MOLECULARLY IMPRINTED MEMBRANES OBTAINED VIA WET PHASE INVERSION FOR EPHEDRINE RETENTION

Elena-Bianca (GEORGESCU) STOICA[1], Ana-Mihaela (FLOREA) GAVRILA[2], Tanta-Verona IORDACHE[3], Andrei SARBU[4]*, Horia IOVU[5]*, Teodor SANDU[6], Hugues BRISSET[7]

The main advantages of using molecularly imprinted polymer (MIP)sensitive layers for drug retention refer to the versatility and cost – effectiveness ratio but especially to the speed of adsorption. Besides the low cost, the sensitive layer will be endowed with: good resistance over time due to the use of synthetic polymer, permanent monitoring and reuse. The preparation of MIP was carried out by wet phase inversion. In this way, thin membranes were obtained by water immersion of the support with the MIP precursors or control solutions, noted non-imprinted polymers (NIP). The ephedrine was used as template in the imprinting process. The obtained membranes were analyzed both before and after removal of the template molecule, using specific characterization techniques.

Keywords: wet phase inversion, MIP membranes, ephedrine retention.

1. Introduction

The molecular imprinting technique is used to synthesize a wide range of polymers with molecular memory, which implies the design of recognition sites for various target molecules and, thus, generating the so called molecularly imprinted polymers (MIPs) [1,2]. Using this technique, a crosslinked polymer matrix is created in the presence of a template molecule [3-5]. After template

[1] PhD.Eng, Dept.of Polymer Science, University POLITEHNICA of Bucharest, Romania, National Institute for Research & Development in Chemistry and Petrochemistry ICECHIM-Romania, Universite de Toulon, France, e-mail: georgescu_elena_bianca@yahoo.com
[2] Eng., National Institute for Research & Development in Chemistry and Petrochemistry ICECHIM - Romania, e-mail: anamihaela.florea@gmail.com
[3] Eng., National Institute for Research & Development in Chemistry and Petrochemistry ICECHIM - Romania, e-mail: iordachev.icechim@gmail.com
[4] Eng., National Institute for Research & Development in Chemistry and Petrochemistry ICECHIM - Romania, e-mail: andr.sarbu@gmail.com
[5] Prof., University POLITEHNICA of Bucharest, Romania, e-mail: horia.iovu@upb.ro
[6] Eng., National Institute for Research & Development in Chemistry and Petrochemistry ICECHIM - Romania, sanduteodor85@gmail.com
[7] Prof., Universite de Toulon, France, e-mail: huguess.brisset@univ-tln.fr
* Corresponding authors: andr.sarbu@gmail.com and horia.iovu@upb.ro

removal, recognition cavities with complementary cavities in size, shape and functionality with the template are generated. Hence, the polymers prepared this way are able to recognize and rebind the same molecule used as template and also structurally related molecules. Due to their advantages, such as affinity, selectivity, low cost, stability and simple preparation, the MIPs have found use in several analytical applications, as membranes or films for sensors and as adsorbents for separations and purification procedures [6-12]. In this study the targeted analyte was ephedrine, a sympathomimetic substance that can be found in plants (Ephedra genus). It is usually used as an anesthetic or in treating conditions such as high blood pressure, asthma or nasal decongestion. The major problems of this drug refer to fact that it is a precursor of methamphetamine, a very high-risk drug [13-15], which is also used as doping agent by athletes.

In general, a membrane or a film is defined as the separation area between two phases, a selective barrier [16]. A MIP membrane or film can be used to separate the target molecule from a complex solution, *via* permeation processes or in batch mode [17-19]. Due to enhanced selectivity they are more efficient compared to classical membranes or films, leading to several applications in sensors or food industry and wastewater purification [20-23]. Membranes or films can be obtained using various methods, e.g. phase inversion, sol-gel, stretching or interface reaction. Yet, the most preferred method is the phase inversion process [24]. Phase inversion involves the transformation of a polymer from the liquid phase in the solid phase and this process can be carried out using two routes, *wet* or *dry*. In the *wet* phase inversion approach the membrane formation is achieved by coagulation and refers to casting the polymer solution on the support followed by immersion into a non-solvent of the polymer. The *dry* phase inversion method assumes the film formation by solvent evaporation, after the polymer solution has been casted on the support [25, 26].

In the context of increased illicit trafficking and consumption of drugs, the purpose of this paper was to obtain molecularly imprinted thin membranes for ephedrine retention *via* the wet phase inversion technique with application in sensors for illicit drugs detection. The membranes were tested for specific recognition of the template, in order to determine their potential in bio mimetic sensors development.

