Talanta 94 (2012) 133–139

Contents lists available at SciVerse ScienceDirect

Talanta

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

Design of molecularly imprinted polymers for diphenylamine sensing

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ARTICLE INFO

Article history:
Received 21 December 2011
Received in revised form 28 February 2012
Accepted 5 March 2012
Available online 12 March 2012

Keywords:
Molecularly imprinted polymers
Potentiometric sensors
Diphenylamine

ABSTRACT

A series of the polymers imprinted with diphenylamine (DPA) and respective non imprinted polymers were synthesized using precipitation polymerization. Synthesized polymers were characterized by Fourier Transform Infra-Red spectroscopy with Total Attenuated Reflectance (FTIR–ATR), Scanning Electron Microscopy (SEM) and equilibrium batch re-binding experiments. Influence of the synthesis conditions, namely monomer/template ratio and reaction duration, on the polymer binding capacity and selectivity towards aromatic compounds was investigated. Binding behavior of MIP was described using Freundlich isotherm. Significance of the effects of the synthesis conditions on the polymer properties was evaluated using ANOVA. MIPs synthesized at different conditions, which displayed different properties (binding capacity and selectivity), and respective non-imprinted polymers were employed for the fabrication of the potentiometric sensors. While sensors prepared using imprinted polymers had higher sensitivity and selectivity compared to the ones containing non-imprinted polymer, no difference was observed between sensors containing different imprinted polymers. No correspondence between polymers' characteristics obtained in the equilibrium re-binding studies and potentiometric behavior of the sensors based on the same polymers was observed. Therefore, equilibrium re-binding studies cannot be used for predicting sensor behavior.

1. Introduction

Molecular imprinting has gained popularity during last decades as a technique of synthesizing polymer materials with chemically selective recognition sites [1–3]. Molecular imprinting consists in the polymerization of the monomer mixture in the presence of target molecule or template in the inert solvent. Prior to polymerization, template interacts with functional monomer or precursor in the solution and the structure of these pre-polymerization complexes is preserved by copolymerization in the presence of an excess amount of a cross-linker. After polymerization, the template is removed from the polymer matrix, thus leaving cavities or specific binding sites in the material. Interaction between template and monomer can be covalent and non-covalent and, consequently, molecular imprinting can be divided into covalent imprinting (pre-organized approach), and non-covalent imprinting (self-assembly approach). The latter approach is more widely used as it allows preparation of the imprinted polymers for virtually any type of substance. Successful polymer imprinting with inorganic ions, low molecular weight organic substances, proteins and even cells and viruses have been reported [1–5]. Such versatility in conjunction with high stability, low cost and easy preparation make molecular imprinting an attractive technique for the fabrication of ionofores for chemical sensing.

MIP performance may be influenced by a variety of factors including type and concentration of a monomer [6,7], template concentration [8], type of solvent [6,9] and synthesis conditions (temperature, pressure, reaction duration, etc.) [9–12]. Though significant progress has been achieved in the understanding of the effects of these factors on MIP properties, no general recommendations on MIP synthesis procedure were developed up to date. That means that optimization has to be done for each template individually. Optimization of the imprinted polymer compositions and synthesis conditions is usually done using combinatorial approach, which can be realized either computationally [13–15] or using semi-automated experimental protocols allowing synthesizing in parallel large number of polymers [6]. Despite being successful in practice, combinatorial approach does not add to the understanding of the physical mechanisms related to MIP formation and ligand recognition and obtained results usually cannot be generalized to the other templates. Only a few studies deal with the design of MIP specifically to be used in chemical sensors [16,17]. Limitations associated specifically with MIP use as active substances in chemical sensing, namely difficulties with integrating MIPs with

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doi:10.1016/j.talanta.2012.03.007

transducers and with transforming binding event into an analytical signal and poor performance of many MIPs in aqueous media, are also rarely addressed [18].

The purpose of the present study was optimization of synthesis conditions and preparation of MIP imprinted by diphenylamine (DPA), a scald inhibitor in apples and pears [19,20], and application of this MIP as an active substance for potentiometric chemical sensors. No reports on chemical sensors for DPA detection or polymers imprinted with DPA were found in the literature.

2. Experimental

2.1. Materials

Methacrylic acid monomer (MAA, 99%), trimethylolpropane trimethacrylate (TRIM, tech), 2,2′-azobis(2-methylpropionitrile) (AIBN), diphenylamine (99+%), 3,4-ethylenedioxythiophene (97%), tris(hydroxymetyl)aminometane (TRIS), phenol, catechin and acetonitrile (Chromasolv grade) were from Sigma–Aldrich. Ethanol and methanol (both for analysis grade) were from Merck. Nitric acid, potassium nitrate and sodium hydroxide were from Panreac. Aniline was from May and Baker Ltd., Dagenham, acetic acid (99.8+%) and magnesium perchlorate hydrate (for analysis grade) were from Riedel-de Haen. Ultrapure water was used throughout experiments.

2.2. Apparatus

Scanning electron microscopy (SEM) imaging was carried out on a SU-70 Hitachi Field Emission-Scanning Electron Microscope equipped with Schottky electron gun. Polymer microspheres were sputter coated with gold prior to the SEM measurement.

