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Design of molecularly imprinted polymers for diphenylamine sensing

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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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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:

$$Q = \frac{m(\mathrm{DPA_{bound}})}{m_{\mathrm{MIP}}} = \frac{(C_i - C_f)V_s}{m_{\mathrm{MIP}}},$$

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 (μmol g^{-1}), F is the concentration of free DPA in solution (mmol L^{-1}), a is the Freundlich parameter related to the binding affinity (μmol g^{-1}/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:

$$K_{\mathrm{DPA/Int}} = \frac{[\mathrm{DPA_{bound}}][\mathrm{Int_{free}}]}{[\mathrm{DPA_{free}}][\mathrm{Int_{bound}}]},$$

where $[\mathrm{DPA_{bound}}]$ and $[\mathrm{DPA_{free}}]$, mmol L^{-1}, are bound to the polymer and free concentrations of diphenylamine and $[\mathrm{Int_{bound}}]$ and $[\mathrm{Int_{free}}]$, 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.