2. Materials and methods

2.1 Materials

Acrylonitrile (AN, 98%) was purchased from ACROS Organics and methacrylic acid (MA, 99%) was purchased from Fluka. The copolymers, noted C3 (with content of 80% AN and 20% AM, wt.%) and C4 (with content of 75% AN and 25% AM, wt.%) were obtained using the recipes described in a previous paper [27]. Dimethylformamide (DMF, analytical grade) was purchased from

Sigma Aldrich and used as received and ethanol, 99.5%, was purchased from ChimReactiv. The template, ephedrine hydrochloride 50 mg/mL was purchased from Zentiva and used as received.

2.2 Instruments and methods

The copolymer precursor solutions were rheologically characterized using Rheotest 2.1 (VEB Medingen) viscometer with coaxial cylinders, at room temperature, aiming to see the influence of the shear gradient upon the dynamic viscosity.

The structure of obtained MIP and NIP membranes was analyzed using Nicolet iS50 FT-IR device with ATR - SMART iTR (Termo SCIENTIFIC). FT-IR spectra were collected using the ATR mode, in the 4000-500 cm^{-1} region, after the membranes were dried and milled. The spectra of MIPs were recorded after template extraction.

The thermal analysis of MIP and NIP membranes was carried using SDT Q5000 IR Thermo-Gravimetric Device (TA Instrument). TGA and DTA diagrams were recorded under nitrogen atmosphere in a temperature range of 25-700°C, with a heating rate of 10°C/min.

The rebinding experiments of ephedrine were carried out using UV-vis 500 Thermo Nicolet Evolution Instrument (Termo SCIENTIFIC) using the methodology described in Section 2.4.

2.3 Preparation of MIP and NIP thin membranes

2.3.1. Copolymer synthesis

For the preparation of MIP membranes *via* wet phase inversion, acrylonitrile and methacrylic acid copolymers were used. This system generates soluble copolymers in DMF, which allows for the preparation of copolymer precursor solutions able to generate thin MIPs or NIPs membranes. It can be noted that DMF is also a very good solvent for ephedrine. Acrylonitrile (AN) was used as structural monomer, due to CN polar groups that renders stability to the imprinted cavities *via* strong hydrogen bonding. Methacrylic acid (AM) was chosen as functional monomer for its ability to bind polar molecules (such as ephedrine). The synthesis of acrylonitrile - methacrylic acid copolymers was carried out by emulsion polymerization without emulsifier, initiated by the redox system (potassium persulfate – PK and sodium metabisulfite – MS) at acid pH [27]. Using this method, two copolymers with different compositions were prepared, noted C3 (80-20 AN-AM, wt.%) and C4 (75-25 AN- AM, wt.%).

2.3.2. Preparation of precursor copolymer solutions and membranes

Two identical solutions of C3 and two of C4 were prepared by dissolving 4 g of C3 or C4 in 42 mL of DMF. Ephedrine hydrochloride (0.3 ml) was added under heating (80°C) and mechanical stirring (300 rot/min) in only two of the

copolymer solutions to prepare the C3-MIP and C4-MIP membranes (see Fig.1 a and b). The other two solutions of copolymers derived from C3 and C4 were used to prepare control membranes, noted C3-NIP and C4-NIP4, respectively.

The membranes were prepared (according to **Fig.1** c-e) by casting the solution onto a glass support and spreading with a knife (casting knife – with a gap of 200 μm). Immediately, the plates were immersed in a phase inversion bath, consisting of distilled water. The phase inversion occurred at room temperature. For stabilization, the membranes were maintained in the coagulation bath for 24 hours. In order to perform the thermal and structural analysis (TGA and FT-IR) the membranes were dried for 48 hours at 60 ºC in the oven and, then, milled in order to obtain a homogenous powder. For the rebinding studies, the membranes were used in their wet form to prevent shrinkage of the imprinted cavities.

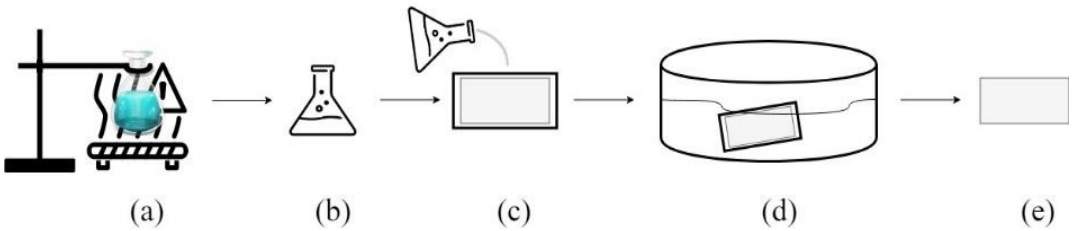


Fig.1 Preparation of MIP/NIP thin membranes: (a) C3 or C4 dissolution, (b) copolymer precursor solution, (c) casting precursor solution on glass supports and spreading with a casting knife, (d) immersion in distilled water, (e) MIP/NIP thin membrane