FTIR-ATR spectra were recorded using ABB MB3000 spectrometer in the wavenumber range of 4000–500 cm^{-1} with resolution of 4 cm^{-1} and acquiring 64 scans.

UV-spectra were recorded using Shimadzu UV-2101PC UV-Vis scanning spectrophotometer using respective solvent (methanol/acetic acid or ethanol/water mixture) as a blank.

Electropolymerization was done using potentiostat/glavanostat EZstat Pro (NuVant Systems Inc., IN, USA).

Potentiometric measurements were made using custom-made multichannel digital voltmeter with high input impedance, which was connected to the PC for data acquisition.

2.3. Polymer synthesis

A series of polymer microparticles imprinted with dipehnylamine and respective non imprinted polymers were synthesized by thermal precipitation polymerization. Methacrylic acid (MAA) was used as a monomer, trimethylolpropane trimethacrylate (TRIM) as a cross-linker, 2,2′-azobis(2-methylpropionitrile) as a catalyst and acetonitrile as a solvent. Amount of template and reaction time were varied with the aim to optimize polymer selectivity towards diphenylamine (Table 1). Synthetic conditions were adapted from [21]. Synthesis was carried out in the 50 ml glass reactor under nitrogen atmosphere with stirring. Forty millilitre of acetonitrile were placed in the reactor and 1.5 mmol of monomer MAA and varying amounts (see Table 1) of template diphenylamine were added. Mixture was left equilibrating for 15 min and 2 mmol of cross-linker TRIM and 0.5 mmol of initiator AIBN were added. Mixture was further degassed with nitrogen for 15 min, after which temperature was increased from 20 °C to 50 °C and maintained at 50 °C for the duration of the reaction (Table 1). After the end of the reaction polymer microparticles were collected by centrifugation. Template was extracted in Soxhlet using a mixture of methanol with acetic acid (9:1). Washing continued until no DPA could be detected by UV-spectrophotometer at 280 nm, which took ca. 12 h. Microparticles were dried at 50 °C until constant weight was achieved. The same conditions were used for the synthesis of non-imprinted polymer particles, except for the addition of the template. Non-imprinted polymer particles were collected by centrifugation, washed with acetonitrile and dried.

Table 1
Preparation conditions of diphenylamine imprinted polymers.

Polymer	Monomer/template ratio	Reaction time (h)
MIP1	7.5	10
MIP2	7.5	24
MIP3	4.3	17
MIP4	3	10
MIP5	3	24
MIP6	2	24
NIP1	–	10
NIP2	–	24

2.4. Re-binding experiments

Washed and dried polymer particles (1 mg) were soaked in 5 ml of ethanol (35%)/water (65%) solutions of diphenylamine, aniline, phenol and catechin. Diphenylamine concentrations varied from 0.01 to 1.2 mmol L^{-1} while concentrations of the other compounds were 0.1 mmol L^{-1}. Polymers were incubated for 24 h at 20 °C at static equilibrium. Remaining free concentration in the solution after incubation were measured using UV-spectrophotometer at 280 nm for diphenylamine, 230 nm for aniline, 329 nm for chlorogenic acid, 210 nm for phenol and 212 nm for catechin. At least three replicate experiments were run. Obtained values of free and bound diphenylamine were used for calculating binding capacity, polymer binding parameters and selectivity coefficients. Binding capacity Q was calculated using the following expression:

$$Q = \frac{m(\text{DPA}_{\text{bound}})}{m_{\text{MIP}}} = \frac{(C_i - C_f)V_s}{m_{\text{MIP}}},$$

where C_i is the initial diphenylamine concentration (mmol L^{-1}), C_f is the final diphenylamine concentration (mmol L^{-1}), V_s is the solution volume (L) and m_{MIP} is the polymer mass (g).

Binding isotherms were fitted using Freundlich equations [22,23]:

$$B = aF^m,$$

where B is the amount of bound DPA per gram of polymer ($\mu\text{mol g}^{-1}$), F is the concentration of free DPA in solution (mmol L^{-1}), a is the Freundlich parameter related to the binding affinity ($\mu\text{mol g}^{-1}/\text{mmol L}^{-1}$), and m is the heterogeneity index.

Selectivity coefficients of the polymer nanoparticles towards diphenylamine compared to the other phenolic compounds were calculated using the following equation:

$$K_{\text{DPA/Int}} = \frac{[\text{DPA}_{\text{bound}}][\text{Int}_{\text{free}}]}{[\text{DPA}_{\text{free}}][\text{Int}_{\text{bound}}]},$$

where $[\text{DPA}_{\text{bound}}]$ and $[\text{DPA}_{\text{free}}]$, mmol L^{-1}, are bound to the polymer and free concentrations of diphenylamine and $[\text{Int}_{\text{bound}}]$ and $[\text{Int}_{\text{free}}]$, mmol L^{-1}, and bound to the polymer and free concentrations of the interferents, which were aniline, catechin, phenol and chlorogenic acid. Data from the re-binding experiments with the total concentration of DPA or interferent of 0.1 mmol L^{-1} were used for the calculations.

