (2019) 3406–3424, https://doi.org/10.1039/c9cs00203k.
[21] B.I. Ipe, S. Mahima, K.G. Thomas, Light–induced modulation of self–assembly on spiropyran–capped gold nanoparticles: a potential system for the controlled release of amino acid derivatives, J. Am. Chem. Soc. 125 (2003) 7174–7175, https://doi.org/10.1021/ja0341182.

Ana I. Furtado: Ana I. Furtado is currently a Ph.D. candidate in Sustainable Chemistry at NOVA School of Science & Technology (NOVA SST) from NOVA University of Lisbon (Portugal), in the aim of The Emerging Periodic Table from the International Year of the Periodic Table – Protocol Established between the Portuguese Chemical Society (SPQ) and the Portuguese Foundation for Science and Technology (FCT–MCTES). She is developing her PhD work in collaboration between CleanMIPTech group from NOVA SST and Institute for Bioengineering and Biosciences (iBB), Institute for Health and Bioeconomy (i4HB) from Instituto Superior Técnico – University of Lisbon (IST/UL). She received a Master's degree in Chemical Engineering (2019) from NOVA SST. At this moment, she has 3 publications, including 1 review and 1 book chapter. Her research focuses on green chemistry, supercritical fluid technology, mechanochemistry, dendrimers, rational design, molecular recognition, molecular imprinting, polymer synthesis & processing, and biopurification processes.

Diogo Lobato: Diogo Lobato received a Master's degree in the Department of Chemical and Biochemical Engineering from NOVA School of Science & Technology (NOVA SST) from NOVA University of Lisbon (Portugal) in 2022. Since 2023, He is a Settlement Technician in Portuguese Transport System Operator (TSO), REN. His work is focused on optimization and developing new procedures for natural gas liquified materials in the Market Operations area.

Sandra Gago: S. Gago received her PhD degree in Chemistry in 2006 from University of Aveiro, (CICECO). Since 2010, she has been an assistant researcher at the Associated Laboratory for Green Chemistry (LAQV) at NOVA School of Science & Technology (NOVA SST) from NOVA University of Lisbon (Portugal). She published more than 70 papers in international journals. Her research interests comprise the design, characterization and modification of new functional materials based on mesoporous silica and magnetic nanoparticles, inorganic/organometallic complexes, ionic and photoresponsive molecules for several applications: antimicrobial systems or drug delivery, nano electrochromic/photochromic devices, oxidative catalysis or photo/electrocatalysis for hydrogen production.

Vasco Bonifácio: Vasco D. B. Bonifácio is currently Professor of Biomaterials at IST–UNL (Lisbon, PT). He has a PhD in Organic Chemistry by NOVA SST (Lisbon, PT) and made his Post–doc in Molecular Electronics at Institüt fur Polymertechnologie (Wuppertal, DE). He was a Visiting Scientist at MIT (Boston, USA) and U. Bordeaux (Bordeaux, FR), and a Visiting Professor at Faculdad de Medicina, U. Castilla–La Mancha (SP) and Fraunhofer Institute for Silicate Research (DE). His main research interests are related with sustainable synthesis of dendrimer therapeutics, using supercritical carbon dioxide and mechanosynthesis, for regenerative medicine and the treatment of cancer and MDR microbial infections.

Raquel Viveiros: Raquel Viveiros is currently a Researcher at LAQV–REQUIMTE|NOVA School of Science & Technology (NOVA SST) from NOVA University of Lisbon (Portugal). She received a PhD degree in Sustainable Chemistry (2017), under a close collaboration between NOVA SST and a pharmaceutical company, HOVIONE; and received a Masterś degree in Chemical and Biochemical Engineering at NOVA SST (2010). Her research focuses on green chemistry, clean technologies, organic/analytical chemistry, molecular recognition, molecular imprinting, polymer synthesis and processing, process development, catalysis and (bio)purification processes. She has a total of 27 publications (Scopus, *h*–index 15), including 2 reviews and 1 book chapter.

Teresa Casimiro: Teresa Casimiro is Professor at the NOVA School of Science & Technology (NOVA SST) from NOVA University of Lisbon (Portugal), in the area of Chemical and Biological Engineering, and Principal Investigator at LAQV–REQUIMTE (https://laqv.requimte.pt/people/978-teresa_casimiro). She is leader of the CleanMIPTech Group at the Green Polymer Synthesis & Processing Laboratory at LAQV–REQUIMTE, Chemistry Department at NOVA SST. T. Casimiro is co–author of over 90 scientific papers, with *h*–index: 29 (WoS). Her research is focused on the synthesis and processing of polymers using green technologies, in particular, supercritical fluid technology, molecular imprinting and plasma technology. The combination of these technologies has led to the development of new affinity polymers – Molecularly Imprinted Polymers (MIPs) – for different applications (https://sites.fct.unl.pt/clean-mip-tech/).