2.4 Rebinding of ephedrine in batch mode

For determining the biding capacity and the imprinting effect of ephedrine, MIP and NIP membranes were washed with ethanol several times (each membrane 6 times with 35 mL ethanol for extracting the template and for removing the traces of DMF). After washing, the membranes were contacted with an aqueous solution of 2.74 mmol/L ephedrine. Before starting the experiments, the measurements were calibrated using several ephedrine solutions of different concentration (in the range of 5.48 mmol/L-0.137 mmol/L), in order to obtain the calibration curve. The method used for determining the rebinding capacity is based on measuring the initial concentration of ephedrine solution (before contact with the membranes) and the final concentration of ephedrine solution (after contact with the membranes) using UV-VIS Spectrophotometry. The equations used to calculate the binding capacity, *Q*, and the imprinting factor, *IF* are presented in eq. 1 and eq.2, respectively.

$$Q = \frac{(c_{N,i} - c_{N,f}) \cdot V_s}{m_p} \text{(eq.1)}$$

where:

- Q is the binding capacity of membrane, (g of ephedrine/ g of membrane);
- $C_{N,i}$ (0.55 g/L) and $C_{N,f}$ (g/L) are the initial and final concentrations of ephedrine in solutions;
- V_s (0.003 L) is the initial volume of the solution;
- m_p (0.01 g) is the quantity of the polymer membrane.

$$IF = \frac{Q_{MIP}}{Q_{NIP}} \text{(eq.2)}$$

where:

- IF is the imprinting factor, which quantifies the specificity of adsorption;
- Q_{MIP} (g ephedrine/g of MIP) is the adsorption capacity of MIP;
- Q_{NIP} (g ephedrine/g of NIP) is the adsorption capacity of NIP.

The calibration curve is required to calculate the concentration of ephedrine before and after contact with the membrane. In this case, the final concentration of the solution is calculated using eq.3, where A is the absorbance at the maximum wavelength, λ_{max}=256 nm.

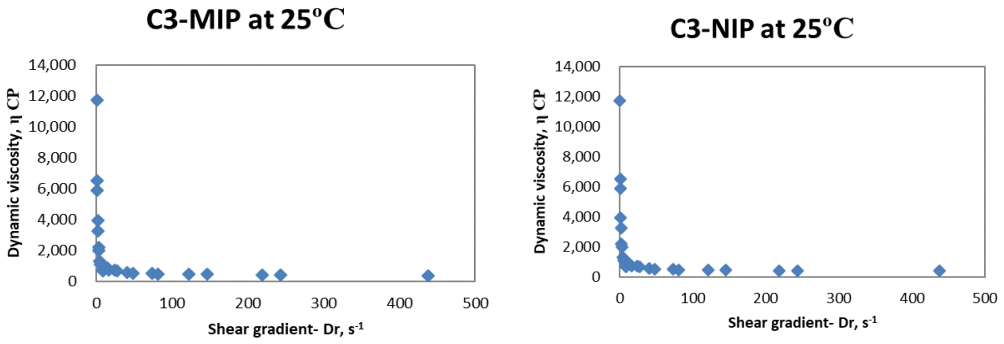
$$C_{N,f} = \frac{A - 0.192}{116.35} \text{(eq.3)}$$

3. Results and discussions

3.1. Rheological characterization

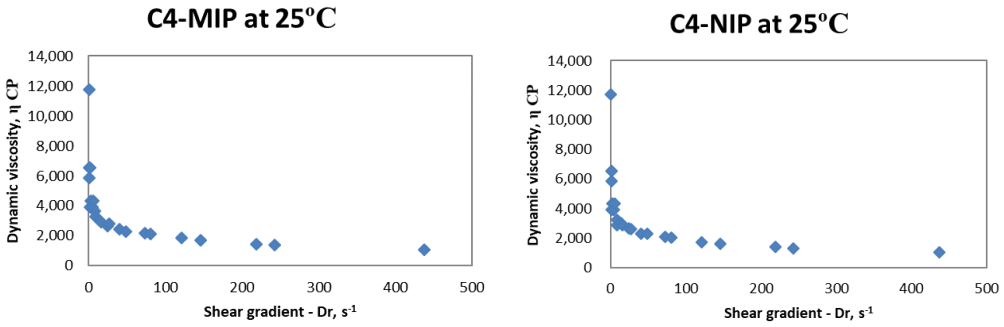
The rheological behavior of the precursor solutions for C3-MIP/C3-NIP and C4-MIP/C4-NIP pairs is presented in Figs. 2 and 3, respectively.