Significance of the effects of the monomer/template ratio and reaction duration on the binding capacity and selectivity of the polymers was evaluated using two-way ANOVA with interaction.

2.5. Sensor preparation

Sensor membranes containing MIP particles were prepared by incorporating MIP particles into electropolymerized poly-3,4-ethylenedioxythiophene (PEDOT). Membranes were deposited on the surface of the platinum electrode fabricated using EasyCon kit (Autolab B.V.). Prior to the membrane deposition surface of the platinum electrode was polished with 4000 grit abrasive paper followed by 6 μm diamond polish, thoroughly washed with ethanol and ultrapure water and dried.

Electrochemical polymerization was performed by using a one-compartment, three electrode electrochemical cell. The working electrode was a Pt disc electrode (area 0.126 cm^2), the auxiliary electrode was a glassy carbon and the reference electrode was a Ag/AgCl/KCl (3 mol L^{-1}) electrode. The cell solution was initially purged with nitrogen, and all experiments were performed under a nitrogen atmosphere, at room temperature (20 °C). PEDOT film was deposited on the Pt electrode by electrochemical polymerization at 1.2 V until 15 mC charge was passed. Film thickness can be estimated to be ca. 0.8 μm assuming 2.25 electrons per monomer and PEDOT film density of 1 g cm^{-3} [24]. Electrosynthesis solution contained 0.01 mol L^{-1} of 3,4-ethylenedioxythiophene (EDOT) and 0.1 mol L^{-1} of $Mg(ClO_4)_2$ dissolved in acetonitrile. MIP or NIP particles (10 mg) were added to 5 ml of the electrosynthesis solution and dispersed by sonification during 5 min prior to the electropolymerization. Membranes containing NIP1, MIP1 or MIP5 particles and no particles were prepared. At least three electrodes of each composition were assembled.

2.6. Electrochemical measurements

Sensors with modified PEDOT membranes were used for potentiometric measurements, which were carried out in the following galvanic cell:

$$\text{Ag}|\text{AgCl, KCl}(3\,\text{mol L}^{-1})|\text{sample}|\text{PEDOT}|\text{Pt}$$

Emf values were measured vs. Ag/AgCl reference electrode with precision of 0.1 mV, using custom made multichannel voltmeter with high input impedance connected to the PC for the data acquisition and processing. Sensitivity of the sensors was evaluated by means of the calibration measurements in the DPA solutions in the concentration range from 10^{-7} to 5×10^{-4} mol L^{-1} at pH 2, 4, 6 and 9. Solution of KNO_3 with concentration 0.1 mol L^{-1} was used as a supporting electrolyte at pH 6 and 4, for the latter pH was adjusted to 4 by addition of the nitric acid. Solutions of nitric acid and TRIS with concentrations 0.01 mol L^{-1} were used as supporting electrolytes at pH 2 and 9, respectively. Calibration measurements in DPA solutions at pH 4 were repeated each 5 days during 1 month with the aim to assess sensor lifetime. Response to H^+ was evaluated between pH 2 and 11. Measurements were started in 0.01 mol L^{-1} solution of nitric acid with pH 2 and pH was progressively changed by the additions of NaOH solution till pH 11 was reached. At least 3 replicated calibration measurements were made. After measurements sensors were washed with copious amounts of ultrapure water. Sensors were stored in the ultrapure water between measurements. Parameters of the Nernst equation i.e. slope of the electrode function and standard potential were calculated using linear regression and averaged over replicated calibration runs, performed for each sensor compositions.

Selectivity towards aniline, phenol and catechin were determined using fixed interference method [25]. Concentrations of the interfering ions were 0.5 mol L^{-1} for phenol, 0.05 mol L^{-1} for aniline and 0.005 mol L^{-1} for catechin. Potentiometric selectivity coefficients were calculated using following equation:

$$K^{\text{pot}}_{\text{DPA/Int}} = \frac{a_{\text{DPA}}}{a_{\text{Int}}^{z_{\text{DPA}}/z_{\text{Int}}}},$$

where a_{DPA} and a_{Int} are activities (mol L^{-1}), and z_{DPA} and z_{Int} are charges of diphenylamine and interferent, respectively.

3. Results and discussion

3.1. Re-binding performance and selectivity of MIP particles

Molecular imprinting consists in the synthesis of the highly cross-linked polymer in the presence of a template. Template removal leaves in the polymer binding cavities or sites with affinity to the template molecule. However, imprinted polymers are not homogeneous and contain a range of binding sites, both selective and non-selective, which affects the affinity of the material. This is a result of the complex nature of the formation of cross-linked polymers, which can be influenced by several physical factors often interrelated [9]. In the present study two of them, namely monomer/template ratio and reaction duration, were varied with the aim to optimize synthesis conditions, using selectivity as optimization criteria. It is consensual that prolonged reaction times lead to a higher degree of cross-linkage and, consequently, to more rigid polymers with better defined imprinting cavities, which results in higher specificity [9]. However, increase of the polymer rigidness results in the hindered mass transfer and slow binding kinetics. Optimal duration of the polymerization reaction should be established empirically for each polymerization system. Another parameter which is known to affect the polymerization outcome, although the nature of this effect is not well understood, is monomer/template ratio. Dependence of the polymer specificity on the template concentration, which can be predicted theoretically on the basis of the stoichiometry of the pre-polymerization template/monomer complex, is not always observed in practice [26]. Influence of the interactions taking place during polymerization process on the binding sites' structure was suggested as an explanation for the observed deviation from the theory. Therefore, as actual events determining binding sites structure remain unknown, optimal template/monomer ratio for the given system should also be found experimentally.

In the present study influence of the conditions namely template concentration and reaction duration on the MIP properties was assessed. Properties of the MIPs that were considered were biding capacity and selectivity obtained from the equilibrium re-binding studies. Binding isotherms for all studied polymers are shown in Fig. 1. Binding capacity was calculated for the maximum concentration of DPA used, which was 1.2 mmol L^{-1} (Table 2). Experimental data were fitted using Freundlich model, which was demonstrated to be generally applicable to non-covalently imprinted polymers in the intermediate concentration range [22]. Freundlich equation parameters, heterogeneity index m and pre-exponential factor a, are shown in Table 2. R^2 of the fit was 0.99 for all polymers. Both of the Freundlich model parameters have a direct physical meaning. The pre-exponential factor a is a measure of the binding capacity, i.e. number of binding sites and average affinity. Heterogeneity index m describes binding sites properties and takes values from zero to one, with one corresponding to the homogeneous and values approaching zero to increasingly heterogeneous binding sites.

Significance of the effect of the template concentration, reaction time and their interaction on Q, a and m was assessed using ANOVA (Table 3). Both effects and their interaction were significant for binding capacity, effect of template concentration being the largest followed by the interaction effect. Contour plot of the

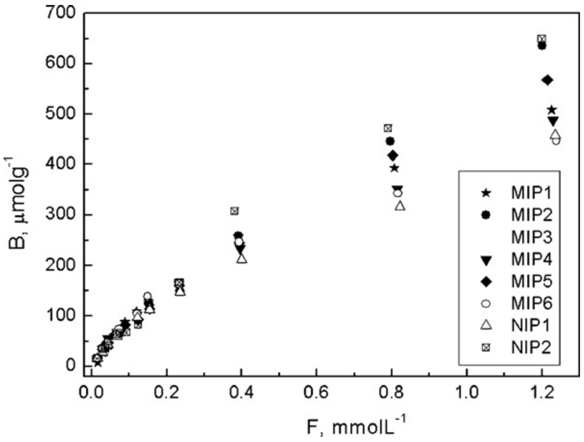

Fig. 1. Binding isotherms for synthesized polymers (see Table 1 for codes). F is the free DPA concentration in solution (mmol L^{-1}); B is the amount of DPA bound per 1 g of the polymer (μmol g^{-1}).

Table 2
Binding capacity and binding characteristics calculated using Freundlich isotherm of diphenylamine imprinted and respective non-imprinted polymers. Standard deviations ($n = 3$) are shown in parentheses.

Polymer	Q (μmol g^{-1})	a (μmol g^{-1}/mmol L^{-1})	m
MIP1	488(12)	420(5)	0.67(0.02)
MIP2	517(8)	451(8)	0.69(0.02)
MIP3	648(3)	567(11)	0.81(0.03)
MIP4	479(6)	430(7)	0.75(0.02
MIP5	636(7)	536(10)	0.81(0.02)
MIP6	555(3)	492(2)	0.76(0.01)
NIP1	460(8)	396(8)	0.62(0.02)
NIP2	458(6)	387(6)	0.69(0.02)

interaction effect (Fig. 2) shows that dependence of the binding capacity on the reaction duration and template concentration was non-linear with maximum at the middle level of both parameters. This non-linearity was confirmed by the synthesizing polymer with template concentration higher than MIP5–MIP6. MIP6 was found to have binding capacity lower than MIP5.

Effect of template concentration and interaction were significant for the pre-exponential factor, which increased concurrently with the increase of template concentration, this effect being more pronounced at longer reaction time. None of the studied experimental factors had significant effect on the heterogeneity of the polymer binding sites.

Selectivity of the polymers was assessed towards substances with similar structure to the template (aniline) and phenolic compounds (catechin, chlorogenic acid and phenol). Selectivity coefficients are shown in the Table 4. According to ANOVA (Table 3), effect of the template concentration was significant for the selectivity to all substances except catechin, effect of reaction time was significant for the selectivity to all substances except aniline and the interaction effect was significant for the selectivity to all four compounds. As contour plots of the interaction effects show (Fig. 3a–d), relationship between the selectivity and synthetic conditions is nonlinear and dependent on the interferent. In the case of aniline, template concentration had largest effect on the polymer selectivity, which was highest at the lower template load, independently on the reaction duration (Fig. 3a). On the contrary, selectivity towards chlorogenic acid and phenol was highest for longer reaction time and did not depend on the template load (Fig. 3c and d). Dependence of the selectivity towards catechin on the synthesis conditions was more complex, with maximum selectivity observed for the polymer with highest template load and shorter reaction times, followed by the polymers with monomer/template ratio of 7.5 independently of the reaction time. No clear conclusion can be made about optimal reaction duration or template concentration on the basis of selectivity data. Two imprinted polymers, MIP1 and MIP5, which displayed highest selectivity towards aniline and chlorogenic acid, respectively, were selected for sensor preparation. Also sensor containing the non-imprinted polymer NIP1 and sensor without any polymer were prepared for the comparison.