As one can see that the precursor solutions with ephedrine, C3-MIP and C4-MIP, (Fig.2-a and Fig.3-a) presented a similar rheological behavior to that of solutions without the template, i.e. C3-NIP and C4-NIP (Fig.2-b and Fig.3-b), meaning a pseudoplastic behavior at low shear gradient with passage in quasi-Newtonian behavior at higher shear gradients. However, it should be mentioned that the solutions with C3 copolymer are quasi-Newtonian at lower shear rates, starting from around 10 s^{-1}, unlike the ones with C4 where this behavior is observed at share rates above 100 s^{-1}. Also, it appears that the template does not have a significant effect on the dynamic viscosity of the solutions when compared to the NIPs, in same copolymer series. This difference of behavior may be the result of copolymers compositions. C4 contains more MA and, hence, more intermolecular hydrogen bonds that opposes to the share rate.



a) b)

Fig.2 Rheological behavior of the precursor solution at 25°C used to prepare the following membrane set: a) C3-MIP and b) C3-NIP



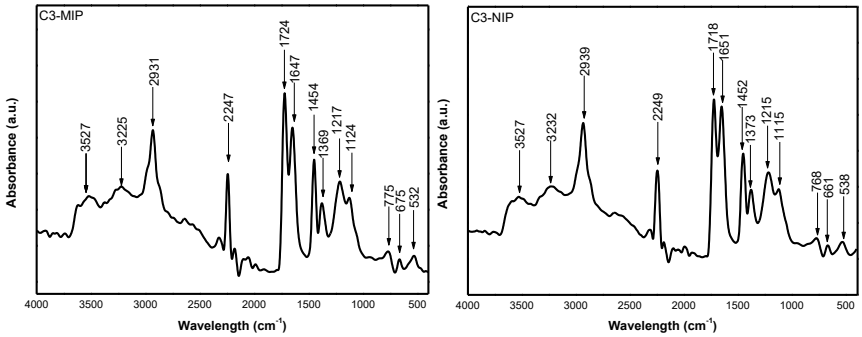
a) b)

Fig.3 Rheological behavior of the precursor solution at 25°C used to prepare the following membrane set: a) C4-MIP and b) C4-NIP

3.2. Structural analysis of the membranes

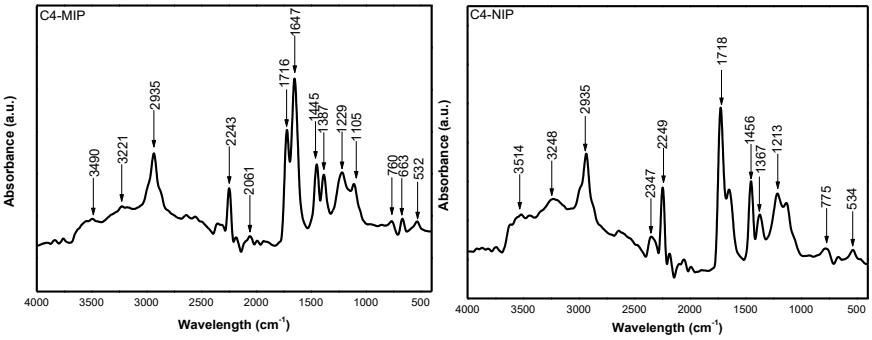
The FT-IR spectra of C3-MIP and C4-MIP membrane series are presented in Fig.4 and Fig.5, respectively, and compared to the corresponding control sample C3-NIP or C4-NIP. Both imprinted and non-imprinted membrane series, presented several commune characteristic bands, including the band from 2243-2249 cm^{-1} assigned to CN from acrylonitrile units and the group of bands from 1716-1724 cm^{-1} and 1450 cm^{-1} assigned to carboxylic groups and CH_2 groups, respectively, from methacrylic acid units in the copolymer. The spectra of the MIPs (after template extraction) and of the NIPs presented similar profiles with no additional bands of ephedrine, which proves that extraction of the template was quantitatively. Ephedrine presents two very sharp bands in the IR spectra at 750 and 700 cm^{-1} attributed to the aromatic ring [28]. In the spectrum of C4-MIP (Fig. 5a) important changes of intensities and shifts were observed for several