Table 3
Sources of variation in the ANOVA models for binding capacity (Q), Freundlich equation parameters (a and m) and selectivity coefficients to aniline ($K_{DPA/An}$), catechin ($K_{DPA/Cat}$), chlorogenic acid ($K_{DPA/CA}$) and phenol ($K_{DPA/Ph}$). Significant sources of variation ($p < 0.05$) are marked in bold. Ratio is monomer/template ratio, time is reaction duration.

Parameters	p-Values		
Effects	Ratio	Time	Conc. × time
Q	**0.0002**	**0.000**	**0.0002**
a	**0.0009**	0.098	**<0.0001**
m	0.061	0.488	0.050
$K_{DPA/An}$	**<0.0001**	0.821	**<0.0001**
$K_{DPA/Cat}$	0.157	**0.005**	**<0.0001**
$K_{DPA/CA}$	**0.038**	**0.002**	**<0.0001**
$K_{DPA/Ph}$	**0.014**	**0.013**	**<0.0001**

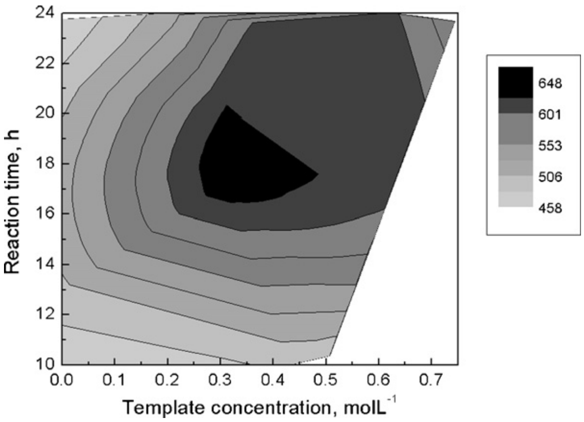

Fig. 2. Contour plot of binding capacity of polymer nanoparticles towards DPA vs. template concentration and reaction time.

3.2. Characterization of MIP particles

In the present study MIPs were synthesized using precipitation polymerization, which produces polymer particles with sizes from hundreds of nanometers to several microns [27–29]. Commonly MIPs are prepared in the form of macroporous monolith, which is subsequently grinded and sieved to obtain particle of desired size. Besides being laborious and have low yield, grinding process produces particles of irregular shape and may be detrimental to the some of the binding sites [28]. Precipitation polymerization has the advantage of producing regular shaped polymer particles, which do

Table 4
Selectivity coefficients $K_{DPA,X}$ of diphenylamine imprinted and respective non-imprinted polymers towards phenol and its derivatives. Standard deviations are shown in parentheses.

Polymer	Aniline	Catechin	Chlorogenic acid	Phenol
MIP1	96 (11)	19 (1)	14 (2)	9 (1)
MIP2	92 (2)	12 (5)	170 (18)	20 (2)
MIP3	59 (16)	4.4 (0.5)	44 (5)	7 (1)
MIP4	52 (4)	34 (1)	31 (4)	6 (1)
MIP5	23 (2)	9.3 (0.7)	193 (20)	14 (2)
MIP6	20 (1)	12 (2)	92 (5)	23 (1)
NIP1	3 (1)	10 (1)	10 (1)	7 (1)
NIP2	7 (2)	6.0 (0.5)	9 (1)	6 (1)

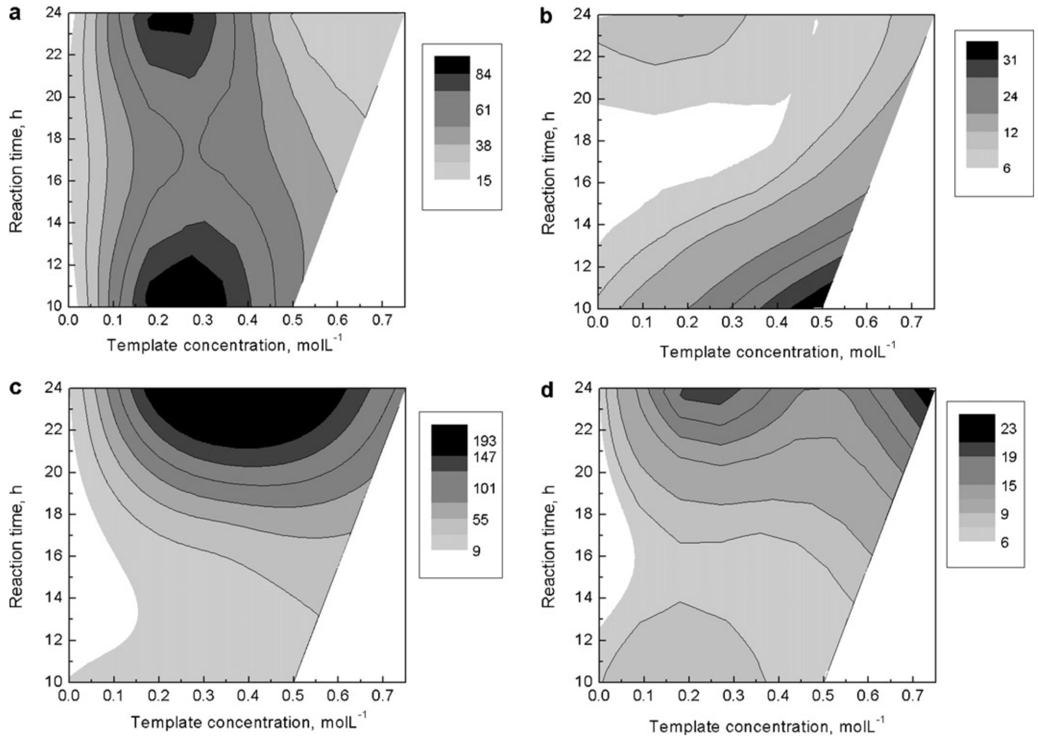

Fig. 3. Contour plots of selectivity coefficients of the polymers towards (a) aniline, (b) catechin, (c) chlorogenic acid and (d) phenol vs. template concentration and reaction time.

not require grinding and can be directly used for the preparation of the sensing membranes.

SEM micrographs (Fig. 4) show size and morphology of the impressed with diphenylamine and non-impressed polymer particles synthesized under different experimental conditions (Table 1). Relatively uniform size distribution of the particles was obtained for each set of the experimental conditions. Size of the imprinted polymer particles varied between 200 and 800 nm while non-imprinted particles were 5–10 times bigger – 1.8 and 2.5 μm for 10 and 24 h of polymerization, respectively. There is a certain disagreement in the literature concerning the influence of the template on the particle size, with some authors reporting no effect at all [21], while others observing the non-imprinted polymer particles to be bigger or more monodisperse compared to the imprinted ones [27,28]. MIPs are macroporous polymers, whose growing start from the nuclei formed around the initiator, which grow to 10–30 nm aggregates to form microspheres, that aggregates into larger clusters, etc. [26]. In this particular case, the presence of the template molecule, diphenylamine, affected polymerization process by slowing down particles growth and aggregation.

The FTIR-ATR spectra of MAA, TRIM, diphenylamine and one of the imprinted polymers (MIP4) are shown in Fig. 5. Band at 1635 cm^{-1} assigned to the carbon double bond stretch, which is clearly seen in the spectra of MAA and TRIM, is practically absent in the spectra of the imprinted polymer, indicating low content of unreacted double bonds. The intensity of this band was about the same in all synthesized polymers, both imprinted and non-imprinted (data not shown), which allows to conclude that similar polymerization degree was obtained in all cases. The band at around 1700 cm^{-1}, corresponding to the C═O stretch of the carboxylic acid, is observed in the spectra of MAA, TRIM and MIP4. However, exact position of this band differs, being at 1691 cm^{-1} for MAA and at 1725 cm^{-1} for MIP4, which can be assigned to the hydrogen-bonded carboxylic dimmers and “free” carboxylic group, respectively [30,31]. This band in the MIP4 spectra larger and less symmetric compared to the MAA spectra. We can hypothesize that carboxylic groups in the MIP are mostly “free”, although some dimers are also present. No differences in the position of this band were observed in different MIPs and NIPs. Bands characteristic for aromatic structure and secondary amine group, namely band at 1588 cm^{-1} and 1485 cm^{-1}, assigned to the aromatic ring stretch, at 3042 cm^{-1}, assigned to C–H stretch, at 744 cm^{-1} and 687 cm^{-1}, assigned to the aromatic C–H out-of-plane bend, and at 3381 cm^{-1}, assigned to the secondary amine aromatic N–H stretch, have been observed in the spectra of diphenylamine, None of these bands is present in the spectra of the imprinted polymer, indicating that diphenylamine did not participate in the polymerization and that it was completely removed in the polymer washing step.

Similarity between FTIR-ATR spectra of all MIPs and NIPs indicate that different experimental conditions and even the presence of the template did not affect polymer chemical composition. They did essentially affect the process of polymer nuclei aggregation, as was evidenced by SEM images resulting in different polymer morphologies.

3.3. Potentiometric detection of diphenylamine

Sensitivity of the sensors based on two imprinted (MIP1 and MIP5) and one non-imprinted (NIP1) polymer in solutions of diphenylamine at different pH are depicted in Fig. 6. Sensors containing imprinted polymers displayed anionic response of 54–58 mV/pX at pH 2 and 4, which is close to the Nernstian for the single charged ion. No deterioration of the sensor characteristics in the DPA solutions at pH 4 was observed after 1 month. Standard deviations of the slope of the electrode function were 1.5 and 2 mV/pX for the sensors MIP1 and MIP5, respectively. Sensors containing non-imprinted polymer and with pure PEDOT membrane did not display any response to diphenylamine. Sensitivity of all sensors decreased with the increase of pH, diminishing ca. 1.5 times at pH 6 and disappearing at pH 9. Sensor with PEDOT membrane without polymer particles did not display any response to diphenylamine. Sensors containing imprinted and non-imprinted