characteristic bands, compared to the control sample C4-NIP (Fig. 5b) and also to the C3-MIP/NIP pair. For instance, the bands registered for C4-NIP at 1213 & 1367 cm^{-1} and 1456 cm^{-1} characteristic for the symmetric and asymmetric stretching vibrations of C-O bond (from O=C-O) and δ_{CH} (-CH2-C=O), respectively, shifted toward 1229 & 1387 $cm^{-1}$and 1445 cm^{-1}. The significant shifts can be attributed to the insufficient removal of ephedrine from the C4-MIP as a result to stronger copolymer-template interactions, which may occur during the imprinting stage due to more methacrylate units present in the C4 copolymer matrix. Although the ephedrine was extracted almost completely, the traces of ephedrine can leach-out in time and lead to false-positive responses. Therefore, it can be noted that C3-MIP was easier to wash and may be reconditioned with minimum of effort if necessary.



a) b)

Fig.4 FT-IR spectra of molecularly imprinted and non-imprinted membranes derived from C3 precursor solution, a) C3-MIP and b) C3-NIP



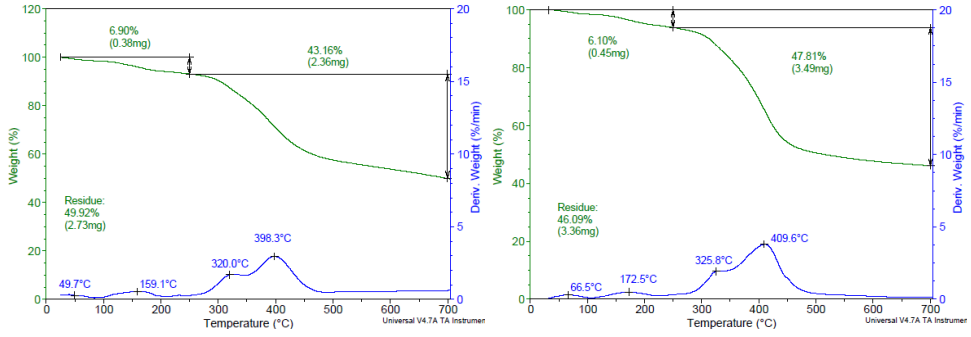
a) b)

Fig.5 FT-IR spectra of molecularly imprinted and non-imprinted membranes derived from C4 precursor solution, a) C4-MIP and b) C4-NIP

3.3. Thermogravimetric analysis

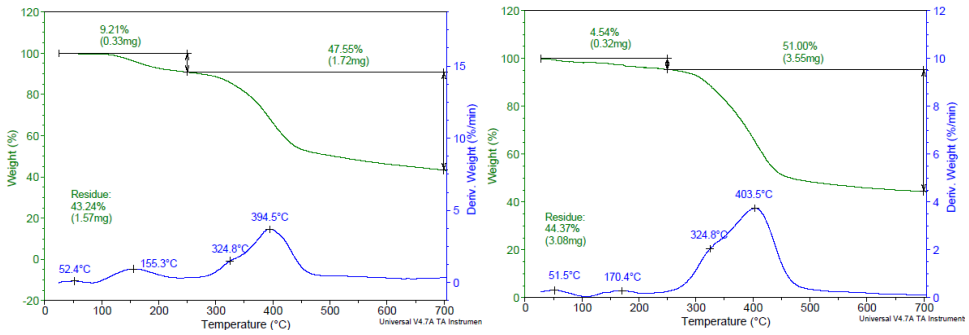
Further on, in Fig.6 a, b and Fig.7 a, b, the TGA and DTA, decomposition profiles of the control membranes and MIP membranes before ephedrine extraction are presented for both series for copolymers.

For all membranes, C3-MIP/C3-NIP and C4-MIP/C4-NIP, the temperature range for water loss was 25-105 °C and for DMF loss was 105-190 °C. In contrast to other studies [29], where similar copolymer compositions were used to prepare pearls, the cyclization step at about 253 °C was not present in the membranes. As expected, the copolymer chain decomposes in several steps with a relative homogeneous degradation. The last decomposition step, for all the MIP (Fig.6b – Fig.7b) and NIP (Fig.6a – Fig.7a) membranes, showed maximum decomposition rates above 398 °C. Yet, it can be mentioned that for the MIPs, this peak was shifted to higher values, which may be due to the hydrogen bonds established between the polymer and the template. The most stabile membrane was however C3-MIP, attaining at peak a temperature of 409.6 °C. This last step was in all cases accompanied by a shoulder around 320-325 °C, which was more pronounced for the C3-MIP/C3-NIP pair, and the residue amount for C3-MIP/C3-NIP pair was also higher relative the one registered for C4-MIP/C4-NIP. Therefore, it can be assumed that the shoulder represented in fact the cyclization of CN groups but shifted towards higher temperature values (according to previously studies reported by this same group [30] and that ephedrine leads to higher stability and more ash formation, particularly for C3-MIP/C3-NIP membranes.



a) b)

Fig.6 TGA/DTA diagrams of molecularly imprinted and non-imprinted membranes derived from C3 precursor solutions: a) C3-NIP and b) C3-MIP before extraction



a) b)

Fig.7 TGA/DTA diagrams of molecularly imprinted and non-imprinted membranes derived from C4 precursor solutions: a) C4-NIP and b) C4-MIP before extraction

3.4. Batch Rebinding experiments

Template re-binding capacity is one of the most important properties of molecularly imprinted polymers. It shows the efficiency of recognition sites formed in the molecular imprinting process. In fig. 8, the binding capacities for each pair, C3-MIP/C3-NIP or C4-MIP/C4-NIP, after contact with the ephedrine solution (at 30, 120 and 180 min) are presented. As can be seen from fig.8, the binding capacities of MIPs are higher than those of NIPs, especially in the first 120 minutes, indicating a good specificity towards ephedrine compared to the control.

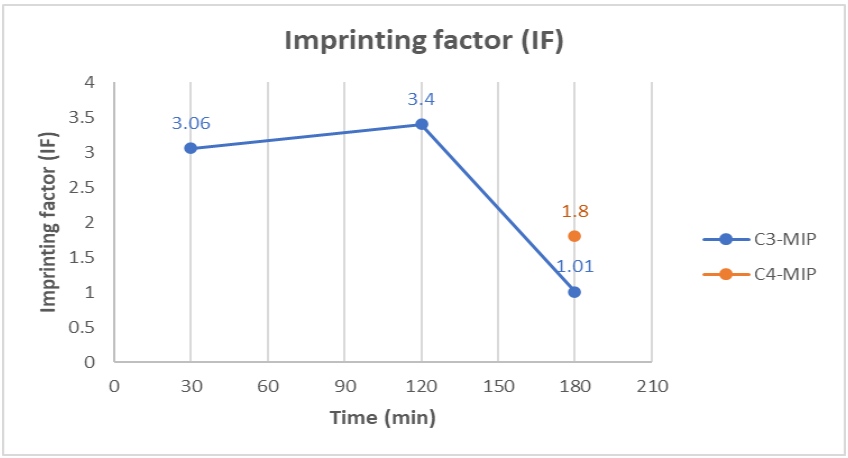


Fig.8 Re-biding capacities for C3-MIP/C3-NIP and C4-MIP/C4-NIP pairs towards ephedrine

For the C3-MIP/C3-NIP pair, the binding capacities were evaluated after 30, 120, and 180 min, while for the C4-MIP/C4-NIP pair just after 180 min, to provide an explanation why the study was further on focused on the C3-MIP/C3-NIP pair. At 180 minutes the adsorption capacity of C3-MIP was around 122 mg ephedrine/g polymer while for C4-MIP was around 70 mg of ephedrine/g polymer. The

superior specificity of C3-MIP over that of C4-MIP was also confirmed by the imprinting factors calculated in time (as presented in Fig..9). From the calculations of IF, resulted that the C3-MIP can recognize and rebind faster the template, after 180 min, compared to C4-MIP, which is more sluggish. Interestingly, C3-MIP also presented a very high specificity, maximum *IF*=3.1, towards ephedrine at only 30 min. This result is very important especially for sensors applications where the response time is crucial.