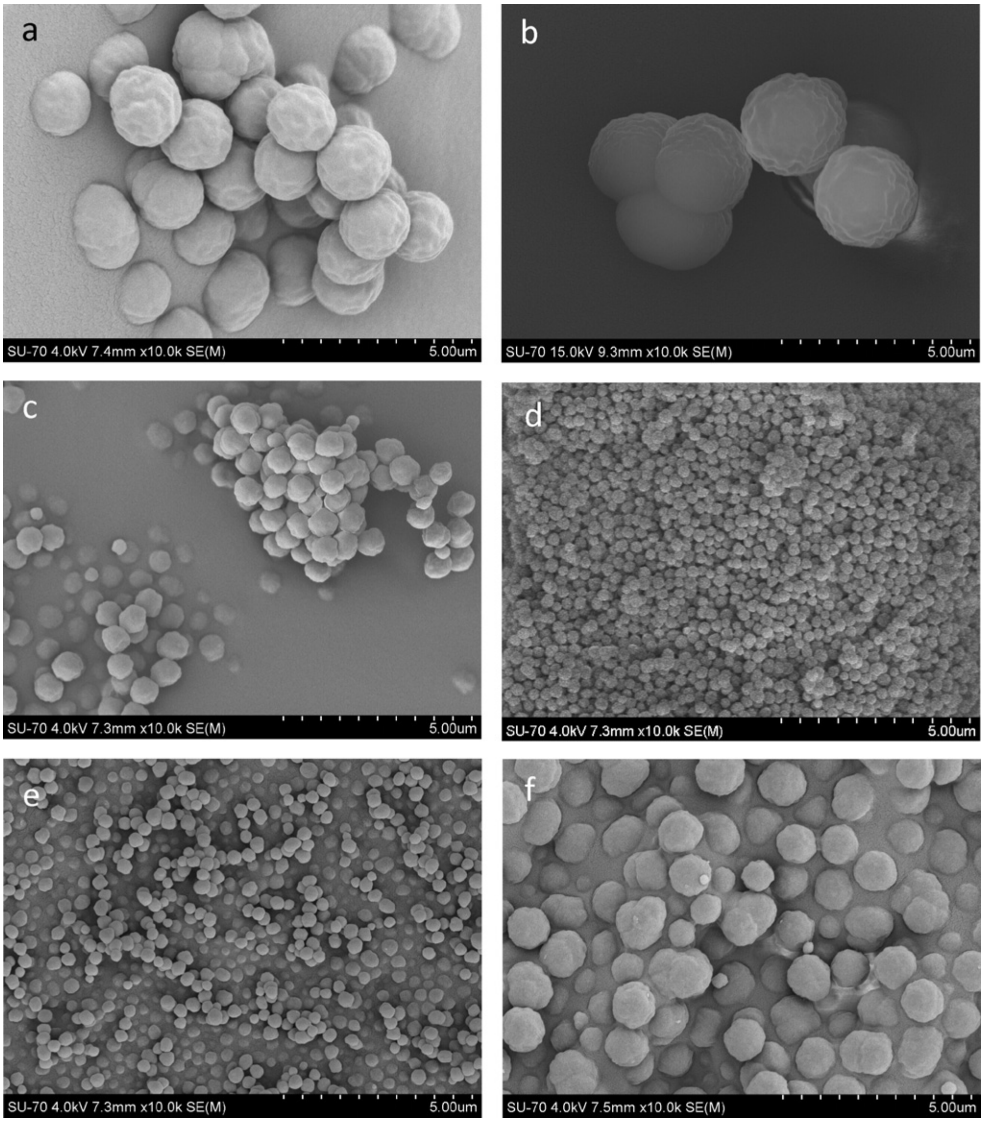

Fig. 4. SEM micrographs of (a) NIP1, (b) NIP2, (c) MIP1, (d) MIP2, (e) MIP4, and (f) MIP6.

polymers and no polymer at all displayed very low sensitivity to H^+, with values of 8 ± 2 mV/pH in the pH range from 2 to 11. As this pH response was the same for all sensors including the one with pure PEDOT membranes, this low pH sensitivity can be attributed to the conducting polymer itself.

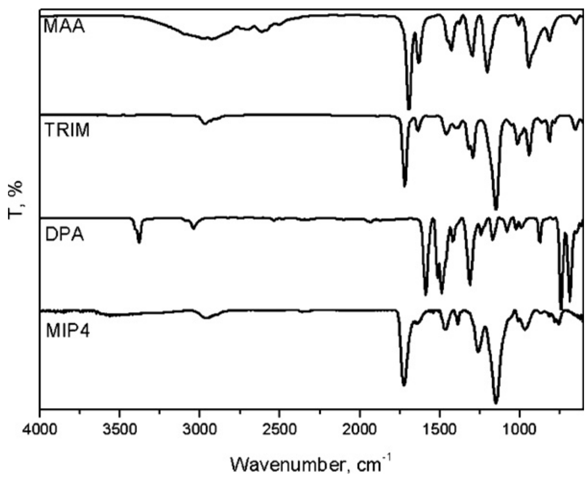

Fig. 5. FTIR-ATR spectra of monomer (MMA), cross-linker (TRIM), template (DPA) and imprinted polymer MIP5.