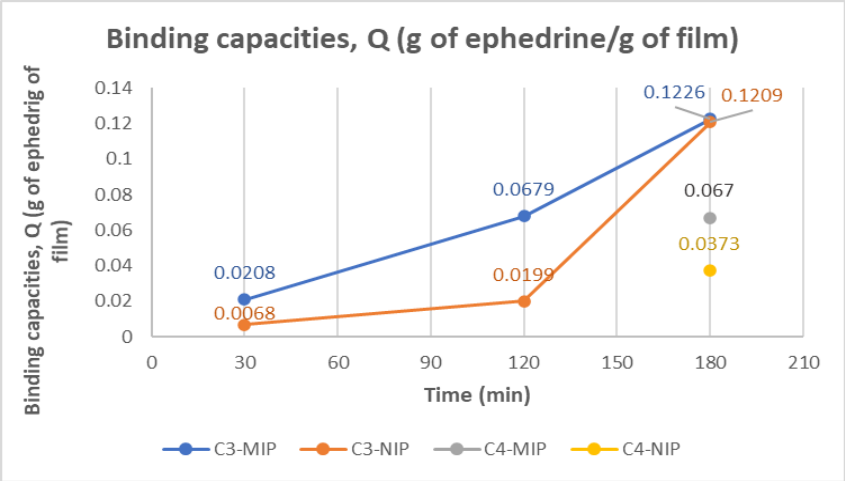


Fig.9 The imprinting factors for C3-MIP and C4-MIP membranes for ephedrine

4. Conclusions

In the present paper, MIP membranes for ephedrine specific retention were successfully prepared *via* the wet phase inversion method, in order to probe their possible application in sensors development. Rheological characterization proved that it was more likely to have variations of viscosity due to the composition of copolymers rather than the presence of the template. The presence of characteristic bands in the copolymers and the absence of ephedrine in the MIPs, after template extraction, was confirmed by FTIR. TGA analysis revealed higher amounts of residue for the C3-MIP/C3-NIP membranes and higher thermal stability. Finally, the rebinding experiments proved that specific binding sites for ephedrine are formed in the imprinting process. Although the rebinding capacities and the imprinting factors indicated that both MIP membrane types were able to recognize and rebind the template, overall, C3-MIP demonstrated better thermal stability, higher adsorption capacities and faster binding of ephedrine than C4-MIP, which makes it a more suitable sensitive element in biosensors development.

Acknowledgements

The authors thank for the help and support of the Romanian Ministry of Research and Innovation (MCI) through Project NUCLEU PN.19.23.02.01. and the Executive Unit for Financing Higher Education, Research, Development and Innovation (UEFISCDI) through Project no. TE123/2018 "BACTERIOSENS".

R E F E R E N C E S

[1]. M. Yoshikawa, "Molecularly imprinted polymeric membranes'' in Bioseparation, **vol. 10**, no.6, Nov. 2001, pp. 277–286.

[2]. R. Zang, T. Zhang, Y. Lv, P. Qin, H. Li, J-p Li, T. Tan, "Selective binding of heparin oligosaccharides in a magnetic thermoresponsive molecularly imprinted polymer'', **vol. 201**, April 2019, pp.441-449.

[3]. J. Wackerlig, R. Schirhagl, "Applications of molecularly imprinted polymer nanoparticles and their advances toward industrial use: a review'', in Anal. Chem. **vol. 88**, Jan. 2016, pp. 250–261.

[4]. Z. Iskierko, P.S. Sharma, K. Bartold, A. Pietrzyk-Le, K. Noworyta, W. Kutner, "Molecularly imprinted polymers for separating and sensing of macromolecular compounds and microorganisms'', in Biotechnol. Adv., **vol. 34**, Jan 2016, pp. 30–46.

[5]. K. Haupt, K. Mosbach, "Molecularly imprinted polymers and their use in biomimetic sensors'', in Chem. Rev., **vol. 100**, June 2000, pp. 2495-2504.

[6]. S.A. Piletsky, A.P.F. Turner, "Electrochemical sensors based on molecularly imprinted polymers'', in Electroanalysis, **vol.14,** no.5**,** March2002, pp. 317-323.

[7]. E.L. Holthoff, F.V. Bright, "Molecularly templated materials in chemical sensing'', in Anal. Chim. Acta, **vol. 594**, May 2007, pp. 147-161.

[8]. E.L. Holthoff, F.V. Bright, "Molecularly imprinted xerogels as platforms for sensing'', in Acc. Chem. Res., **vol.40**, June 2007, pp. 756-767.

[9]. A.L. Hillberg, K.R. Brain, C.J. Allender, "Molecular imprinted polymer sensors: implications for therapeutics'', in Adv. Drug. Deliv. Rev., **vol. 57**, Dec. 2005, pp.1875-1889.

[10]. L. Ye, K., "Mosbach, Molecular imprinting: synthetic materials as substitutes for biological antibodies and receptors'', in Chem. Mater., **vol. 20**, Jan. 2008, pp. 859-868.

[11]. R.J. Ansell, "Molecularly imprinted polymers for the enantioseparation of chiral drug'', in Adv. Drug Deliv. Rev., **vol.57**, Dec. 2005, pp.1809–1835.

[12]. S.A. Piletsky, N.W. Turner, P. Laitenberger, "Molecularly imprinted polymers in clinicaldiagnostics—Future potential and existing problems'', in Med. Eng. Phy., **vol.26**, Dec.2006, pp. 971–977.

[13]. R. Andraws, P. Chawla, D.L. Brown, "Cardiovascular effects of ephedra alkaloids: a comprehensive review'', in Prog. Cardiovas. Dis., **vol. 47**, Feb. 2005, pp. 217–225.