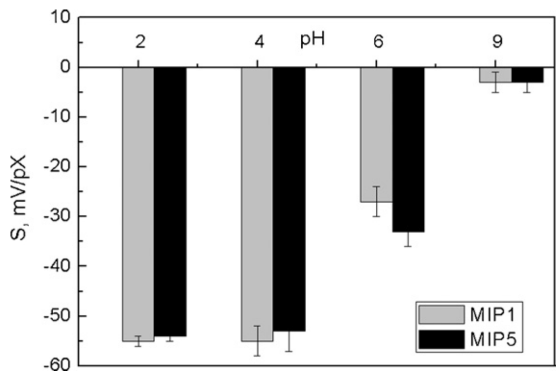

Fig. 6. Sensitivity (slope of the electrode function) to DPA of the sensors with imprinted (MIP1 and MIP5) and non-imprinted (NIP1) polymers at different pH levels.

Table 5
Selectivity coefficients p$K_{\mathrm{DPA,X}}$ of the potentiometric sensors based on diphenylamine imprinted and non-imprinted polymers towards phenol, aniline and catechin.

Polymer	Aniline	Catechin	Phenol
MIP1	−2.14	<−2	<−3.9
MIP5	−2.11	<−2	<−3.9

Observed anionic response to diphenylamine can be explained by the very low basicity of diphenylamine, which has pK_a of 1.03 [19] and therefore is present in the anionic form at all studied pH. Methacrylic acid is a weaker acid than diphenylamine and its pK_a further increases upon polymerization to 6.7 [18]. Thus, at pH 2 and 4 carboxylic groups in the polymer are not dissociated, at pH 6 ca. 17% are dissociated and at pH 9 ca. 67%. Thus, the decrease of the sensor response to diphenylamine at pH 6 and 9 can be explained by the electrostatic repulsing of anion and negatively charged carboxylic groups of the polymer.

Sensor selectivity to phenol and phenolic compounds, aniline and catechin, was estimated using fixed interference method. The resulting values of $\log K^{\mathrm{pot}}_{\mathrm{DPA,X}}$ are presented in Table 5. Sensors containing imprinted polymers displayed high selectivity to DPA in the presence of all studied compounds. Presence of 0.5 mol L^{-1} of phenol and 0.005 mol L^{-1} of catechin did not interfere with the responses of both MIP1 and MIP5 sensors. To the contrary to the binding study, there was no difference in potentiometric selectivity between sensors containing impressed polymers MIP1 and MIP5. Moreover, there was no correspondence between selectivity coefficients determined in the re-binding studies and potentiometric measurements. For example, polymers displayed the highest selectivity to aniline according to the re-binding studies while potentiometric selectivity to this compound was the lowest. It was reported in the literature that results of the equilibrium re-binding studies could not be used for the prediction of the polymer selectivity in the chromatographic mode [6]. The similar conclusion can be made for the potentiometric selectivity of the sensors with membranes containing MIPs as no correlation between potentiometric and equilibrium re-binding data was observed.

4. Conclusion

Optimization of the synthetic conditions for the polymer imprinted with diphenylamine was carried out using as optimization criteria binding capacity and selectivity calculated from equilibrium re-binding data. Effects of both template concentration and reaction duration and their interaction were significant for binding capacity. Dependence of the binding capacity on these two parameters was non-linear with the highest binding capacity was achieved for the polymer with template/monomer ratio of 4.3 and reaction duration 17 h. Effect of template concentration and template concentration/reaction duration interaction were significant for the pre-exponential factor of the Freundlich isotherm, which characterized binding capacity and number of binding sites of the polymer. None of the studied experimental factors had significant effect on the other Freundlich parameter – heterogeneity. Relationship between selectivity coefficients calculated from equilibrium re-binding studies and synthetic conditions was found to be nonlinear and dependent on the interferent. Effect of template concentration was significant for the selectivity to all substances except catechin, effect of reaction time was significant for the selectivity to all substances except aniline and the interaction effect was significant for the selectivity to all four compounds. Variation of the synthesis conditions did not affect polymerization degree and chemical compositions of the polymers as was evidenced by the FTIR-ATR spectra. Difference in the polymer particle morphology namely particle size were observed using SEM, non-imprinted polymers forming particles 5–10 times bigger compared to the imprinted ones.

MIPs synthesized at different conditions with different properties (binding capacity and selectivity) were selected for the preparation of the potentiometric sensors. However, no difference in potentiometric sensitivity and selectivity towards DPA was observed for the sensors containing different imprinted polymers. Also, no correspondence between selectivity or other parameters from re-binding studies and potentiometric selectivity was found, which indicates a need to develop other approaches to MIP characterization.

Acknowledgments

Financial support of Portuguese Science and Technology Foundation, through the European Social Fund (ESF) and “Programa Operacional Potencial Humano – POPH” is gratefully acknowledged.

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