[14]. S.Tulgar, E.A. Alasehir, O. Selvi, "The antimicrobial activity of ephedrine and admixture of ephedrine and propofol: an in vitro study'', in Revista Brasileira de anestesiologia, **vol. 68**, June 2018, pp. 69-74.

[15]. R.F. Ludlow, S. Otto, "Two-vial, LC-MS identification of ephedrine receptors from a solution-phase dynamic combinatorial library of over 9000 components'', Journal of the American Chemical Society, **vol. 130**, Aug. 2008, pp.12218–12219.

[16]. C. Algieri, E. Drioli, L. Guzzo, L. Donato, "Bio-mimetic sensors based on molecularly imprinted polymers'', in Sensors, **vol. 14**, July 2014, pp. 13863-13912.

[17]. L. Donato, A. Figoli, E. Drioli,"Novel composite poly(4vinylpiridine)/polypropylene membranes with recognition properties for (S)-naproxen'', in J. Pharmaceut. Biomed. Anal., **vol. 37**, Apr. 2005, pp. 1003–1008.

[18]. R. Suedee, N. Bodhibukkana, N. Tangthong, C. Amnuaikit, S. Kaewnopparat, T. Srichana, "Development of a reservoir-type transdermal enantioselective controlled delivery system forracemic propanolol using a molecularly imprinted polymer composite membrane'', in J. Control.Release, **vol. 129**, Aug. 2008, pp. 170–178.

[19]. J.-Y. Wang, F. Liu, Z.-L Xu, K. Li, "Theophylline molecular imprint composite membranesprepared from poly(vinylidene fluoride) (PVDF) substrate'', in Chem. Eng. Sci., vol. 65,May 2010, pp. 3322–3330.

[20]. G.P. González, P.F. Hernando, J.S.D Alegría, "An optical sensor for the determination ofdigoxin in serum samples based on a molecularly imprinted polymer membrane'', in Anal. Chim. Acta, **vol. 638**, Apr. 2009, pp. 209–212.

[21]. L. Donato, F. Tasselli, E. Drioli, "Molecularly imprinted membranes with affinity propertiesfor folic acid'', in Separ. Sci. Technol., **vol. 45**, June 2010, pp. 2273–2279.

[22]. F. Trotta, E. Drioli, C. Baggiani, D. Lacopo, "Molecular imprinted polymeric membrane for naringin recognition'', in J. Membrane Sci., **vol. 201**, 2002, pp.77–84.

[23]. D.Y. Hou, J. Wang, D. Qu, Z.K Luan, C.W. Zhao, X.J. Ren, "Desalination of brackish groundwater by direct contact membrane distillation'', in Water Sci. Technol., **vol. 61**, Apr. 2010, pp. 2013–2020.

[24]. M. Mulder, Membrane Processes in Basic Principles of Membrane Technology. Springer International Publishing AG, 1996.

[25]. E. Drioli, M. Romano, "Progress and new perspectives on integrated membrane operations for sustainable industrial growth'', in Ind. Eng. Chem. Rev., **vol. 40**, Feb. 2001, pp.1277–1300.

[26]. M. Ulbricht, "Advanced functional polymer membrane'', in Polymer, **vol. 47**, March 2006, pp. 2217–2262.

[27]. A.M. Florea, T.V. Iordache, C. Branger, M. Ghiurea, S. Avramescu, G. Hubca, A Sarbu, "An innovative approach to prepare hypericin molecularly imprinted pearls using a "phyto-templat", in Talanta, **vol.148**, Feb. 2016, pp.37-45.

[28]. Riyanto and S.W. Nas, "Validation of Analytical Methods for Determination of Methamphetamine Using Fourier Transform Infrared (FTIR) Spectroscopy", in IOSR Journal of Pharmacy and Biological Sciences (IOSR-JPBS)**, Vol. II,** no. 5, Sep. 2016, pp. 51-59.

[29]. J. Yang, Y. Liu, J. Liu, Z. Shen, J. Liang, X. Wang, "Rapid and Continuous Preparation of Polyacrylonitrile-Based Carbon Fibers with Electron-Beam Irradiation Pretreatment", in Materials (Basel), **vol. 11**, Aug. 2018, pp. 1270.

[30]. A.M. Florea, T.V. Iordache, A. Zaharia, B. Georgescu, A.E. Voicu, B. Tsyntsarski, G. Hubca, A. Sarbu, "Evaluation of Molecularly Imprinted Polymer Pearls for Selective Isolation of Hypericins", in Materiale Plastice, **vol. 54**, Aug. 2017, pp. 495-501.